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Article

Synthesis of Hydroxy Acids of Dinorcholane and 5β-Cholane

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Uma mistura diastereomérica (55:45) de 3-oxo-23,24-dinorcol-4-en-22-hydroxi ácidos (**4A** e **4B**) foi sintetizada a partir de 3-oxo-23,24-dinorcol-4-en-22-al (**1**) em 3 etapas. Similarmente, 3-oxo-5β-cholano-24-hidroxi ácido (**9**) foi sintetizado a partir de 5β-cholano-3α,24-diol (**5**) em 4 etapas. Dados espectroscópicos completos para estes compostos são apresentados.

A diastereomeric mixture (55:45) of 3-oxo-23,24-dinorchol-4-en-22-hydroxy acids (**4A** and **4B**) was synthesized from 3-oxo-23,24-dinorchol-4-en-22-al (**1**) in 3 steps. Similarly, 3-oxo-5β-cholane-24-hydroxy acid (**9**) was synthesized from 5β-cholane-3α,24-diol (**5**) in 4 steps. Full spectroscopic data for these compounds are presented.

Keywords: 3-oxo-23,24-dinorchol-4-en-22-al, 3-oxo-5β-cholane-24-al, cyanohydrin silyl ether, hydroxy acid, NMR

Introduction

Enantiopure α-hydroxy acids (AHAs) are versatile building blocks in organic synthesis. They are used for obtaining pharmaceutically and biologically active substances, such as vitamins. α-Hydroxy acids are easily synthesized from cyanohydrins. Thus, molecules with cyanohydrin substructures are interesting intermediates for the synthesis of AHAs.[1] Usually, cyanohydrins are prepared by the addition of a cyanide group to the carbonyl carbon of aldehydes or ketones resulting in enantiomeric mixtures of optically active cyanohydrins when aldehydes or asymmetric ketones are employed. However, if a chiral centre already exists next to the carbonyl carbon, two possible diastereomeric products are formed, and they are not of equal amounts. As part of our on-going studies on the synthesis and reactions of steroid monomers and dimers,[2-8] we now report on the development of a convenient and general method for the synthesis of diasteriomerc (55:45) dinorcholane hydroxy acids and 5β-cholane hydroxy acid using readily available dinorcholanal and 5β-cholane-3α,24-diol as the starting materials.

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Results and Discussion

The cyanation reaction of carbonyl compounds is one of the most powerful procedures for the synthesis of cyanohydrins or cyanohydrin trimethylsilyl ethers. These products can easily be converted to a variety of other acid derivatives, such as α-hydroxy carboxylic acids, α-hydroxy aldehydes, β-amino alcohols and α-amino acids.[1] Several useful cyanating reagents are available, but among them, trimethylsilyl cyanide (TMSCN) is one of the most effective and safe cyanating sources for nucleophilic addition to carbonyl compounds.[9]

When an achiral nucleophile adds to an achiral aldehyde, a chiral centre is formed, which is a racemic mixture. However, when a chiral centre is present next to the carbonyl carbon, two possible diastereomeric products are formed. Thus, the reaction of 3-oxo-23,24-dinorchol-4-en-22-al (**1**) with TMSCN produced a diastereomeric mixture (55:45) of 22-cyanohydrin silyl ethers (**2A** and **2B**) (Scheme 1).

The FABMS spectrum of the mixture revealed the $[M+H]^+$ and $[M+Na]^+$ ions, respectively, at *m/z* 428 and 450. The presence of a C≡N moiety was evident from the IR absorption band at 2229 cm^{-1}. Among the ^1H NMR

signals, the two most important peaks were at δ 0.17 (s, 9H, 3 × Me), 4.42 and 4.38 (bd, 1H), which confirmed the presence of a TMS unit at C-22. In the ^{13}C NMR spectrum of this mixture, apart from the signals confirming the above findings, the most revealing signals were a pair of signals at δ 64.8 and 65.0 for the oxymethine (C-22, **2A** and **2B**) and another pair of signals at δ 120.1 and 118.3 for the C≡N carbon of two diastereomeric isomers **2A** and **2B**. The diastereomeric ratio was determined by ^{13}C NMR spectroscopic integration.

