Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 529–538

BIOORGANIC & MEDICINAL CHEMISTRY

Synthetic and Novel Biocatalytic Resolution Studies on (±)-5/6/7-Acetoxy-4-aryl-3,4-dihydrocoumarins

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Received 27 August 2002; accepted 17 September 2002

Abstract—Eleven (±)-5/6/7-acetoxy-4-aryl-3,4-dihydrocoumarins have been synthesised in two steps starting from the coupling of cinnamic acid/substituted cinnamic acid with appropriate phenols, followed by acetylation in 50–83% overall yields. All hydroxy- and acetoxycoumarins were unambiguously identified on the basis of their spectral data. *Candida antarctica* lipase-catalysed deacetylation of these racemic acetoxydihydrocoumarins in dioxane occurred with moderate enantioselectivity. This is one of the rare examples of resolution using phenolic ester moiety as a remote handle for chiral recognition by a lipase.

Introduction

4-Phenyldihydrocoumarins are naturally occurring compounds and this skeleton is also common in various classes of other natural products, for example flavonoids, tannins, etc.[1–4] The compounds of this class are indicated to be involved in the defence mechanism of plants by interfering with signal transduction in fungal pathogens and herbivores.[5] Few compounds containing 4-phenyl-3,4-dihydrocoumarin nucleus possess important biological activities, for example inhibitors of aldose reductase[2] and protein kinases,[3] antiherpetic,[6] etc. The 4-phenyldihydrocoumarins contain an asymmetric centre at the C-4 position and thus their naturally occurring analogues are optically active. In recent years, there has been a considerable effort towards the synthesis of optically enriched compounds because different enantiomers usually have quite different physiological activities. One of the recent developments in this direction is the application of biocatalysts for carrying out enantioselective reactions with an added advantage of environmental friendliness. Among the different biocatalytic processes, lipase-catalysed selective acylation/deacylation reactions represent an important class of enzymatic transformations in organic synthesis which is mainly attributed to the low cost of lipases and their wide tolerance towards a variety of reaction conditions and substrates.[7,8] We have successfully demonstrated the applications of lipases from porcine pancreas *Candida, Aspergillus* and *Pseudomonas* species for carrying out regio- and stereoselective acylation/deacylation reactions on polyphenolics,[9–12] carbohydrates[13–16] and polyols.[17,18] We report herein the synthesis of eleven 5/6/7-acetoxy-4-aryl-3,4-dihydrocoumarins and their novel enantioselective deacetylation catalysed by *Candida antarctica* lipase in dioxane.

Results and Discussion

Three 4-phenyldihydrocoumarins, that is, (±)-7-hydroxy-4-phenyl-3,4-dihydrocoumarin (**8**), (±)-6-hydroxy-4-phenyl-3,4-dihydrocoumarin (**9**) and (±)-5-hydroxy-7-methyl-4-phenyl-3,4-dihydrocoumarin (**10**), have been synthesised by the condensation of cinnamic acid (**1**) with corresponding phenols, that is, resorcinol (**5**), *p*-benzoquinol (**6**) and orcinol (**7**), in the presence of concentrated HCl–HCl gas in 91, 89 and 90% yields,

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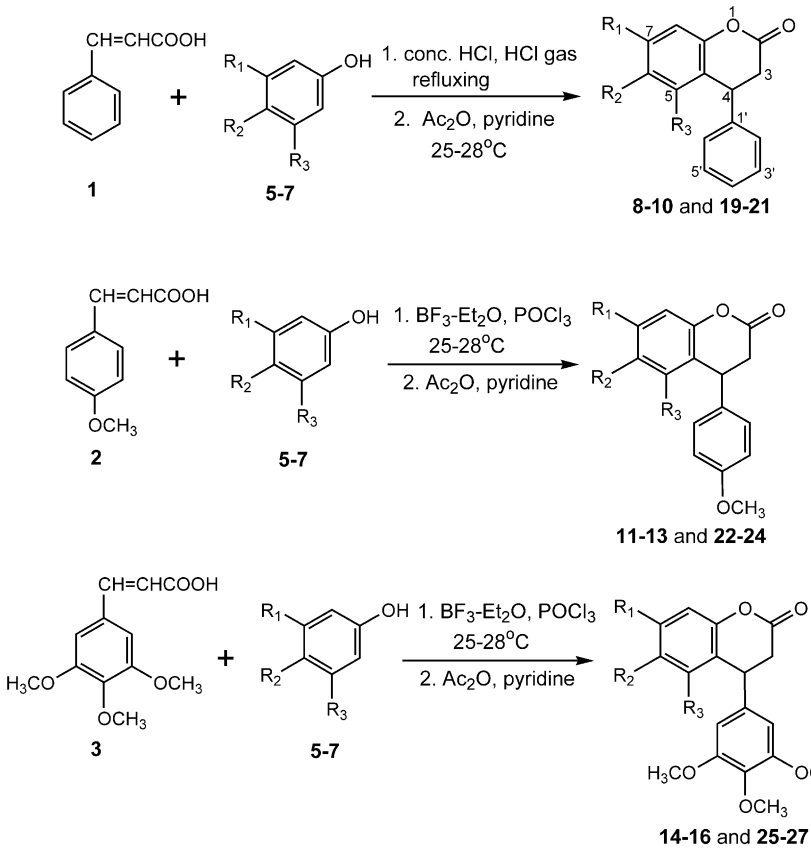
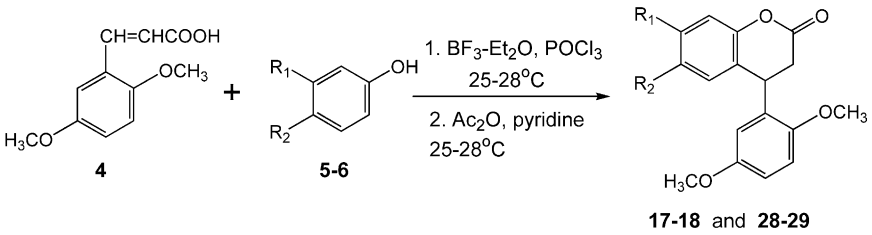
PII: S0968-0896(02)00454-6



