Short Communication

A Facile Synthesis of Ethyl (3-Oxo-5β-Cholan)-24-yl Oxalate

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Abstract

A facile synthesis of ethyl (3-oxo-5β-cholan)-24-yl oxalate (**8**) has been achieved from lithocholic acid (**1**) in 5 steps with a 64% yield. Comprehensive NMR spectroscopic data interpretations for all new compounds are presented.

Keywords: lithocholic acid, methyl lithocholate, oxalyl chloride, esterification, NMR.

1. Introduction

Steroids are a group of pharmacologically active molecules widely found in both plant and animal kingdoms. Lithocholic acid (**1**), a secondary bile acid, is a biologically important molecule which has one functional group at each end of the molecule, and can be functionalised selectively after protecting one functional group or may be functionalised in both reactive sites.[1] As part of our continuing studies on the synthesis and reactions of steroidal derivatives,[2-10] we report now on an efficient synthetic method for the synthesis of ethyl (3-oxo-5β-cholan)-24-yl oxalate (**8**) in 5 steps starting from lithocholic acid (**1**).

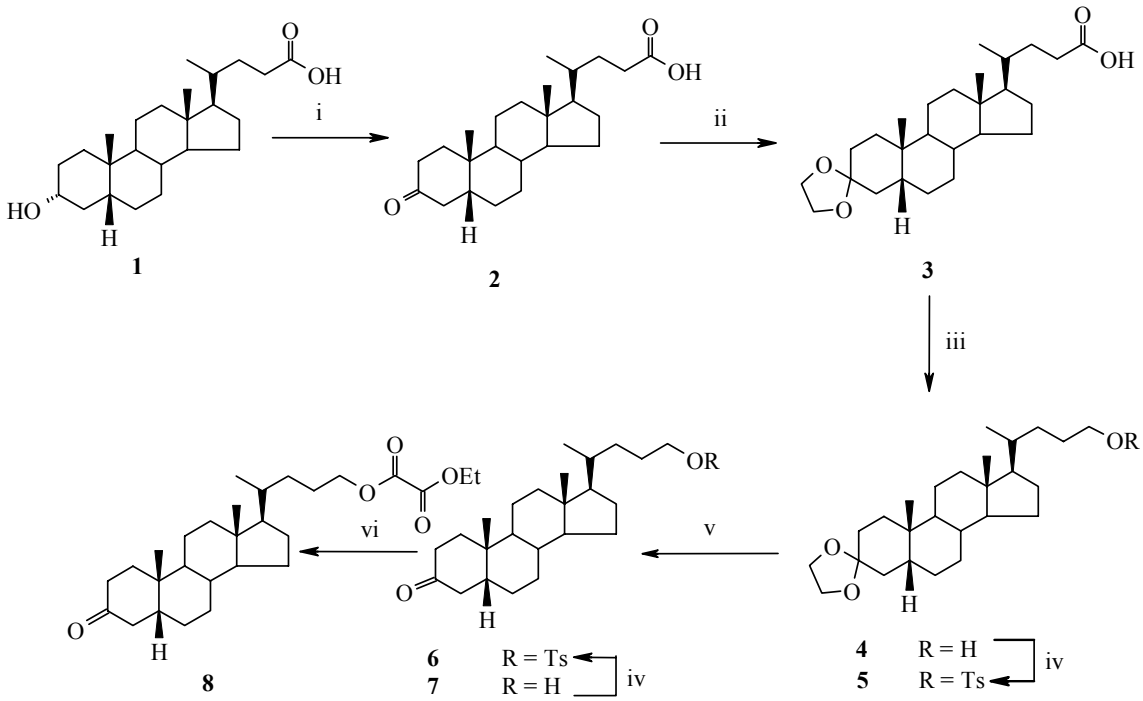
2. Results and Discussion

Secondary alcohols are easy to oxidise to corresponding ketones by the treatment of Jones' reagent. Lithocholic acid (**1**) was oxidised to 3-oxo acid **2**[11] (Scheme 1). The structure of **2** was confirmed by NMR (^{1}H and ^{13}C) and ESIMS analyses. The $[M+H]^+$ and $[M+Na]^+$ ions were observed, respectively, at *m/z* 375 and 397 in its ESIMS spectrum. The presence of the ketonic carbonyl (C-3) and acid carbonyl (C-24) was confirmed from the peaks, respectively, at δ 203.8 and 180.1 in its ^{13}C NMR spectrum. The downfield shift (0.10 ppm) of the protons of C-19 methyl group (δ 1.01) compared to that of lithocholic acid (δ 0.91) also supported the presence of an oxo group at C-3.

