Pergamon

SCIENCE DIRECT®

Tetrahedron: *Asymmetry* 14 (2003) 3111–3115

TETRAHEDRON: *ASYMMETRY*

Resolution of α-methylene-β-hydroxy esters catalyzed by free and immobilized *Pseudomonas* sp. lipase

M. G. Nascimento,* Sandra P. Zanotto, Sílvia P. Melegari, Luciano Fernandes and Marcus Mandolesi Sá

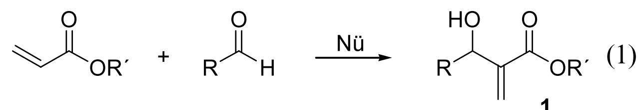
Departamento de Química, Universidade Federal de Santa Catarina, Trindade, Florianópolis, SC, Brazil 88040-900

Received 7 May 2003; accepted 7 August 2003

Abstract—Kinetic resolutions of α-methylene-β-hydroxy esters (Baylis–Hillman products) have been performed via enzymatic enantioselective transesterification with *Pseudomonas* sp. lipase (PSL), free or immobilized in poly(ethylene) oxide (PEO), silica gel and montmorillonite K10, under different reaction conditions. The corresponding (*R*)-(+)-acetates from alkyl-substituted racemic alcohols were obtained with e.e. >99% and excellent to moderate conversions using the PSL/PEO system and vinyl acetate as acylating agent, in hexane. A naphthyl-substituted hydroxy ester was inert under these experimental conditions.
© 2003 Elsevier Ltd. All rights reserved.

1. Introduction

α-Methylene-β-hydroxy esters **1** are versatile building blocks for the synthesis of many important compounds such as natural products (kijanolide,[1] mycestericin E,[2] terpenticin,[3] necic acids,[4] terpenoids,[5] insect pheromones),[6,7] nitrogen-containing heterocycles[8–10] and other biologically-active molecules.[11,12] These multifunctional compounds **1** are readily available by a unique synthetic transformation involving a nucleophile-catalyzed reaction of α,β-unsaturated esters with aldehydes, the Baylis–Hillman reaction (Eq. (1)).[13–15] A convenient access to optically-active α-methylene-β-hydroxy esters **1**, however, represents a challenging issue that has received increasing interest.[2,16] Asymmetric versions of the Baylis–Hillman reaction using either chiral auxiliaries or chiral catalysts have been developed, but only in a few cases were the chemical yields and enantiomeric excess high.[17–20] In addition, these methodologies usually employ rather expensive chiral sources and difficult experimental protocols with multi-step transformations that restrict their use. Racemic α-methylene-β-hydroxy esters **1** are easily prepared from inexpensive reagents, and therefore they are good candidates for kinetic resolution. Although chemical resolution carried out by selective hydrogenation or epoxidation of the C=C double bond present in racemic **1** have been reported, these methods also present restrictions concerning the availability of the resolving reagents, multi-step synthesis, and low yields.[20–23]



(1)

Lipases (glycerol ester hydrolases E.C. 3.1.1.3) are established catalysts for the stereoselective resolution of synthetic or semi-synthetic chiral alcohols.[24,25] They accept a wide range of substrates, which are usually converted with high enantioselectivity. These enzymes also exhibit high stability in non-aqueous solvents. In most cases, enantiomerically pure alcohols are prepared from racemic or pro-stereogenic precursors and reactions are often performed via transesterification in organic solvents. To increase the reaction rate and to shift the equilibrium towards product synthesis, activated esters such as vinyl acetate are routinely employed.[26] Among the available lipases, those from *Pseudomonas* sp. have been used to enhance kinetic resolutions of ferrocene derivatives and starting materials for the synthesis of terpenoids and carotenoids, as well as in the stereoselective preparation of polyesters[27] and polycarbonates.[28]

* Corresponding author. Tel.: +55-48-3319219; fax: +55-48-3319711; e-mail: graca@qmc.ufsc.br

