Pergamon

Tetrahedron, Vol. 52, No. 16, pp. 5845-5856, 1996

0040-4020/96 $15.00 + 0.00

S0040-4020(96)00214-1

A NEW METHOD FOR THE SYNTHESIS OF 2-GLYCOSYLAMINO PYRIDINES.[1]

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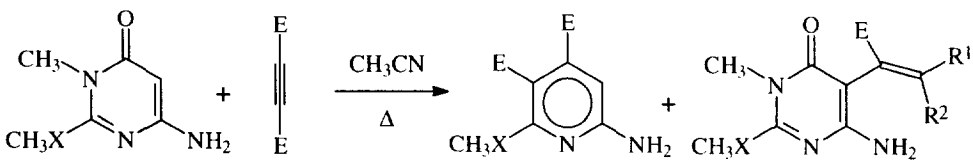
Abstract: Several 2-glycosylaminopyridines **7**, were synthesized through a tandem Diels-Alder/Retro Diels-Alder (DA/RDA) reaction starting from 6-glycosylaminopyrimidines, **6**, with dimethyl acetylenedicarboxylate (DMAD) as dienophile. This approach represents a new method for the synthesis of 2-aminopyridine nucleoside analogues. The compounds were tested for their antiviral activity but did not prove active.

INTRODUCTION

In a previous paper[2], we reported a study on the reactivity of several 6-aminopyrimidin-4-(3H)-ones **1a-d** towards DMAD, resulting in 2-aminopyridines **3a,b** as main products, which were formed from DA/RDA and 6-amino-5-(1',2'-dicarbomethoxyethenyl)pyrimidin-4(3H)-ones **4a,b** and **5a,b** by Michael addition (see Scheme 1).



1a X=O
1b X=S

2

3a X=O (79%)
3b X=S (64%)

4a,b: R^1 = E; R^2 = H
5a,b: R^1 = H; R^2 = E

Scheme 1

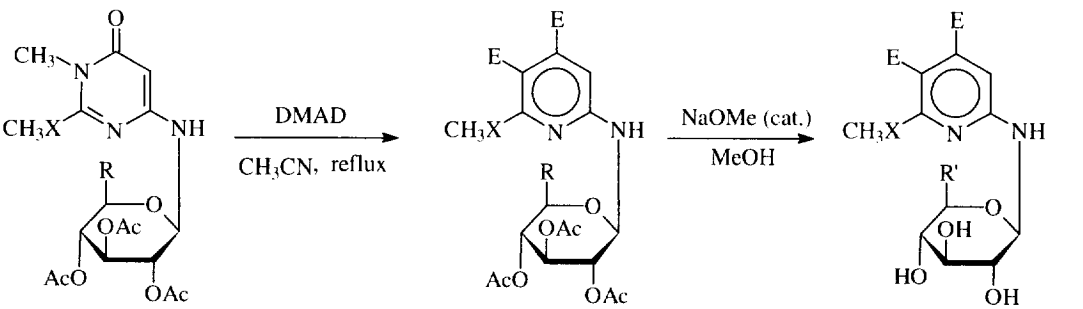
That study revealed the importance of blocking the N(3)-position at the pyrimidine ring to improve the yields of the products formed in the DA/RDA reaction, as well as the importance of using a polar and aprotic solvent such as acetonitrile.

Herein, we present the extension of the above method, using the optimal conditions to obtain DA products in those reactions, that is, pyridine nucleosides, for the first time through pyrimidine-pyridine transformation by DA/RDA. In addition, we include the antiviral results of some of these new pyridine nucleosides. Their biological interest is evident, because they are deazapyrimidine analogues, or deazapurine precursors, which have been used in a great variety of biological studies.[3]

The chosen nucleosidic derivatives were 6-(per-O-acetyl-(or per-O-methyl)-D-glycosyl)amino-3-methylpyrimidin-4(3H)-ones. In these compounds the glycoside moieties were attached to an exocyclic amino group to maintain the electronic characteristics of the 2-azadienic system in the heterocyclic moiety. The glycoside moieties were protected at their hydroxyl groups to improve the solubility in the reaction medium and to avoid undesired reactions of those groups.

RESULTS AND DISCUSSION

Therefore, the reactions of several xylo and glucopyranosyl aminopyridimines **6** with DMAD in acetonitrile under reflux were accomplished, affording, after chromatographic purification, good to excellent yield of glycosylaminopyridines **7**.[4] These compounds were deacetylated using sodium methoxide as catalyst to afford **8** (see Scheme 2 and Table1).