Ketones are more easily reduced than acids or esters. Therefore, any reducing agent that can reduce acid or ester functionalities can also reduce the keto group. If the keto group is protected by the formation of ketal, stable in basic condition, then the acid or ester functionalities can be easily reduced without affecting the ketal. Later, the ketal can be removed by the treatment with aqueous acid. The 3-oxo group in **2** was protected by the formation of a ketal. 3,3-Ethylenedioxy acid (**3**)[12] was obtained by treating **2** in orthoformate $(CH_3O)_3CH$ with ethylene glycol $(CH_2OH)_2$ and *p*-TsOH.H_2O (Scheme 1). The identity of compound **3** was established by ^{13}C NMR (Table 1) and ESIMS data analyses. The ESIMS spectrum revealed the $[M+H]^+$ and $[M+Na]^+$ ions, respectively, at *m/z* 419 and 441. The presence of the ethylenedioxy moiety was apparent from the presence of a ^{1}H NMR signal at δ 3.90 (s, 4H) and also from the ^{13}C NMR signals at δ 64.2 and 64.0.

The reduction of **3** with excess lithium aluminium hydride provided 3,3-ethylenedioxy alcohol **4** (Scheme 1). The IR spectrum displayed absorption band at 3377 cm^{-1} which was characteristic for alcoholic OH stretching. In the ^{1}H NMR spectrum, a 2H multiplet at δ 3.60, corresponding to an oxymethylene, indicated the presence of an OH group at C-24. This fact was corroborated further from the ^{13}C NMR (Table 1) signal at δ 63.6 for the C-24 oxymethylene carbon. The $[M+H]^+$, and $[M+Na]^+$ ions at *m/z* 405 and 427 observed in its ESIMS spectrum confirmed the molecular formula $C_{26}H_{44}O_3$.

3,3-Ethylenedioxy alcohol **4** was treated selectively with 1.2 molar excess of *p*-TsCl at 4 °C for 2h to give 3,3-ethylenedioxy tosylate **5** which was refluxed with 80% aqueous AcOH for 18h at 120 °C to yield 3-oxo tosylate **6** (Scheme 1). The ESIMS spectrum of **5** revealed the $[M+H]^+$ and $[M+Na]^+$ ions, respectively at *m/z* 559 and 581.



Scheme 1. (i) Jones' reagents, acetone, 4 °C, 40 min. (ii) Ethylene glycol, orthoformate, *p*-TsOH.H_2O, r.t., 2h. (iii) LAH, THF, r.t., 18h. (iv) *p*-TsCl, Pyridine, 4 °C, 2h. (v) AcOH (80%), MeOH, refluxed at 120 °C, 18h. (vi) $(COCl)_2$, DCM, r.t., 18h, then $CHCl_3$ (1% EtOH), 40 °C, 1h.

Table 1: ^{13}C NMR ($CDCl_3$, 100 MHz) data of compounds **3-5**

No	δ in ppm			No	δ in ppm		
	6	**7**	**8**		**6**	**7**	**8**
1	37.2	37.0	37.3	17	56.0	56.1	56.0
2	37.0	37.0	37.0	18	12.0	12.0	12.1
3	213.4	213.4	213.5	19	22.6	23.1	22.7
4	42.3	42.4	42.4	20	35.2	35.6	35.4
5	44.3	44.3	44.4	21	18.4	18.6	18.3
6	25.8	25.8	25.8	22	31.4	31.8	31.3
7	26.6	26.6	26.6	23	25.6	29.4	31.0
8	35.5	35.6	35.5	24	71.2	63.6	174.4
9	40.7	40.8	40.7	24-O**C**$H_2$$CH_3$	-	-	60.1
10	34.9	34.2	34.9	24-OCH_2**C**H_3	-	-	14.0
11	21.2	21.2	21.2	*24-OTs*			
12	40.1	40.1	40.1	1	144.6	-	-
13	42.7	42.7	42.8	2,6	129.8	-	-
14	56.4	56.5	56.4	3,5	127.9	-	-
15	24.1	24.2	24.2	4	133.3	-	-
16	28.2	28.3	28.3	4-Me	21.6	-	-