0957-4166/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2003.08.015

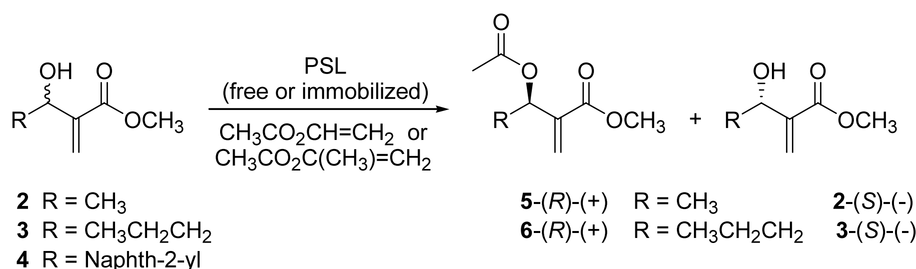
The preparative-scale resolutions of (*RS*)-methyl mandelate and some bicyclic compounds by transesterification with vinyl acetate catalyzed by *Pseudomonas* sp. lipase (PSL) have recently been described.[29,30] However, enzymatic kinetic resolution of racemic α-methylene-β-hydroxy esters[31,32] using lipases such as PSL and *Pseudomonas* AK is limited to a few examples.[33,34] While good to excellent enantiomeric excess (e.e.) was achieved in such resolutions, only modest enantiomeric ratio (*E*) values[35] were observed for alcohols **1** containing carbomethoxy groups ($R'=CH_3$)[33] or large side chains ($R > C_2H_5$).[34] Additionally, high quantities of enzyme were usually employed in these specific transformations without recycling the biocatalyst, therefore restricting the above methodologies.

Herein, we report the preparation of racemic α-methylene-β-hydroxy esters and their resolution via enzymatic enantioselective transesterification with PSL, free or immobilized in poly(ethylene) oxide (PEO), silica gel and montmorillonite K10, under different conditions.

2. Results and discussion

Compounds **2** and **3** were prepared according to the literature by reacting methyl acrylate with the appropriate aldehyde in the presence of catalytic DABCO at room temperature for 5–7 days.[1,5,7] The previously unreported naphthyl allylic alcohol **4** was similarly obtained and was fully characterized by spectroscopic data. The racemic acetate derivatives **5** and **6** used as standards for GC chiral analysis were prepared by treating the corresponding alcohols **2** and **3** with acetyl chloride and triethylamine in CH_2Cl_2 at 0°C for 2 h.[4,5,8] All compounds were isolated in good yields after purification by flash chromatography.

Enzymatic transesterification of compounds **2**–**4** was then investigated (Scheme 1). The reactions were monitored by gas chromatography using a chiral column (vide infra). A preliminary evaluation of various experimental conditions such as substrate structure, acylating agent, support for lipase, solvent and time revealed that transesterification was remarkably dependent on these reaction parameters (Table 1). The kinetic resolution of **2** was better achieved by using PSL immobilized in PEO, vinyl acetate as acylating reagent, and hexane as solvent, obtaining a conversion (%c) of 50% and the corresponding acetate with e.e. higher than 99%.[36] On the other hand, immobilization of PSL in silica gel or montmorillonite K10 greatly decreased the enzymatic activity and no product was formed. For the resolution of substrates **2** and **3** using the PSL/free system, poorer conversions and e.e. values were achieved, even after a 168 h reaction. Furthermore, substrate **4** was inert under these experimental conditions.



Scheme 1.

Table 1. Preliminary screening for the biocatalytic resolutions of compounds **2**, **3** and **4**

Compound–catalyst	Solvent	Acylating reagent	Time (h)				
			24 e.e.[a] (%)	48 e.e. (%)	72 e.e. (%)	168 e.e. (%)	% c[b]
2–PSL/free[c]	Hexane	Vinyl acetate	52	77	87	91	39
	CH_3CN	Vinyl acetate	1	3	3	20	0.5
	Hexane	Isopropenyl acetate	17	27	40	90	27
2–PSL/PEO[d]	Hexane	Vinyl acetate	57	90	99	99	50
2–PSL/silica[d]	Hexane	Vinyl acetate	0	0	0	0	0
2–PSL/K10[d]	Hexane	Vinyl acetate	0	0	0	0	0
3–PSL/free[c]	Hexane	Vinyl acetate	1	5	8	14	12
4–PSL/free[e]	Hexane	Vinyl acetate	0	0	0	0	0
	Hexane	Isopropenyl acetate	0	0	0	0	0

[a] Enantiomeric excess for the acetate, determined by chiral CG.
[b] c = conversion determined by ^{1}H NMR (200 MHz).
[c] PSL = 500 mg; substrate **2** or **3** = 7.8 mmol.
[d] PSL = 100 mg; support (PEO, silica gel or montmorillonite K10) = 500 mg; substrate **2** = 1.4 mmol.
[e] PSL = 500 mg; substrate **4** = 0.8 mmol.