6	X	R
a	O	H
b	O	CH_2OAc
c	S	H
d	S	CH_2OAc

7	X	R
a	O	H
b	O	CH_2OAc
c	S	H
d	S	CH_2OAc

8	X	R'
a	O	H
b	O	CH_2OH
c	S	H
d	S	CH_2OH

Scheme 2

Table 1: Analytical and UV spectroscopic data

Comp.	react. time	Yield %	Mp °C	$[\alpha]^{20}_D$ Solvent (g/100 ml)	M.F.[a]	UV λ_{max} (log ε) solvent
7a	7 h	85	164	28 MeOH (0.1)	$C_{21}H_{26}N_2O_{12}$	251.5 (4.00) 318.0 (3.91) MeOH
7b	21 h	88.5	102	28 MeOH (0.1)	$C_{24}H_{30}N_2O_{14}$	251.5 (4.02) 318.0 (3.94) MeOH
7c	22 h	76	158	-63 MeOH (0.1)	$C_{21}H_{26}N_2O_{11}S$	242.2 (4.15) 279.5 (4.02) 327.7 (3.88) MeOH
7d	39 h	82	124	-44 MeOH (0.1)	$C_{24}H_{30}N_2O_{13}S$	241.4 (4.15) 279.5 (4.02) 327.7 (3.87) MeOH
7e	21 h	80	-[b]	-	-[b]	253.2 (4.02) 319.5 (3.94) MeOH
7f	21 h	64	-[b]	-	-[b]	253.9 (4.04) 319.5 (3.92) MeOH
8a	0.5 h	83	110	-13.5 H_2O (1)	$C_{15}H_{20}N_2O_9$ 1 1/3 H_2O	254.3 (4.065) 318.5 (4.01) MeOH
8b	1 h	81	184	-87 H_2O (1)	$C_{16}H_{22}N_2O_{10}$	254.3 (4.06) 318.3 (4.02) MeOH
8c	1 h	80	-[b]	7.5 DMSO (1)	-[b]	242.5 (4.15) 282.0 (4.03) 327.3 (3.895) MeOH
8d	2 h	79	127	127 H_2O (0.5)	$C_{16}H_{22}N_2O_9S$	243.0 (4.13) 282.0 (4.02) 327.5 (3.89) MeOH

a. Determined by elemental analysis and MS spectrum.
b. The Mp and M.F. could not be determined because of they were obtained as an oil.

Through ^{1}H-NMR (see Table 2), we detected similar signals for **7a-d** as for pyridine derivatives **3a,b**, and the maintenance of the chemical shift for the protons of the glycoside moiety for **6a-d**.[5] In DMSO-d_6, in most cases we could measure the coupling constant for $J_{1'-2'}$ after D_2O exchange. Those constants around 9 Hz agree with the ß-configuration in pyranosyl derivatives, which was afterwards confirmed by single crystal x-ray diffraction analysis[6] of the xylosyl derivatives **7a** and **7c**, supporting that the glycoside moiety was not involved in this type of reaction.

After obtaining these good results with compounds **7a-d**, we carried out the reaction of DMAD with other glycosidic derivatives such as per-O-acetylribofuranosyl and per-O-methylxylopyranoside derivatives, to prove the generality of these reactions with different sugar rings or protective groups.

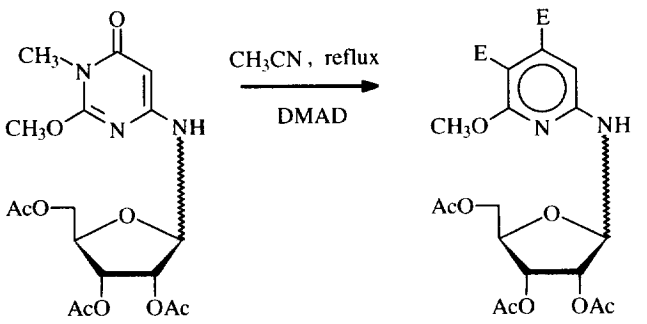
Table 2: ^{1}H- and ^{13}C-NMR data.

