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ESI for paper

The Use of Polymer-supported *Candida antarctica* Lipase B to Achieve the Entropically-driven Ring-opening Polymerization of Macrocyclic Bile Acid Derivatives via Transesterification: Selectivity of the Reactions and the Structures of the Polymers Produced

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The compound/formulae/table and reference numbers in this ESI are the same as/or a continuation of those included in the full paper.

RESULTS AND DISCUSSION

1. Synthesis of compounds 26 – 29

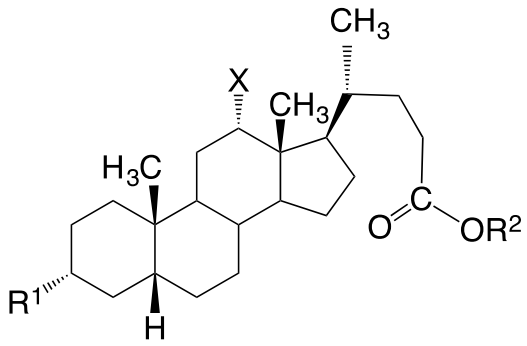
The syntheses of these simple bile acid derivatives used standard methods. Full details are given in the Experimental section.

Compound **28** was prepared by esterifying lithocholic acid (**35**) with methanol using a sulphuric acid catalyst. The product (**36**) was acetylated by treatment with acetyl chloride and pyridine.

Compound **26** was prepared by esterifying lithocholic acid (**35**) with ethanol using a sulphuric acid catalyst. The product (**37**) was acetylated by treatment with acetyl chloride and pyridine.

Compound **27** was prepared by esterifying deoxycholic acid (**38**) with methanol using a sulphuric acid catalyst. The product (**39**) was acetylated by treatment with acetyl chloride and pyridine.

Compound **29** was prepared by reaction of lithocholic acid (**35**) with an excess of ethylene glycol in the presence of sulphuric acid.[5] The product **40** was diacetylated by treatment with acetyl chloride and pyridine.



(**26**): $R^1 = CH_3CO_2$; $R^2 = C_2H_5$; X = H

(**27**): $R^1 = CH_3CO_2$; $R^2 = CH_3$; X = OH

(**28**): $R^1 = CH_3CO_2$; $R^2 = CH_3$; X = H

(**35**): R^1 = OH; R^2 = H; X = H

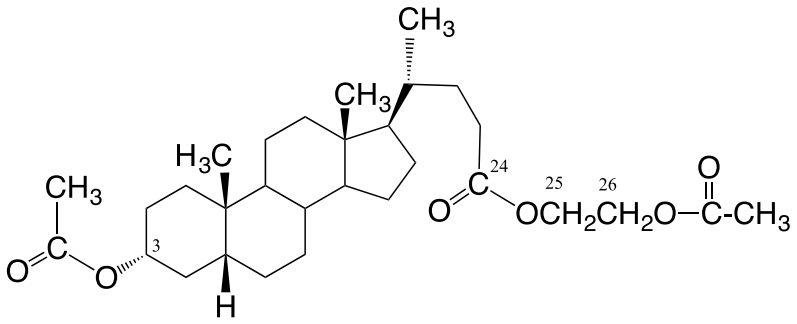
(**36**): R^1 = OH; $R^2 = CH_3$; X = H

(**37**): R^1 = OH; $R^2 = C_2H_5$; X = H

(**38**): R^1 = OH; R^2 = H; X = OH

(**39**): R^1 = OH; $R^2 = CH_3$; X = OH

(**40**): R^1 = OH; $R^2 = CH_2CH_2OH$; X = H



(**29**)

2. Acetylation of steroidal alcohols 36, 37 and 39 using CALB.

These reactions were carried out partly as syntheses of the final products and partly to demonstrate how easy it is to acylate alcohols using volatile esters and PS-CALB. The experiments confirm that it is not necessary to use an activated ester such as vinyl acetate to achieve esterifications, and that whilst the 3α-hydroxyl groups react well, no reaction of the 12α-hydroxyl group was detected. Thus, using PS-CALB allows selective reactions to be carried out.

The monoesters **36**, **37** and **39** were acetylated by treatment with a 9-fold excess of ethyl acetate in the presence of PS-CALB. The products were recovered and retreated with ethyl acetate under the same reaction conditions. By ^{1}H NMR spectroscopy this gave diesters **28**, **26** and **27**, respectively, in high yields. Full details are given in the Experimental section.

3. Typical characterization data for polymers prepared by ED-ROP.

The typical data given are for the ED-ROP summarized in Table 1, entry 7. They concern the polymerization of MCOs **11**. Figure 1 shows the FT-IR spectrum of the polymer. For comparison Figure 2 shows the FT-IR spectrum of the starting MCOs **11**. The few differences there are occur in the region below 1250 cm^{-1}. Figure 3 shows

the ^{1}H NMR spectrum of the polymer for a solution in deuteriochloroform. Finally Figure 4 shows the readout from the SEC analysis. This includes details of the columns and elution solvent. The signal at 27.03 ml is an *n*-dodecane standard.

Figure 1: ATR IR spectrum of polymer from MCOs **11**.

