

SYNTHESIS OF 17-AZACHOLESTEROL

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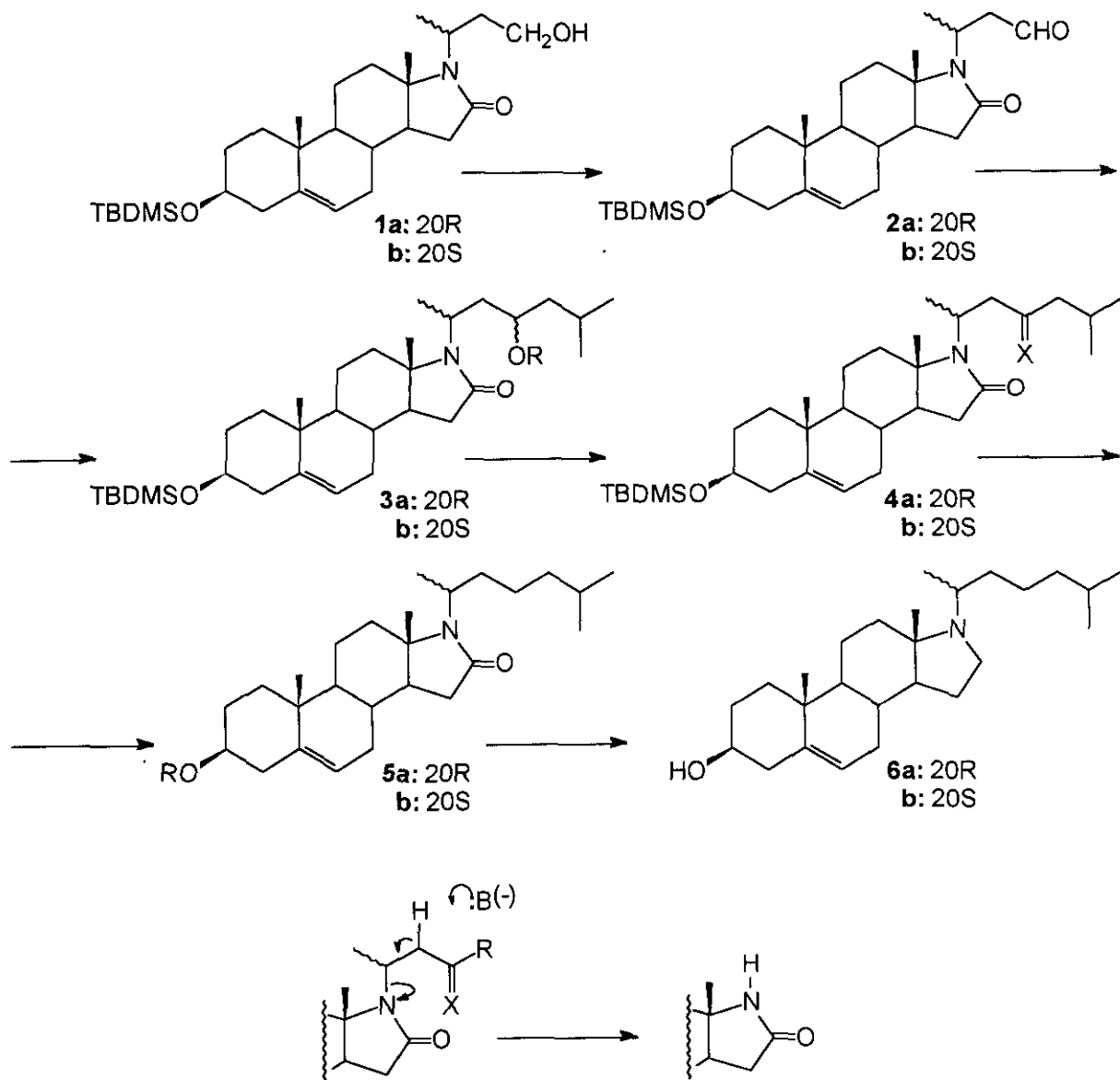
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Abstract - The synthesis of 17-azacholesterol from the *N*-(3-hydroxy-1-methylpropyl)-17-aza lactam is described. The starting alcohol was oxidized to aldehyde which was treated with isobutylmagnesium bromide. The secondary alcohol obtained was oxidized to ketone. Both ketone and lactam carbonyl groups were consecutively reduced to CH₂.