Comp.	Solvent	$\delta_{HN\text{-}2'}$ [a] ($J_{HN,1'}$)	$\delta_{H\text{-}3}$	$\delta_{H3'X'\text{-}6}$	$\delta_{H\text{-}1'}$ ($J_{1'\text{-}2'}$)	$\delta_{C\text{-}6'}$	$\delta_{C\text{-}5'}$	$\delta_{C\text{-}1'}$	$\delta_{C\text{-}3}$	$\delta_{C\text{-}5}$	$\delta_{C\text{-}4}$	$\delta_{C\text{-}2/C\text{-}6}$
7a	$CDCl_3$	5.9 d (9 Hz)	6.5 s	3.95 s	--	--	--	--	--	--	--	--
	DMSO-d_6	8.1 d (9.6 Hz)	6.5 s	3.92 s	5.9-5.5 m	--	63.2 t	79.9 d	99.7 d	103.2 s	142.3 s	157.4 s 160.55 s
7b	$CDCl_3$	5.9 d (9.3 Hz)	6.5 s	4.0 s	--	62.1 t	70.7 d	81.2 d	99.6 d	106.3 s	141.9 s	155.8 161.1 s
	DMSO-d_6	8.1 d (9.3 Hz)	6.5 s	3.9 s	5.7 pt + D_2O, d, (9.5 Hz)	--	--	--	--	--	--	--
7c	$CDCl_3$	5.9 d (8.9 Hz)	6.4 s	2.5 s	--	--	64.1 t	81.4 d	102.7 d	114.6 s	142.6 s	156.1 s 161.2 s
	DMSO-d_6	8.3 d (9.5 Hz)	6.6 s	2.5 s	5.9-5.5 pt + D_2O, d, (8.9 Hz)	--	--	--	--	--	--	--
7d	$CDCl_3$	6.0 d (9 Hz)	6.4 s	2.5 s	--	62.2 t	70.7 d	81.0 d	102.85 d	114.6 s	142.7 s	155.9 s 161.15 s
	DMSO-d_6	8.3 d (9.2 Hz)	6.6 s	2.5 s	5.8 pt + D_2O, d, (8.6 Hz)	--	--	--	--	--	--	--
7e	DMSO-d_6	8.3 d (9.2 Hz)	6.5 s	3.85 s	5.8 dd + D_2O, d (4.8 Hz)	--	--	--	--	--	--	--
7f	DMSO-d_6	7.6 d (8.9 Hz)	6.7 s	3.85 s	6.4 dd + D_2O, d (4.1 Hz)	--	--	--	--	--	--	--
8a	DMSO-d_6	7.9 d (8.2 Hz)	6.45 s	3.8 s	[b] + D_2O 4.9, dm (6.8 Hz)	60.8 t	72.5 d	82.0 d	99.0 d	101.2 s	142.3 s	158.05 s 160.5 s
8b	DMSO-d_6	7.9 d (8.2 Hz)	6.45 s	3.8 s	[b] + D_2O 4.9, m	--	--	--	--	--	--	--
8c	DMSO-d_6	8.1 d (8.3 Hz)	6.48 s	2.42 s	[b] + D_2O 5.05, m	--	--	--	--	--	--	--
8d	DMSO-d_6	8.1 d (8.9 Hz)	6.45 s	2.35 s	[b] + D_2O 5.0, m	--	--	--	--	--	--	--

a. Deuterium exchangeable.
b. It appears together with the hydroxyls signal, being able to distinguish when the hydroxyl protons are changed with D_2O.

Therefore, first a mixture of α and β 6-(per-O-acetyl-D-ribofuranosyl)amino-2-methoxy-3-methylpyrimidin-4(3H)-ones **7e,f** were treated in the same conditions as above, giving the same reaction type and affording a mixture in a similar proportion, of α– and β–2-ribofuranosylaminopyridine derivatives (see Scheme 3).

Then we monitored the reaction with the isolated anomeric compounds after complex chromatographic separation of the above mixture of **6e,f**.



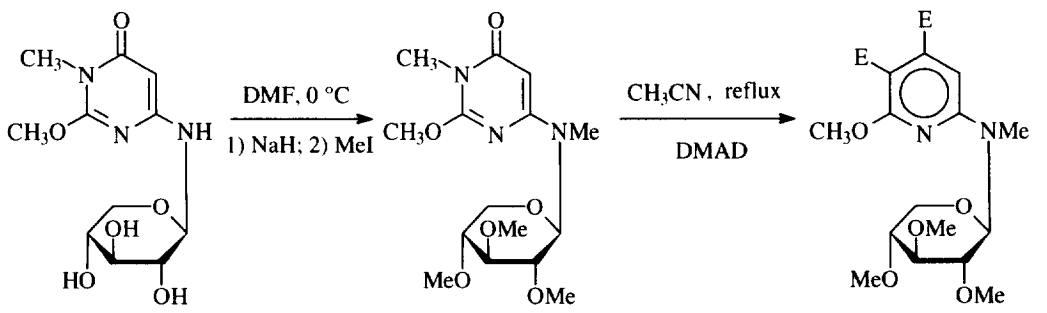
6e: β
6f: α

7e: β
7f: α

Scheme 3

The reaction of DMAD with each pure anomer revealed no anomerization to obtain the ribofuranosylaminopyridine derivatives, which is quite clear by ^{1}H-NMR (see Table 2).[7] The chemical shift for H-1' and the remaining signal for the ribofuranosyl moiety was maintained irrespective of the precursor.[8] In the spectra a different chemical shift for H-3 in the pyridine ring was noted, which was slightly higher for the α-anomer.