In its ^1H NMR spectrum, in addition to the signals associated with the protons of the starting material **4**, signals at δ 7.75 (2H, J = 8.2 Hz) and δ 7.30 (2H, J = 8.2 Hz) for the *p*-di-substituted benzene ring system and at δ 2.41 for the methyl group of the tosyl moiety were observed. The downfield shift (δ 3.96) of the resonance for the C-24 methylene protons of 3,3-ethylenedioxy tosylate **5** (compared to that of **4** at δ 3.60) confirmed the attachment of the tosyl unit at C-24. Also in its ^{13}C NMR spectrum (Table 1), the C-24 carbon resonance was observed at a further deshielded position (δ 71.2) compared to that of **4** (δ 63.6).

Table 2: ^{13}C NMR ($CDCl_3$, 100 MHz) data of compounds **6-8**

No	δ in ppm			No	δ in ppm		
	6	**7**	**8**		**6**	**7**	**8**
1	37.2	37.0	37.3	17	56.0	56.1	56.0
2	37.0	37.0	37.0	18	12.0	12.0	12.1
3	213.4	213.4	213.5	19	22.6	23.1	22.7
4	42.3	42.4	42.4	20	35.2	35.6	35.4
5	44.3	44.3	44.4	21	18.4	18.6	18.3
6	25.8	25.8	25.8	22	31.4	31.8	31.3
7	26.6	26.6	26.6	23	25.6	29.4	31.0
8	35.5	35.6	35.5	24	71.2	63.6	174.4
9	40.7	40.8	40.7	24-O**C**$H_2$$CH_3$	-	-	60.1
10	34.9	34.2	34.9	24-OCH_2**C**H_3	-	-	14.0
11	21.2	21.2	21.2	*24-OTs*			
12	40.1	40.1	40.1	1	144.6	-	-
13	42.7	42.7	42.8	2,6	129.8	-	-
14	56.4	56.5	56.4	3,5	127.9	-	-
15	24.1	24.2	24.2	4	133.3	-	-
16	28.2	28.3	28.3	4-Me	21.6	-	-

The IR spectrum of **6** displayed absorption band at 1714 cm^{-1} characteristic for a ketonic carbonyl functionality and thus supported the presence of an oxo group at C-3. In its HRESIMS spectrum, the $[M+Na]^+$ ion was observed at *m/z* 537.30146 calculated for $C_{31}H_{46}SO_4Na$ which indicated the presence of an oxo group, instead of an ethylenedioxy group, in the molecule. In the ^{1}H NMR spectrum of **6**, the signal for the ethylendioxy protons (as in **5**) was absent. The downfield shift (0.08 ppm) of the protons of C-19 methyl group (δ 0.97) compared to that of **5** (δ 0.89) supported the presence of an oxo group at C-3. This fact was confirmed further from the ^{13}C NMR spectrum (Table 2), where a signal at δ 213.3 owing to the ketonic carbonyl carbon at C-3 was present.

Acid hydrolysis of **4** yielded 3-oxo alcohol **7** which was tosylated to obtain **6** (Scheme 1). The compound **7** was identified primarily by comparing its melting point and IR data with published data.[13] In its ESIMS spectrum, the $[M+H]^+$ and $[M+Na]^+$ ions, respectively, at *m/z* 361 and 383 which indicated the presence of an oxo group, instead of an ethylenedioxy group, in the molecule. In the ^{1}H NMR spectrum of **7**, the signal for the ethylendioxy protons (as in **4**) was absent. The downfield shift (0.08 ppm) of the protons of C-19 methyl group (δ 0.98) compared to that of **4** (δ 0.90) supported the presence of an oxo group at C-3. The ^{13}C NMR spectrum (Table 2) of **7** displayed the expected resonance at δ 213.4 for the ketonic carbonyl (C-3).