We have recently described¹ the synthesis of the epimeric at C-20 lactam alcohols (**1a**) and (**1b**). Both primary alcohols were transformed into the corresponding iodides, and then subjected to lithium isobutylcuprate. However, the coupling reaction was only partially successful in the case of 20S iodide due to a low yield and completely failed in 20R series. That is why the studies on the side chain construction in 17-azasteroids were continued. It is expected that 17-azacholesterol (**6**) may act as a hypocholesterolemic agent.²

Now we report the more efficient method for the construction of the 2-isooctyl side chain in 17-azasteroids. The epimeric primary alcohols (**1a** and **1b**) were separately oxidized with CrO₃/pyridine to the corresponding aldehydes (**2a**) and (**2b**). The extension of the side chain by a four carbon atom fragment was performed using Grignard reaction. The reactions of aldehydes (**2a**) or (**2b**) with isobutylmagnesium bromide afforded the epimeric mixtures of C-23 alcohols (**3**) (R=H; **a** or **b**) in high yields (the ratio of C-23 epimers was found to be almost 1:1 in both cases). The minor product (about 10%) of the reaction appeared to be the compound (**1**) (**a** or **b**). There was no need to separate the epimeric secondary alcohols (**3**) since according to the synthetic plan the next step was deoxygenation. However, all methods that were tried to accomplish this transformation failed due to the problem with derivatization (e.g. the reaction with TsCl/pyridine yielded instead of tosylate (**3**) (R=Ts) the corresponding chloride in low yield and an unidentified very polar material). Therefore

the epimeric mixture of C-23 alcohols (**3**) ($R=H$; **a** or **b**) was oxidized with $\text{CrO}_3/\text{pyridine}$ to ketone (**4**) ($X=O$, **a** or **b**, respectively) which in turn, was subjected to Wolff-Kischner reduction.³



The hydrazone was easily formed but its decomposition required strongly basic agent. Under these conditions a proton from α position was abstracted and the fragmentation to the parent lactam with no substituent on nitrogen atom took place instead of deoxygenation. A similar fragmentation was also observed when alkylation of aldehyde (**2**) tosylhydrazone with isobutyllithium was attempted.⁴ Eventually the desired transformation was accomplished by reduction of tosylhydrazone (**4**) ($X = \text{NNHTs}$) with sodium cyanoborohydride in the presence

of *p*-TsOH.⁵ The product obtained in 20S series was proved identical in all respects with the previously described compound (5b) (R=TBDMS).¹ The reduction of the lactam carbonyl group of (5a) (R=H) with lithium aluminum hydride afforded 17-azacholesterol (6). Molecular mechanics studies⁶ show that its preferred side chain conformation *in vacuo* (torsion angle C13-N17-C20-C21 amounts -51.9°) differs only slightly from that of cholesterol itself (the angle -57.3°). The analysis of the compound (6) conformation in the solution and the evaluation of its biological activity are under way.

EXPERIMENTAL SECTION

Melting points were determined on Kofler apparatus of Boetius type and were uncorrected. Nmr spectra were recorded with Bruker AC 200F Spectrometer using CDCl₃ solutions with TMS as an internal standard. Infrared spectra were recorded on a Specord 75 IR as CCl₄ or CHCl₃ solutions unless otherwise stated. Mass spectra were obtained at 70 eV with AMD-604 spectrometer. The reaction products were isolated by column chromatography performed on silica gel 70-230 or 230-400 mesh ASTM (Merck). Thin layer chromatograms were developed on aluminum tic sheets pre-coated with silica gel 60 F₂₅₄ and visualized with 50% sulfuric acid after heating. All solvents were dried and freshly distilled prior to use.

