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Stereoselective synthesis of 3β-bile acid derivatives from the 3α-analog

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Abstract

The stereoselective transformation from the commonly occurring 3α-bile acids and derivatives to their 3β-analogs is important for certain applications of these amphiphilic natural compounds, especially in the preparation of polymers. We report here the stereoselective conversion of methyl cholate to its 3β-hydroxy epimer by the use of the Mitsunobu reaction and to a 3β-amino cholic acid analog via a tosylation followed by a S_n2 displacement with sodium azide and a hydrogenation of the azide group with overall yields of 86% and 78%, respectively. The preparation of the methacrylate and methacrylamide derivatives from these compounds is also described.

Keywords: Stereoselective synthesis; Cholic acid derivatives; Bile acids

1. Introduction

Bile acids are natural compounds stored in the gallbladder and aid in the digestion of food in the gastro-intestinal tract. In their salt form, bile acids can emulsify and solubilize, by the formation of micelles, hydrophobic substances such as fat and other lipid molecules and ultimately help in the absorption of these substances in the intestine. They are a group of compounds consisting of a steroid backbone, an extended carboxylic acid group and different numbers of hydroxyl groups. They are also found to be conjugated with taurine or glycine and the conjugates usually have lower pK_a values and can be easily solubilized in an aqueous environment. The use, binding and modification of bile acids have stimulated a lot of research interests in both biomedical and chemical fields because of their biological importance and their amphiphilic and acid-base properties. The functional groups of bile acids render various chemical modifications possible. For example, aliphatic chains can be attached to bile acids to obtain organized structures [1] or photolabile groups can be added to identify bile salt carriers [2,3]. They can also be used as a building compo-

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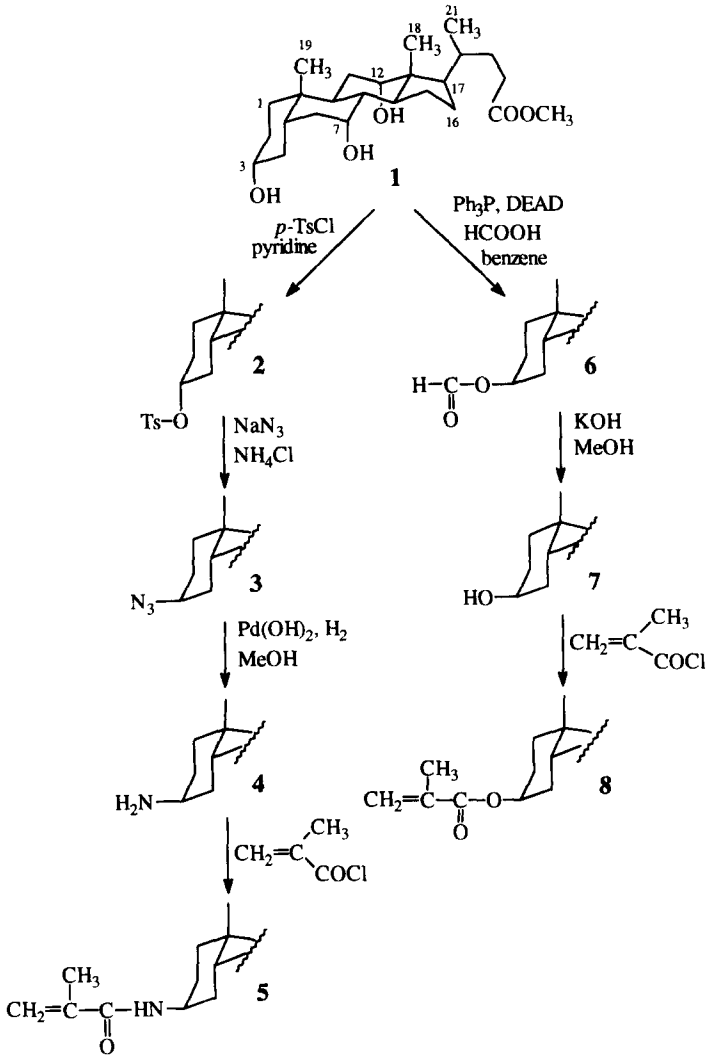

Fig. 1. Stereoselective synthesis of 3β-amino-cholic acid methyl ester, transformation from 3α- to 3β-cholic acid methyl ester and the preparation of methacrylic monomers.