The superior resolution obtained for (*RS*)-methyl 3-hydroxy-2-methylenebutanoate **2** using PSL/PEO is worthy of note. A clean conversion to the corresponding (*R*)-acetate **5** was observed, thus evidencing the high molecular recognition performed by the enzyme.

These preliminary findings prompted a more detailed investigation on the catalytic activity of PSL/PEO compared with free enzyme. Therefore, resolution of substrates **2** and **3** using vinyl acetate as the acylating agent and hexane as the solvent was evaluated with both PSL/PEO and PSL/free systems. As depicted in Table 2, excellent results were obtained for the resolution of the allylic alcohol **2** employing either PSL/free or PSL/PEO systems. In all cases, acylated products **5**-(*R*)-(+) or **6**-(*R*)-(+) were obtained with e.e. >99% and E >200 regardless of the time or the catalytic system employed. The use of polymer-supported enzyme PSL/PEO (100 mg of catalyst for 1.4 mmol of substrate) is noteworthy, improving the rate, the extent of conversion and the selectivity for both hydroxy esters **2** and **3**, in comparison with PSL/free mediated transformations (500 mg of catalyst for 7.8 mmol of substrate). In addition, when resolution promoted by PSL/PEO was stopped at a maximum conversion of 50% (by a simple filtration to separate the catalyst), enantiomerically pure alcohol **2**-(*S*)-(−) and acetate **5**-(*R*)-(+) ($[\alpha]_D = +18.0$; $CHCl_3$, *c* 5.0, 25°C) were produced and easily separated by preparative silica gel chromatography (entry 15 in Table 2). The fact that the resolution is effected under very mild conditions was demonstrated by entry 16 in Table 2. The reaction was carried out for longer periods after reaching the maximum conversion of 50%, but no racemization was detected as the e.e. for substrate and product were unchanged from 96 to 168 h.

While substrate **2** was completely resolved in 3–4 days using PSL/PEO system, the chain-extended derivative **3** reached only 36% of conversion after 7 days. The corresponding (*R*)-(+)-acetate **6** was isolated in high enantiomeric purity, but the e.e. for the unreactive (*S*)-(−)-alcohol was poor (entry 24 in Table 2). These observations clearly show that the size of the R groups strongly influences the resolution using lipases as biocatalyst.[34]

Another interesting feature presented by biocatalysis is the possibility of recycling and re-using the enzymes, undoubtedly a desirable property for economical and environmental concerns. Accordingly, when PSL/free and PSL/PEO were re-used twice to resolve hydroxy ester **2**, enantioselectivities comparable with those observed for freshly-used catalytic systems were attained. PSL/PEO was particularly advantageous in this case, since this catalyst can be easily recycled by simple filtration followed by thorough washings with hexane. Moreover, resolution of hydroxy ester **2** employing a re-used PSL/free stored for 30 days at low temperature (∼10°C) furnished alcohol **2**-(*S*)-(−) with e.e. = 65%. Conversely, PSL/PEO under similar conditions promoted the resolution of **2**-(*S*)-(−) with e.e. = 99%, therefore attesting the superior stability and efficiency of the polymer-supported enzyme. Possible explanations for the enhancement of catalytic activity with PSL/PEO system could be related to a better diffusion of substrates and products through the poly-

Table 2. Biocatalytic resolutions of compounds **2** and **3** using free and immobilized PSL with vinyl acetate in hexane

Entry	Substrate (mmol)	PSL/support (mg of enzyme)	Time (h)	E.e.s. (%)[a]	E.e.p. (%)[a]	Conversion (%)[b]	*E*
1	**2** (7.8)	PSL/free (500)	24	52	99	34	335
2			48	77	99	44	466
3			96	90	99	48	617
4			168	91	99	48	637
5	**2** (1.4)	PSL/PEO (50)	24	23	99	19	249
6			48	38	99	28	289
7			96	65	99	40	391
8			168	81	99	45	500
9	**2** (1.4)	PSL/PEO (75)	24	41	99	29	298
10			48	72	99	42	431
11			96	80	99	45	491
12			168	83	99	46	520
13	**2** (1.4)	PSL/PEO (100)	24	57	99	37	354
14			48	90	99	48	617
15			96	99	99	50	1057
16			168	99	99	50	1057
17	**3** (7.8)	PSL/free (500)	24	1	99	1	200
18			48	5	99	5	209
19			96	10	99	9	219
20			168	14	99	12	228
21	**3** (1.4)	PSL/PEO (100)	24	29	99	23	264
22			48	40	99	29	295
23			96	54	99	35	342
24			168	56	99	36	350

