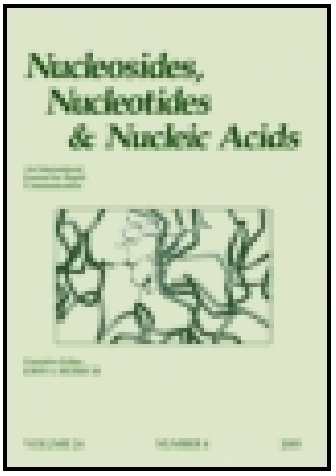

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Nucleocyclitols. Synthests of Ethanolamino-Purinyl Inositol Derivatives and Their Reaction With Thionyl Chloride

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NUCLEOCYCLITOLS. SYNTHESIS OF ETHANOLAMINO-PURINYL INOSITOL DERIVATIVES AND THEIR REACTION WITH THIONYL CHLORIDE

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Abstract: The reaction of mono- and diethanolamine with a 6-chloropurinyl inositol gave 6-ethanolaminopurinyl-inositol derivatives which by reaction with thionyl chloride generated, through quaternization of N^1 of the purine, condensed imidazo-purinyl carbocyclic nucleoside analogs.

INTRODUCTION

In recent years the search for carbocyclic analogs of nucleosides has elicited ample interest, motivated by their diverse physiological activities in such different aspects as those of antitumor, antibiotic or antiviral agents.[1] In the inositol field, previous studies[2,3] showed compound 2, (see Scheme 1) to be a useful sustrate for coupling reactions with purines to give carbocyclic nucleoside analogs funtionalized both in the purine and in the inositol moieties. Whereas the synthesis of derivatives by substitution in the purine ring was relatively straightforward, the modification of the inositol portion through displacements of the mesyloxy groups proved to be very difficult.[4]

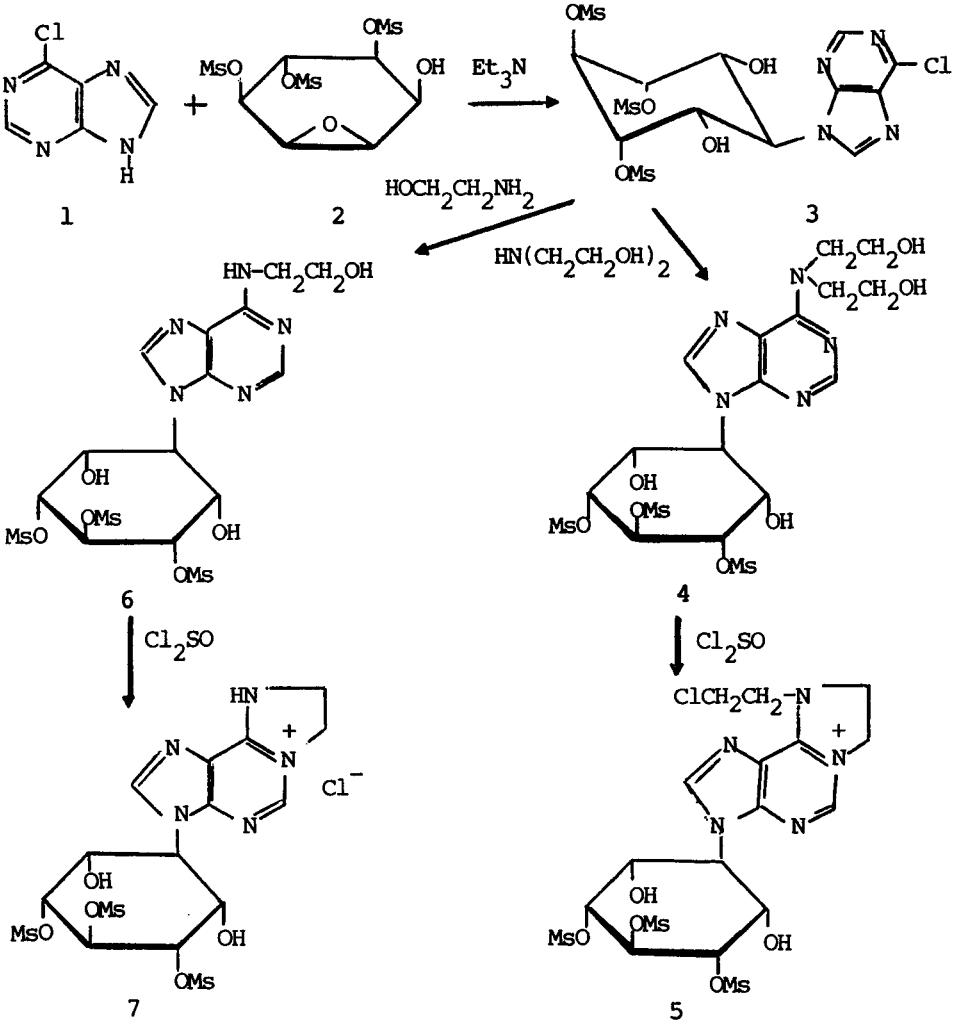
The previously described[3] 6-chloropurinyl inositol 3 was a good starting material for substitution at the 6-position of the purine ring. The unusual resistance of the mesyl groups to the alkaline conditions required for that reaction favored a clean procedure, without side reactions.