nent in the preparation of macrocycles in molecular recognition studies [4]. Various interesting polymers can be prepared from bile acids [5–7] and they can be attached to polymers for slow release in pharmaceutical applications [8]. Because of their biological importance, the stereoselective transformation [9–11] and the chemical modifications [12] of bile acids were extensively studied. Cholic acid, or 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid, one of the most common bile acids, has three hydroxyl groups (its methyl ester **1** is shown in Fig. 1), which exhibit very different reactivities depending to their location on the steroid skeleton. Indeed, the OH group on the C3 position is more reactive than the other OH groups [13,14]. Recently we reported the preparation of polymers from the 3-amino derivatives of cholic acid [7], since the transformation from 3-hydroxy to 3-amino cholic acid may improve the hydrophilicity of the monomers. We have found that the stereochemistry of the functional group on position 3 is quite important to the properties of the polymers, e.g., the 3β-methacrylamide monomer of cholic acid produces a polymer with higher molecular

weight and better solubility than the 3α-monomer [7]. For this reason, we have sought the stereoselective synthesis of 3β-derivatives of bile acids. Although the synthesis of 3-amino-cholic acid was previously reported [15], two stereoisomers were formed and chromatographic separation of the 3α- and 3β-amino cholic acid epimers was needed. Stereoselective transformation from hydroxy to amino groups has been reported in the synthesis of sinefungin by Maguire et al. [16]. This method was used in this study for the synthesis of the 3β-amino cholic acid derivative. We have not yet seen any other report on the stereoselective synthesis of 3β-amino-bile acid analogs in literature. On the other hand, the stereoselective transformation between 3α- and 3β-hydroxy isomers for steroids is also possible as summarized in Mitsunobu's review [17]. However, whether or not the transformation would occur depends largely on the reaction conditions and the solvent [18,19] and the yields were often less than satisfactory, even if the reaction takes place at all [9–11,17–19]. To synthesize the monomers from the bile acid derivatives used in the preparation of amphiphilic polymers, we have modified or improved the existing synthetic procedures and successfully transformed the 3α-bile acid into 3β-bile acid analogs, including the 3-hydroxy and 3-amino groups. This report provides a simple route to obtain only the 3β-epimers with high yields. The methods reported here will be of interest to researchers working with these or other related lipid molecules.

2. Experimental and results

3α-Cholic acid methyl ester (3α-CAME) was purchased from Sigma and used as received. All other chemicals, including formic acid, triphenylphosphine (Ph_3P), diethyl azodicarboxylate (DEAD), triethylamine and palladium hydroxide on carbon (Pearlman's catalyst) were purchased from Aldrich. Methacryloyl chloride was prepared from methacrylic acid and benzoyl chloride [20], both from Aldrich. All solvents were also purchased from Aldrich and some of them, such as pyridine, tetrahydrofuran (THF), dimethylformamide (DMF) and dichloromethane (DCM), were redistilled prior to use.

NMR spectra were recorded at 23°C on a Braker AMX-300 operating at 300.1 MHz for ^1H and 75.5 MHz for ^{13}C, in deuterated chloroform, which also served as an internal reference (7.27 ppm for ^1H and 77.00 ppm for the center peak of ^{13}C, with all chemical shifts reported relative to tetramethylsilane). The NMR samples were prepared by adding ~10–15 mg of the compound in ~0.5 ml of the deuterated solvent. Particulars regarding the pulse width (14 μs), pulse angle (50°), recycle delay (3 s), spectral width (3 kHz and 17 kHz), number of data points (20–40 K) and number of acquisitions (32 and 256) are those noted in the parentheses, for ^1H and ^{13}C respectively, unless otherwise mentioned. The ^{13}C-NMR assignments were made by comparison with results in literature [21,22] and with the help of distortionless enhancement by polarization transfer (DEPT) for all the major products and two-dimensional ^1H-^{13}C correlation experiments for selected products. The reported chemical shifts are generally accurate to ±0.05 ppm, but the chemical shift of one peak relative to another of the same compound is accurate to ±0.01 ppm. The elemental analyses of selected compounds were performed by GLC Ltd. (Guelph, Ontario). GLC estimated the error of the analyses to be ±0.3% for hydrogen and nitrogen and ±0.4% for the other elements analyzed.

The synthetic routes for the preparation of the 3β-amino and 3β-hydroxy cholic acid analogs are shown schematically in Fig. 1.