The diastereomeric 22-cyanohydrin silyl ethers (**2A** and **2B**) were refluxed 18 h using NH_3 (25%) in THF and the reaction mixture was washed with dil. HCl to obtain the 22-cyanohydrin. The products obtained were found to be a diastereomeric mixture of 22-cyanohydrins **3A** and **3B** (Scheme 1). A portion of the mixture was subjected to preparative reversed-phase HPLC resulting in the isolation of pure **3A** and **3B**. The isomer **3A** was eluted first with the retention time of 17.1 min, followed by the isomer **3B** having the retention time of 18.3 min. The diastereomeric ratio (55:45) was obtained from the HPLC signals. The compound **3A** and **3B** had different melting points, and they were 230 °C and 189-190 °C, respectively.

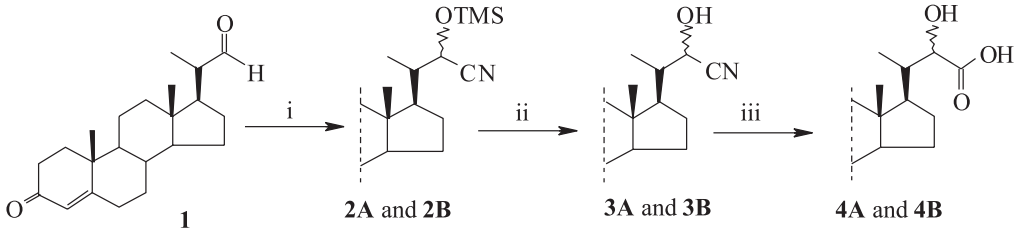
In the ^{1}H NMR of **3A**, the most important peak was a doublet at δ 4.53 (*J* 4.1 Hz) for the oxymethine at C-22, indicating the presence of a cyanohydrin unit at C-22. The ^{13}C NMR showed characteristic signals for the carbons, C-22 and C≡N at δ 64.9 and 122.4, respectively.[3] The ^{1}H NMR of **3B** was similar to that of **3A** with the exception that the resonance for the C-22 oxymethine (δ 4.48, *J* 4.1 Hz) was slightly less deshielded than **3A**. The ^{13}C NMR of **3B** was also similar to that of **3A** with the exception that the characteristic signals for the carbons, C-22 and C≡N were observed at δ 65.2 and 118.4, respectively. Unambiguous assignment of all ^{1}H and ^{13}C resonances were achieved by ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC experiments. The $[M+H]^+$ and $[M+Na]^+$ ions of both isomers were observed, respectively, at *m/z* 356 and 378 in their FABMS spectra. The presence of OH and C≡N functionalities, in both cases, was supported from the IR absorption bands at 3336 and 2235 cm^{-1}, respectively.

Acid hydrolysis of the diastereomeric mixture **3A** and **3B** in MeOH yielded the diastereomeric mixture of 22-hydroxy acids **4A** and **4B** (Scheme 1). The IR spectrum of this mixture showed the absorption bands characteristic for acid and alcohol hydroxyls at 3453 (acidic O-H), 3383 (alcoholic O-H), and acid carbonyl at 1720 cm^{-1}. In the ^{1}H NMR spectrum, a broad doublet at δ 3.68/3.63 for an oxymethine proton indicated the presence of a hydroxyl group at C-22 of diastereomeric isomers **4A** and **4B**. The ^{13}C NMR spectrum of this mixture displayed a pair of signals at δ 161.2/161.0 for the acid carbonyl carbon at C-22, and a pair of signals at δ 64.9 and 65.2 for the oxymethine (C-22) of diastereomeric isomers **4A** and **4B**. The diastereomeric ratio was determined by ^{13}C NMR spectroscopic integration. The FABMS spectrum revealed the $[M+H]^+$ and $[M+Na]^+$ ions, respectively, at *m/z* 375 and 397.