Ethyl (3-oxo-5β-cholan)-24-yl oxalate (**8**) was synthesised from **7** using excess oxalyl chloride and $CHCl_3$ (1% EtOH) at 40 °C (Scheme 1). The IR spectrum of **8** exhibited three absorption bands at 1765 and 1742 cm^{-1} for the oxalate carbonyls and 1710 cm^{-1} for the ketonic carbonyl. The HRFABMS spectrum of **8** exhibited the $[M+H]^+$ ion at *m/z* 461.336678 corresponding to $C_{28}H_{45}O_5$. The ^{1}H and ^{13}C NMR of **8** (Table 2), in addition to the signals assignable to the starting material, exhibited signals for an ethyl oxalate moiety. The deshielded ^{1}H and ^{13}C chemical shifts of C-24 oxymethylene (δ_H 4.22 and δ_C 67.6) confirmed the attachment of the ethyl oxalate group at C-24.

3. Experimental

The steroid starting material (Lithocholic acid, **1**), oxalyl chloride and LAH were purchased from Aldrich and used as received. All chemicals and solvents were used throughout without further purification. 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 visualised under UV illumination and/or by I_2 vapour. Vacuum liquid chromatography (VLC) was performed on silica gel 60H. 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 as KBr pellets. 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 ^{1}H 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.

3-Oxo-5β-cholan-24-oic acid (2):
Lithocholic acid (**1**, 500 g, 1.33 mmol) was oxidised using freshly prepared Jones' reagents (2 ml) to obtain the title compound **2** as a white solid (463 mg, 93%), mp: 122-123 °C (*lit.*[11] mp: 121-122 °C and IR). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.68 (s, 3H, 18-Me), 0.92 (d, *J* = 6.1 Hz, 3H, 21-Me), 1.01 (s, 3H, 19-Me). ^{13}C NMR (100 MHz, $CDCl_3$): δ 37.5 (C-1), 37.3 (C-2), 203.8 (C-3), 42.6 (C-4), 44.6 (C-5), 26.1 (C-6), 26.9 (C-7), 35.6 (C-8), 41.1 (C-9), 35.2 (C-10), 21.5 (C-11), 40.3 (C-12), 43.1 (C-13), 56.7 (C-14), 24.4 (C-15), 28.4 (C-16), 56.3 (C-17), 12.4 (C-18), 22.9 (C-19), 35.8 (C-20), 18.5 (C-21), 31.2 (C-22), 31.0 (C-23), 180.1 (C-24). ESIMS: *m/z* 375 $[M+H]^+$, 397 $[M+Na]^+$.

3,3-Ethylenedioxy-5β-cholan-24-oic acid (3):
To a stirred solution of **2** (350 mg, 0.93 mmol) in toluene (10 ml) containing 4Å molecular sieves, ethylene glycol (10 ml) and *p*-TsOH.H_2O (177 mg, 0.93 mmol) was added at r.t. and the mixture was stirred for 2h. The reaction mixture was carefully poured into a mixture of ice and saturated $NaHCO_3$ solution. A white precipitation was formed and the mixture was extracted with EtOAc (3 x 20 ml) and the organic layer was washed with brine, separated, dried ($MgSO_4$), filtered and evaporated to dryness. The crude product was purified by VLC (eluted with 15% EtOAc in pet-ether) to obtain the title compound **3** (232 mg, 60%), mp: 179-180 °C (*lit.*[12] mp: 181-182 °C, IR and ^{1}H NMR) and ^{13}C NMR (100 MHz, $CDCl_3$): Table 1. ESIMS: *m/z* 419 $[M+H]^+$, 441 $[M+Na]^+$.

