# Synthesis of Steroidal Nitrosoureas as Antitumor Activity

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**Abstract** □ Steroidal nitrosoureas have been synthesized and their antitumor activity on L1210 cells was evaluated. N-(2-Chloroethyl)-N-nitrosocarbamoyl-3-aza-A-homo-5α-cholestane (5a) showed significantly low $ED_{50}$ value of 1.6 μg/ml whose activity is equivalent to that of methyl-CCNU ($ED_{50}$ = 1.7mg/ml).

**Keywords** □ Steroidal nitrosoureas, Antitumor activity on L1210 cells, Chloroethyl- and methylnitrosourea analogs of 3-aza-A-homo-5α-cholestane, $ED_{50}$.

Several steroid derivatives with attached alkylating moieties have been found to be active against selected animal tumor systems. These include the cholesterol derivative, phenesterin (1) (NSC-104469) and two estradiol derivatives, estradiol mustard (2) (NSC-112259) and estracyt (3) (NSC-89199) which have been used in clinical trial. A steroid nitrosourea agent has also been synthesized and demonstrated to be active against the growth of the DMBA-induced transplantable rat mammary tumor 13762 (4).

Based on the antitumor activity of these compounds, we have synthesized chloroethyl- and methylnitrosourea analogs of 3-aza-A-homo-5α-cholestane.

## EXPERIMENTAL METHODS

All melting points were uncorrected. IR spectra were recorded on a Perkin-Elmer 710 B spectrophotometer. NMR Spectra were obtained with a Varian EM-360 A using $CDCl_3$ as a solvent unless specified otherwise and tetramethylsilane as internal standard. Thin layer chromatography plates (3×9cm) were made with slurry medium of 30g of silica gel G. Type 60 and 100 ml of $CHCl_3$ : $CH_3OH$ (2 : 1, v/v) and their chromatograms were developed with $C_6H_6$ : $CH_3OH$ (8 : 2, v/v).

***3-Aza-A-homo-5α-cholestan-4-one* (2)**

A mixture of 5α-cholestan-3-one oxime (5g, 1.24 m mol) and triphenyl phosphine (7.25g, 10 m mol) in 100 ml of $CCl_4$ was refluxed for 4 h. The mixture was evaporated *in vacuo* and crystallized from MeOH (charcoal) to afford **2** (3.8g, 88% yield) as a colorless crystal. mp 275-277℃, lit. (6) mp 277.5-277.6℃ ; IR (KBr) 1650cm$^{-1}$ (lactam C=O). Anal. ($C_{27}H_{47}NO$) C,H,N.

***3-Aza-A-homo-5α-cholestane* (2)**

A mixture of lactam **2** (3g, 7.3 mmol) and $LiAlH_4$ (3g, 8.0 m mol) in dry dioxane (450 ml) was refluxed for 48 h. The excess $LiAlH_4$ was decomposed with $H_2O$, and filtered. The filtrate was extracted with $CHCl_3$, and the extract was washed (water and brine), dried ($MgSO_4$), and evaporated to dryness. Crystallization from acetone furnished **3** as a colorless solid (2.2g, 78% yield); mp 132-134℃ ; NMR ($CDCl_3$, δ ppm) 4.3 (m, 1H, NH), 2.6-3.5 (m, 4H, C-2, C-3), 0.65-2.0 (m, streoid-H), Anal. ($C_{27}H_{49}N$) C, H,N.

***N-(2-Chloreothyl) carbamoyl-3-aza-A-homo-5α-cholestane* (4a)**

To a solution of **3** (0.8g, 2 mmol) in dry $CHCl_3$ (15 ml) was added 2-chloroethyl isocyanate (0.4 ml, 5 mmol) for a period of 3 h. at room temperature. The solvent was evaporated *in vacuo* to an oily residue which was chromatographed on alumina to yield pure **4a** as an oily product (0.7g, 69% yield); IR (neat) 1665cm$^{-1}$ (urea C=O), NMR ($CDCl_3$, δ ppm) 4.9 (br s, H, NH), 3.1-3.5 (m, 4H, C-2, C-3), 3.5-3.8 (m, 4H, $ClCH_2CH_2N$-), 0.65-2.0 (m, steroid-H). Anal. ($C_{30}H_{53}ClN_2O$) C,H,N.

***N-Methylcarbamoyl-3-aza-A-Homo-5α-cholestane* (4b)**

Methyl isocyanate (3 ml, 5.1 mmol) was added to a solution of **3** (0.8g, 2 mmol) in dry $CHCl_3$ (15 ml) and reacted at room temperature for 3 h. The reaction mixture was evaporated to dryness

under reduced pressure at a temperature not exceeing 40℃, and the residue was then crystallized from $CH_3OH$ to give 4b as white solids (0.75g, 83% yield), mp 158-60℃ ; IR(KBr) 1620cm$^{-1}$ (urea C=O); NMR ($CDCl_3$, $\delta$ ppm) 2.8(d, 3H, NMe), 4.2(q, 1H, NH), 0.65-2.0(m, steroid-H). Anal.($C_{29}H_{53}N_2O$) C,H,N.

***N*-(2-Chloroethyl)-*N*-nitrosocarbamoyl-3-aza-A-homo-5α-cholestane (5a)**

Sodium nitrite (0.5g, 7 m mol) was added slowly to an ice-cold solution (—5℃) of 4a (0.5g, 1 m mol) in 20 m*l* of glacial acetic acid. The reaction mixture was stirred at 0℃ for 3 h, and it was then poured into ice-water and extracted with $CHCl_3$. The extract was washed ($H_2O$ and brine), dried ($MgSO_4$), and evaporated to oily residues which were chromatographed on alumina to afford pure 5a as an oily product. (0.48g, 70% yield). IR (neat) 1690cm$^{-1}$ (urea C=O); NMR ($CDCl_3$, $\delta$ ppm) 4.6-4.9(t, 2H, $CH_2Cl$), 3.85-4.15(t, 2H, $-CH_2-N-NO$), 0.65-2.0(n, steroid-H). Anal.($C_{30}H_{52}ClN_3O_2$) C,H,N.