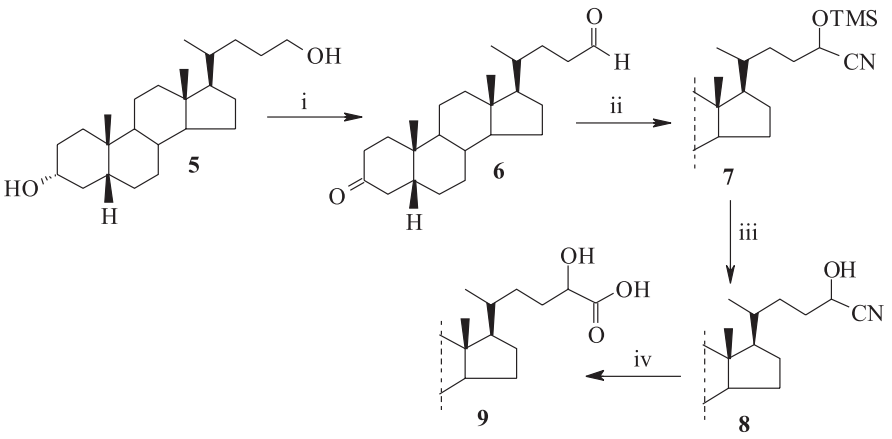
As outlined in Scheme 2, 3-oxo-5β-cholane-24-hydroxy acid (**9**) was synthesized from the diol **5** in 4 steps. In the first step, diol **5** was oxidized using PCC in DCM to obtain 5β-cholanal **6**.[10] The FABMS spectrum of **6** revealed the $[M+H]^+$ and $[M+Na]^+$ ions, respectively, at *m/z* 359 and 381. In its ^{13}C NMR spectrum, the signals at δ 213.4 and 203.1 established, respectively, the presence of 3-oxo and C-24 aldehyde functionalities.

3-Oxo-24-cyanohydrin silyl ether **7** was obtained from **6** in MeCN using TMSCN (Scheme 2). The FABMS spectrum of **7** revealed the $[M+H]^+$ and $[M+Na]^+$ ions at *m/z* 458 and 480, respectively. In its IR spectrum the absorption band at 2238 cm^{-1} was indicative of a C≡N stretching. In the ^{1}H NMR spectrum the signals for the protons associated with C-1 to C-24 of **7** were almost similar to those of **6**, except that a signal at δ 4.31 for the oxymethine proton of C-24 (instead of an aldehyde proton at δ 9.50 in **6**) and a 9H singlet for the TMS group were present at δ 0.19. The ^{13}C NMR spectrum also corroborated this fact by showing signals at δ 61.9, 120.2 and -0.4, respectively, for the C-24 oxymethine, nitrile and TMS carbons.

The 24-cyanohydrin silyl ether **7** was treated with NH_3 (25%) and refluxed for 18h then washed with dil. HCl to yield 24-cyanohydrin **8** (Scheme 2). The FABMS spectrum of **8** revealed the $[M+H]^+$ and $[M+Na]^+$ ions at *m/z* 386 and



Scheme 1. (i) TMSCN, MeCN, 12h; (ii) NH_3 (25%), THF, 18h, then washed with dil. HCl; (iii) conc. HCl, MeOH, THF, 24h.



Scheme 2. (i) PCC, DCM, 18h; (ii) TMSCN, MeCN, 12h; (iii) NH_3 (25%), THF, 18h, then washed with dil. HCl; (iv) conc. HCl, MeOH, THF, 24h.

408, respectively. In its IR spectrum the absorption bands at 3452 and 2241 cm^{-1} were indicative of alcohol OH and C≡N stretchings. The ^1H and ^{13}C NMR spectra of **8** were similar to those of **7** with the exception that there was no signal for a TMS group, and both C-24 oxymethine and nitrile signals were slightly deshielded.

Acid hydrolysis of the 24-cyanohydrin **8** in MeOH yielded 24-hydroxy acid **9** (Scheme 2). The IR spectrum showed the absorption bands characteristic for acid and alcohol hydroxyls, respectively, at 3458 and 3454 cm^{-1}, and an acid carbonyl at 1728 cm^{-1}. In the ^1H NMR spectrum of **9**, a broad doublet at δ 3.62 for an oxymethine proton indicated the presence of a hydroxyl group and an acid group at C-24. The ^{13}C NMR spectrum displayed a signal at δ 162.0 for the acid carbonyl carbon at C-24, and a signal at δ 62.4 for the oxymethine (C-24). The FABMS spectrum revealed the $[M+H]^+$ and $[M+Na]^+$ ions, respectively, at m/z 405 and 427.