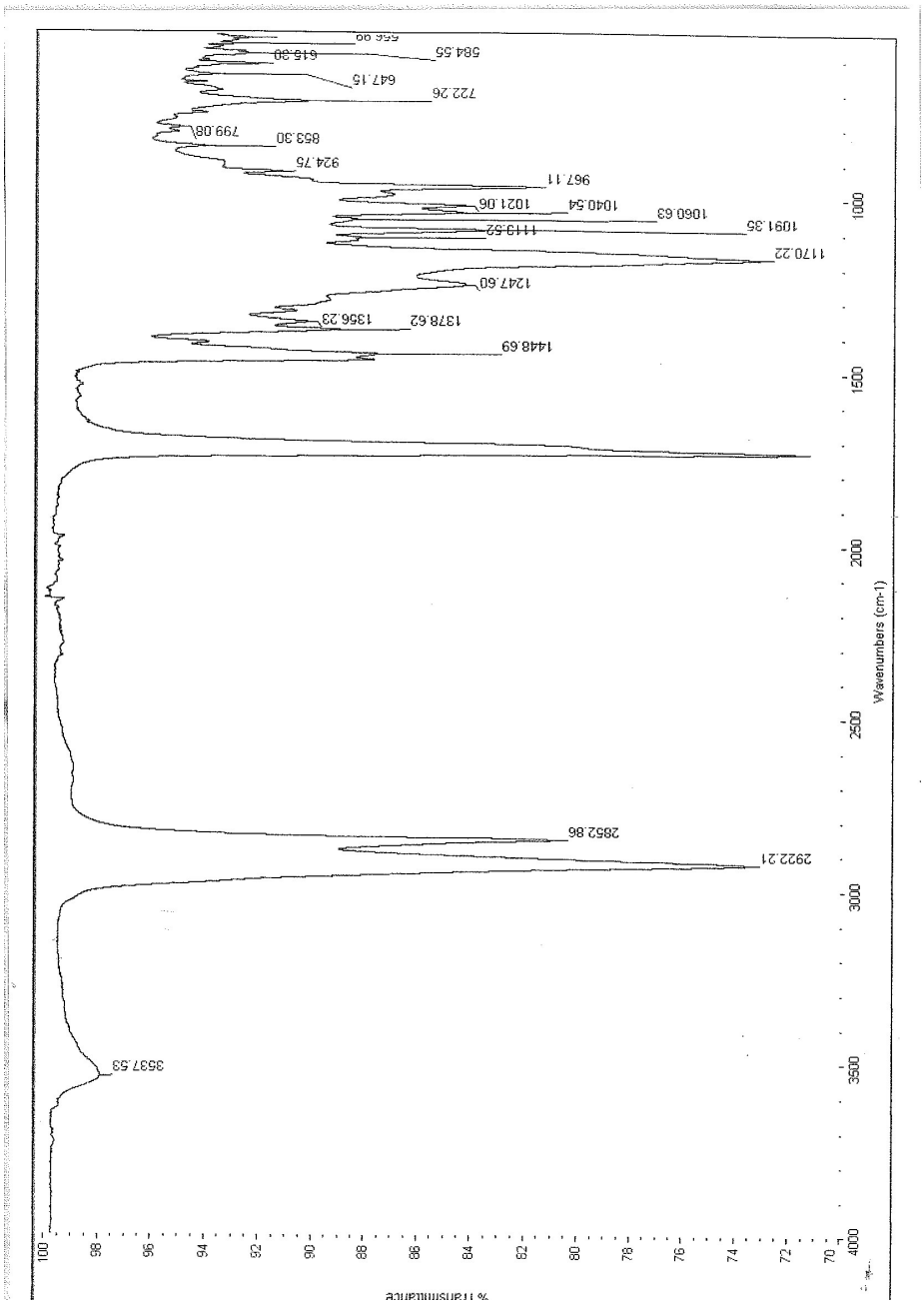


Figure 2: ATR IR spectrum of MCOs **11**.

