N-Chloroazasteroids: Preparation of a Novel Class of Thiophilic Steroids

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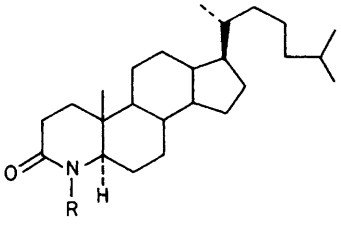
The chlorination of various azasteroid lactams with *N*-chlorosuccinimide afforded novel *N*-chloroazasteroids which reacted with a model thiol to produce *N*-thioalkylated products and, in one case, a sulphenate ester.

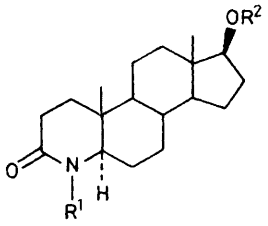
Modified steroids containing alkylating or other reactive functional groups capable of bonding covalently to their receptor proteins, or to enzymes which transform them, are of biological and medicinal interest. For example, reactive mimics of natural steroids have been employed in affinity-labelling studies of steroid receptors,[1] and as enzyme inhibitors.[2] Such compounds also act as selective, cytotoxic agents towards cells containing receptor proteins. Thus, steroids with appended nitrogen-mustard side chains have been used in the treatment of breast and prostate cancer.[3] Since thiol (sulphydryl) groups are known to play a crucial role in the binding of estradiol in the active site,[4] and also presumably of other steroid hormones, steroid mimics capable of reacting specifically with thiols[1d,1e,5] are of special interest.

We now report the preparation of several novel *N*-chloroazasteroid analogues of cholestanes, androstanes, and estranes, which were found to display high reactivity towards a model thiol.

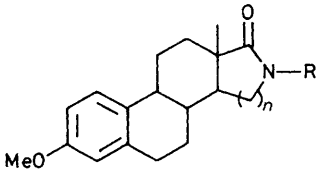
Azasteroid lactams[6] (**1**), (**4**), (**8**), (**11**), and (**14**) were efficiently *N*-chlorinated with *N*-chlorosuccinimide (NCS) to



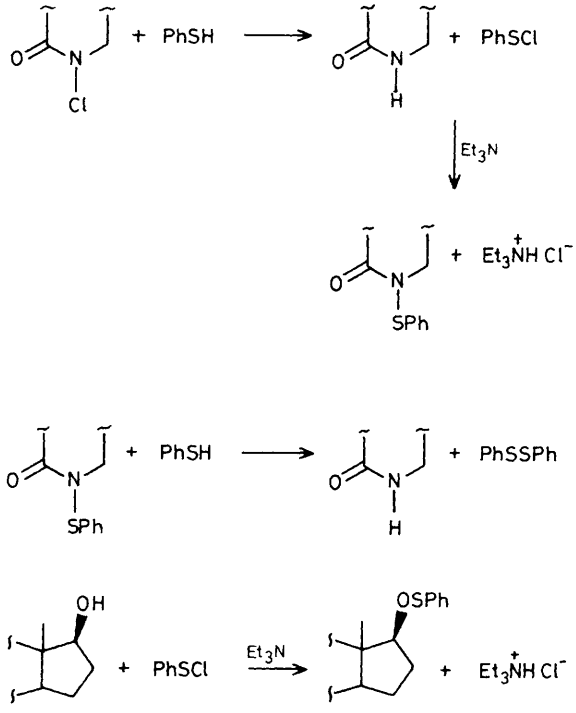
(1) R = H
(2) R = Cl
(3) R = SPh



(4) R^1 = H, R^2 = H
(5) R^1 = Cl, R^2 = H
(6) R^1 = H, R^2 = SPh
(7) R^1 = SPh, R^2 = H
(8) R^1 = H, R^2 = Ac
(9) R^1 = Cl, R^2 = Ac
(10) R^1 = SPh, R^2 = Ac