This study established facial synthetic routes for the synthesis of diastereomeric mixture (55:45) of 3-oxo-23,24-dinorchol-4-en-22-hydroxy acids (4A and 4B) from 3-oxo-23,24-dinorchol-4-en-22-al (**1**), and 3-oxo-5β-cholane-24-hydroxy acid (**9**) from 5β-cholane-3α,24-diol (**5**). On the basis of spectroscopic data obtained for compound **9**, it was not possible to confirm if this compound was a pure enantiomer or a racemic mixture. However it was certain that this compound (**9**) could not be a diastereomeric mixture, because there was no stereo centre next to the carbonyl (reactive centre).

Experimental

General

The starting material 5β-cholane-3α,24-diol (**5**) was previously synthesized and identified in our lab.[2] 3-Oxo-23,24-dinorchol-4-en-22-al (**1**), pyridinium chlorochromate (PCC) and trimethylsilyl cyanide (TMSCN) were purchased from Aldrich and used as received. The reactions were monitored and the purity of the products was assessed by thin-layer chromatography (TLC) performed on silica gel (Merck type 60) and visualized under UV illumination and/or by I_2 vapor. Melting points of the products were determined on a Gallen-kamp melting point apparatus. Infrared spectra (wave numbers in cm^{-1}) were recorded on an ATI Mattson Genesis FTIR spectrophotometer either as KBr pellets or in $CHCl_3$. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Unity INOVA 400 MHz NMR spectrometer. NMR spectra were obtained in $CDCl_3$. Chemical shifts (δ) are reported in ppm downfield from TMS, using the residual solvent peak (7.25 ppm for ^1H and 77.23 ppm for ^{13}C) as an internal standard and coupling constants (*J*) in Hz. Mass spectroscopic analyses were performed at the EPSRC Mass Spectrometry Service at Swansea.

*Synthesis of diastereomeric 3-oxo-23,24-dinorchol-4-ene-22-cyanohydrin silyl ether (**2A** and **2B**)*

To a stirred solution of 3-oxo-23,24-dinorchol-4-en-22-al (**1**, 200 mg, 0.61 mmol) in MeCN (8 mL), TMSCN (266 mg, 4.4 equiv.) was added under N_2 and the reaction was monitored by TLC. After 12 h the reaction mixture was evaporated to dryness to obtain a dark yellow oil, which was purified by Vacuum Liquid Chromatography (VLC) (30% EtOAc in pet-ether) to yield a white solid as a diastereomeric mixture (55:45) of the title compound **2A** and **2B**. Yield: 246 mg (94%), mp 140-141 °C. IR ($CHCl_3$) ν_{max}/cm^{-1}: 2935 (C-H), 2852 (C-H), 2229 (C≡N), 1666 (ketonic C=O), 1616 (C=C), 1460, 1253 (ether C-O), 1186, 1105, 1040, 937, 879, 847, 752 and 654. ^1H NMR (400 MHz, $CDCl_3$): δ 0.17 (s, 9H, OTMS), 0.69 (s, 3H, 18-Me), 1.10 (d, *J* 5.2 Hz, 3H, 21-Me), 1.15 (s, 3H, 19-Me), 4.42 (br d, 1H, 22-OCH, **2A**) and 4.38 (br d, 1H, 22-OCH, **2B**),

5.68 (s, 1H, 4-CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 35.4 (C-1), 33.6 (C-2), 199.2 (C-3), 123.5 (C-4), 171.0 (C-5), 32.5 (C-6), 31.6 (C-7), 35.3 (C-8), 51.1 (C-9), 38.2 (C-10), 20.7 (C-11), 39.0 (C-12), 42.3 (C-13), 53.3 (C-14), 24.0 (C-15), 27.2 (C-16), 55.1 (C-17), 11.8 (C-18), 17.1 (C-19), 42.0 (C-20), 13.4 (C-21), 64.8 (C-22, **2A**) and 65.0 (C-22, **2B**), 120.1 (C-23, **2A**) and 118.3 (C-23, **2B**), -0.3 (OTMS). FABMS m/z: 428 $[M+H]^+$, 450 $[M+Na]^+$. HR-FABMS *m/z*: $[M+H]^+$ 428.2979; calc. 428.2979 for $C_{26}H_{42}NO_2Si$.

