Nucleosteroids: Synthesis of Purinyl-steroid Derivatives under Mitsunobu Reaction Conditions

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The feasibility of using the Mitsunobu reaction for coupling of purines with steroids to obtain nucleosteroids was investigated in the particular case of 6-chloropurine and cholesterol. Three principal products were obtained: 3β-(6-chloropurin-9-yl)-5-cholestene (**1**), 3α-(6-chloropurin-9-yl)-5-cholestene (**2**), and 6β-(6-chloropurin-9-yl)-3,5-cyclocholestane (**3**), isolated with 10.5%, 22.7%, and 12.6% yield, respectively. The stereochemical and structural diversity of the coupling products are explained by the formation of a homoallylic carbocation through participation of the double bond of cholesterol.

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The synthesis of carbocyclic nucleoside analogs covers a wide range of substances in which an ample variety of carbocyclic moieties has been displayed [1-3]. The development of this type of compound points to the search for new perspectives for antiviral, antibiotic, or antitumoral applications, in which the use of nucleoside analogs has been significant [3-4].

We already investigated the synthesis of several steroid-purine coupling products [5,6] through an approach based on nucleophilic reactions, as was previously described by van Lier *et al*. [7]. To broaden the range of steroids potentially useful as starting materials for coupling with nitrogenated bases, we studied the feasibility of the conditions of the Mitsunobu reaction applied to the particular case of cholesterol. The mildness and simplicity of the procedure is an important aspect of this reaction. The biologic importance of cholesterol made it an attractive substrate to develop related nucleosteroids potentially useful in the above mentioned therapeutic fields.

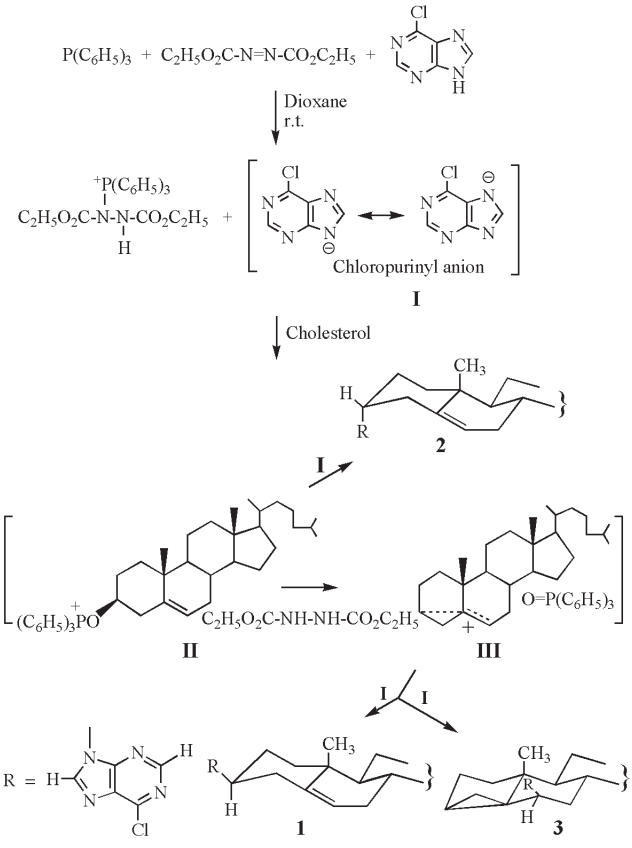
In this report, we describe the coupling of 6-chloropurine with cholesterol through substitution of the hydroxyl group by employing conditions of the Mitsunobu reaction. The experiment was conducted at room temperature, and 3β-(6-chloropurin-9-yl)-5-cholestene (**1**), 3α-(6-chloropurin-9-yl)-5-cholestene (**2**), and 6β-(6-chloropurin-9-yl)-3,5-cyclocholestane (**3**) were isolated in 10.6%, 22.8%, and 12.6% yields, respectively (total yield, 46.0%). The course and products of the reaction are shown in Scheme 1, which is similar to the results by Aneja *et al.* [8] dealing with the Mitsunobu reaction of cholesterol [9].