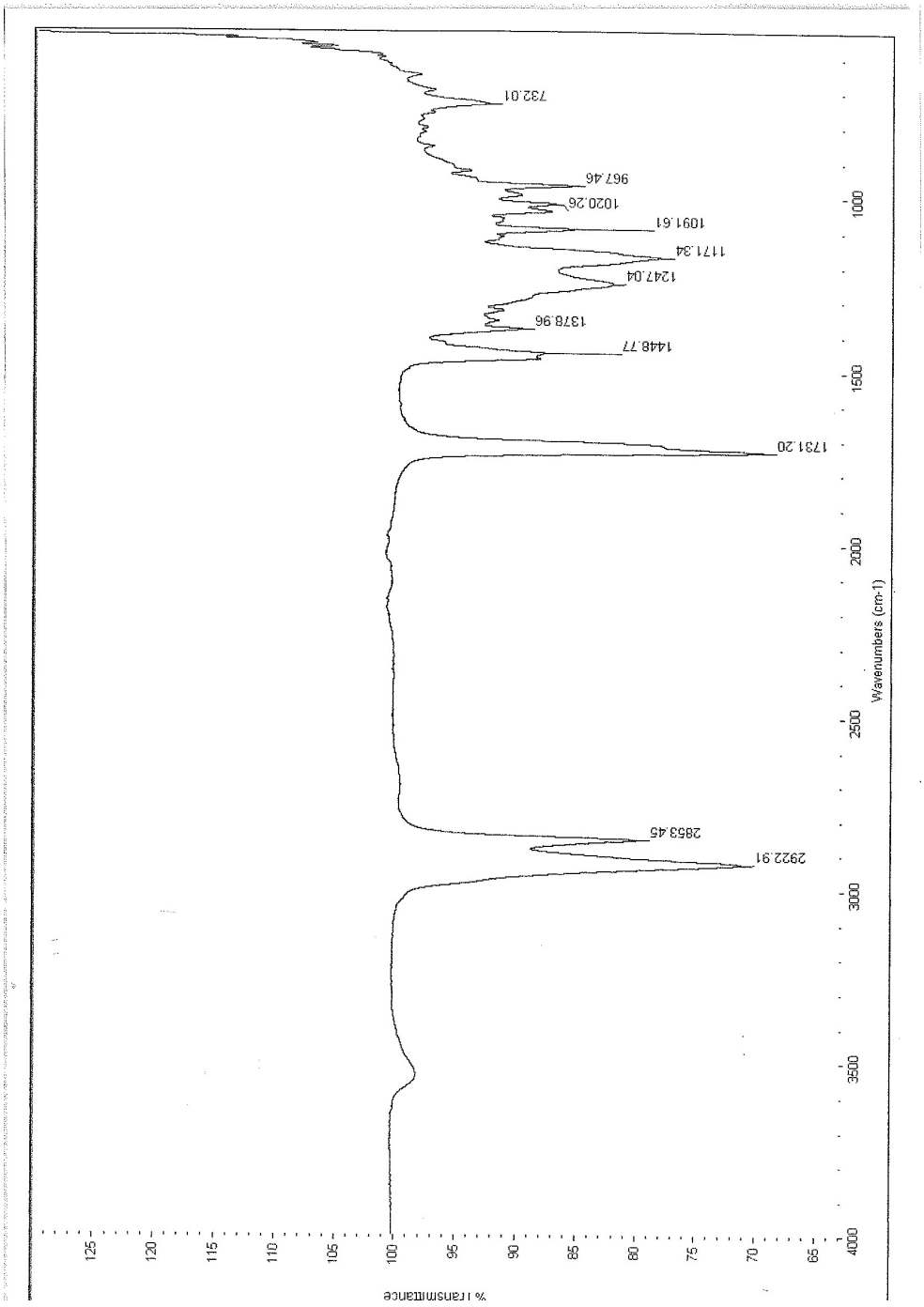


Figure 3: ^{1}H NMR spectrum of the polymer from the ED-ROP of MCOs **11**. For a solution in $CDCl_3$.

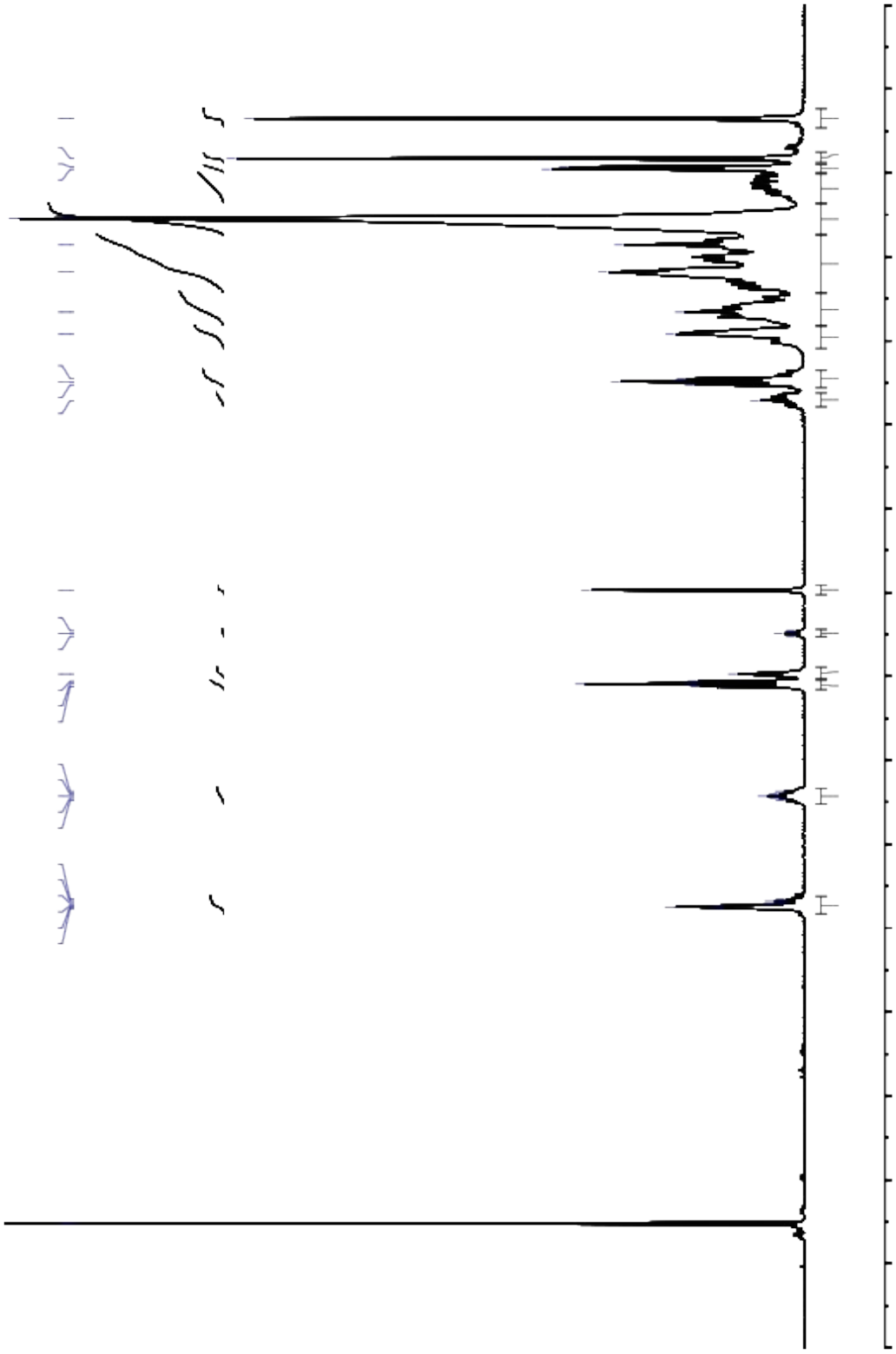
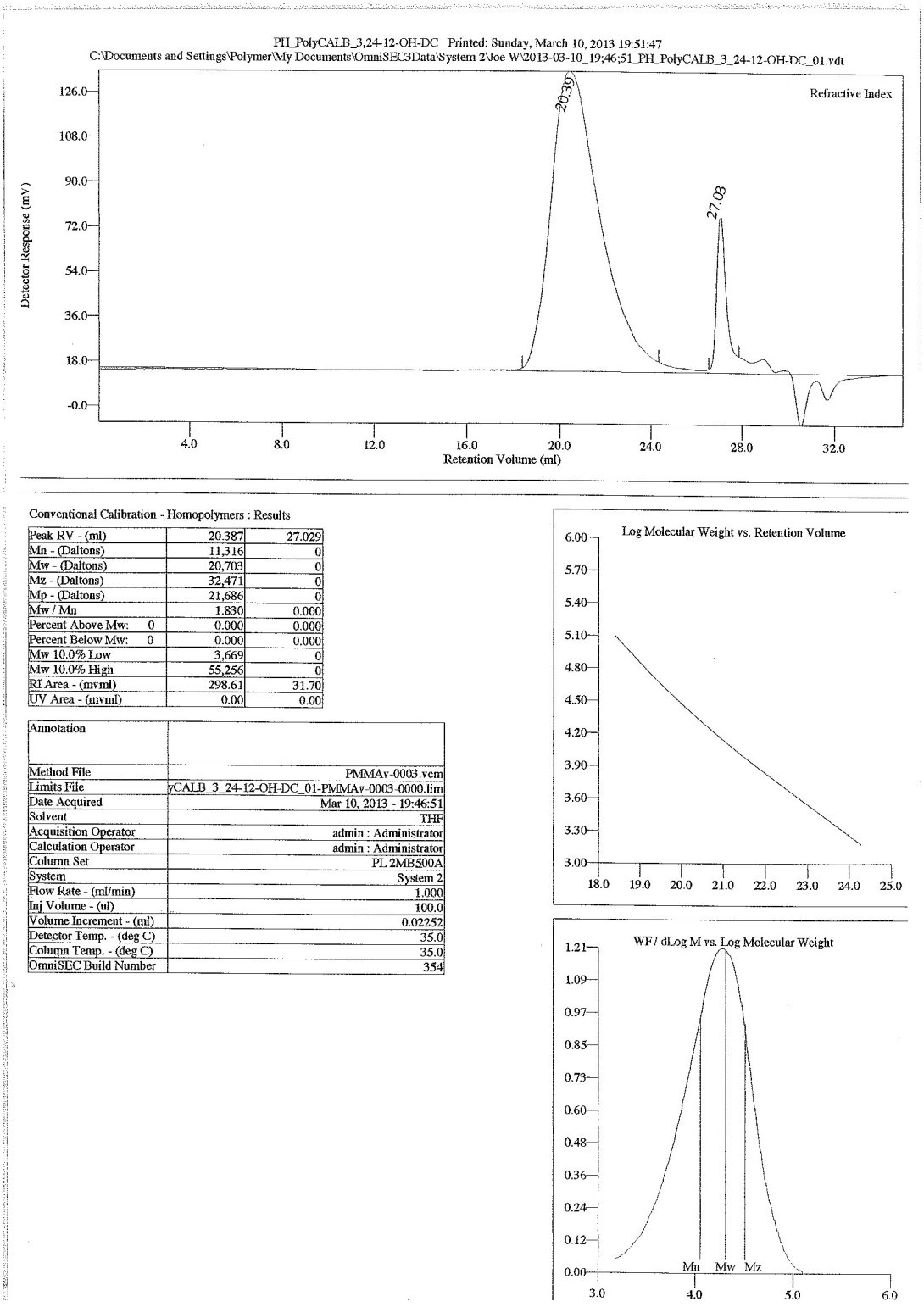


