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SYNTHESIS OF 5-GLYCOSYLAMINO PYRIMIDINES. A NEW CLASS OF COMPOUNDS WITH POTENTIAL ANTI-AIDS ACTIVITY.

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Abstract: Reaction of the diamino pyrimidine derivative, **1**, with D-ribose and D-xylose produces 7-polihydroxyalkylpteridines or 5-glycosylaminopyrimidines depending on the presence or absence of acetic acid. The preliminary results in tests "in vitro" against HIV for compounds **5** and **6** seem to be promising.

Amongst the many and varied substances that have been synthesized and evaluated in recent years as potential biologically active agents, pteridines and pyrimidine nucleoside analogues are promising compound types.[1-8]

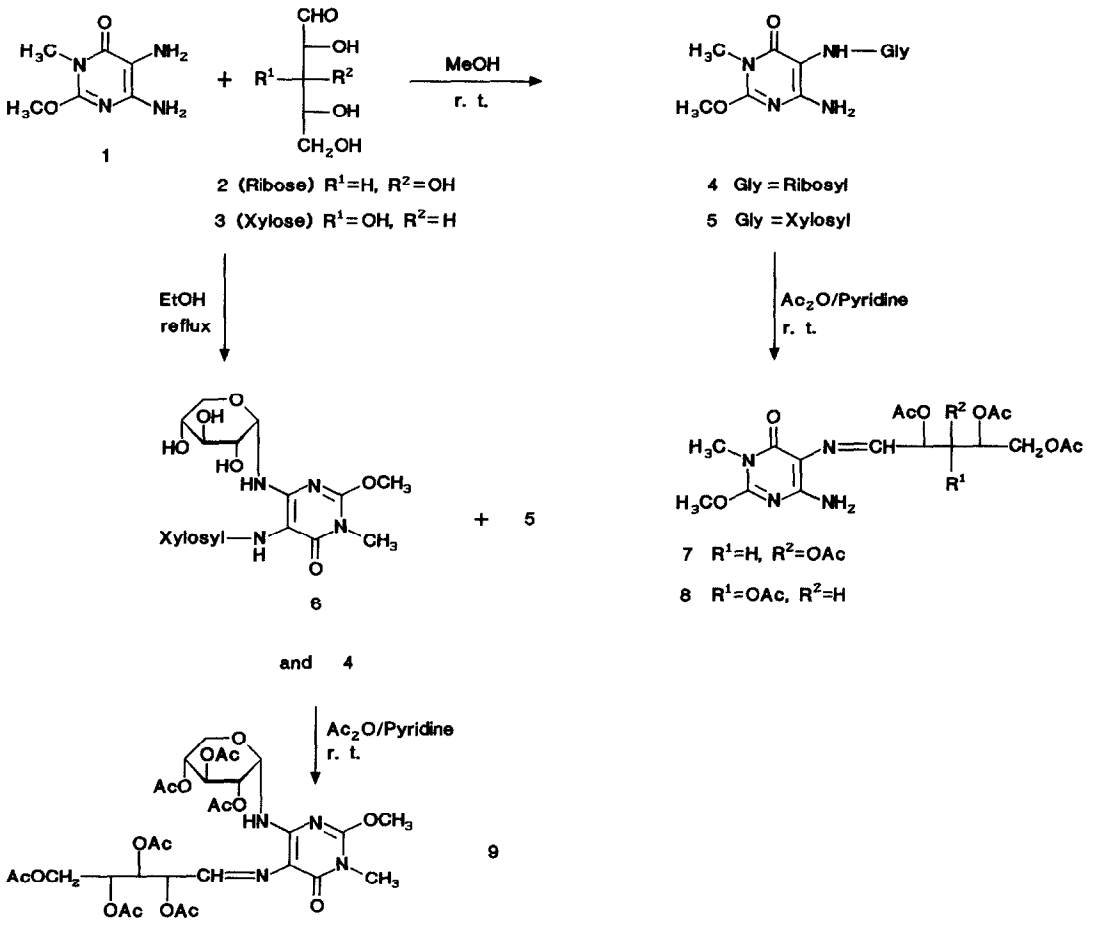
As part of our programme of research on the synthesis and biological activity of nucleoside analogues, we became interested in the preparation of nucleosides derived from 5,6-diaminopyrimidines[9] as well as 6- and 7-polihydroxyalkyl substituted pteridines. These compound types have not been intensively studied against HIV and, on the other hand, 5-glycosylamino pyrimidines constitute useful intermediates for the synthesis of other nucleoside analogues.[10]