The interaction of diethyl azodicarboxylate, triphenylphosphine, and 6-chloropurine with cholesterol would form intermediates **I-III** [8]. The nucleophilic attack of the purinyl anion **I** to intermediates **II**, and **III**, gives the 3α-(9-purinyl)-5-cholestene **2**, 3β-(9-purinyl)-5-cholestene **1**, and 6β-(9-purinyl)-3,5-cyclocholestane **3**, respectively.

The structure of compound **1** was assigned based on the following evidence. The significant features of its ^{1}H-nmr spectrum showed two aromatic protons corresponding to the purine nucleus (8.73 and 8.18 ppm for H-2'and H-8') and two low-field steroid resonances for H-3 (4.46 ppm) and H-6 (5.48 ppm). This shows the presence of the 5-ene linkage, which was confirmed by the ^{13}C-nmr resonances for C-5 (137.1 ppm) and C-6 (121.7 ppm). The broad multiplet for the H-3 resonance indicates the diaxial splitting to C-2 and C-4 protons.

The purinyl moiety presented two alternatives for its attachment to C-3 of the steroid portion. The uv spectrum of **1** in ethanol exhibited a maximum at 265 nm, suggesting the 3β-(6-chloropurin-9-yl)-5-cholestene structure. Namely, the absorption maximum of **1** corresponded to that of 6-chloro-9-methylpurine (λ_{max} 268 nm at pH 11) [10a], but not to that of 6-chloro-7-methylpurine (λ_{max} 271 at pH 11) [10b].

Scheme 1



This was confirmed by the ^{13}C-nmr data from the following procedure. When the free pair of electrons on the nitrogen atom in the anion was substituted, an upfield shift for the carbon α to that nitrogen atom and a downfield shift for the β- and γ-carbon atoms were observed [11] in comparison with the base anion. On this basis, the ^{13}C-chemical shifts of **1** were compared with those of the 6-chloropurine anion formed by treatment of 6-chloropurine with lithium hydroxide in deuteriodimethyl sulfoxide.

Thus,Table **1** shows the large upfield shifts for C-4 and C-8 of purine ring, which are in the α-position relative to the substituted nitrogen atom (N^9). In certain cases, a reverse behavior from the downfield β shift predicted was observed for C-5, which is explained by the bridgehead nature of this carbon atom [12].

The structure of compound **3** assigned as 6β-(6-chloropurin-9-yl)-3,5-cyclocholestane was based on the absence of the vinylic H-6 proton signal and the olefinic C-5 and C-6 carbon signals in the ^{1}H- and ^{13}C-nmr spectra, respectively. To define the stereochemistry of the insertion of the purine in C-6 of cholesterol, a HH NOESY pulse sequence was applied to detect an eventual NOE between CH_3-19 and H_6, if this proton was β-oriented. Such interaction could not be observed but instead, in the two-dimensional spectrum, the cross-signals attributable to the interaction of H-8' of the purine with CH_3-19 were present, indicating thus the β-orientation of the purine group in compound **3**. This is supported by the value of the coupling constants of H-6 (1.6 and 4.6 ppm) with the neighboring CH_2-7, which reflects the bisecting character of that proton in α-orientation with the vicinal methylene group.

Table 1

^{13}C-nmr Chemical Shifts for 6-Chloropurinyl Anion and the 6-Chloropurinyl Moiety in Compound **1**

	Chemical Shifts [a]				
Compound	**C-2**	**C-4**	**C-5**	**C-6**	**C-8**
6-Chloropurinyl anion (**I**)	147.0	163.1	132.5	144.3	157.2
6-Chloropurinyl moiety in **1**	149.7	149.2	130.1	150.0	141.2
Δδ **I – 1**	-2.7	+13.9	+2.4	-5.7	+16.0

[a] Shifts given in ppm downfield from tetramethyl silane for solutions in deuteriodimethyl sulfoxide.

With respect to the stereochemistry of C-3 of the steroid, it can also be determined by ^{13}C-nmr employing the DEPT sequence of pulses. Compound **1** possessing the chloropurinyl group in pseudo-equatorial orientation (*exo*), exhibits a deshielded value at C-3 (54.6 ppm) with respect to that of compound **2** having the purinyl group in a pseudo-axial orientation (*endo*), appearing at a higher field (49.8 ppm).