Figure 4: SEC trace of polymer from MCOs **11**. The polymerization reaction was carried out for 10 h. The peak at 27.03 ml is due to *n*-dodecane, introduced as an internal standard.



Conventional Calibration - Homopolymers : Results

Peak RV - (ml)	20.387	27.029
Mn - (Daltons)	11,316	0
Mw - (Daltons)	20,703	0
Mz - (Daltons)	32,471	0
Mp - (Daltons)	21,686	0
Mw / Mn	1.830	0.000
Percent Above Mw: 0	0.000	0.000
Percent Below Mw: 0	0.000	0.000
Mw 10.0% Low	3,669	0
Mw 10.0% High	55,256	0
RI Area - (mvml)	298.61	31.70
UV Area - (mvml)	0.00	0.00

Annotation	
Method File	PMMAv-0003.vcm
Limits File	yCALB_3_24-12-OH-DC_01-PMMAv-0003-0000.lim
Date Acquired	Mar 10, 2013 - 19:46:51
Solvent	THF
Acquisition Operator	admin : Administrator
Calculation Operator	admin : Administrator
Column Set	PL 2MB500A
System	System 2
Flow Rate - (ml/min)	1.000
Inj Volume - (ul)	100.0
Volume Increment - (ml)	0.02252
Detector Temp. - (deg C)	35.0
Column Temp. - (deg C)	35.0
OmniSEC Build Number	354

4. Examples of ^{1}H NMR spectra of transesterifications.

The following ^{1}H NMR spectra are typical for the experiments summarized in Table 2. Signal assignments were made on the basis that at the 3-position acetates give a 3 H singlet near δ 2.00 ppm, and propionates a 2 H quartet near δ 2.30 ppm and a 3 H triplet near δ 1.10 ppm. At the 24-position the methyl ester give a 3 H singlet near δ 3.60 ppm, whilst an ethyl ester gives a 2 H quartet near δ 4.1 ppm and a 3 H triplet near δ 1.2 ppm.

Figure 5: This shows the final spectrum for the experiment summarized in Table 2, entry 1, i.e. the final product from the reaction of diester **26** with ethyl propionate.