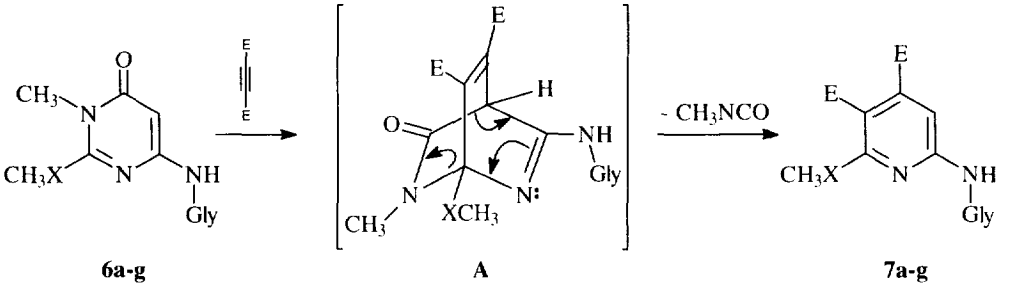
Finally, we carried out the reactions with 6-(tri-O-methyl-β-D-xylopyranosyl)methylamino-2-methoxy-3-methylpyrimidin-4(3H)-ones, **6h**, which has methyls instead of acetyl protecting groups in the sugar ring. Compound **6h** was prepared in a 93% yield starting from 2-methoxy-3-methyl-6-(ß-D-xylopyranosyl)aminopyrimidin-4(3H)-one **6g** upon reaction with sodium hydride and methyl iodide in dry DMF. The reaction of **6h** with DMAD for 3.5 hours gave the desired pyridine derivative **7h** in a yield of 70 % (see Scheme 4).



6g **6h** **7h**

Scheme 4

The reaction of pyrimidine nucleosides **6** with DMAD only affects the heterocyclic ring, leading to pyridine nucleosides **7**, in the same way as for the base ring, that is through a tandem DA/RDA reaction (see Scheme 5). This proves that the inclusion of the glycosidic moiety at the amino group does not change the behavior of the heterocyclic ring, as we predicted.



6a-g **A** **7a-g**

Scheme 5

If we compare the yields of reactions **6a-d** with those of the corresponding pyrimidines **1a-b**, it is evident that the yields of the pyridine derivatives are increased (85-88% for **7a,b** against 79% for **3a**, X=O, and 76-82% for **7c-d** against 64% for **1b**, X=S). In the previous paper[2], we postulated these reactions as a competition between a DA/RDA reaction and Michael addition. The Michael addition is facilitated through proton migration by the amino group, which in the nucleoside derivatives is not as available due to the presence of a bulky substituent.

In the reactions with the ribofuranosyl derivatives the less reactivity of the α-anomer than that of the ß-anomer was showed. That seems to be due to the higher steric hindrance of 2'-acetyl group on DMAD in its approach towards the pyrimidine ring, because they are in the "cis-conformation" in the α-anomer. A similar explanation can be used for the longer reaction time in the case of glucopyranosyl respect to xylopyranosyl derivatives, where the acetyl-O-methylene is responsible of the steric hindrance.

BIOLOGICAL RESULTS

The antiviral activity was determined for compounds **3a,b** and **7a-d** according to previously established procedures.[9] At the highest concentration tested (varying from 100 to 400 µg/ml, depending on the assay, compounds **3a,b** and **7a-d** did not prove active against the following viruses: herpes simplex virus type 1 (HSV-1, strain KOS), ,herpes simplex virus type 2 (HSV-2, strain G), vaccinia virus, vesicular stomatitis virus (VSV), thymidine kinase (TK)- deficient HSV-1 (strain B2006) in human embryonic skin-muscle (ESM) fibroblasts; influenza A and B virus in Madin-Darby canine kidney (MDCK) cells; Coxsackie B4 and polio-1 virus, as well as respiratory syncytial virus (RSV) in Hella cells; human immunodeficiency virus type 1 (HIV-1, strain III_B) and type 2 (HIV-2, strain ROD) in CEM cells; human cytomegalovirus (HCM, strain AD-169 and Davis) in human

embryonic lung (HEL) cells; and reo-1 virus, parainfluenza-3 virus, Sindbis virus, Semliki forest virus, and arenaviruses Junin and Tacaribe in African green monkey kidney (Vero) cells.