Compound **2** showed similar features to compound **1**, *i.e*., the purinyl peaks and the H-6 (5.60 ppm) and H-3 (5.00 ppm) resonances. The resonance corresponding to H-3 appeared as a narrow doublet due to its small dihedral angle with the vicinal protons. The 5-ene linkage was also demonstrated by ^{13}C-nmr at 136.9 ppm (C-5) and 123.1 ppm (C-6). The ultraviolet absorbance at 266 nm suggested the linkage of the purine (N^9) with the steroid C-3, which was confirmed by the ^{13}C-nmr data (Experimental) for C-4 and C-8 of the purinyl moiety. They showed displacements to higher field with respect to the chloropurinyl anion (13.9 and 14.1 ppm, respectively), as described above for compound **1**.

The stereochemistry of C-3 was determined through the DEPT technique applied to a bidimensional spectrum for the C-H resonances. Compound **2** showed a frequency of 49.8 for C-3, which was shielded with respect to that of C-3 in compound **1**, indicating the presence of the chloropurinyl group in a pseudo-axial (*endo*) orientation.

Aneja *et al* [8] suggested that the formation of a C-6 benzoate derivative of β-configuration could be explained through an initial isomerization to the homoallylic carbocation **III**. This would be followed by the attack to C-6 by the nucleophile, which in our case was the chloropurinyl anion.

In a similar way to compounds **1** and **2**, the ^{13}C-nmr data for C-4 and C-8 of the purinyl moiety (Experimental) in compound **3** showed shielding (14.1 and 13.4 ppm, respectively) with respect to the chloropurinyl anion, indicating the bond of the purine N^9 with the steroid C-6.

EXPERIMENTAL

Melting points (Kofler hot-stage) are uncorrected. Thin-layer chromatography (tlc) was conducted on silica gel G (Merck) plates (0.25 mm layer thickness) with the following solvents: A) 99.5:0.5 (v/v) chloroform- methanol, B) 98:2 (v/v) chloroform-methanol. The spots were detected with iodine vapor. The uv spectra were recorded with a Hewlett Packard 8453 spectrophotometer and were measured in ethanol. ^{1}H-nmr spectra were recorded at 20-25 °C with a Brucker ACE spectrometer at 200 (^{1}H) MHz and with tetramethyl silane as the internal reference standard. The ^{13}C-H DEPT experiments were conducted with a Brucker AM-500 spectrometer at 125.7 (^{13}C) MHz. Optical rotations were measured with a 343 Perkin-Elmer automatic polarimeter at 20° C.

Reaction of Cholesterol with 6-Chloropurine: Coupling under Mitsunobu Reaction Conditions.

Cholesterol (38.6 mg, 0.1 mmol), triphenylphosphine (52.4 mg, 0.2 mmol), and 6-chloropurine (30.8 mg, 0.2 mmol) were suspended in dried dioxane (3 mL), and diethyl azodicarboxylate (DEAD, 0.1 ml) in dioxane (1 mL) was added dropwise while magnetic stirring was continued. The solids were dissolved, and the solution was stirred for 48 hours at room temperature. Then, the solvent was evaporated to dryness, and the residue was dried under vacuum.

The residual powder was macerated at room temperature with chloroform (4 mL), a solid was collected by filtration, and the chloroform solution was evaporated to half volume to give a further amount of solid which was collected by filtration. The tlc (solvent B) of the solid (43.3 mg) was pure diethyl hydrazine-1,2-dicarboxylate.

The above filtrate (chloroform) solution was evaporated, and the residue was dried in a vacuum dessicator. The tlc (solvent A) of the final solid (133.2 mg) showed the spots corresponding to the coupling products with R_f's between 0.40-0.60. Other fast-moving spots corresponded to unreacted starting material or secondary products.

Isolation of the Coupling Products.