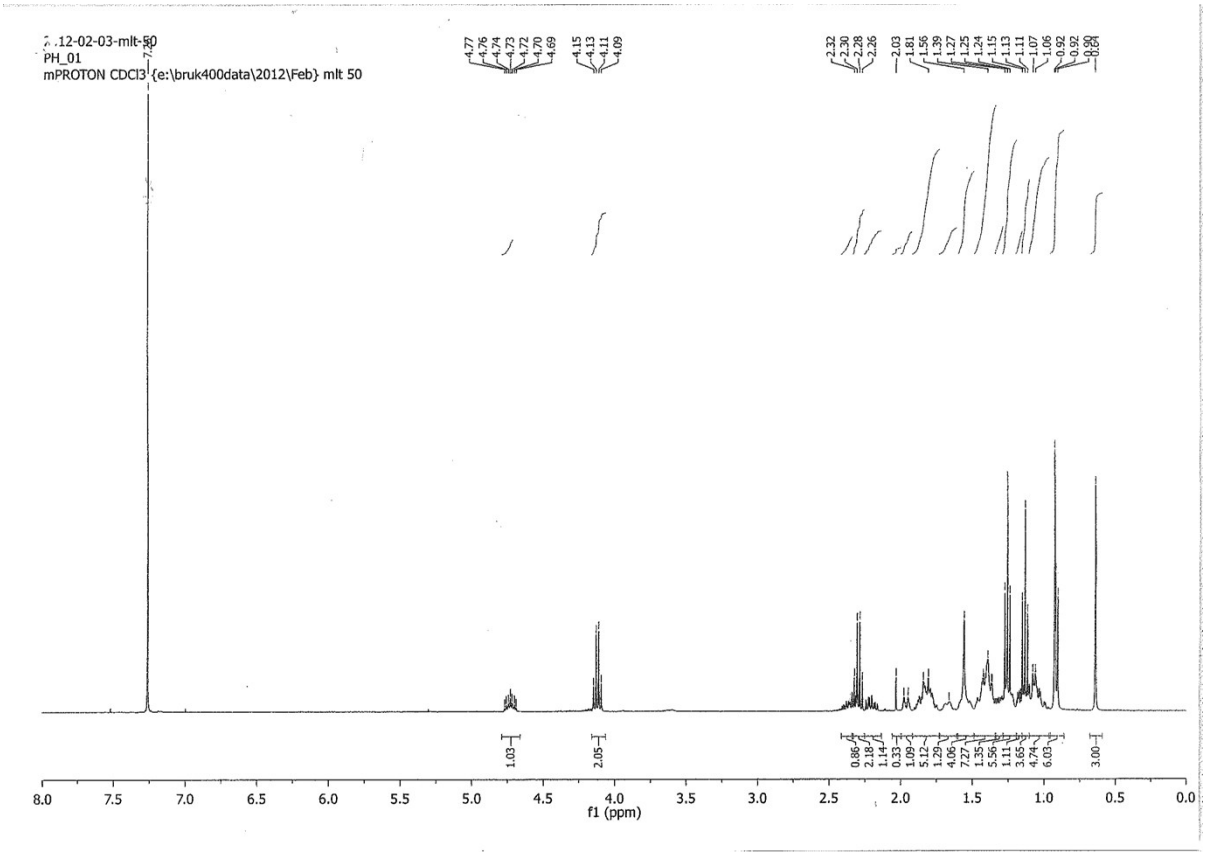


Figure 6: This shows the final spectrum for the experiment summarized in Table 2, entry 4, i.e. the final product from the reaction of diester **27** with methyl propionate (twice).

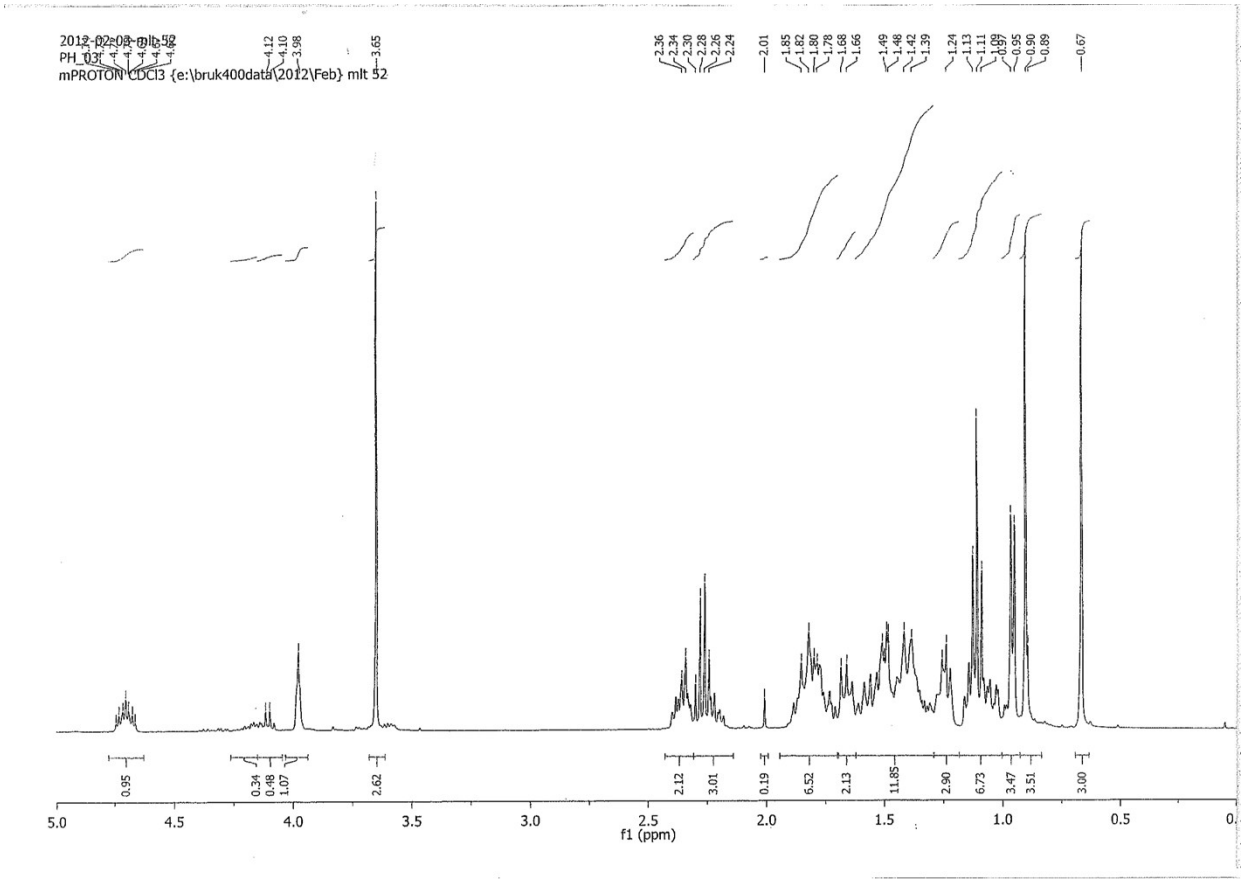


Figure 7: This shows the final spectrum for the experiment summarized in Table 2, entry 5, i.e. the final product from the reaction of diester **28** with ethyl propionate.