EXPERIMENTAL SECTION.

Melting Points were determinated in a Melting Points Apparatus Gallenkamp and are uncorrected. Proton nuclear Magnetic Resonance (^{1}H-NMR) spectra were recorded in a Perkin-Elmer R-600 and using tetramethylsilane as internal standard; the following abbreviations are used to described signal coupling: s= singlet; bs=broad singlet; d=doublet; t=triplet; dd=double doublet; pt=pseudotriplet. Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR) spectra were recorded in a Bruker AM-300 Spectrometer from "Servicios Técnicos de la Universidad de Granada" (STUGRA). Ultraviolet and Visible (UV) spectra were recorded in a GBC UV/VIS 911 spectrophotometer. Infrared spectra were recorded in a Beckman 4250 spectrophotometer (potassium bromide pellets). The following abbreviations are used to described signal strength: b=broad; s=strong; w=weak. Mass spectra were recorded in a Hewlett-Packard HP-5988-A from STUGRA. The analysis C, H and N were performed in a Perkin-Elmer 240 C from STUGRA. Flash column chromatography was performed in an Eyela equipment EF10 on Merck Silica Gel 60 (0.040-0.063 mm) using the solvent system indicated in each case. Reaction progress and products purity were monitored by thin layer chromatography (tlc) on Merck Silica Gel 60GF$_{254}$ (0.2 mm) aluminium precoated sheets with fluorescent indicator, the spots were visualized by ultraviolet irradiation and by spraying with 4% sulphuric acid/methanol solution and subsequent heating. DMAD (99%) was purchased from Aldrich, and directly used without further purification. Sodium hydride (60% dispersion in mineral oil) was purchased from Aldrich, and used after treating with several portions of ethyl ether to eliminate the oil. Compounds **6** were obtained by a previously published procedure.[5, 8]

Preparation of 2-methoxy-3-methyl-6-(tri-O-methyl-ß-D-xylopyranosyl)methylaminopyrimidin-4(3H)-one (6h). To a suspension of 1.435 g of *2-methoxy-3-methyl-6-(β-D-xylopyranosyl)aminopyrimidin-4(3H)-one*, **6g**, in 25 ml of dry DMF, 1.5 g of sodium hydride were added. The mixture was maintained stirring at room temperature for 1 h. Then it was cooled in a salt-ice bath, and added another 25 ml of dry DMF, adding then 2.5 ml of methyl iodide in portions of 0.5 ml each 5 minutes. Once the addition was completed the mixture was maintained with stirring in salt-ice bath for 1 h. Thereafter methanol was added to eliminate the excess of NaH, evaporated at reduced pressure, and 80 ml of methylene chloride and 80 ml of water were added. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4 and evaporated to dryness at reduced pressure, and 1.59 g (93 %) of **6h** were obtained as oil which could not be crystallized. tlc: Rf =0.20 (methylene chloride/ethyl ether 9:1, two elutions); ^{1}H-NMR (dimethyl sulfoxide-d_6) (ppm):): 2.8 (s, 3H, 6-NCH$_3$), 3.2, 3.35, 3.4, 3.5 (N(3)-CH$_3$, three OCH$_3$, and two xylose protons); 3.95 (s, 3H, 2-OCH$_3$); 3.6-4.1 (m, 2H, xylose protons); 5.1 (s, 1H, H-5,); 5.6 (dm, 1H, H-1'); ^{13}C-NMR ($CDCl_3$): 27.3 (N(3)-$\underline{C}H_3$), 30.3 (6-NCH$_3$), 55.4 (2-OCH$_3$), 59.0, 60.1, 61.0 (three O$\underline{C}$H$_3$);

65.7 (C-5'), 79.7, 80.0 (C-4', C-3'), 82.2 (C-5), 83.9 (C-1'), 87.0 (C-2'), 156.0, 160.7, 164.3 (C-2, C-4, C-6); Ms m/z (abundance %); 343 (M^+, 9), 198 (28), 174 (99), 88 (100), 45 (46).

General procedure to obtain 4,5-dicarbomethoxy-6-methoxy-(or 6-methylthio)-2-(per-O-acetyl (or -O-methyl)-D-glycosyl)aminopyridines, 7. To a solution of 3-methyl-6--(per-O-acetyl (or per-O-methyl)-D-glycosyl)aminopyrimidin-4(3H)-ones **6** in dry acetonitrile (5 ml/mmol **6**) was added DMAD **2** (molar ratio 1:2), and stirred under reflux until the starting compound **6** was not detected in tlc (methylene chloride/methanol 9:1). After solvent evaporation, the main reaction product **7** was purified by flash column chromatography (dimensions will be given in each case) and elution in polar gradient (methylene chloride to methylene chloride/ethyl ether 9:1 in the most cases), and finally crystallized from methanol.