3β-Hydroxy-16-oxo-24-nor-17-azachol-5-enaldehyde *tert*-butyldimethylsilyl ether (2)

Chromic anhydride (180 mg, 1.8 mmol) was dissolved in dichloromethane (5 ml) - pyridine (1.4 ml) mixture and stirred at room temperature for 0.5 h. To this reagent a solution of primary alcohol (1a) (78 mg, 0.164 mmol) in dichloromethane (1 ml) was added and the reaction mixture was stirred 1 h at room temperature, filtered through silica gel (elution with 25% ethyl acetate-benzene) and evaporated. Yield of aldehyde (2a): 65 mg (84%), mp 135-138°C (from hexane); ir (CHCl₃) 1724, 1668, 1100, 841 cm⁻¹; ¹H-nmr δ 9.76 (almost s, 1H, -CHO), 5.32 (m, 1H, 6-H), 3.88 (m, 1H, 20-H), 3.48 (m, 1H, 3α-H), 3.32 (dd, J_{gem} = 17.7 Hz, J_{vic} = 8.0 Hz, 1H, 22-H), 2.88 (dd, J_{gem} = 17.7 Hz, J_{vic} = 4.7 Hz, 1H, 22-H), 1.37 (d, J = 6.8 Hz, 3H, 21-H), 1.05 (s, 3H, 18-H), 1.02 (s, 3H, 19-H), 0.89 (s, 9H, *t*-Bu-Si), 0.06 (s, 6H, Me-Si); ¹³C-nmr δ 200.7 (CH), 175.5 (C), 141.8 (C), 120.2 (CH), 72.3 (CH), 63.6 (C), 51.9 (CH), 50.0 (CH), 49.0 (CH₂), 42.6 (CH₂), 42.5 (CH), 37.0 (CH₂), 36.7 (C), 35.7 (CH₂), 33.5 (CH₂), 31.9 (CH₂), 31.0 (CH₂), 30.5 (CH), 25.9 (3 × CH₃), 21.2 (CH₂), 20.4 (CH₃), 19.3 (CH₃), 18.2 (C), 17.1 (CH₃), -4.6 (2 × CH₃); mass spectrum 473 (M⁺, 1), 458 (7), 416 (100), 388 (10), 346 (41). Anal. Calcd for C₂₈H₄₇NO₃Si: C, 70.99; H, 10.00; N, 2.96. Found: C, 70.67; H, 9.97; N, 2.97.

A similar reaction of 20S epimer (1b) afforded aldehyde (2b), mp 175-178°C (from pentane-dichloromethane), ir (CHCl₃) 1722, 1669, 1100, 841 cm⁻¹; ¹H-nmr δ 9.74 (t, J = 1.3 Hz, 1H, -CHO), 5.31 (m, 1H,

6-H), 3.74 (m, 1H, 20-H), 3.48 (m, 1H, 3 α -H), 3.30 (ddd, $J_{gem} = 17.5$ Hz, $J_{vic} = 7.4$ Hz, $J_{ald} = 1.3$ Hz, 1H, 22-H), 2.85 (ddd, $J_{gem} = 17.5$ Hz, $J_{vic} = 6.2$ Hz, $J_{ald} = 1.3$ Hz, 1H, 22-H), 1.43 (d, $J = 6.7$ Hz, 3H, 21-H), 1.11 (s, 3H, 18-H), 1.02 (s, 3H, 19-H), 0.89 (s, 9H, *t*-Bu-Si), 0.06 (s, 6H, Me-Si); mass spectrum 473 (M^+ , 0.2), 458 (3), 430 (2), 416 (28), 388 (6), 346 (100). Anal. Calcd for $C_{28}H_{47}NO_3Si$: C, 70.99; H, 10.00; N, 2.96. Found: C, 70.65; H, 9.98, N, 3.02.