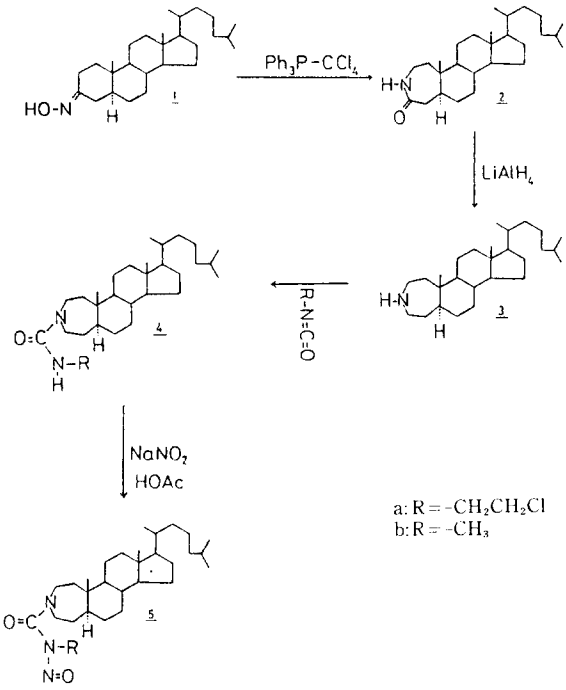
***N*-Methyl-*N*-nitrosocarbamoyl-3-aza-A-homo-5α-cholestane (5b)**

Following the general procedure described above, reaction of sodium nitrite (0.5g, 7 mmol) and 4b (0.5g, 1 mmol) in 20 m*l* of glacial acetic acid furnished 5b (0.46g, 87% yirld); mp 101-102℃ ; IR(KBr) 1700cm$^{-1}$ (urea C=O); NMR ($CDCl_3$, $\delta$ ppm) 3.1(s, 3H, N-Me), 0.65-2.0(m, steroid-H). Anal.($C_{29}H_{51}N_3O_2$) C,H,N.

## RESULTS AND DISCUSSION

N-(2-Chloroethyl)-N-nitrosocarbamoyl-and N-methyl-N-nitrosocarbamoyl groups substituted at the 3-aza-A-homo-5α-cholestane were prepared by a series of conversions starting from 5α-cholestan-3-one oxime (1) (Scheme 1). Beckmann rearrangement of the oxime 1 with a carbon tetrachloride-triphenyl phosphine combination gave 3-aza-A-homo-5α-cholestan-3-one (2) (5) in moderate yield. Reduction of the lactam 2 by $LiAlH_4$ afforded 3-aza-A-homo-5α-cholestane (3) (6) which was reacted with 2-chloroethyl isocyanate and methyl isocyanate to yield N-(2-chloroethyl) carbamoyl-3-aza-A-homo-5α-cholestane (4a) and N-methyl-carbamoyl-3-aza-A-homo-5α-cholestane (4b), respectively.

Nitrosation of the unsymmetrical 1,3-disubstituted ureas, 4a and 4b, can theoretically give two isomeric nitrosoureas. However, sterically bulky 3-aza-A-homo-5α-cholestane moiety made the nitrosation (under 99% HCOOH and dry $NaNO_2$) regioselective to yield exclusively the N-(2-chloroethyl)-N-nitrosocarbamoyl-3-aza-A-homo-5α-cholestane (5a) and N-methyl-N-nitrosocarbamoyl-A-homo-5α-cholestane (5b). The purity of a nitrosourea has been shown by Montgomery, *et al.* (7) to be most clearly established by NMR spectroscopy. The spectral asymmetry of the -N(NO)-CONHCH$_2$CH$_2$Cl ($A_2B_2X$ system) group due to the NH coupling of the adjacent methylene group can be clearly distinguished from the spectral symmetry of the -NHCON(NO)CH$_2$-CH$_2$Cl ($A_2B_2$ system) group.




**Scheme 1**

***Biological Data***

Antitumor activity of compounds, 5a and 5b, was evaluated against the murine leukemic lymphoblast L1210 cell and as a positive control test methyl-CCNU was also applied to the present testing cells of L1210. The growth ratio for each dose of testing substance, Y was calculated by the following

$$\frac{T - C_o}{C - C_o} \times 100 = Y\,(\%)$$

where T=mean cell count for each dose of testin substance after 48 hours incubation; C=mean ce count for control after 48 hours incubation; $C_0$=

**Table I. Antitumor Activity of Steroidal Nitrosoureas.**

| Comp. No. | 5a | 5b | methyl-CCNU |
|---|---|---|---|
| $ED_{50}$ ($\mu g/ml$) | 1.6 | 5.4 | 1.7 |

mean cell count at the start of incubation. When Y values were plotted against doses of methyl-CCNU semilogarithmically, a straight line could be obtained; a concentration of methyl-CCNU which could inhibit the growth of L1210 cells by 50% ($ED_{50}$) was estimated as 1.7 mg/m*l*. The synthesized compounds, 5a and 5b, showed $ED_{50}$ values in Table I. The compound 5a showed significantly low $ED_{50}$ of 1.6 mg/m*l*.

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