2.1. Methyl 3α-tosyloxy-7α,12α-dihydroxy-5β-cholanoate (3α-tosyloxy-cholic acid methyl ester) (2)

This product **2** was synthesized by the use of a modified procedure of the one published in the literature [2,23]. A solution of **1** (4.23 g, 10 mmol) in dry pyridine (10 ml) was cooled to ~0°C with an ice-water bath under dry inert gas. A solution of *p*-toluene sulfonyl chloride (2.15 g, 12 mmol) in pyridine was added dropwise over a period of 10–15 min. The mixture was allowed to warm gradually to room temperature and stirred for 20 h. The salt formed during the reaction was filtered out and the solvent was removed by rotary evaporation under reduced pressure. The crude product

was dissolved in diethyl ether and the insoluble salt was removed. Finally, the product **2** was obtained by re-crystallization from ethyl acetate and petroleum ether with a yield of 4.98 g (86%).

2.2. Methyl 3β-azido-7α,12α-dihydroxy-5β-cholanoate (3β-azido-cholic acid methyl ester) (3)

This compound was prepared according the methods reported by Kramer and Kurtz [2] with some modifications. First, 1.0 g of **2** (1.7 mmol), an excess of sodium azide (5.6 g, 86 mmol) and ammonium chloride (4.6 g, 86 mmol) were dissolved in 50 ml of dry DMF and refluxed for 5 h. The mixture was then cooled to room temperature and added to a separatory funnel containing 50 ml of a saturated aqueous solution of NaCl and extracted with DCM (3 × 50 ml). The solvent was removed and the crude product was dissolved in 50 ml DCM and washed with water (2 × 25 ml) to remove any salt left. The solvent was then evaporated and the product was then purified by chromatography on a silica gel column with 4 vol% butanol in DCM as an eluent which yielded 0.70 g (91%) of the desired product **3** (m.p. 143–144°C).

2.3. Methyl 3β-amino-7α,12α-dihydroxy-5β-cholanoate (3β-amino-cholic acid methyl ester) (4)

First, 1.0 g of **3** (2.2 mmol) and palladium hydroxide on carbon (0.87 g, weight includes 45% moisture; palladium hydroxide content 20% on a dry weight basis) were added to methanol (40 ml). The mixture was stirred under H_2 at 5 atm for 72 h. After the addition of ammonium hydroxide (20 ml), the mixture was stirred for another 30 min. It was then filtered through celite. After removing the solvent, 0.92 g of product **4** were obtained with a yield close to 100% since the conversion was practically complete. The NMR chemical shift of the 3-CH group was changed to 3.33 ppm for ^1H and 48.47 ppm for ^{13}C. In deuterated dimethylsulfoxide, the amino protons can be observed at 4.15 ppm. The product was found to be stable up to ~230°C, at which temperature it started to decompose.

2.4. Methyl 3β,7α,12α-trihydroxy-5β-cholanoate (3β-hydroxy-cholic acid methyl ester) (7)

Cholic acid methyl ester **1** (4.2 g, 10 mmol) was dissolved in 100 ml of dry benzene-THF (20:1 v/v). Ph_3P (5.3 g, 20 mmol) and formic acid (0.79 ml, 20 mmol) were added subsequently to the solution followed by agitation at room temperature for 15 min. A solution of DEAD in benzene (3.15 ml or 20 mmol in 20 ml) was added dropwise to the mixture during a period of 30 min and the mixture was stirred overnight. After filtering off the solid formed, the solution was evaporated under reduced pressure and a syrup-like product was obtained. This 3β-formoxy product (**6**) can be separated by column chromatography as reported [18] and the yield was ~95%. The ^1H-NMR chemical shift for the C-3 proton was changed from 3.48 ppm to 5.14 ppm and the formyl proton peak appeared at 8.06 ppm, providing direct evidence for the obtained product. It is of note that the hydrolysis of the formyl group can be performed on the crude product without purification. The product **6** was dissolved in ~75 ml of methanol. After addition of 15 ml of 1 M KOH methanolic solution, the mixture was stirred for 8 min at room temperature. Then, 500 ml of 1 M HCl aqueous solution was added and DCM was used to extract the product (3 × 100 ml). The solvent was evaporated and the product was recrystallized in DCM (m.p. 179–181°C); 3.6 g of product **7** was obtained for the two-step reaction (overall yield 86%). Elemental analysis was carried out for **7**. Calculated for $C_{25}H_{42}O_5$: C, 71.05; H, 10.02; O, 18.93. Found: C, 71.00; H, 10.26; O, 18.68.