3 β .23 ξ -Dihydroxy-17-azacholest-5-en-16-one 3-*tert*-butyldimethylsilyl ether (3) (R=H)

To the stirred solution of aldehyde (2a) (41 mg, 0.087 mmol) in anhydrous ether (3 ml) under nitrogen at 0°C 0.5 ml (1.15 mmol) of isobutylmagnesium bromide (2.3 M solution in ether) was dropwise added. The reaction mixture was stirred 1 h at 0°C, quenched with water, dried over $MgSO_4$ and evaporated. Column chromatography over silica gel afforded C-23 epimers of (3a) (R=H; 40 mg; 87%) eluted with 20% ethyl acetate-benzene. Further elution with benzene-ethyl acetate (6:4) yielded primary alcohol (1a) (4 mg).

3a (crystalline mixture of C-23 epimers); ir ($CHCl_3$) 3380, 1665, 1099, 841 cm^{-1} ; 1H -nmr δ 5.31 (m, 1H, 6-H), 4.36 and 3.61 (2 x m, 2 x 0.5H, 23-H), 3.49 (m, 2H, 3 α -H and 20-H), 1.36 and 1.35 (2 x d, $J = 6.7$ Hz and 7.1 Hz, 3H, 21-H), 1.24 (s, 1.5H, 18-H), 1.12 (s, 1.5H, 18-H), 1.02 (s, 3H, 19-H), 0.90 (m) and 0.89 (s) - overlapped (15H, 26-H, 27-H, and *t*-Bu-Si), 0.06 (s, 6H, Me-Si); mass spectrum 531 (M^+ , 4), 516 (29), 513 (18), 474 (55), 431 (100), 416 (26), 346 (21). Anal. Calcd for $C_{32}H_{57}NO_3Si$. C, 72.26; H, 10.80; N, 2.63 Found: C, 71.99; H, 10.63; N, 2.61.

Aldehyde (2b) similarly treated with isobutylmagnesium bromide afforded the epimeric mixture of C-23 alcohols (3b); ir ($CHCl_3$) 3395, 1668, 1099, 841 cm^{-1} ; 1H -nmr δ 5.32 (m, 1H, 6-H), 3.65 (m, 1H, 23-H), 3.48 (m, 2H, 3 α -H and 20-H), 1.44 and 1.39 (2 x d, $J = 6.8$ Hz and 7.0 Hz, 3H, 21-H), 1.02 (s, 3H, 19-H), 0.89 (m and s, 15H, 26-H, 27-H, and *t*-Bu-Si), 0.06 (s, 6H, Me-Si); mass spectrum 531 (M^+ , 4), 516 (35), 513 (26), 474 (51), 431 (100), 416 (28), 346 (99). Exact mass calcd for $C_{32}H_{57}NO_3Si$: 531.4108. Found: 531.4107

3 β -Hydroxy-17-azacholest-5-en-16,23-dione *tert*-butyldimethylsilyl ether (4) (X = O)

Chromic anhydride (180 mg, 1.8 mmol) was dissolved in dichloromethane (5 ml) - pyridine (1.4 ml) mixture and stirred 0.5 h at room temperature. A solution of secondary alcohol (3a) (R=H; 40 mg, 0.075 mmol) in 1 ml of dichloromethane was added and stirring was continued for 2 h. The product was purified by filtration through silica gel (elution with 10% ethyl acetate-benzene). Yield of ketone (4a) (X=O). 32 mg (80%), mp 128-131°C (from hexane); ir ($CHCl_3$) 1707, 1670, 1100, 842 cm^{-1} ; 1H -nmr δ 5.31 (m, 1H, 6-H), 3.77 (m, 1H, 20-H), 3.47 (m, 1H, 3 α -H), 3.36 (dd, $J_{gem} = 17.9$ Hz, $J_{vic} = 8.9$ Hz, 1H, 22-H), 2.73 (dd, $J_{gem} = 17.9$ Hz, $J_{vic} = 3.8$ Hz, 1H, 22-H), 1.29 (d, $J = 6.7$ Hz, 3H, 21-H), 1.02 (s, 6H, 18-H and 19-H), 0.90 (m) and 0.89 (s) - overlapped (15H,