5,6-diaminopyrimidines **4** and **5** have showed activity against HIV. These products possess structures relatively unusual for well established active compounds in this area; for this reason, these studies should make a new route in search of antiviral agents.

Reaction of 5,6-diamino-2-methoxy-3-methylpyrimidin-4(3H)-one, **1**, with molar amount of D-ribose or D-xylose in methanol at room temperature yield the 6-amino-5-glycosylaminopyrimidine derivatives **4** and **5** respectively (Scheme I). Higher reactivity at C_5-NH_2[11] lead to the glycosidation at this position instead C_6-NH_2. The 5-N-glycosidic structure of **4** and **5** is definitively proved by comparing their structural data with the corresponding to the C_6-NH_2 glycosyl derivatives previously published.[12]

A study of the specific rotation values for compounds **4** and **5** in H_2O and DMSO demonstrated that these products exist in solution in such solvents as a mixture of the α and β pyranosyl anomers together with the azomethine isomer, as we have previously observed in other similar syntheses.[9]

SCHEME I



Nevertheless, formation of 2-methoxy-3-methyl-6-(α-D-xylopyranosylamino)-5-xylosylaminopyrimidin-4(3H)-one, **6**, was observed when reaction with xylose was carried out in refluxing ethanol; compound **5** was also detected by

TLC. In the reaction of **1** with ribose under the same conditions only compound **4** was isolated (Table); however, a compound with Rf similar to **6** was detected by TLC, this fact could indicate that the riboside homologous is also formed although such compound was not possible to isolate presumably due to its high solubility. Probably, compound **6** is formed by condensation of the C_6-NH_2 group in **5** with an additional molecule of D-xylose; products of this type are obtained by reaction of 6-aminopyrimidines with aldoses.[12] The study of the rotation values of compound **6** showed that for the glycosidic moiety at C-5, it exists as a mixture of α and β pyranosides and the azomethine isomer, whereas, on the basis of the $J_{1',2'}$ value (3.4 Hz), the anomeric centre at C-6 clearly posses α configuration.

TABLE

Comp.	Yield(%)	Mol. Formula[a]	M.P.(°C)[b]	IC_{50} (M)	EC_{50} (M)	TI_{50} (IC/EC)
4	77.0 (MeOH) 70.5 (EtOH)	$C_{11}H_{18}N_4O_6$	154-6 [c]	$>8.30x10^{-4}$	$6.40x10^{-5}$	> 13.0
5	45.0 (MeOH) [d] (EtOH)	$C_{11}H_{18}N_4O_6$	128-30(A)	$>2.20x10^{-4}$	$1.40x10^{-4}$	> 1.60
6	10.0 (EtOH)	$C_{16}H_{26}N_4O_{10}$	184-6[c](dec)	$>5.80x10^{-4}$	-----	-----
7	12.0 [g]	$C_{19}H_{26}N_4O_{10}$	140 (B)			
8	34.0 [g]	$C_{19}H_{26}N_4O_{10}$	143-5 (A)			
9	36.0 [g]	$C_{30}H_{40}N_4O_{17}$	[e]			
10	29.0	$C_{11}H_{14}N_4O_5$	170-2 (B)	$>8.90x10^{-4}$	-----	-----
11	43.0	$C_{11}H_{14}N_4O_5$	210-2 (B)	$>2.20x10^{-4}$	-----	-----
12	22.0 [g]	$C_{17}H_{20}N_4O_8$	[f]			
13	36.0 [g]	$C_{17}H_{20}N_4O_8$	[f]			

[a] Satisfactory microanalysis and spectroscopic data were obtained for all new compounds

[b] Solvent for crystallization: A, Methanol; B, Ethanol.

