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SYNTHESIS OF 3-KETO-4-DIAZO-5-α-DIHYDROSTEROIDS AS POTENTIAL IRREVERSIBLE INHIBITORS OF STEROID 5-α-REDUCTASE

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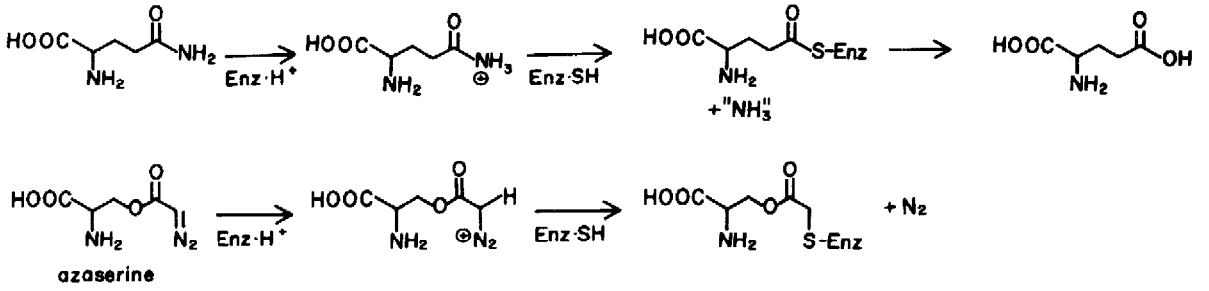
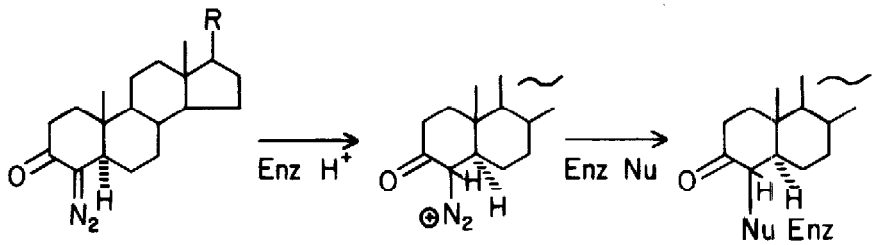
SUMMARY: 3-Keto-4-diazo-5-α-dihydrosteroids are prepared using the Hendrickson diazo transfer reaction on the corresponding β-diketones, which are available from 3-keto-Δ-4-steroids via Li/NH_3 reduction and acylation of the kinetic enolate.

The NADPH-dependent enzyme steroid 5-α-reductase catalyzes the reduction of 3-keto-Δ-4,5-steroids to the corresponding 5-α-dihydro analogues, and is of physiological importance in the conversion of testosterone to the more active androgen, 5-α-dihydrotestosterone.[1] A plausible mechanism is the initial priming of the enone system by protonation,[2] followed by stereospecific hydride addition to the 5-position from the α-face by NADPH.[3] The resultant enol then tautomerizes to the ketone. The enzyme hence is conceivably able to protonate both the 3-ketone (priming step) and carbon 4 (keto-enol tautomerism), and thus may be susceptible to inhibition by a diazoketone analogue of the substrate. This use of diazoketones is suggested by the irreversible inhibition of N-formylglycinamide ribonucleotide (FGAR) amidotransferase by the naturally-occurring diazoester, azaserine.[4] As part of its normal mechanism of action (Scheme 1) FGAR amidotransferase protonates the amido group of the natural substrate glutamine, thereby facilitating nucleophilic displacement by an enzymatic sulfhydryl group to generate enzyme-bound ammonia and glutamate. Buchanan and collaborators have demonstrated that azaserine, a glutamine analogue is protonated by FGAR amidotransferase and the resultant diazonium ion then alkylates the active site sulfhydryl group resulting in inactivation of the enzyme (Scheme 1).[4]



Scheme 1

Based on this analogy we hoped that the 3-keto-4-diazo-5-α-dihydrosteroids 1 would prove to be specific irreversible inhibitors of steroid 5-α-reductase. Thus, if the diazo ketones 1 bind to the active site, the ability of the enzyme to protonate at carbon 4 should lead to a reactive diazonium species which could be alkylated by a nucleophilic residue in the active site, leading to irreversible inhibition (Scheme 2).



1a R = OH

b R = 

Scheme 2

The analogue 1b was chosen as a target compound rather than the 5-α-dihydrotestosterone analogue 1a as it has been shown that modifications of the side chain at 17 can lead to compounds with a higher affinity for the active site than testosterone itself.[5,6] The synthesis of 1b is shown in Scheme 3.