The final solid (133.2 mg) was first purified by preparative tlc (solvent A) in a 20 x 20 cm plate with 1 mm layer thickness. The band containing the coupling products was isolated and chromatographed again under the same conditions as above. Three products were isolated at R_f's 0.45, 0.50, and 0.55 (46.0%). The individual amounts and respective yields obtained were: 3β-(6-chloropurin-9-yl)-β-cholestene (**1**) 5.5 mg (10.6%), 3α-(6-chloropurin-9-yl)-5-cholestene (**2**) 11.9 mg (22.8%), and 6β-(6-chloropurin-9-yl)-3,5-cyclo-cholestane (**3**) 6.5 mg (12.6%).

Structural Data of the Coupling Products.

3β-(6-Chloropurin-9-yl)-5-cholestene (**1**).

This compound was obtained as needles, mp 168-170°, $[\alpha]_D^{20°}$ -28.8° (c 0.3, chlf.), λmax 265 nm (ε mM 9.1). Tlc (solvent A) R_f 0.45. ^{1}H-nmr ($CDCl_3$): δ 8.73 and 8.18 (H-2', H-8', purine), 5.48 (d, H-6, J= 5.41 Hz), 4.46 (broad multiplet, H-3α), 1.00-2.70 (methylene protons), 1.16 (3H, 19-CH_3), 0.85, 0.88, 0.90 (methyl groups), 0.71 (3H, 18-CH_3); ^{13}C-nmr (deuteriodimethyl sulfoxide): δ 137.1 (C-5), 121.7 (C-6), 54.6 (C-3); for purine ring resonances, see Table 1.

Anal. Calcd. for $C_{32}H_{47}ClN_4$: C, 73.46; H, 9.05; Cl, 6.77; N, 10.70. Found: C, 73.15; H, 9.12; Cl, 7.02; N, 10.46.

3α-(6-Chloropurin-9-yl)-5-cholestene (**2**).

This compound was obtained as needles, m.p.185-186°, -95.7° (c 0.33; chlf), λmax 266 nm (ε mM 8.93). Tlc (solvent A) R_f= 0.50. ^{1}H-nmr ($CDCl_3$): δ 8.73 and 8.62 (H-2', H-8', purine), 5.60 (d, H-6, J= 3.8 Hz), 5.05 (narrow doublet, H-3), 1.00-2.70 (methylene protons), 1.13 (3H, H-19), 0.85, 0.88, 0.92 (methyl protons), 0.69 (3H, 18-CH_3); ^{13}C-nmr (deuteriodimethyl sulfoxide): δ 136.9 (C-5), 123.1 (C-6), 49.8 (C-3); purine carbon resonances: 149.6 (C-2'), 149.2 (C-4'), 129.4 (C-5'), 150.2 (C-6'), 143.1 (C-8').

Anal. Calcd. for $C_{32}H_{47}ClN_4$: C, 73.46; H, 9.05; Cl, 6.77; N, 10.70. Found: C, 73.31; H, 9.19; Cl, 6.76; N, 10.54.

6β-(6-Chloropurin-9-yl)-3,5-cyclocholestane (**3**).

This compound was obtained as a syrup, $[\alpha]_D^{20}$ -12.4° (c 0.5; chlf.), λmax 266 nm (ε mM 9.0). Tlc (solvent A) R_f= 0.55. ^{1}H-nmr ($CDCl_3$): δ 8.70 and 8.65 (H-2', H-8', purine), 4.15 (H-6, $J_{6\text{-}7\text{-}CH2}$= 1.6 and 4.6 Hz), 1.00-2.60 (methylene protons), 1.04 (3H, 19-CH_3), 0.91, 0.87, 0.80 (methyl protons), 0.61 (3H, 18-CH_3), 0.65-0.78 (cyclopropyl protons); ^{13}C-nmr (deuteriodimethyl sulfoxide): δ 57.9 (C-6); purine carbon resonances: 149.7 (C-2'), 149.0 (C-4'); 129.9 (C-5'), 150.5 (C-6'), 143.8 (C-8').

Anal. Calcd. for $C_{32}H_{47}ClN_4$: C, 73.46; H, 9.05; Cl, 6.77; N, 10.70. Found: C, 73.20; H, 9.25; Cl, 6.62; N, 10.56.

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REFERENCES AND NOTES

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