[c] Compound **4** was purified by refluxing in ethanol several hours.

[d] Detected by TLC.

[e] This compound was purified by dissolving in diethyl ether and hexane and keeping overnight in fridge.

[f] Purified by preparative TLC.

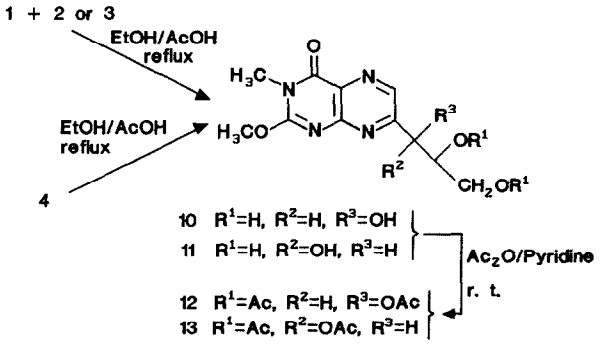
[g] Reactions were carried out by dissolving in a 1:1 (V/V) mixture of Ac_2O/Py (10 ml/g) and stirring at room temperature for 24 hours; then evaporated under reduced pressure, coevaporated with MeOH several times and the residue purified as indicated.

When reaction between **1** and **2** or **3** was carried out in ethanol or methanol in the presence of molar amount of acetic acid, the 7-trihydroxypropyl pteridines **10** and **11** were obtained (Scheme II).

Compounds **4**, **5**, **6**, **10** and **11** were converted for detailed characterization into their acetyl derivatives **7**, **8**, **9**, **12** and **13** respectively. The fact that compounds **4**, **5** and **6** do not denote any change for their rotation values in pyridine and that acetylation in this medium leads to the acetyl azomethinic derivatives **7**, **8**, and **9**, support the assignment as azomethine structure for the mentioned compounds in solid state.

Formation of pteridine derivatives **10** and **11** can be explained by an Amadori rearrangement[13] from **4** and **5**, as it has been evidenced by heating under reflux compound **4** with acetic acid in absolute ethanol (11% yield). Substitution at C-7 position in pteridines **10** and **11** have been demonstrated by analogy with other similar situations.[9] On the other hand, we are working now in the synthesis of the 6-substituted isomers of **10** and **11**. The preliminary results indicated that the proposed structures are the correct ones.

SCHEME II



The "in vitro" anti-HIV activity of compounds **4**, **5**, **6**, **10** and **11** was determined by the NCI against T4 lymphocytes (CEM cell line) exposed to HIV at a virus-to-cell ratio of approximately 0.05. Cultures were incubated at 37°C in a 5% carbon dioxide atmosphere for 6 days. Individual wells were analyzed by addition of the tetrazolium salt XTT and spectrophotometrycally

studies were performed to quantize formazan production. Drug-treated virus-infected cells were compared with drug-treated non-infected cells and with other appropriate controls (untreated infected and untreated noninfected cells, drug-containing wells without cells, etc.) on the same plate. Approximate values for 50% effective concentration (EC_{50}), 50% inhibitory concentration (IC_{50}) and therapeutic index ($TI=IC_{50}/EC_{50}$) were calculated for each test.[14] Since compounds **4** and **5** have showed a moderate activity (see Table), in order to improve the biological activity, additional biological and chemical aspects, as structural modifications and extension of this methodology to other 5,6-diaminopyrimidines and aldoses, are currently under investigation.

Acknowledgements

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