The (20-R) alcohol 2,[7] (m.p. 138°C) available via $NaBH_4$ reduction of the commercially available aldehyde, was transformed to the t-butyldimethylsilyl ether 3[8] (m.p. 108°C) in 82% yield using t-butyldimethylsilyl chloride and imidazole in DMF.[9] 3 was then reduced according to Stork and d'Angelo[10] with lithium in ammonia using aniline as a proton donor, and the resulting enolate trapped with trimethylsilyl chloride to afford the enol ether 4[8] (m.p. 113°C) in 52% yield after purification by recrystallization from ethyl acetate. Treatment of the enol ether 4 in ether with methyl lithium regenerated the enolate which was trapped at -78° with benzoyl chloride[11] to afford the β-diketone 5[8] (m.p. 161-163°C) in 53% yield. The 4-H appears as a doublet (J = 12 Hz) in the 60 M Hz p.m.r. spectrum demonstrating that the 4-benzoyl group is in the α-position.[12] The silyl ether protecting group was then removed in 86% yield with lithium tetrafluoroborate (25°, 24 hours, CH_3CN/CH_2Cl_2)[13] to afford the alcohol 6[8] (m.p. 234-236°C). The diazoketone 1b is then generated from 6 by treatment with sodium hydride and p-toluenesulfonyl azide.[14] An analytically pure sample,[8] m.p. 210°C (decomp) was obtained in 32% yield by HPLC followed by crystallization from methylene chloride-heptane.

The diazoketone 1b has been found to inactivate steroid 5-α-reductase from rat prostate in vitro in a time-dependent manner and this will be reported elsewhere.[15]

Acknowledgments

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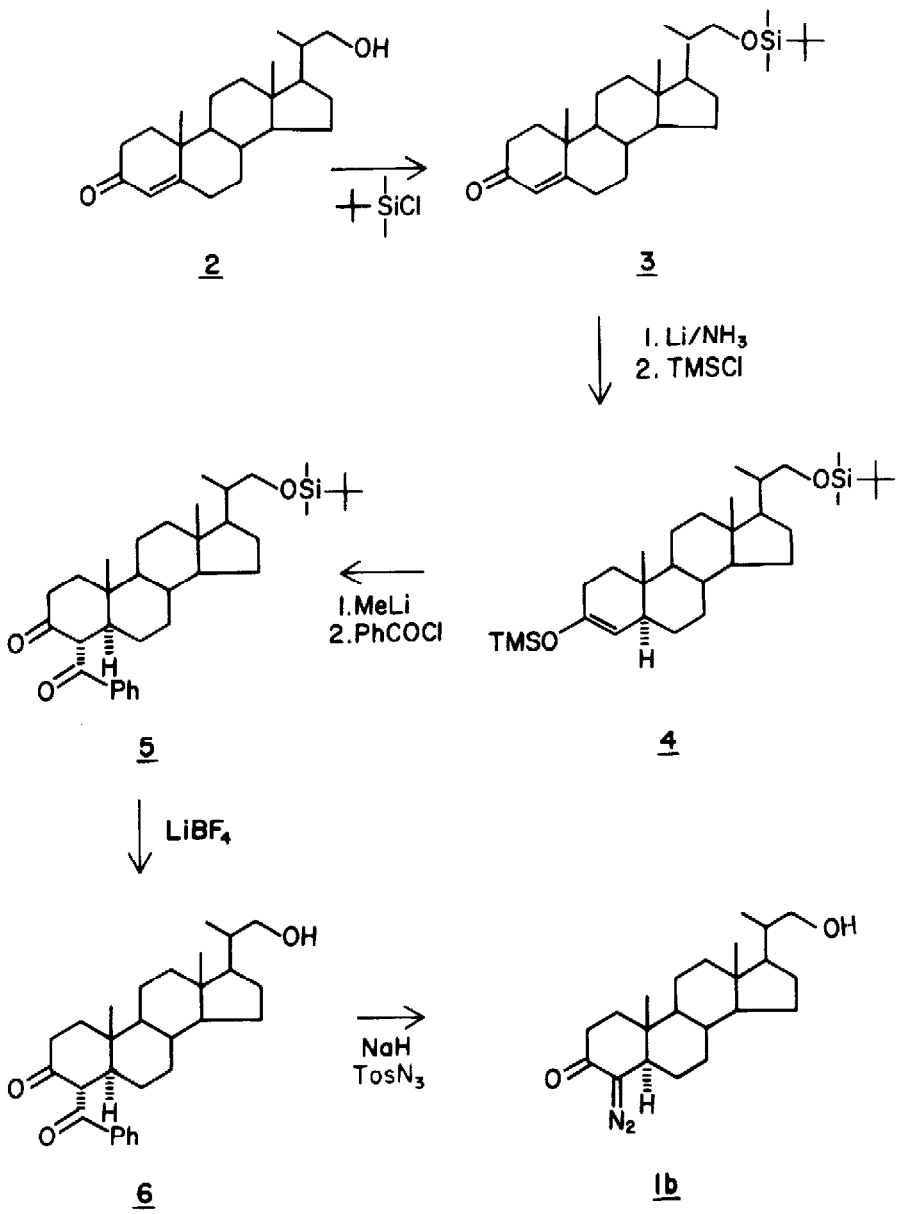
OH
SiCl
OSi
2
3
1. Li/NH3
2. TMSCl
1. MeLi
2. PhCOCl
TMSO
H
Ph
5
4
LiBF4
NaH
TosN3
N2
6
Ib

Scheme 3

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