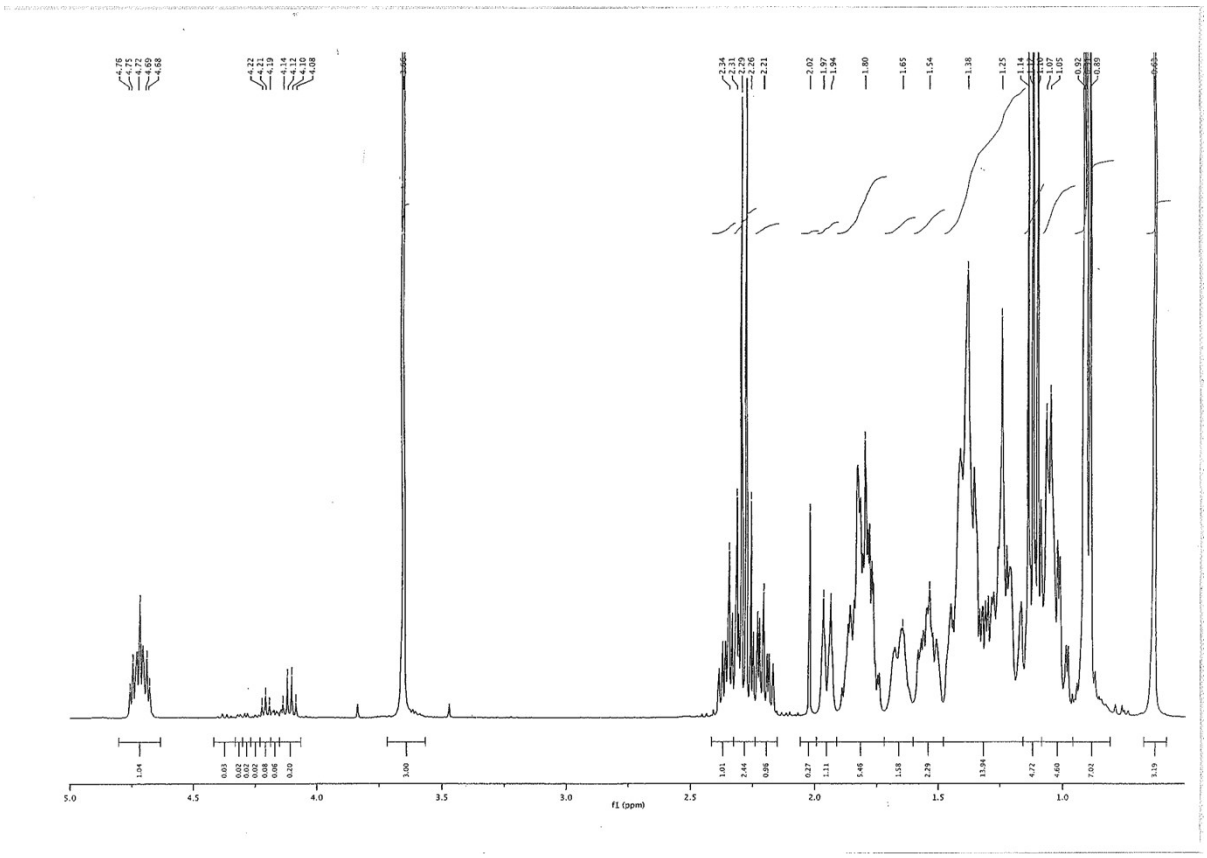
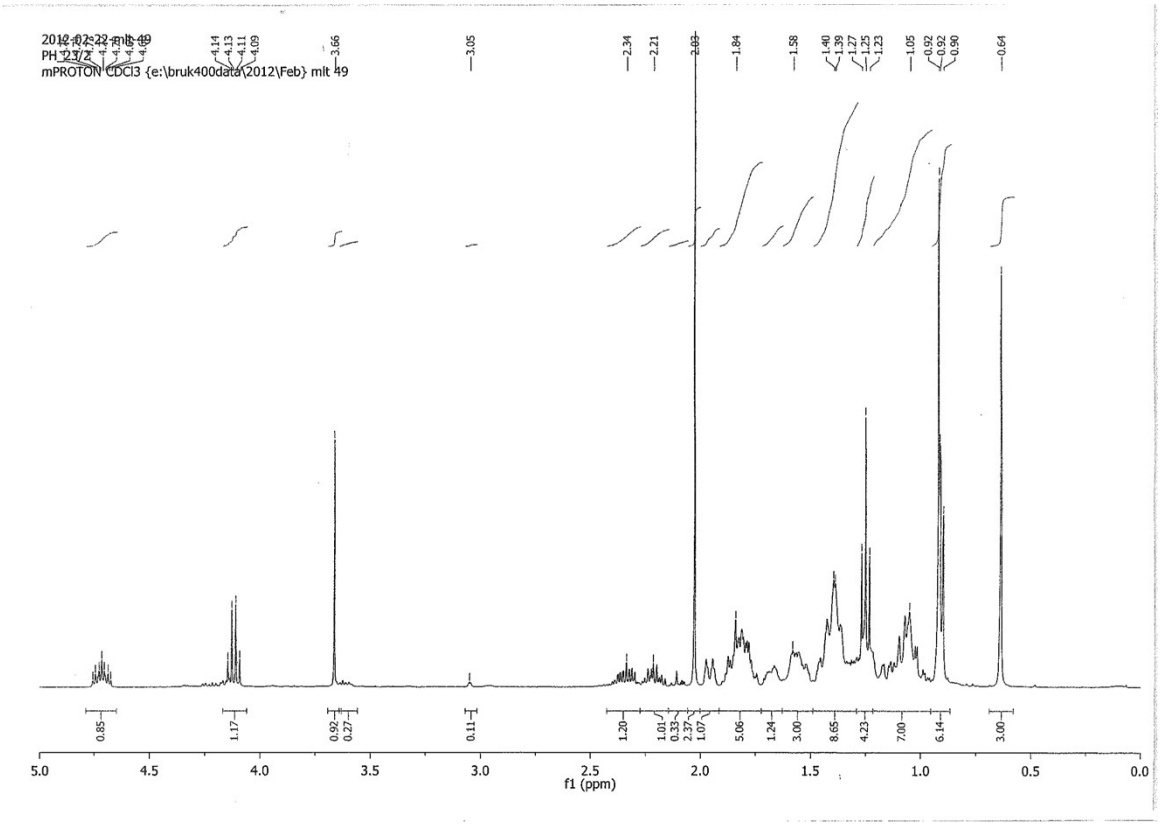


Figure 8: This shows the final spectrum for the experiment summarized in Table 2, entry 8, i.e. the final product from the reaction of diester **28** with ethyl acetate.