*Synthesis of diastereomeric 3-oxo-23,24-dinorchol-4-ene-22-cyanohydrin (**3A** and **3B**)*

To a stirred solution of the mixture of **2A** and **2B** (225 mg, 0.53 mmol) in THF (8 mL), 25% NH_3 (5 mL) was added and the mixture was refluxed for 18 h. Dilute HCl was added to the reaction mixture, extracted with $CHCl_3$ and washed with H_2O to obtain 22-cyanohydrin (**3**). The solid was cleaned up by VLC (40% EtOAc in pet-ether) to yield as a diastereomeric mixture (55:45) of the title compound **3A** and **3B**. Yield: 155 mg (82%). Small portion of the mixture of **3A** and **3B** was separated by preparative reversed-phase HPLC using a LUNA C_{18} preparative column (250 mm × 21.2 mm, 10 µm), eluted with a linear gradient: 45-100% acetonitrile in water in 40 minutes, followed by 100% acetonitrile for 10 min, flow rate = 20 mL min^{-1}, monitored by a photo-diode-array detector (Figure 1).

The purified 22-cyanohydrin **3A** was obtained as crystals, mp 230 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.53 (d, *J* 4.1 Hz, 1H, 22-OCH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 64.9 (C-22), 122.4 (C-23) (lit. mp 230 °C, IR, ^{1}H NMR, ^{13}C NMR and FABMS).[3]

The purified 22-cyanohydrin **3B** was obtained as a white amorphous solid, mp 189-190 °C. IR ($CHCl_3$) ν_{max}/cm^{-1}: 3336 (O-H), 2945 (C-H), 2873 (C-H), 2235 (C≡N), 1670 (ketonic C=O), 1616 (C=C), 1448, 1379, 1254, 1072, 935, 847 and 756. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.76 (s, 3H, 18-Me), 1.09 (d, *J* 6.5 Hz, 3H, 21-Me), 1.19 (s, 3H, 19-Me), 4.48 (d, *J* 4.1 Hz, 1H, 22-OCH), 5.67 (s, 1H, 4-CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 35.5 (C-1), 33.9 (C-2), 199.7 (C-3), 123.9 (C-4), 171.3 (C-5), 32.8 (C-6), 31.9 (C-7), 35.6 (C-8), 52.0 (C-9), 38.5 (C-10), 20.9 (C-11), 39.3 (C-12), 42.8 (C-13), 53.6 (C-14), 24.2 (C-15), 27.4 (C-16), 55.3 (C-17), 12.1 (C-18), 17.4 (C-19), 41.1 (C-20), 13.2 (C-21), 65.2 (C-22), 118.4 (C-23). FABMS *m/z*: 356 $[M+H]^+$, 378 $[M+Na]^+$. HR-FABMS *m/z*: $[M+H]^+$ 356.2583; calc. 356.2584 for $C_{23}H_{34}NO_2$.

*Synthesis of diastereomeric 3-oxo-23,24-dinorchol-4-ene-22-hydroxy acid (**4A** and **4B**)*

To a solution of **2** (100 mg, 0.28 mmol) in THF (8 mL), conc. HCl (2 mL) was added, and the mixture was stirred under reflux for 24h. Solvent was removed under reduced pressure. The residue was dissolved in DCM and saturated $NaHCO_3$ was added to neutralize the HCl, and the mixture was extracted with DCM, washed with H_2O and dried over $MgSO_4$. The solid was identified as a diastereomeric mixture (55:45) of the title compound **4A** and **4B** as slowly solidifying yellow oil. Yield: 56 mg (53%), mp 179-180 °C. IR ($CHCl_3$) ν_{max}/cm^{-1}: 3453 (acidic O-H), 3383 (alcoholic O-H), 2938 (C-H), 2860 (C-H), 1720 (acidic C=O), 1674 (ketonic C=O), 1618 (C=C), 1464, 1378, 1269, 1223, 1188, 1060, 867 and 750. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.73 (s, 3H, 18-Me) and 0.72 (s, 3H, 18-Me), 1.15 (d, *J* 6.7 Hz, 3H, 21-Me), 1.17 (s, 3H, 19-Me), 3.68 (br d, 1H, 22-OCH, **4A**) and 3.63 (br d, 1H, 22-OCH, **4B**), 5.72 (s, 1H, 4-CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 35.7 (C-1), 34.1 (C-2), 200.7 (C-3), 125.9 (C-4), 172.6 (C-5), 33.1 (C-6), 32.1 (C-7), 35.6 (C-8), 52.2 (C-9), 39.0 (C-10), 21.1 (C-11), 39.5 (C-12), 43.2 (C-13), 53.9 (C-14), 24.3 (C-15), 27.5 (C-16), 56.2 (C-17), 12.2 (C-18), 17.8 (C-19), 41.3 (C-20), 13.9 (C-21), 64.9 (C-22, **4A**) and 65.2 (C-22, **4B**), 161.2 (C-23, **4A**) and 161.0 (C-23, **4B**). FABMS *m/z*: 375 $[M+H]^+$, 397 $[M+Na]^+$. HR-FABMS *m/z*: $[M+H]^+$ 375.25298; calc. 375.25299 for $C_{23}H_{35}O_4$.