4,5-Dicarbomethoxy-6-methoxy-2-(tri-O-acetyl-ß-D-xylopyranosylamino)pyridine (7a). Starting from 2.07 g (5 mmol) of *2-methoxy-3-methyl-6-(tri-O-acetyl-ß-D-xylopyranosylamino)pyrimidin-4(3H)-one*, **6a**. After 7 hours under reflux, solvent removing and purifying by flash chromatography column (5 × 16 cm) 2.12 g (85 %) of **7a** were obtained. tlc: Rf =0.23 (methylene chloride/ethyl ether 9:1). Anal. calcd. for $C_{21}H_{26}N_2O_{12}$: C, 50.60; H, 5.25; N, 5.62. Found: C, 50.60; H, 5.31; N, 5.69; IR_{max} (cm^{-1}): 3365, s; 3010, w; 2955, s; 2868, s; 1757, s; 1737, s; 1609, s; 1525, s; 1459, s; 1368, s; 1218, s; Ms m/z (abundance %); 498.4 (M^+, 5), 467 (4), 240 (13), 209 (41), 92(2), 59(2), 43 (100).

4,5-Dicarbomethoxy-6-methoxy-2-(tetra-O-acetyl-ß-D-glucopyranosylamino)pyridine (7b). Starting from 1.94 g (4 mmol) of *2-methoxy-3-methyl-6-(tetra-O-acetyl-ß-D-glucopyranosylamino)pyrimidin-4(3H)-one*, **6b**. After 21 hours under reflux, solvent removing and purifying by flash chromatography column (5 × 16 cm) 2.02 g (88.5 %) of **7b** were obtained. tlc: Rf =0.28 (methylene chloride/ethyl ether 9:1). Anal. calcd. for $C_{24}H_{30}N_2O_{14}$: C, 50.53; H, 5.30; N, 4.91. Found: C, 50.25; H, 5.44; N, 4.69; IR_{max} (cm^{-1}): 3357, s; 3090, w; 2955, w; 2868, w; 1745, s; 1703, s; 1601, s; 1536, s; 1460, s; 1369, s; 1237, s; Ms m/z (abundance %); 570.4 (M^+, 5), 539.4 (2), 240 (4), 209 (13), 59(1), 43 (100).

4,5-Dicarbomethoxy-6-methylthio-2-(tri-O-acetyl-ß-D-xylopyranosylamino)pyridine (7c). Starting from 2.15 g (5 mmol) of *3-methyl-2-methylthio-6-(tri-O-acetyl-ß-D-xylopyranosylamino)pyrimidin-4(3H)-one*, **6c**. After 22 hours under reflux, solvent removing and purifying by flash chromatography column (5 × 16 cm) 1.96 g (76 %) of **7c** were obtained. tlc: Rf =0.27 (methylene chloride/ethyl ether 9:1). Anal. calcd. for $C_{21}H_{26}N_2O_{11}S$: 49.02; H, 5.09; N, 5.44. Found: C, 48.93; H, 5.13; N, 5.64; IR_{max} (cm^{-1}): 3368, s; 3003, w; 2951, s; 2866, s; 1760, s; 1740, s; 1591, s; 1510, s; 1461, s; 1368, s; 1217, s; Ms m/z (abundance %); 514 (M^+, 4), 483 (1), 256 (0.5), 225 (5), 92(1), 59(1), 43 (100).

4,5-Dicarbomethoxy-6-methylthio-2-(tetra-O-acetyl-ß-D-glucopyranosylamino)pyridine (7d). Starting from 2.01 g (4 mmol) of *3-methyl-2-methylthio-6-(tetra-O-acetyl-ß-D-glucopyranosylamino)pyrimidin-4(3H)-one*, **6d**. After 39 hours under reflux, solvent removing and purifying by flash chromatography column (5 × 16 cm) 1.92 g (82 %) of **7d** were obtained. tlc: Rf =0.22 (methylene chloride/ethyl ether 9:1). Anal. calcd. for $C_{24}H_{30}N_2O_{13}S$: C, 49.14; H, 5.15; N, 4.77. Found: C, 48.78; H, 5.19; N, 4.78; IR_{max} (cm^{-1}): 3431, s; 3081, w; 2952, w; 2932, w; 1753, s; 1733, s; 1596, s; 1515, s; 1439, s; 1367, s; 1221, s; Ms m/z (abundance %); 586.4 (M^+, 3), 555.4 (2), 256 (2), 225 (14) 59(2), 43 (100).