Published[5] and current experiments in our laboratory showed interesting effects of some of the nucleocyclitols thus far synthesized as growth promoters or senescense retarders upon vegetal cells. Effects upon animal cells were observable in assays on tumors and on the inmunological system, and this led us to synthesize nitrogen mustard derivatives of nucleocyclitols as potentially stronger, physiologically active molecules.

RESULTS AND DISCUSSION

When compound **3** was heated with diethanolamine in methanol for 88 h, the 6-diethanolamino derivative **4** was obtained in 86.2% yield. Refluxing compound **4** with pure thionyl chloride for 9 h led to a dichloro derivative in 71.7 % yield. This substance showed excellent solubility in water and presented on TLC an unexpected slow-moving behavior, compared with the presumably more polar starting material **4**. This behavior was in contrast with that expected for a N,N-bis(2-chloroethyl)amino derivative. Microanalysis showed the presence of two differently bonded chlorine atoms. One of them was directly titratable at room temperature in aqueous solution and the other was quantified only after mineralization of the sample. This reflects the different character, ionic and covalent respectively, of the two chlorine bonds in the molecule. The structure depicted in **5** for this derivative, accounts for this behavior and is supported by NMR data. The stability of the chloroethyl group in protic solvents was observed in other intramolecular cyclization products of bis chloroethylamino derivatives.[6]

Analogous reactions conducted with monoethanolamine gave the 6-ethanolamino derivative **6**, which by reflux with pure thionyl chloride led to compound **7**. This substance was readily soluble in water and insoluble in most organic solvents. Its chlorine content was directly quantified in aqueous solution, and on TLC showed an R_F value smaller than



SCHEME 1

that of 6. The chromatographic patterns of 5 and 7 did not change even with long standing in solution.

The usual chlorination procedure with thionyl chloride in pyridine of compounds 4 and 6 afforded dark, intractable syrups.

With regard to the methylene protons the ^{1}H NMR spectra in D_2O of 5 and 7 are consistent with these structures . In

compound 5 the methylene group linked to the quaternary nitrogen atom appears as the more deshielded, at 4.82 ppm, then at higher field (4.40 ppm) appears the signal of the chloromethylene group. The two methylenes bonded to the nitrogen atom on C-6 resonate at 4.26 and 3.98 ppm. The former value is ascribable to the group in the pentaatomic cycle, which would be the more deshielded owing to the neighboring positive charge. The chloroethylamino group typically showed[7] the $N-CH_2$ and $Cl-CH_2$ resonances at about 3.50 and 4.30 ppm, respectively.

In the case of the two methylene groups in compound 7, that linked to the quaternary nitrogen atom resonates at 4.75 ppm and the other at 4.12 ppm. Comparison with the corresponding values for 5 (4.82 and 4.26 ppm respectively) indicate, for this compound, an additional deshielding influence by the N-chloroethyl group, specially on the vicinal methylene group.

The formation of aziridinium ions was considered as another structural alternative, but the stability of compound 7, which after ten days in aqueous solution was recovered quantitatively unaltered, exclude such a possibility. Aziridines hydrolyze easily affording an ethanolamine structure. On the other hand their formation from tertiary nitrogen atoms seem poorly favored[7].