2.5. Methacrylate and methacrylamide of cholic acid analogs

The methacrylate derivatives of 3(α or β)-hydroxy-cholic acid methyl esters and the methacrylarnide derivatives of 3(α or β)-amino-cholic acid methyl esters can be prepared easily by reacting the respective derivatives of cholic acid methyl esters with methacryloyl chloride at 0°C. The synthetic procedure described previously [4,7] was used for the preparation of the 3-methacrylic derivatives of cholic acid analogs. In the synthesis of methyl 3β-methacryloyloxy-7α,12α-dihydroxy-

Table 1
^{1}H-NMR chemical shifts of the cholic acid derivatives in $CDCl_3$

Proton	1	7	3	9	8
3-CH	3.48	4.08	3.89	4.64	5.09
7-CH	3.85	3.87	3.85	3.87	3.88
12-CH	3.97	4.00	3.97	4.00	4.00
18-CH_3	0.68	0.71	0.68	0.71	0.71
19-CH_3	0.88	0.95	0.92	0.92	0.95
21-CH_3	0.98	0.99	0.97	0.99	0.99
	(d, $J = 6.1$)	(d, $J = 6.4$)	(d, $J = 6.2$)	(d, $J = 6.2$)	(d, $J = 6.4$)
OCH_3	3.66	3.67	3.66	3.67	3.67
MA-CH_3	—	—	—	1.92	1.95
MA-CH_2	—	—	—	5.51;6.08	5.53;6.10

MA, methacryloyl.
$\delta(CHCl_3) = 7.27$ ppm.
Coupling constants J are given in Hz.

5β-cholanoate (**8**), 2 g of **7** (4.7 mmol) and 0.9 ml of triethylamine was added in 30 ml of chloroform and the mixture was place in an ice-water bath followed by adding a solution of 0.7 ml of methacryloyl chloride in 2 ml chloroform during a period of 20 min. The reaction mixture at 0°C was allowed to warm up gradually to room temperature overnight and was poured into an acidic ice-water (mixed with 0.5 ml concentrated HCl). Chloroform was used for the extraction and the organic phase was washed to neutral and dried with calcium sulfate followed by the evaporation of solvents. After recrystallisation in acetone/petroleum ether, 2.1 g of product **8** was obtained as identified by NMR (yield 90%, m.p. 150–151°C). An identical procedure was used for the preparation of compounds **5** and **9** (methyl 3β-methacryloylamino-7α,12α-dihydroxy-5β-cholanoate and methyl 3α-methacryloyloxy-7α,12α-dihydroxy-5β-cholanoate) with a yield of 81% and 80%, respectively (m.p. 198–200°C and 180–181°C for **5** and **9**, respectively). Elemental analyses were carried out for **5** and **8**. Calculated for $C_{29}H_{47}NO_5$ (**5**): C, 71.17; H, 9.61; N, 2.86; O, 16.36. Found: C, 71.00; H, 9.89; N, 2.85; O, 16.32. Calculated for $C_{29}H_{46}O_6$ (**8**): C, 71.02; H, 9.39; O, 19.59. Found: C, 70.70; H, 9.63; O, 19.59.

The ^{1}H- and ^{13}C-NMR chemical shifts for the major products are summarized in Tables 1 and 2. The ^{1}H- and ^{13}C-NMR chemical shifts of 3α- and 3β-amino-cholic acid derivatives have already appeared in a previous report [7] and thus are not listed in these tables. We have found the chemical shifts observed for compounds **1** and **7** are somewhat at variance with results already published [21,22]. Therefore, they are listed in the tables and, in addition, serve as a comparison to the chemicals shifts of the 3-methacryloyloxy cholic acid methyl esters (**8** and **9**) observed under identical conditions. The chemical structure of **9** is shown in Fig. 2.