[a] Enantiomeric excesses for the substrate (e.e.s.) and for the product (e.e.p.) were determined by chiral CG.
[b] Determined by ^1H NMR (200 MHz).

meric matrix and to conformational constraints adopted by the immobilized enzyme which might be closer to the transition state.[29,37]

3. Conclusion

The enzymatic resolution of α-methylene-β-hydroxy esters with PSL/PEO presented here is a simple, mild and economically-important method, since the enantiomeric ratio (*E*) is excellent and enzymes can be recycled for further reutilization without significant loss of their catalytic activity. The influence of side chain groups in substrates **2**–**4** was also observed, but still requires additional investigations. This methodology should be applied successfully to other racemic Baylis–Hillman adducts and important synthetic targets such as hydroxyl-containing building blocks and insect pheromones.

4. Experimental

4.1. General considerations

All chemicals were of reagent grade and were used as received. Melting points are uncorrected. ^{1}H NMR (200 MHz) spectra were recorded in $CDCl_3$ solution, using tetramethylsilane as the internal standard. Infrared spectra were acquired using KBr for solids and film for liquid samples. Column chromatography utilized silica gel (Aldrich, 60–120 mesh particle size). PS lipase (30,000 U/g, Amano 30) was obtained from Amano Enzyme USA Co., Ltd.; PEO (300,000 g/mol) was purchased from SIGMA; montmorillonite K10 was supplied by Fluka. The reaction's progress and enantiomeric excess were determined by gas chromatography using a Shimadzu CG-14B equipped with a chiral column (CP-chirasil-Dex CB), and H_2 as a carrier gas, with a detector, an injection set at 275°C and a column set to temperatures of 80–140°C (2°C/min). The extent of conversion (%c) was obtained by ^{1}H NMR integrals (200 MHz, $CDCl_3$). The enantiomeric ratio (*E*) values were calculated from the degree of conversion and the e.e. of the product, according to the Sih equation.[35]

4.2. Preparation of the PSL/PEO film

The enzyme immobilization in PEO was performed by dissolving 500 mg of polymer and 50–100 mg of PSL in 20 mL of H_2O with further solvent evaporation at room temperature forming a film, which was then cut into several regular sections.

4.3. Immobilization of PSL in silica and K10

PSL (100 mg) was suspended in H_2O (10 mL), and the suspension was mixed with silica gel (500 mg) or K10 (500 mg) in 10 mL H_2O at room temperature. After 5 h of stirring, the mixture was filtered and the resulting system (PSL/silica or PSL/K10) was dried in the oven (100°C) and then stored in a desiccator, ready for use.

4.4. Preparation of racemic α-methylene-β-hydroxy esters and acetates

Compounds **2**, **3**, **5** and **6** were prepared according to the literature and purified by flash chromatography (hexane/AcOEt 9:1); their spectroscopic characterizations were in agreement with published data.[1,4,5,7,8]

4.5. Methyl 3-hydroxy-2-methylenebutanoate 2[1]

IR: 3426, 1734 and 1645 cm^{-1}; ^{1}H NMR: δ 1.37 (d, 3H, $J=6.5$ Hz), 2.79 (s, 1H), 3.78 (s, 3H), 4.62 (q, 1H, $J=6.5$ Hz), 5.83 (s, 1H) and 6.21 (s, 1H).

4.6. Methyl 3-hydroxy-2-methylenehexanoate 3[5,7]

IR: 3440, 2958, 2874, 1718 and 1630 cm^{-1}; ^{1}H NMR: δ 0.94 (t, 3H, $J=7.0$ Hz), 1.26–1.68 (m, 4H), 2.66 (broad s, 1H), 3.78 (s, 3H), 4.41 (t, 1H, $J=6.0$ Hz), 5.80 (s, 1H) and 6.22 (s, 1H).

4.7. Methyl 3-acetoxy-2-methylenebutanoate 5[4,8]

IR: 1742 and 1634 cm^{-1}; ^{1}H NMR: δ 1.40 (d, 3H, $J=6.5$ Hz), 2.07 (s, 3H), 3.78 (s, 3H), 5.71 (q, 1H, $J=6.5$ Hz), 5.82 (s, 1H) and 6.29 (s, 1H).