*Synthesis of 3-oxo-5β-cholan-24-al (**6**)*

A solution of 5β-cholan-3α,24-diol (**5**, 370 mg, 1.03 mg) in dry DCM (40 mL) was treated with PCC (440 mg, 2 molar equiv.), and the mixture was refluxed for 18h under N_2. The reaction was quenched with H_2O and extracted with ether. The ethereal solution was washed with H_2O, $NaHCO_3$ and brine, dried over $MgSO_4$. The compound was purified by VLC (20% EtOAc in pet-ether) and identified as the title compound **6**. Yield: 287 mg (78%), mp 81-82 °C (lit. mp 82-83 °C, IR and ^{1}H NMR).[10] ^{13}C NMR (100 MHz, $CDCl_3$): δ 37.2 (C-1), 37.0 (C -2), 213.4 (C-3), 42.3 (C-4), 44.3 (C-5), 25.8 (C-6), 26.6 (C-7), 35.3 (C-8), 40.7 (C-9), 34.9 (C-10), 21.2 (C-11), 40.3 (C-12), 42.8 (C-13), 56.4 (C-14), 24.1 (C-15), 28.1 (C-16), 56.0 (C-17), 12.1 (C-18), 22.6 (C-19), 35.5 (C-20), 18.2 (C-21), 27.9 (C-22), 30.7 (C-23), 203.1 (C-24). FABMS *m/z*: 359 $[M+H]^+$, 381 $[M+Na]^+$.

*Synthesis of 3-oxo-5β-cholane-24-cyanohydrin silyl ether (**7**)*

A solution of **6** (200 mg, 0.56 mmol) in dry MeCN (8 mL) was treated with TMSCN (244 mg, 4.4 molar equiv.), and the mixture was refluxed for 12 h under N_2. After rotary evaporation, the crude solid was purified by Preparative Thin Layer Chromatography (PTLC) (30% EtOAc in pet-ether) and the title compound **7** was obtained as a yellowish thick gum. Yield: 160 mg (62%). IR ($CHCl_3$) ν_{max}/cm^{-1}: 2953 (C-H), 2856 (C-H), 2238 (C≡N), 1726 (ketonic C=O), 1460, 1379, 1277, 1120, 1076, 847 and

744. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.19 (s, 9H, OTMS), 0.63 (s, 3H, 18-Me), 0.88 (d, *J* 6.5 Hz, 3H, 21-Me), 0.97 (s, 3H, 19-Me), 4.31 (br t, 1H, 24-CH-O). ^{13}C NMR (100 MHz, $CDCl_3$): δ 37.3 (C-1), 37.1 (C-2), 213.5 (C-3), 41.0 (C-4), 44.4 (C-5), 25.8 (C-6), 26.7 (C-7), 35.4 (C-8), 40.8 (C-9), 35.0 (C-10), 21.3 (C-11), 40.1 (C-12), 42.4 (C-13), 56.5 (C-14), 24.2 (C-15), 28.3 (C-16), 56.1 (C-17), 12.1 (C-18), 22.7 (C-19), 35.6 (C-20), 18.5 (C-21), 33.0 (C-22), 30.7 (C-23), 61.9 (C-24), 120.2 (C-25), -0.4 (24-OTMS). FABMS *m/z*: 458 $[M+H]^+$, 480 $[M+Na]^+$. HR-FABMS *m/z*: $[M+H]^+$ 458.3448; calc. 458.3449 for $C_{28}H_{48}NO_2Si$.