3. Discussion

The elimination of the chromatographic purification of the tosylate **2** not only saves time and labor in the synthesis of **2** but also improves the yield. In the preparation of 3β-amino-cholic acid methyl ester **4**, the inversion of the configuration from 3α to 3β occurred during the reaction of the tosylate **2** with sodium azide, which is a typical S_n2 substitution. This 3β-configuration remained unchanged during the hydrogenation to obtain **4** and later the preparation of the methacrylate **5**. The hydrogenation can also be accomplished at atmospheric pressure for a prolonged period of time. Compound **3** can be obtained alternatively by a Mitsunobu reaction with hydrazoic acid [19]. However, the procedure was not used here because of the potential hazard in handling the hydrazoic acid. In the preparation of 3β-cholic acid methyl ester **7**, a Mitsunobu reaction was

carried out. We chose to use formic acid instead of benzoic acid used by Loibner and Zbiral [19], since it can be more easily removed and separated. A very small amount of THF was added to facilitate the dissolution of the reaction mixture. The yield of the 3β-formoxy cholic acid derivative **6** is also higher than that reported with benzoic acid [19]. It is not clear to us, due to the lack of experimental details, why the same reaction did not take place in some cases as reported previously [9]. Obviously the same transformation from 3α to 3β can be accomplished by a two-step reaction via a tosylate as in the synthesis of **3**, which has already been reported by Chang and Iida [9–11]. The Mitsunobu reaction provides a valuable shortcut to the same products.

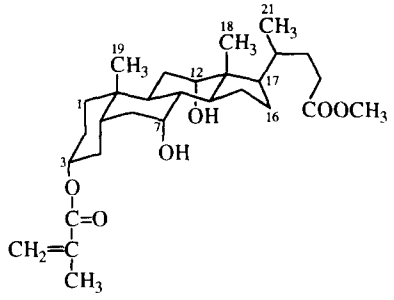

Fig. 2. Chemical structure of 3α-methacryloyloxy-cholic acid methyl ester (**9**).

Table 2
^{13}C-NMR chemical shifts of the cholic acid derivatives in $CDCl_3$

Carbon	**1**	**7**	**3**	**9**	**8**
1-CH_2	35.65	29.69	30.46	35.04	30.70
2-CH_2	30.26	27.63	24.51	26.64	24.83
3-CHOH	71.80	66.86	58.69	74.55	70.82
4-CH_2	39.30	36.49	32.98	34.51	33.51
5-CH	41.46	35.90	36.72	41.87	36.99
6-CH_2	34.61	34.27	34.14	34.84	34.20
7-CHOH	68.35	68.57	68.39	68.27	68.43
8-CH	39.38	39.41	39.36	39.28	39.33
9-CH	26.17	25.77	26.07	26.49	25.75
10-C	34.71	35.18	35.05	34.64	35.01
11-CH_2	28.05	28.52	28.42	28.18	28.46
12-CHOH	73.01	73.02	72.96	73.02	72.99
13-C	46.31	46.53	46.51	46.47	46.44
14-CH	41.41	41.56	41.83	41.16	41.73
15-CH_2	23.15	23.21	23.16	23.11	23.18
16-CH_2	27.46	27.44	27.43	27.42	27.43
17-CH	46.87	47.19	47.22	47.17	47.13
18-CH_3	12.37	12.50	12.45	12.40	12.42
19-CH_3	22.37	22.92	22.79	22.35	22.98
20-CH	35.25	35.23	35.17	35.21	35.21
21-CH_3	17.22	17.31	17.28	17.28	17.24
22-CH_2	30.84	30.83	30.80	30.75	30.76
23-CH_2	31.05	31.08	31.34	30.97	31.04
24-COOH	174.81	174.74	174.67	174.72	174.72
OCH_3	51.43	51.51	51.46	51.42	51.43
MA-CO	—	—	—	166.95	166.78
MA-C=	—	—	—	136.88	137.08
MA-CH_2=	—	—	—	124.73	124.78
MA-CH_3	—	—	—	18.22	18.27

MA, methacryloyl.
$\delta(CDCl_3) = 77.00$ ppm.

The ^{1}H-NMR signal of the CH group at position **3** serves as a clear indicator of the stereochemistry of the molecules. In addition to the chemical shift changes (as indicated in Table 1), the ^{1}H-NMR signal of the 3α proton, which is on an equatorial position, is a rather broad multiplet, while that of the 3β axial proton, is much sharper, similar to the other two axial protons on positions 7 and 12.

Throughout the synthesis, the methyl esters of cholic acid and of its derivatives were always used, since the esters have much better solubilities in organic solvent than the free acids. The methyl esters of the bile acids derivatives are readily soluble in a variety of organic solvents, including chloroform, DCM, THF, methanol, ethanol, etc, while the solubilities of the free acids are much lower in the above-mentioned solvents. The methyl ester bond can be hydrolyzed by reacting with a base in an aqueous methanolic medium. The hydrolysis can be completed within a short period of time depending on the conditions of hydrolysis (such as the base and its concentration, temperature and time). We found that a reaction time of 2 h was sufficient to selectively hydrolyze the methyl ester group of the methacrylate of 3α-cholic acid methyl ester when 2 N NaOH was used at temperature of $\sim$70°C [24]. This was done by monitoring the ^{1}H-NMR chemical shift of the CH signal at position 3. Apparently, the α-methyl group of the methacrylic residue and the bulkiness of the steroid backbone create a steric

hindrance to the attack on this carboxylic acid ester group, thus, stabilizing the methacrylate ester bond. Therefore, the corresponding free acids including those of the methacrylic monomers can be obtained with relative ease.