5. Preparation of poly(pentadecanolactone) (18).

Pentadecanolactone (**17**) was obtained from Aldrich and was used without further purification. It was polymerized by treatment of a solution in toluene at 70 ^{o}C with PS-CALB for 20 h using the standard method described in the full paper. The results are shown in Table 4, entry 1. The ED-ROP was then repeated using different solvents, entries 2 and 3, and in the presence of 2 molar equivalents of soluble amides,

entries 4 and 5. In one case, entry 6, 2 molar equivalents of a soluble nitrile were added to the reaction mixture.

It will be noted that the molecular weights were excellent except in the presence of the amides.

Table 4: Polymerization of pentadecanolactone (**17**)[a]

Entry	Additive	Yield (%)	Molecular weights[b] M_n	M_w	$Đ$[c]
1	none	93	39,100	69,600	1.78
2	Di-i-propyl ether[d]	95	95,900	132,100	1.37
3	$CH_3CON(CH_3)_2$[d]	0	--	--	--
4	*n*-$C_{17}H_{35}CONH_2$[e]	90	1,600	2,500	1.56
5	*n*-$C_{17}H_{35}CON(CH_3)_2$[e]	91	2,500	4,400	1.76
6	*n*-$C_{17}H_{35}CN$[e]	92	32,900	40,700	1.97

[a] See Experimental for standard procedure. Unless indicated otherwise solvent was toluene. Reaction time 20 h. [b] By SEC.

[c] $Đ$ = dispersity. See reference 26 for IUPAC's recent recommendations on dispersity.

[d] Used as solvent. [e] 2.0 molar equivalents added to the reaction mixture.

EXPERIMENTAL

Materials and Methods. Materials and methods are as given in our previous publication.[17]

1. Preparation of substrates for model studies.

*Methyl lithocholate (**36**).* This ester was prepared by heating a solution of commercial lithocholic acid (**35**) (20 g) in methanol (370 mL) under reflux for 20 h in the presence of concentrated sulfuric acid (2% w/v). The cooled reaction mixture was reduced to ca. 50 mL using a rotary evaporator, the concentrate added to a large volume of water, and the mixture extracted with ether. Evaporation of the ether from dried extracts gave the crude product. The crude product was recrystallized from 60 – 80 petroleum ether (92 % yield). This gave white crystals, m.p. 125 - 126 ^{o}C (lit., 130 - 132 ^{o}C[29]; and 125 – 127.5 ^{o}C[30]); FT-IR (KBr) ν_{max} 3402 br. (OH) and 1728 cm^{-1} (C=O); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) 3.62 (m, 1H, 3β-CH), 3.66 (s, 3H, OCH_3), 2.35 (m, 1H, H-23), 2.21 (m, 1H, H-23), 2.0 – 1.0 (m, 26H, various CH), 0.92 (s, 3H, C19), 0.90 (d, 3H, C21 methyl), 0.64 (s, 3H, C18).

*Methyl 3a-acetoxycholanate (**28**).* The above methyl ester **36** (2.5 g) in pyridine (8.0 ml) was carefully treated with acetyl chloride (4.0 ml) and the mixture was left at room temperature for 5 d. The crude product was recrystallized from methanol (75 %). The white crystals had m.p. 133 - 134 ^{o}C; (lit.,[29] 135 – 136 ^{o}C); FT-IR (KBr) 1725 cm^{-1} (C=O); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) 4.72 (m, 1H, 3β-CH), 3.66 (s, 3H, OCH_3), 2.35 (m, 1H, H-23), 2.21 (m, 1H, H-23), 2.03 (s, 3H, CH_3CO_2), 2.0 – 1.0 (m, 26H, various CH), 0.92 (s, 3H, C19), 0.90 (d, 3H, C21 methyl), 0.64 (s, 3H, C18).

*Ethyl lithocholate (**37**).* This ester was prepared analogously to the methyl ester described above. The product (68 % yield) had m.p. 93 - 95 ^{o}C (lit.,[30] 94.5 - 96 ^{o}C); FT-IR ν_{max} 3410 br. (OH) and 1728 cm^{-1} (C=O); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) 4.12 (m, 2H, OCH_2), 3.62 (m, 1H, 3β-CH), 2.34 (m, 1H, H-23), 2.19 (m, 1H, H-23),

2.0 – 1.0 (m, 28H, various CH), 1.25 (t, 3H, OCH_2CH_3), 0.91 (s, 3H, C19), 0.90 (d, 3H, C21 methyl), 0.63 (s, 3H, C18).

*Ethyl 3a-acetoxycholanate (**26**)*. Recrystallization of the crude product from aqueous ethanol gave white crystals (85 %), m.p. 94.5 - 96 ^oC; (lit.,[30] 95 – 98 ^oC); FT-IR (KBr) ν_{max} 1736 cm^{-1} (C=O); ^1H NMR (400 MHz, $CDCl_3$, δ, ppm) 4.72 (m, 1 H, 3β-CH), 4.12 (q, 2 H, OCH_2), 2.34 (m, 1 H, H-23), 2.20 (m, 1 H, H-23), 2.03 (s, 3 H, CH_3CO_2), 1.9 – 1.0 (m, 26 H, various CH), 0.92 (s, 3 H, C19), 0.91 (d, 3 H, C21 methyl), 0.64 (s, 3H, C18).