4.8. Methyl 3-acetoxy-2-methylenehexanoate 6[5,8]

IR: 1726 and 1634 cm^{-1}; ^{1}H NMR: δ 0.94 (t, 3H, $J=7.0$ Hz), 1.30–1.70 (m, 4H), 2.08 (s, 3H), 3.71 (s, 3H), 5.62 (t, 1H, $J=7.5$ Hz), 5.72 (s, 1H) and 6.27 (s, 1H).

The previously unreported naphthyl allylic alcohol **4** was prepared as follows:

4.9. Methyl 3-hydroxy-2-methylene-3-(2-naphthyl)-propanoate 4

55 mg of DABCO (0.50 mmol) was added to a solution containing 260 mg of 2-naphthaldehyde (1.66 mmol) in 0.30 mL of methyl acrylate (3.33 mmol) and the mixture was allowed to stir for 72 h at 25°C. The reaction was then diluted in CH_2Cl_2 (10 mL), washed with 5% HCl (5 mL) and H_2O (5 mL), dried with Na_2SO_4, and concentrated under reduced pressure. The solid residue obtained was filtered in a plug of silica gel (hexane/AcOEt 9:1) and the solvents were removed in vacuo to give 325 mg of compound **5**, (85%); mp 98–99°C; IR: 3330, 3045, 1734 and 1645 cm^{-1}; ^{1}H NMR: δ 3.71 (s, 3H), 5.73 (s, 1H), 5.87 (s, 1H), 6.37 (s, 1H), 7.47 (m, 3H) and 7.84 (m, 4H). Anal. calcd for $C_{15}H_{14}O_3$ (%): C, 74.36; H, 5.82. Found: C, 74.03; H, 5.90.

4.10. General procedure for enzymatic resolution of racemic α-methylene-β-hydroxy esters with polymer-supported PSL

PSL/PEO system (75–100 mg) was added to a solution of racemic **2** or **3** (1.4 mmol) and vinyl acetate (21.6 mmol) in hexane (50 mL). The mixture was gently stirred at 35°C for 168 h, and was then filtered and

washed thoroughly with hexane. The filtrate was evaporated and the resulting residue was purified by column chromatography (ethyl eter/ethyl acetate 9:1) to give the corresponding (*R*)-α-methylene-β-acetoxy esters and (*S*)-α-methylene-β-hydroxy esters as colourless oils.

4.11. General procedure for enzymatic resolution of racemic α-methylene-β-hydroxy esters with free PSL

Free PSL (500 mg) was added to a solution of racemic **2** or **3** (7.8 mmol) and the acylating agent (vinyl or isopropenyl acetate, 21.6 mmol) in hexane (50 mL). After stirring at 35°C for 168 h, the reaction mixture was treated as above to give the corresponding α-methylene-β-acetoxy esters and α-methylene-β-hydroxy esters.

Acknowledgements

The authors are grateful to Central de Análises (Departamento de Química, UFSC) for spectroscopic analysis and to Amano Enzyme USA Co., Ltd., for kindly furnishing PSL. Financial support and scholarships to SPZ, SPM and MGN by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and to LF by CAPES are also acknowledged.