3,3-Ethylenedioxy-5β-cholan-24-ol (4):
Reduction of **3** (200 mg, 0.46 mmol) using excess lithium aluminium hydride (70 mg, 4 equiv.) in dry THF yielded crude solid. The crude product was purified by VLC using step gradient elution (pet-ether: EtOAc = 90:10, 85:15, 20:80 and finally, 100% pet-ether) to get the title compound **4** (139 mg, 75%), semi-solid, mp: 98 °C. IR ($CHCl_3$): ν_{max} cm^{-1} 3377br (alcoholic O-H), 2939s (C-H), 2866s (C-H), 1446m, 1377m, 1364m, 1182m, 1095s (alcoholic C-O), 945w and 756m. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.61 (s, 3H, 18-Me), 0.91 (d, *J* = 6.8 Hz, 3H, 21-Me), 0.90 (s, 3H, 19-Me), 3.60 (m, 2H, 24-OCH_2), 3.92 (s, 4H, OCH_2-CH_2O) and ^{13}C NMR (100 MHz, $CDCl_3$): Table 1. ESIMS: *m/z* 405 $[M+H]^+$, 427 $[M+Na]^+$. HRESIMS: Found: 427.31882; calc 427.31881 for $C_{26}H_{44}O_3Na$.

3,3-Ethylenedioxy-5β-cholan-24-yl tosylate (5):
Tosylation of **4** (50 mg, 0.12 mmol) was carried out using 1.2 molar excess of *p*-TsCl at 4 °C for 2h. The crude oil was purified by PTLC (20% EtOAc in pet-ether) to obtain the title compound **5** (29 mg, 43%), colourless oil. IR ($CHCl_3$): ν_{max} cm^{-1} 2940s (C-H), 2869s (C-H), 1598m (aromatic C=C), 1448m, 1362m, 1290m, 1177s (O-SO_2), 1097s (diol C-O), 968m, 915m, 814m, 755m and 664m. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.56 (s, 3H, 18-Me), 0.89 (s, 3H, 19-Me), 0.80 (d, *J* = 6.2 Hz, 3H, 21-Me), 3.89 (s, 4H, OCH_2-CH_2O), 3.96 (m, 2H, 24-OCH_2), 24-OTs: 7.75 (d, *J* = 8.2 Hz, 2H, 2 x Ph-**H**), 7.30 (d, *J* = 8.2 Hz, 2H, 2 x Ph-**H**), 2.41 (s, 3H, Ph-**Me** and ^{13}C NMR (100 MHz, $CDCl_3$): Table 1. ESIMS: *m/z* 559 $[M+H]^+$, 581 $[M+Na]^+$. HRESIMS: Found: 581.32766; calc 581.32765 for $C_{33}H_{50}SO_5Na$.

3-Oxo-5β-cholan-24-yl tosylate (6):
A stirred solution of **5** (50 mg, 0.09 mmol) was suspended in 80% AcOH (1 ml), and the reaction mixture was refluxed for 18h. The residue was subjected to PTLC (eluted with 20% EtOAc in pet-ether) to afford the title compound **6** (17 mg, 37%), colourless oil. IR ($CHCl_3$): ν_{max} cm^{-1} 2950vs (C-H), 2864s (C-H), 1714vs (ketonic C=O), 1598m (Ph C=C), 1453m, 1361m, 1176vs (O-SO_2), 1098m, 967m, 917m, 815m, 754m, 664m and 555m. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.61 (s, 3H, 18-Me), 0.83 (d, *J* = 6.5 Hz, 3H, 21-Me), 0.97 (s, 3H, 19-Me), 3.97 (m, 2H, 24-OCH_2), 24-OTs: 7.75 (d, *J* = 8.2 Hz, 2H, 2 x Ph-**H**), 7.30 (d, *J* = 7.9 Hz, 2H, 2 x Ph-**H**), 2.41 (s, 3H, Ph-**Me**) and ^{13}C NMR (100 MHz, $CDCl_3$): Table 2. ESIMS: *m/z* 515 $[M+H]^+$, 537 $[M+Na]^+$. HRESIMS: Found: 537.30146; calc 537.30144 for $C_{31}H_{46}SO_4Na$.