*Methyl deoxycholate (**39**)*. This ester was prepared analogously to the methyl lithocholate (**36**). The product (82 % yield) had m.p. 76 - 78 ^oC (lit.,[31] 80 - 81 ^oC; and,[32] 120 ^oC); FT-IR (KBr) ν_{max} 3450 br. (OH), 1736 cm^{-1} (C=O); ^1H NMR (300 MHz, $CDCl_3$, δ, ppm) 3.98 (m, 1H, 12βH), 3.66 (s, 3H, CO_2CH_3), 3.60 (m, 1H, 3β-CH), 2.36 (m, 1H, H-23), 2.23 (m, 1H, H-23), 2.0 – 0.8 (m, 26H, various CH), 0.97 (d, J = 10 Hz, 3H, C21 methyl), 0.91 (s, 3H, C19), 0.68 (s, 3H, C18).

*Methyl 3α-acetoxydeoxycholate (**27**)*. Recrystallization of the crude product from aqueous ethanol gave white crystals (80 %), m.p. 104 – 106 ^oC (lit.,[31] 128 – 128.5 ^oC); FT-IR (KBr) ν_{max} 1733 cm^{-1} (C=O); ^1H NMR (300 MHz, $CDCl_3$, δ, ppm) 4.67 (m, 1 H, 3β-CH), 3.96 (m, 1 H, 12βH), 3.63 (s, 3 H, OCH_3), 2.33 (m, 1 H, H-23), 2.21 (m, 1 H, H-23), 1.99 (s, 3 H, CH_3CO_2), 1.9 – 0.9 (m, 26 H, various CH), 0.94 (d, J = 10 Hz, 3 H, C21 methyl), 0.89 (s, 3 H, C19), 0.65 (s, 3H, C18).

*Ethylene glycol lithocholate (**40**)*. Following the procedure described in the literature[5,32] a mixture of lithocholic acid (**35**) (6.50 g, 17.3 mmol), ethylene glycol (50 ml), and 5 drops of concentrated hydrochloric acid (5 drops) was stirred and

heated at 80°C for 18 h. Work up gave ethylene glycol lithocholate as a white solid (6.99 g, 96%). Recrystallization from ethyl acetate afforded white crystals with m.p. 156-157 °C; ν_{max} (ATR) 3298 br. (OH), 1731 cm^{-1} (C=O); ^1H NMR (400 MHz, $CDCl_3$, δ, ppm) 4.21 (m, 2H, COOC*H*$_2$CH$_2$OH), 3.82 (m, 2H, COOCH$_2$C*H*$_2$OH), 3.62 (m, 1H, 3β-CH), 2.38 (m, 1H, 23-CH_2), 2.26 (m, 1H, 23-CH_2), 1.97-0.95 (m, 28H), 0.91 (d, $^3J_{HH}$ = 6.4 Hz, 3H, 21-CH_3), 0.91 (s, 3H, 19-CH_3), 0.64 (s, 3H, 18-CH_3). This spectrum is in excellent agreement with that reported in the literature.[5] Calculated for $C_{26}H_{44}O_4$: C, 74.28 %; H, 10.47 %. Found: C, 74.33 %; H, 10.60 %.

*Diacetate of ethylene glycol lithocholate (**29**)*. Pyridine (10.0 mL) was added to a solution of ethylene glycol lithocholate (6.00 g) in dichloromethane (100 mL). The mixture was cooled down to 0 °C and, with stirring, acetyl chloride (10.0 mL) was added dropwise. When the addition was complete the mixture was allowed to warm to room temperature and was then stirred for 20 h. The mixture was filtered and the filtrate poured over crushed ice. The slush was extracted with dichloromethane and the dried extracts evaporated to dryness. The crude product was purified by column chromatography (silica gel, petroleum ether/ethyl acetate - 9/1). This afforded **29** as a white solid (57%). It had m.p. 107-108 °C; FT-IR (ATR) ν_{max} 1731 cm^{-1} (C=O); ^1H NMR (400 MHz, $CDCl_3$, δ, ppm) 4.61 (m, 1 H, 3β-CH), 4.26 (s, 4 H, COOCH$_2$CH$_2$OOC), 2.28 (m, 1 H, 23-CH_2), 2.19 (m, 1 H, 23-CH_2), 2.08 (s, 3 H, CH_3COO), 2.02 (s, 3 H, CH_3COO), 1.96 – 0.96 (m, 28 H), 0.92 (s, 3 H, 19-CH_3), 0.91 (d, J_{HH} = 6.0 Hz, 3 H, 21-CH_3), 0.64 (s, 3 H, 18-CH_3). Calculated for $C_{30}H_{48}O_6$: C, 71.42 %; H, 9.52 %. Found: C, 71.34 %; H, 9.60 %.

2. Acetylation of 3α-alcohols using PS-CALB.

*Methyl 3a-acetoxycholanate (**28**).* Methyl ester **36** (600 mg) in toluene (4.0 ml) at 70 ^{o}C was treated for 20 h with ethyl acetate (2.0 ml) and PS-CALB (300 mg) under an atmosphere of dry nitrogen. The beads were then filtered off and the solution evaporated to dryness. The residue was retreated with ethyl acetate and PS-CALB under the above conditions. Recrystallization of the crude product from methanol gave compound **28** (95%), m.p. 132 - 134 ^{o}C; FT-IR and ^{1}H NMR spectra as reported in above.

*Ethyl 3a-acetoxycholanate (**26**).* Ethyl ester **37** was acetylated using the same procedure as that used in the preparation of **28** using PS-CALB. Recrystallization of the crude product from aqueous ethanol gave compound **26** (88 % yield). It had m.p. 94 – 95 ^{o}C; FT-IR and ^{1}H NMR spectra same as reported above.

*Methyl 3α-acetoxydeoxycholate (**27**).* Methyl ester **39** was acetylated using the same procedure as that used in the preparation of **28** using PS-CALB. Compound **27** (87 % yield) had m.p. 104 – 107 ^{o}C; FT-IR and ^{1}H NMR spectra same as reported above.

ACKNOWLEDGEMENTS

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