(11) R = H, n = 1
(12) R = Cl, n = 1
(13) R = SPh, n = 1
(14) R = H, n = 2
(15) R = Cl n = 2
(16) R = SPh, n = 2



Scheme 1

Table 1. Preparation of *N*-chloroazasteroids

Lactam	Product[a]	Method[b]	Yield/%	M.p./°C
(1)	(2)	A	86	149—151
(4)	(5)	B	90	282—284 (decomp.)
(8)	(9)	A	83	decomp. from 250
(11)	(12)	B	69	141—142
(14)	(15)	B	50	145—147

[a] All *N*-chloroazasteroids were characterized by i.r., ^{1}H n.m.r., and mass spectroscopy, and gave satisfactory elemental analyses. [b] Method A: excess of NCS, $CHCl_3$ (reflux), 24—26 h; method B: KOBu^t, THF, 15 min; then NCS, 15—30 min.

afford, in high yield, the *N*-chloroazasteroids (**2**), (**5**), (**9**), (**12**), and (**15**), respectively, under the conditions shown in Table 1. The products are crystalline solids, easily purified by chromatography on silica gel and amenable to long-term storage, although decomposition does occur upon heating or prolonged exposure to light.

When the *N*-chloroazasteroids were treated with an equimolar amount of benzenethiol in chloroform or tetrahydrofuran (THF), the immediate formation of benzenesulphenyl chloride was evident from the appearance of its characteristic orange-red colour. Upon standing, or more rapidly upon addition of triethylamine, the colour disappeared and chromatographic separation afforded the corresponding *N*-thiolactams (**3**), (**10**), (**13**), and (**16**), in yields of 31, 33, 16, and 32%, respectively. In each case, the starting lactams and diphenyl disulphide accounted for nearly all of the remaining mass balance. The product *N*-thiolactams were homogeneous (t.l.c.) gums or solid foams. Their structures were confirmed by i.r., ^{1}H n.m.r., and low and high resolution mass spectroscopy.

An anomalous and surprising result was observed in the reaction of the *N*-chloroazasteroid (**5**) with benzenethiol under the usual conditions. Instead of the expected *N*-thiolactam (**7**), the prinicipal product [apart from lactam (**4**) and diphenyl disulphide] proved to be the sulphenate ester (**6**), isolated in 23% yield. It was a stable, crystalline solid with m.p. 254—255 °C, and i.r., ^{1}H n.m.r., and mass spectra were consistent with its structure. An i.r. absorption at 3397 cm^{-1} (dilute $CHCl_3$) and a D_2O exchangeable ^{1}H n.m.r. resonance at δ 5.86 indicate the presence of the lactam N–H group and rule out the *N*-thiolactam structure (**7**). Furthermore, the C-17 proton resonance is shifted substantially downfield to δ 4.08, in comparison to δ 3.64 in both the lactam (**4**) and the *N*-chloro compound (**5**), and the C-5 proton appears at nearly the same position (δ 3.02) as in lactam (**4**) (δ 3.05).

The steps for the formation of the *N*-thiolactams and the sulphenate ester are shown in Scheme 1, and are reminiscent of the preparation of *N*-thiosuccinimides from the reactions of thiols with NCS.[7] Since *N*-thioimides are themselves known to react with thiols to afford disulphides,[8] the observed formation of the starting lactams and diphenyl disulphide can be similarly explained. Alternatively, the thiol could also react with the sulphenyl chloride produced in the first step to form the disulphide. It is entirely possible that *N*-thiolactam formation (or sulphenate ester formation) would proceed more efficiently *in vivo*, where the further random reaction of the initial thioalkylated product with a second mole of thiol would be less probable in the highly ordered environment of an active site, unless a second thiol residue was present in close proximity and with the correct orientation relative to the first.

We conclude that *N*-chloroazasteroids are highly reactive toward thiol (sulphydryl) groups and are thus expected to bond covalently and irreversibly to such groups in the active site of an enzyme or receptor.

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