4,5-Dicarbomethoxy-6-methoxy-2-(tri-O-acetyl-ß-D-ribofuranosylamino)pyridine (7e). Starting from 0.16 g (0.39 mmol) of *2-methoxy-3-methyl-6-(tri-O-acetyl-ß-D-ribofuranosylamino)pyrimidin-4(3H)-one*, **6e**. After 21 hours under reflux, solvent removing and purifying by flash chromatography column (2 × 16 cm) and eluting in polar gradient (methylene chloride to methylene chloride/ethyl ether 95:5) 0.15 g (78 %) of **7e** were obtained as solid foam which could not be crystallized. tlc: Rf =0.14 (methylene chloride/ethyl ether 9:1, two elutions). IR_{max} (cm^{-1}): 3374, s; 2999, w; 2955, s; 1746, b s; 1606, s; 1520, s; 1458, s; 1369, s; 1245, s; Ms m/z (abundance %); 498.4 (M^+, 2), 467 (2), 240 (4), 209 (16), 92(1), 59(2), 43 (100).

4,5-Dicarbomethoxy-6-methoxy-2-(tri-O-acetyl-α-D-ribofuranosylamino)pyridine (7f). Starting from 0.16 g (0.39 mmol) of *2-methoxy-3-methyl-6-(tri-O-acetyl-α-D-ribofuranosylamino)pyrimidin-4(3H)-one*, **6f**. After 21 hours under reflux, solvent removing and purifying by flash chromatography column (2 × 16 cm) and eluting in polar gradient (methylene chloride to methylene chloride/ethyl ether 95:5) 0.12 g (63 %) of **7f** were obtained as solid foam which could not be crystallized. tlc: Rf =0.20 (methylene chloride/ethyl ether 9:1, two elutions). IR_{max} (cm^{-1}): 3382, s; 2999, w; 2955, s; 1746, b s; 1606, s; 1521, s; 1457, s; 1368, s; 1244, s; Ms m/z (abundance %); 498.5 (M^+, 1), 467.3 (1), 240 (2), 209 (9), 92(1), 59(2), 43 (100).

4,5-Dicarbomethoxy-6-methoxy-2-(tri-O-methyl-ß-D-xylopyranosyl)methylaminopyridine (7h). Starting from 1.43 g (4.34 mmol) of *2-methoxy-3-methyl-6-(tri-O-methyl-ß-D-xylopyranosy)methylamino pyrimidin-4(3H)-one*, **6h**. After 3.5 hours under reflux, solvent removing and purifying by flash chromatography column (5 × 16 cm) and eluting in polar gradient (methylene chloride to methylene chloride/ethyl ether 95:5) 1.30 g (70 %) of **7h** were obtained as oil which could not be crystallized. ^{1}H-NMR (dimethyl sulfoxide-d_6) (ppm): 2.95 (s, 3H, 6-NCH_3), 3.3, 3.35, 3.5 (12H, three OCH_3, H-2', H-3', H-4'); 3.7, 3.8 (2s, 10H, two $COOCH_3$, 2-OCH_3, H-5'e); 4.0-4.3 (m, 1H, H-5'a); 5.6 (dm, 1H, H-1'); 6.65 (s, 1H, H-3); ^{13}C-MNR ($CDCl_3$): 30.3 (6-N<u>C</u>H$_3$), 52.4, 52.8 (two COO<u>C</u>H$_3$), 54.0 (2-O<u>C</u>H$_3$), 58.9, 59.9, 61.0 (three O<u>C</u>H$_3$); 65.7 (<u>C</u>-5'), 79.7, 80.0 (<u>C</u>-4', <u>C</u>-3'), 83.9 (<u>C</u>-1'), 86.8 (<u>C</u>-2'), 97.8 (C-3), 104.3 (C-5), 142.7 (C-4), 158.0, 160.6, 164.3 (C-2, C-6); 166.7 (two <u>C</u>OOCH$_3$); Ms m/z (abundance %); 428.5 (M^+, 3), 279.2 (11), 174 (2), 115 (37), 88 (100), 59 (13), 45 (46).

General procedure to deacetylate 4,5-dicarbomethoxy-6-methoxy-(or 6-methylthio)-2-(per-O-acetyl-ß-D-glycopyranosyl)aminopyridines, 7. To a solution of **7** in absolute methanol (5 ml/mmol **7**) was added sodium methoxide in catalytic amount (1mmol/100 mmol **7**), and stirred at room temperature until the starting compound **7** was not detected in tlc (methylene chloride/methanol 9:1). Following neutralization with methanolic HCl, solvent evaporation, and purification by either flash column chromatography (dimensions will be given in each case) or simply crystallization from ethanol, the deacetylated product **4,5-dicarbomethoxy-6-methoxy-(or 6-methylthio)-2-ß-D-glycopyranosylamino pyridines, 8** was obtained.