The ^1H NMR spectral data described above also excludes that structure, since the methylene protons in aziridines and aziridinium ions typically resonate at δ 1.90-2.80.

The NMR data corresponding to the inositol protons showed for these substances conformations with the bulky heterocycle in an equatorial orientation and, concomitantly, the mesyloxy groups axially disposed, as depicted for compound 3.

Studies conducted[8] on the proliferation of primary cultures of murine breast adenocarcinoma of diverse metastasic capacity[9], showed that compound 5 has a dose dependent DI_{50} inhibitory power at a concentration as low as 2×10^{-5} M. Upon normal cells (mouse embryo) this compound

showed lesser activity (at 2 x 10^{-4} M concentration). Compound 7 did not show activity at all.

EXPERIMENTAL

Melting points (Koffler hot-stage) are uncorrected. TLC was conducted on silica gel G (Merck) plates (0.25 mm layer thickness) with the following solvents: A): 6:4 (v/v) acetonitrile-methanol, B) 13:1:2:4 (v/v) acetonitrile-acetic acid-ethanol-water. The spots were detected with iodine vapor. ^{1}H NMR spectra were recorded at 20-25°C with a Brucker AC spectrophotometer at 200.1 MHz with Me_4Si as the internal reference-standard.

3'-(6-Chloropurin-9-yl)-3'-deoxy-1',5',6'-tri-O-(methyl sulfonyl)-*muco*-inositol (3).- This compound was synthesized from 6-chloropurine (1) and 2,3-anhydro-1,5,6-tri-O-(methyl sulfonyl)-epi-inositol (2) in DMF, as previously described.[3]

3'-Deoxy-3'-[6-(diethanolamino)purin-9-yl)-1',5',6'-tri-O-(methylsulfonyl)-*muco*-inositol (4).- To a suspension of compound 3 (1.25 g, 2.3 mmol) in methanol (57 mL) was added diethanolamine (2.4 mL, 2.5 mmol) and the mixture was heated at reflux for 88 h. Upon cooling, compound **4** was obtained (1.21 g, 86.2% yield) which was recrystallized from methanol to give prisms, mp 146-148°C, λ_{max}^{EtOH} 264 nm (ε_{mM} 6.32). TLC R_F 0.80 (solvent A). ^{1}H NMR data ($MeSO_2$-d_6): δ 8.20 and 8.17 (purine ring protons), 5.71 (broad d, H-2', H-4'), 5.14 (t, H-6', $J_{1',6'}$= $J_{5',6'}$= 2.9 Hz), 5.04 (t, H-1', H-5', spacings 2.9 Hz) 4.80 (m, 4H, $-CH_2O-$), 4.48 (t, H-3', $J_{2',3'}$=$J_{3',4'}$= 10.7 Hz), 3.69 (broad d, 4H, $-CH_2N$), 3.43 (s, 3H, mesyl group), 3.27(s, 6H, mesyl groups).**Anal.** Calc. for $C_{18}H_{29}O_{13}N_5S_3$: C, 34.89; H, 4.71; N, 11.30; S, 15.52. Found: C, 34.58; H, 5.03; N, 11.48; S, 15.75.

3'-(1-Chloroethyl-2,3-dihydro-1*H*-imidazo[2,1-i]purin-4-ium-7yl)-3'-deoxy-1',5',6'-tri-O-(methylsulfonyl)-*muco*-inositol Chloride (5).- A suspension of compound **4** (100 mg, 0.16 mmol) in thionyl chloride (5 mL) was heated at reflux with exclusion of moisture for 9 h. Then the solution