26H, 27H, and *t*-Bu-Si), 0.06 (s, 6H, Me-Si); ^{13}C -nmr δ 209.5 (C), 175.5 (C), 141.8 (C), 120.2 (CH), 72.3 (CH), 63.5 (C), 52.5 (CH₂), 51.9 (CH), 50.0 (CH), 47.8 (CH₂), 43.5 (CH), 42.6 (CH₂), 37.0 (CH₂), 36.7 (C), 35.5 (CH₂), 33.6 (CH₂), 31.9 (CH₂), 31.0 (CH₂), 30.5 (CH), 25.9 (3 x CH₃), 24.6 (CH), 22.51 (CH₃), 22.46 (CH₃), 21.1 (CH₂), 20.3 (CH₃), 19.2 (CH₃), 18.2 (C), 16.7 (CH₃), -4.6 (2 x CH₃); mass spectrum 529 (M⁺, 13), 514 (23), 472 (100), 444 (29), 430 (13), 346 (31). Anal Calcd for C₃₂H₅₅NO₃Si: C, 72.54, H, 10.46; N, 2.64. Found: C, 72.39, H, 10.36; N, 2.68.

A similar oxidation of alcohol (3b) (R=H) afforded 20S epimer of ketone (4b) (X = O), mp 135-138°C (from pentane); ir (CHCl₃) 1708, 1669, 1100, 842 cm⁻¹; ^1H -nmr δ 5.31 (m, 1H, 6-H), 3.72 (m, 1H, 20-H), 3.47 (m, 1H, 3 α -H), 3.20 (dd, $J_{\text{gem}} = 17.3$ Hz, $J_{\text{vic}} = 6.8$ Hz, 1H, 22-H), 2.82 (dd, $J_{\text{gem}} = 17.3$ Hz, $J_{\text{vic}} = 6.8$ Hz, 1H, 22-H), 1.36 (d, $J = 6.7$ Hz, 3H, 21-H), 1.10 (s, 3H, 18-H), 1.02 (s, 3H, 19-H), 0.89 (d and s, 15H, 25-H, 27-H, and *t*-Bu-Si), 0.06 (s, 6H, Me-Si); mass spectrum 529 (M⁺, 7), 514 (11), 472 (33), 444 (10), 346 (100). Anal Calcd for C₃₂H₅₅NO₃Si: C, 72.54; H, 10.46; N, 2.64. Found: C, 72.29; H, 10.29; N, 2.67.

3 β -Hydroxy-17-azacholest-5-en-16-one (5)