3-Oxo-5β-cholan-24-ol (7):
3,3-Ethylenedioxy-5β-cholan-24-ol (**4**, 150 mg, 0.29 mmol) was suspended in MeOH (10 ml) and 80% aqueous AcOH (4 ml), and heated to reflux for 12h (oil bath at 120 °C). The mixture was allowed to cool to r.t., brine (20 ml) was added and thoroughly extracted with EtOAc (3 x 10 ml). The organic extract was washed with saturated $NaHCO_3$ solution (15 ml), dried ($MgSO_4$), filtered and concentrated. The residue (98 mg) was subjected to PTLC (eluted 20% EtOAc in pet-ether) to obtain the title compound **7** (82 mg, 78%), mp: 115-116 °C (*lit.*[13] mp: 115.5-116.5 °C). IR ($CHCl_3$): ν_{max} cm^{-1} 3401br (alcoholic O-H), 2944vs (C-H), 2864s (C-H), 1714vs (ketonic C=O), 1447m, 1377m, 1096s (alcoholic C-O), 945m and 665w (*lit.*[4] IR: partial assignments). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.65 (s, 3H, 18-Me), 0.84 (d, *J* = 6.8 Hz, 3H, 21-Me), 0.98 (s, 3H, 19-Me), 3.60 (m, 2H, 24-OCH_2) and ^{13}C NMR (100 MHz, $CDCl_3$): Table 2. ESIMS: *m/z* 361 $[M+H]^+$, 383 $[M+Na]^+$.

3-Oxo-5β-cholan-24-yl tosylate (6):
Tosylation of **7** (70 mg, 0.19 mmol) was carried out following the same procedure as described for **5**. The crude product was subjected to PTLC (15% EtOAc in

pet-ether). The title compound **6** (38 mg, 39%) was obtained as an oil. The compound was identified by co-TLC and comparison of IR spectrum with that of previously synthesised materials.

Ethyl (3-oxo-5β-cholan)-24-yl oxalate (8):
A solution of 3-oxo-5β-cholan-24-ol (**7**, 50 mg, 0.14 mmol) in dry dichloromethane (40 ml) was treated with oxalyl chloride (71 mg, 0.56 mmol, 4 molar equiv.), and the mixture was stirred under N_2 at r.t. After 18h, the resulting mixture was quenched cautiously with $CHCl_3$ (1% EtOH) and rotary evaporated at 40 °C to dryness. The crude gummy product was subjected to PTLC (eluted with 15% EtOAc in petroleum-ether) to obtain the title compound **8** (41 mg, 64%), yellow oil. IR ($CHCl_3$): ν_{max} cm^{-1} 2965s (C-H), 2938s (C-H), 2863s (C-H), 1765s (oxalate C=O), 1742s (ethyl and oxalate C=O), 1710s (ketonic C=O), 1446m, 1380m, 1308m, 1179s (ester C-O), 1017m and 862w. ^{1}H NMR (400 MHz): δ_H 0.65 (s, 3H, 18-Me), 0.90 (d, J = 6.5 Hz, 3H, 21-Me), 0.98 (s, 3H, 19-Me), 1.34 (t, J = 7.2 Hz, 3H, $OCH_2\mathbf{CH_3}$), 4.22 (m, 2H, 24-OCH_2), 4.32 (q, J = 7.2 Hz, 2H, $O\mathbf{CH_2}CH_3$) and ^{13}C NMR (100 MHz, $CDCl_3$): Table 2. FABMS: *m/z* 461[M+H]$^+$, 483 [M+Na]$^+$. HRFABMS: Found: 461.336678; calc 461.336677 for $C_{28}H_{45}O_5$.

4. Conclusions

Four new lithocholic acid derivatives (**4**, **5**, **6** and **8**) were synthesised starting from cheap and readily available steroid, lithocholic acid (**1**). The target compound **8** was synthesised in 64% yield from 3-oxo-5β-cholan-24-ol (**7**) and identified as ethyl (3-oxo-5β-cholan)-24-yl oxalate by comprehensive spectroscopic data analyses.

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Povzetek

V prispevku je podana kratka in hitra priprava etil (3-okso-5β-holan)-24-il oksalata (**8**) iz litoholne kisline (**1**) v petih stopnjah z 64% izkoristkom. Prav tako je izčrpno predstavljena NMR spektroskopska interpertacija novih spojin.