4,5-Dicarbomethoxy-6-methoxy-2-ß-D-xylopyranosylaminopyridine (8a). Starting from 2.74 g (5.5 mmol) of **7a**. After 30 minutes (the reaction was quantitative), solvent removing and purifying by flash chromatography column (5 × 16 cm) 1.7 g (83 %) of **8a** were obtained. tlc: Rf =0.14 (methylene chloride/methanol 94:6). Anal. calcd. for $C_{15}H_{20}N_2O_9$. $1_{1/3}$ H_2O: C, 45.45; H, 5.72; N, 7.07. Found: C, 45.55; H, 5.65 N, 6.79; IR_{max} (cm^{-1}): 3500-3000, b s; 2955, w; 2924, w; 1734, b s; 1608, b s; 1524, s; 1461, s; 1367, s; 1252, s; Ms m/z (abundance %); 372.2 (M^+, 9), 269 (35), 253.1 (47), 240 (24), 221 (29),209 (100), 92 (16), 60 (58), 59(31), 44 (26), 43 (42).

4,5-Dicarbomethoxy-6-methoxy-2-ß-D-glucopyranosylaminopyridine (8b). Starting from 1.69 g (3.0 mmol) of **7b**. After 1 hour (the reaction was quantitative), solvent removing and purifying by crystallization from ethanol, 0.96 g (81 %) of **8b** were obtained. tlc: Rf =0. (methylene chloride/methanol 94:6). Anal. calcd. for $C_{16}H_{22}O_{10}N_2$: C, 47.76; H, 5.51; N, 6.96. Found: C, 47.72; H, 5.60 N, 6.97; IR_{max} (cm^{-1}): 3500-3000, b s;3306, s; 2950, w; 1729, b s;1712, s; 1613, b s; 1583, s; 1517, s; 1457, s; 1363, s; 1256, s; Ms m/z (abundance %); 402.2 (M^+, 2), 269 (13), 253.1 (100), 240 (1), 221 (56),209 (39), 92 (18), 60 (32), 59 (33), 44 (69), 43 (42).

4,5-Dicarbomethoxy-6-methylthio-2-ß-D-xylopyranosylaminopyridine (8c). Starting from 1.83 g (3.56 mmol) of **7c**. After 1 hour (the reaction was quantitative), solvent removing and purifying by flash chromatography column (6 × 16 cm) 0.9 g (80 %) of **8c** were obtained as solid foam. tlc: Rf =0.18 (methylene chloride/methanol 9:1). IR_{max} (cm^{-1}): 3500-3000, b s; 2952, w; 2924, w; 1718, b s; 1587, b s; 1505, s; 1436, s; 1327, s; 1261, s;

4,5-Dicarbomethoxy-6-methylthio-2-ß-D-glucopyranosylaminopyridine (8d). Starting from 2.00 g (3.4 mmol) of **7d**. After 2 hour (the reaction was quantitative), solvent removing and purifying by crystallization from ethanol, 1.12 g (79 %) of **8d** were obtained. tlc: Rf =0. (methylene chloride/methanol 94:6). Anal. calcd. for $C_{16}H_{22}N_2O_9S$: C, 45.93; H, 5.30; N, 6.73. Found: C, 45.79; H, 5.23 N, 6.63; IR_{max} (cm^{-1}): 3500-3000, b s; 3307, s; 2950, w; 1736, s;1720, s; 1637, w; 1618, w; 1589, s; 1507, s; 1440, s; 1362, s; 1233, s; Ms m/z (abundance %); 418.3 (M^+, 2), 285 (13), 269 (27), 256 (3), 237 (100),225 (24), 92 (11), 60 (37), 59 (28), 44 (23), 43 (67).

ACKNOWLEDGEMENTS

We thank the "Consejería de Educación y Ciencia, Junta de Andalucía, Spain" for the award of a scholarship in the "Plan de F.P.D e I." to J. Cobo, and Dr J. Low of the University of Dundee and Prof. G. Ferguson of the University of Guelph for the structural resolution of some structures, through x-ray diffraction. These investigations were supported in part by the Biomedical Research Programme of the European Community and the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek. The technical assistance of Ann Absillis, Anita Camps, Frieda De Meyer and Anita Van Lierde is gratefully acknowledged.

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(*Received in UK* 18 *December* 1995; *revised* 19 *February* 1996; *accepted* 22 *February* 1996)