References

1. Roush, W. R.; Brown, B. B. *J. Org. Chem.* **1993**, *58*, 2151–2161.
2. Iwabuchi, Y.; Furukawa, M.; Esumi, T.; Hatakeyama, S. *J. Chem. Soc., Chem. Commun.* **2001**, 2030–2031.
3. Bailey, M.; Markó, I. E.; Ollis, W. D.; Rasmussen, P. R. *Tetrahedron Lett.* **1990**, *31*, 4509–4512.
4. Ameer, F.; Drewes, S. E.; Hoole, R.; Kaye, P. T.; Pitchford, A. T. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2713–2717.
5. (a) Hoffmann, H. M. R.; Rabe, J. *J. Org. Chem.* **1985**, *50*, 3849–3859; (b) Hoffmann, H. M. R.; Rabe, J. *Helv. Chim. Acta* **1984**, *2*, 413–415.
6. (a) Cheskis, B. A.; Moiseenkov, A. M.; Shpiro, N. A.; Stashina, G. A.; Zhulin, V. M. *Bull. Acad. Sci. USSR, Div. Chem. Sci. (Engl. Transl.)* **1990**, *39*, 716–720; (b) Moiseenkov, A. M.; Cheskis, B. A.; Shpiro, N. A.; Stashina, G. A.; Zhulin, V. M. *Bull. Acad. Sci. USSR, Div. Chem. Sci. (Engl. Transl.)* **1990**, *39*, 517–521.
7. Mateus, C. R.; Feltrin, M. P.; Costa, A. M.; Coelho, F.; Almeida, W. P. *Tetrahedron* **2001**, *57*, 6901–6908.
8. Kim, J. N.; Chung, Y. M.; Im, Y. J. *Tetrahedron Lett.* **2002**, *43*, 6209–6211.
9. Annunziata, R.; Benaglia, M.; Cinquini, M.; Cozzi, F.; Raimondi, L. *J. Org. Chem.* **1995**, *60*, 4697–4706.
10. Räcker, R.; Döring, K.; Reiser, O. *J. Org. Chem.* **2000**, *65*, 6932–6939.
11. Basavaiah, D.; Kumaragurubaran, N. *Tetrahedron Lett.* **2001**, *42*, 477–479.
12. Kundu, M. K.; Sundar, N.; Kumar, S. K.; Bhat, S. V.; Biswas, S.; Valecha, N. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 731–736.
13. (a) Basavaiah, D.; Rao, A. J.; Satyanarayana, T. *Chem. Rev.* **2003**, *103*, 811–891; (b) Basavaiah, D.; Rao, P. D.; Hyma, R. S. *Tetrahedron* **1996**, *52*, 8001–8062.
14. Ciganek, E. *Organic Reactions*; John Wiley & Sons: New York, 1997; Vol. 51, pp. 201–350.
15. Drewes, S. E.; Roos, G. H. P. *Tetrahedron* **1988**, *44*, 4653–4670.
16. Langer, P. *Angew. Chem., Int. Ed.* **2000**, *39*, 3049–3052.
17. Brzezinsky, L. J.; Rafel, S.; Leahy, J. W. *J. Am. Chem. Soc.* **1997**, *119*, 4317–4318.
18. Yang, K.-S.; Chen, K. *Org. Lett.* **2000**, *2*, 729–731.
19. Krishna, P. R.; Kannan, V.; Ilangovan, A.; Sharma, G. V. M. *Tetrahedron: Asymmetry* **2001**, *12*, 829–837.
20. Iwabuchi, Y.; Nakatani, M.; Yokoyama, N.; Hatakeyama, S. *J. Am. Chem. Soc.* **1999**, *121*, 10219–10220.
21. Trost, B. M.; Tsui, H.-C.; Toste, F. D. *J. Am. Chem. Soc.* **2000**, *122*, 3534–3535.
22. Kitamura, M.; Kasahara, I.; Manabe, K.; Noyori, R. *J. Org. Chem.* **1988**, *53*, 708–710.
23. Brown, J. M. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 190–203.
24. Roberts, S. M. *J. Chem. Soc., Perkin Trans. 1* **1999**, *1*, 1–21.
25. Roberts, S. M. *Biocatalysis for Fine Chemical Synthesis*; John Wiley & Sons: New York, 1999.
26. Yang, H.; Henke, E.; Bornscheur, U. T. *J. Org. Chem.* **1999**, *64*, 1709–1712.
27. Kobayashi, S.; Uyama, H.; Namekawa, S.; Hayakawa, H. *Macromolecules* **1998**, 5655–5659.
28. Al-Azemi, T. F.; Bisht, K. S. *Macromolecules* **1998**, 6536–6540.
29. Morisso, F. D. P.; Costa, V. E. U. *Tetrahedron: Asymmetry* **2001**, *12*, 2641–2647.
30. Queiroz, N.; Nascimento, M. G. *Tetrahedron Lett.* **2002**, *43*, 5225–5227.
31. Adam, W.; Hoch, U.; Saha-Möller, C. R.; Schreier, P. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1737–1739.
32. Basavaiah, D.; Rao, P. D. *Synth. Commun.* **1994**, *24*, 913–917.
33. Burgess, K.; Jennings, L. D. *J. Org. Chem.* **1990**, *55*, 1138–1139.
34. Hayashi, N.; Yanagihara, K.; Tsuboi, S. *Tetrahedron: Asymmetry* **1998**, *9*, 3825–3830.
35. Sih, C. J.; Wu, S.-H. *Top. Stereochem.* **1989**, *19*, 63–125.
36. The absolute configurations tentatively assigned to hydroxy esters **2** and **3** and to acetates **5** and **6**, in Scheme 1, were based on related work[33] in which hydrogenation of the double bond afforded the corresponding α-methyl-β-hydroxy (or β-acetoxy) esters with known stereochemistry.[22]
37. Pleiss, J.; Fischer, M.; Schimidt, R. D. *Chem. Phys. Lipids* **1998**, *93*, 67–80.