was evaporated to dryness and dried for 24 h in a vacuum dessicator over sodium hydroxide. The residual solid was recrystallized from 1:1 methanol-ethanol affording **5** (76 mg, 71.7% yield), mp 235-237°C, λ_{max}^{EtOH} 272 nm(ε_{mM} 10.8). TLC R_F 0.10 (solvent A), R_F 0.56 (solvent B). ^{1}H NMR data (D_2O): δ 8.52 and 8.46 (purine ring protons), 5.42 (t, H-6 , $J_{1',6'}$ = $J_{5',6'}$ = 3.0 Hz), 5.25 (broad s, H-1 , H-5), 4.82 (m, 5H, H-2', H-3 , H-4 ,-CH_2N^+), 4.40 (t, CH_2Cl, spacings 5.3 Hz), 4.26 (t, CH_2-N of the pentaatomic cycle), 3.98 (t,CH_2-N of chloroethylamino group, spacings 5.3 Hz), 3.37 (s, 3H, mesyl group), 3.32 (s, 6H, mesyl groups). **Anal.** Calc. for $C_{18}H_{27}O_{11}N_5S_3Cl_2$: C, 32.93; H, 4.14; N, 10.66; S, 14.65; Cl, 10.80. Found: C, 32.65; H, 3.81; N, 10.67; S, 15.08; Cl, 10.52.

3'-Deoxy-3'-[6-(ethanolamino)purin-9-yl]-1',5',6'-tri-O-(methylsulfonyl)-*muco*-inositol (6).- To a suspension of compound **3** (300 mg, 0.54 mmol) in methanol (150 mL) monoethanolamine (0.15 mL, 2.5 mmol) was added and the mixture was heated at reflux for 72 h. Then the solution was evaporated to dryness, dried overnight in a vacuum dessicator and the residual solid was recrystallized from methanol affording **6** (245 mg, 75.8% yield) as prisms mp 165-167°C, λ_{max}^{MeOH} 252 nm (ε_{mM} 7.29). TLC R_F 0.61 (solvent A). ^{1}H NMR data ($MeSO_2$-d_6): δ 8.20 and 8.17 (purine ring protons), 5.75 (broad d, H-2', H-4'), 5.14 (t, H-6', $J_{1',6'}$=$J_{5',6'}$= 2.7 Hz), 5.04 (t, H-1', H-5', spacings 2.8 Hz), 4.80 (broad m. -CH_2O-), 4.47 (t, H-3', $J_{2',3'}$=$J_{3',4'}$= 10.8 Hz), 3.58 (broad s, -CH_2-N), 3.44 (s, 3H, mesyl group), 3.28 (s, 6H, mesyl groups). **Anal.** Calc. for $C_{16}H_{25}O_{12}N_5S_3.H_2O$: C, 32.37; H, 4.58; N, 11.79; S, 16.20. Found: C, 32.66; H, 4.93; N, 11.61; S, 15.93.

To a boiling suspension of compound **6** (12 mg) in 2-propyl alcohol (2 mL) was added concentrated hydrogen chloride until dissolution was achieved. The solution was enriched in 2-propanol (1 mL) and kept overnight at 0°C. The hydrochloride salt of **6** was obtained as an amorphous solid (11.2 mg, 89%), mp 200-202°C.

3'-Deoxy-3'-(2,3-dihydro-1*H*-imidazo[2,1-i]purin-4-ium-7-yl)- 1',5',6'-tri-O-(methylsulfonyl)-*muco*-inositol Chloride (7). A suspension of compound **6** (61.9 mg, 0.1 mmol) in thionyl chloride (3 mL) was heated at reflux with the exclusion of moisture for 2 h. The solution was evaporated to dryness and the residual solid was dried overnight in a vacuum dessicator over sodium hydroxide. The residue was taken up with little acetone and filtered to give a pure substance on TLC (48.7 mg, 78.6% yield), R_F 0.15 (solvent A), R_F 0.45 (solvent B). Recrystallization from 2:1 methanol-ethanol gave mp 222-224°C, λ_{max}^{EtOH} 264 nm (ε_{mM} 13.7). ^{1}H NMR data (D_2O): δ 8.46 and 8.35 (purine ring protons), 5.35 (t, H-6', $J_{1',6'}=J_{5',6'}=$ 3.0 Hz), 5.18 (broad s, H-1', H-5'), 4.75 (m, 5H, H-2', H-3', H-4', $-CH_2N^+$), 4.12 (t, CH_2N), 3.29 (s, 3H, mesyl group), 3.23 (s, 6H, mesyl groups). **Anal.** Calc. for $C_{16}H_{24}O_{11}N_5S_3Cl.\ H_2O$: C, 31.39; H, 4.28; N, 11.44; S, 15.71; Cl, 5.79. Found: C, 31.70; H, 4.19; N, 11.79; S, 15.91; Cl, 5.70.

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