*Synthesis of 3-oxo-5*β*-cholane-24-cyanohydrin (**8**)*

A solution of **7** (150 mg, 0.33 mmol) in dry THF (5 mL) was treated with 25% NH_3 (5 mL), and the mixture was refluxed for 18 h, extracted with $CHCl_3$ and washed with H_2O, dil. HCl and brine, dried over $MgSO_4$. The solvent was evaporated at reduced pressure to afford a crude solid which was purified by PTLC (40% EtOAc in pet-ether) and the title compound **8** was obtained as an oil. Yield: 81 mg (64%). IR ($CHCl_3$) ν_{max}/cm^{-1}: 3452 (O-H), 2939 (C-H), 2862 (C-H), 2241 (C≡N), 1726 (ketonic C=O), 1450, 1379, 1254, 1119, 1078, 866 and 755. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.68 (s, 3H, 18-Me), 0.93 (d, *J* 6.5 Hz, 3H, 21-Me), 0.99 (s, 3H, 19-Me), 4.41 (br t, 1H, 24-OCH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 37.3 (C-1), 37.1 (C -2), 213.5 (C-3), 41.0 (C-4), 44.3 (C-5), 25.8 (C-6), 26.7 (C-7), 35.2 (C-8), 42.3 (C-9), 35.2 (C-10), 21.5 (C-11), 40.1 (C-12), 42.4 (C-13), 56.6 (C-14), 24.3 (C-15), 28.2 (C-16), 56.2 (C-17), 12.3 (C-18), 22.9 (C-19), 35.7 (C-20), 18.8 (C-21), 33.2 (C-22), 30.9 (C-23), 30.9 (C-23), 62.3 (C-24), 122.0 (C-25). FABMS *m/z*: 386 $[M+H]^+$, 408 $[M+Na]^+$. HR-FABMS *m/z*: $[M+H]^+$ 386.3053; calc. 386.3054 for $C_{25}H_{40}NO_2$.

*Synthesis of 3-oxo-5*β*-cholane-24-hydroxy acid (**9**)*

To a solution of **8** (75 mg, 0.19 mmol) in THF (5 mL), conc. HCl (2 mL) was added, and the mixture was stirred under reflux for 24h. The solvent was removed under reduced pressure. The residue was dissolved in DCM and saturated $NaHCO_3$ was added to neutralize the HCl, and the mixture was extracted with DCM, washed with H_2O, dried over $MgSO_4$. The solvent was evaporated at reduced pressure to afford a crude product which was purified by PTLC (40% EtOAc in pet-ether) and the title compound **9** was obtained as an oil. Yield: 53 mg (69%). IR ($CHCl_3$) ν_{max}/cm^{-1}: 3458 (acidic O-H), 3454 (alcoholic O-H), 2938 (C-H), 2863 (C-H), 1728 (ketonic C=O), 1452, 1378, 1255, 1118, 1079, 868 and 756. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.68 (s, 3H, 18-Me), 0.92 (d, *J* 6.5 Hz, 3H, 21-Me), 0.98 (s, 3H, 19-Me), 3.62 (br d, 1H, 24-OCH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 37.3 (C-1), 37.1 (C -2), 213.5 (C-3), 41.0 (C-4), 44.3 (C-5), 25.8 (C-6), 26.7 (C-7), 35.2 (C-8), 42.3 (C-9), 35.2 (C-10), 21.5 (C-11), 40.1 (C-12), 42.4 (C-13), 56.6 (C-14), 24.3 (C-15), 28.2 (C-16), 56.2 (C-17), 12.3 (C-18), 22.9 (C-19), 35.7 (C-20), 18.8 (C-21), 33.2 (C-22), 30.9 (C-23), 62.4 (C-24), 162.0 (C-25). FABMS *m/z*: 405 $[M+H]^+$, 427 $[M+Na]^+$. HR-FABMS *m/z*: $[M+H]^+$ 405.2999; calc. 405.2999 for $C_{25}H_{41}O_4$.

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