A solution of compound (4a) (X = O; 43 mg, 0.081 mmol) and *p*-toluenesulfonylhydrazide (25 mg, 0.134 mmol) in ethanol (1 ml) was refluxed 2 h. Ethanol was removed *in vacuo* and the crude tosylhydrazone (4a) (X = NNHTs) was dissolved in DMF (0.5 ml) and sulfolane (0.5 ml), then *p*-TsOH (50 mg, 0.263 mmol) and sodium cyanoborohydride (40 mg, 0.635 mmol) were added. The reaction mixture was heated 24 h at 105°C under a layer of refluxing cyclohexane (0.5 ml). The reaction mixture was poured into water containing a few drops of hydrochloric acid and extracted with chloroform. The extract was dried over MgSO₄, evaporated and the residue was chromatographed on a silica gel column. Elution with 60% ethyl acetate-benzene yielded 10 mg (31%) of compound (5a) (R = H); ir (CHCl₃) 3610, 1669, 1179, 1100 cm⁻¹; ^1H -nmr δ 5.36 (m, 1H, 6-H), 3.3-3.7 (2 x m, 2 x 1H, 3 α -H and 20-H), 1.30 (d, $J = 6.8$ Hz, 3H, 21-H), 1.12 (s, 3H, 18-H), 1.03 (s, 3H, 19-H), 0.86 (d, $J = 6.5$ Hz, 6H, 26-H and 27-H), ^{13}C -nmr δ 175.1 (C), 141.0 (C), 120.8 (CH), 71.5 (CH), 63.6 (C), 52.0 (CH), 49.9 (CH), 48.9 (CH), 42.1 (CH₂), 38.8 (CH₂), 36.9 (CH₂), 36.6 (C), 36.0 (CH₂), 35.0 (CH₂), 33.6 (CH₂), 31.4 (CH₂), 31.0 (CH₂), 30.5 (CH), 27.8 (CH), 25.3 (CH₂), 22.6 (CH₃), 22.5 (CH₃), 21.2 (CH₂), 19.6 (CH₃), 19.2 (CH₃), 17.7 (CH₃).

A similar reaction was also carried out with 20S epimer of ketone (4b) (X = O). The crude product (5b) (R = H) was subjected to *tert*-butyldimethylsilyl chloride/imidazole/DMF and the TBDMS derivative obtained was proved identical in all respects with the previously described compound (5b) (R = TBDMS).¹

17-Azacholest-5-en-3 β -ol (17-azacholesterol; 6a)

A stirred solution of compound (5a) (R = H; 20 mg, 0.05 mmol) in anhydrous THF (5 ml) was heated with lithium aluminum hydride (100 mg, 2.7 mmol) at reflux 4 h. The reaction was quenched with a few drops of water, dried over MgSO₄, and all inorganic material was filtered off. The crude product was purified on a silica gel column. Elution with chloroform-methanol (9:1) afforded 17-azacholesterol (6a), (7 mg; 41%), mp 152-155°C (from benzene); ir (CHCl₃) 3610, 3400, 1061 cm⁻¹; ¹H-nmr δ 5.33 (m, 1H, 6-H), 3.94 (m, 2H, 16-H and OH), 3.54 (m, 1H, 3 α -H), 3.03 (m, 1H, 20-H), 2.96 (m, 1H, 16-H), 1.50 (d, J = 6.3 Hz, 3H, 21-H), 1.18 (s, 3H, 18-H), 0.99 (s, 3H, 19-H), 0.86 and 0.87 (2 x d, 2 x J = 6.5 Hz, 6H, 26-H and 27-H); ¹³C-nmr δ 140.7 (C), 120.5 (CH), 71.3 (CH), 62.7 (C), 59.8 (CH), 51.7 (CH), 51.1 (CH₂), 48.1 (CH), 41.9 (CH₂), 38.4 (CH₂), 36.9 (CH₂), 36.2 (C), 35.8 (CH₂), 32.8 (CH₂), 32.7 (CH), 31.4 (CH₂), 30.6 (CH₂), 27.8 (CH), 24.3 (CH₂), 23.3 (CH₂), 22.6 (CH₃), 22.3 (CH₃), 21.8 (CH₂), 19.3 (CH₃), 17.4 (CH₃), 11.9 (CH₃); mass spectrum 387 (M⁺, 2), 372 (44), 370 (9), 302 (100), 260 (10). Exact mass calcd for C₂₅H₄₂NO (M⁺ - CH₃): 372.3266. Found: 372.3261. Anal Calcd for C₂₆H₄₅NO: C, 80.56; H, 11.70, N, 3.61. Found: C, 80.39; H, 11.69; N, 3.65.

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