

## SYNTHESIS OF 1,2-<sup>3</sup>H-DEOXYCORTICOSTERONE AZIDOFORMATE

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### SUMMARY

*An efficient synthesis of 1,2-<sup>3</sup>H-deoxycorticosterone azidoformate from 1,2-<sup>3</sup>H-deoxycorticosterone via the chloroformate, adaptable to microgram quantities, is described.*

### INTRODUCTION

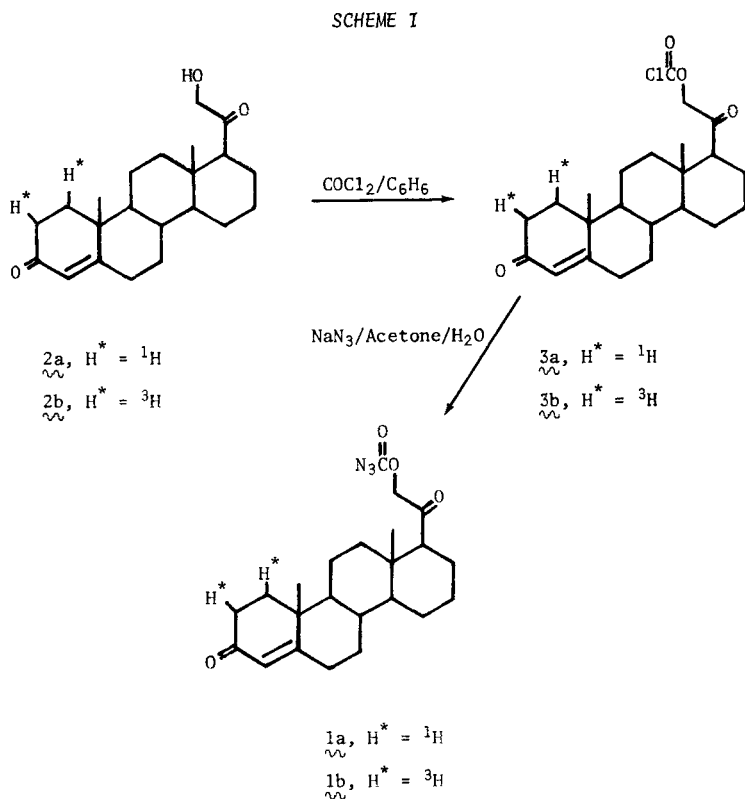
We have examined numerous synthetic progestagens and related steroids as competitors with progesterone in binding to two progesterone receptor proteins, one found in chick oviduct and the other in human uterine cytosol (1). A major objective of these studies was to find potential substrates for affinity labeling of the proteins. Required is a steroid which competes reasonably well with progesterone for specific binding sites and also possesses a substituent capable of reacting with an amino acid residue at the active site to form a covalent bond (2). The most promising class of substrates are photoaffinity labels such as diazo or azido steroids which on irradiation lose nitrogen to give carbenes (3) and nitrenes (4), respectively. Both of the latter will undergo insertion reactions with any amino acid residue in sufficient proximity. Of a number of such steroids synthesized and subjected to competitive binding studies (5), deoxycorticosterone azidoformate (1<sub>a</sub>) was the best competitor (binding affinity 40-50 relative to progesterone 100) (1) amenable to synthesis from an available isotopically labeled precursor.

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## RESULTS AND DISCUSSION

Treatment of deoxycorticosterone (2a) with excess phosgene in benzene gave deoxycorticosterone chloroformate (3a) which was converted to deoxycorticosterone azidoformate (1a) with sodium azide in aqueous acetone (Scheme I).



On a one mmol scale, formation of 3a was complete after 20 hr, and pure 1a was obtained in 72% overall yield after recrystallization. However, on the small scale required for synthesis of tritiated azidoformate 1b (1 mg), only a trace of chloroformate 3a was formed in 20 hr and even after 14 days a small amount of unchanged 2a remained. Compounds 2a, 3a, and 1a, however, were readily separated by tlc. Accordingly, pure 1b of specific activity 3.2 mCi/mg was obtained after preparative tlc in 67% overall yield from 2b.

## EXPERIMENTAL

A Beckman LS-100 scintillation counter was used for radioactivity determinations. Melting points were taken in open capillary tubes and are corrected. Silica gel (HF-254) was used for tlc separations in benzene-methanol (9:1).

Deoxycorticosterone Azidoformate (1a)

A solution of 2a (330 mg, 1.00 mmol) in phosgene-saturated benzene (25 ml) was stirred 20 hr at room temperature. The solution was evaporated to dryness under reduced pressure and the residual chloroformate 3a (6), homogeneous to tlc ( $R_f$  0.7), was dissolved in acetone (15 ml) and water (0.5 ml) and treated with sodium azide (0.10 g, 1.5 mmol). The mixture was stirred 2 hr at room temperature, then concentrated under reduced pressure and partitioned between methylene chloride and water. Evaporation of the methylene chloride and re-crystallization of the residue from methanol gave 1a (289 mg, 72%), homogeneous to tlc ( $R_f$  0.8), mp 117-119° dec; uv (methanol) 290 ( $\epsilon$  140, sh), 240 nm ( $\epsilon$  17,500);  $[\alpha]^{25}_D +213^\circ$  ( $c$  1.22,  $\text{CHCl}_3$ ). Ir and nmr spectra were in accord with the assigned structure.

*Anal.* Calcd for  $\text{C}_{22}\text{H}_{29}\text{N}_3\text{O}_4$ : C, 66.14; H, 7.32; N, 10.52. Found: C, 66.17; H, 7.30; N, 10.55.

1,2-<sup>3</sup>H-Deoxycorticosterone Azidoformate (1b)

1,2-<sup>3</sup>H-Deoxycorticosterone (2b) (0.03 mg, 3.9 mCi) (New England Nuclear, Boston, Mass. 02118), diluted with 1.0 mg of 2a, was dissolved in phosgene-saturated benzene (0.5 ml) and allowed to stand 16 days at room temperature. The solution was evaporated to dryness and the residue was dissolved in acetone (0.5 ml) and water (2 drops) and treated with sodium azide as described above. Pure 1b (0.8 mg, 2.5 mCi, 67% yield) was separated from unchanged 2b ( $R_f$  0.5) and an unidentified trace contaminant by preparative tlc.

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