Most of the final products can be purified by simple recrystallization so that lengthy chromatographic separations are unnecessary, especially when relatively large quantities are prepared. However, it is of interest to note that, in general, the 3β-cholic acid analogs are more soluble in organic solvents and are more difficult to crystallize than the 3α-analogs. X-ray grade crystals were obtained for 3α-methacrylate and 3α-methacrylamide derivatives of cholic acid methyl ester and their structures were determined also by X-ray diffraction experiments [24], but we have not yet been able to obtain crystals large enough from the β-bile acid analogs for X-ray diffraction studies.

Although the reactions reported here were performed only on cholic acid, one of the most commonly occurring bile acids, such reactions can be easily applied to other bile acids, all of which possess even fewer hydroxyl groups than cholic acid.

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References

[1] M. Ahlheim and M.L. Hallensleben (1992) Makromol. Chem. 193, 779–797.
[2] W. Kramer and G. Kurz (1983) J. Lipid Res. 26, 910–923.
[3] W. Kramer and S. Schneider (1989) J. Lipid Res. 30, 1281–1288.
[4] R.P. Bonar-Law and A.P. Davis (1993) Tetrahedron 49, 9829–9844.
[5] M. Ahlheim, M.L. Hallensleben and H. Wurm (1986) Polym. Bull. 15, 497–501.
[6] M. Ahlheim, M.L. Hallensleben and L. Manfred (1988) Makromol. Chem., Rapid Commun. 9, 299–302.
[7] J.K. Denike and X.X. Zhu (1994) Macromol. Chem. Rapid Commun. 15, 459–465.
[8] N. Ghedini, P. Ferruti, V. Andrisano, M.R. Cesaroni and G. Scapini (1983) Synth. Commun. 13, 701–706.
[9] F.C. Chang (1979) J. Org. Chem. 44, 4567–4572;
[10] T. Iida and F.C. Chang (1982) J. Org. Chem. 47, 2966–2981;
[11] T. Iida and F.C. Chang (1983) J. Org. Chem. 48, 1194–1197.
[12] W. Kramer, G. Wess, S. Müllner and H. Neubauer (1992) European Patent 0489423 A1.
[13] D. Kritchevsky and P.P. Nair (1971) in: P.P. Nair and D. Kritchevsky (Eds.), The Bile Acids, Vol. 1, Plenum Press New York, pp. 1–10.
[14] A. Radominska-Pyrek, P. Zimniak, M. Chari, E. Golunski, R. Lester and J. St. Pyrek (1986) J. Lipid Res. 27, 89–101.
[15] S. Schneider, U. Schramm, A. Schreyer, H.-P. Buscher, W. Gerok and G. Kurz (1991) J. Lipid Res. 32, 1755–1767.
[16] M.P. Maguire, P.L. Feldman and H. Rapoport (1990) J. Org. Chem. 55, 948–955.
[17] O. Mitsunobu (1981) Synthesis 1–28.
[18] A.K. Bose, B. Lal, W.A. Hoffman and M.S. Manhas (1973) Tetrahedron Lett. 18, 1619–1622.
[19] H. Loibner and E. Zbiral (1977) Helv. Chim. Acta 60, 417–425.
[20] H.C. Brown (1938) J. Am. Chem. Soc. 60, 1325–1328.
[21] T. Iida, T. Tamura and T. Matsumoto and F.C. Chang (1983) Org. Magn. Reson. 21, 305–309.
[22] S. Barnes and D.N. Kirk (1988) in: K.D.R. Setchell, D. Kritchevsky and P.P. Nair (Eds.), The Bile Acids, Vol. 4, Plenum Press, New York, pp. 65–136.
[23] J. Barnett and T. Reichstein (1938) Helv. Chim. Acta 21, 926–938.
[24] M. Moskova (1994) Mémoire de maîtrise, Université de Montréal, pp. 52–55.