	5, 8, 11 & 14	6, 9, 12 & 15	7, 10, 13 & 16	19, 22 & 25	20, 23 & 26	21, 24 & 27
R_1	OH	H	CH_3	OAc	H	CH_3
R_2	H	OH	H	H	OAc	H
R_3	H	H	OH	H	H	OAc

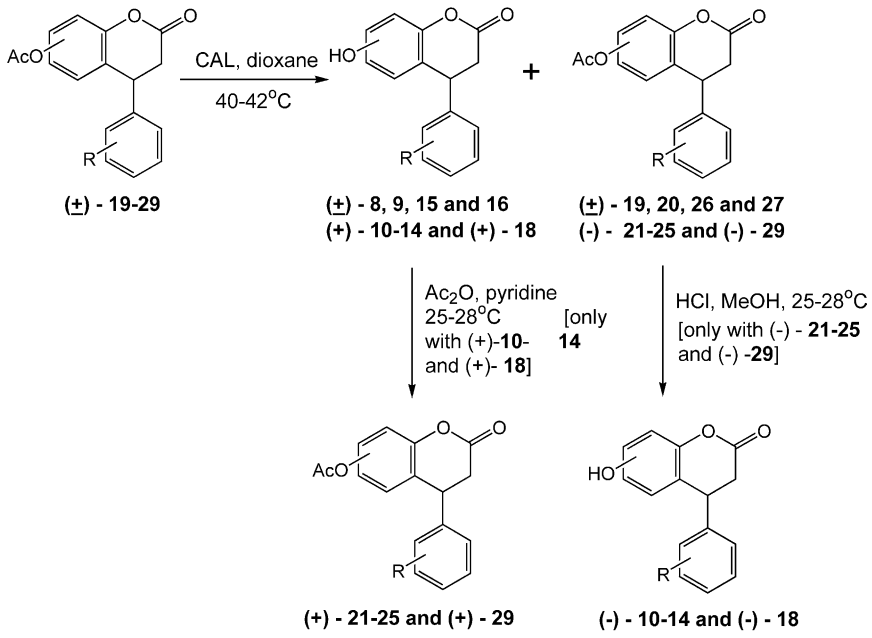
Scheme 1.

respectively (Scheme 1).[19] The other eight dihydrocoumarins, that is, (±)-7-hydroxy-4-(4′-methoxyphenyl)-3,4-dihydrocoumarin (**11**), (±)-6-hydroxy-4-(4′-methoxyphenyl)-3,4-dihydrocoumarin (**12**), (±)-5-hydroxy-7-methyl-4-(4′-methoxyphenyl)-3,4-dihydrocoumarin (**13**), (±)-7-hydroxy-4-(3′,4′,5′-trimethoxyphenyl)-3,4-dihydrocoumarin (**14**), (±)-6-hydroxy-4-(3′,4′,5′-trimethoxyphenyl)-3,4-dihydrocoumarin (**15**), (±)-5-hydroxy-7-methyl-4-(3′,4′,5′-trimethoxyphenyl)-3,4-dihydrocoumarin (**16**), (±)-7-hydroxy-4-(2′,5′-dimethoxyphenyl)-3,4-dihydrocoumarin (**17**) and (±)-6-hydroxy-4-(2′,5′-dimethoxyphenyl)-3,4-dihydrocoumarin (**18**) have been synthesised by the condensation of substituted cinnamic acids **2**–**4** with the corresponding phenols **5**–**7** in the presence of milder Friedel–Crafts catalyst BF_3–Et_2O and $POCl_3$ in 54–75% yields (Schemes 1 and 2).[20] Attempts towards condensation of substituted cinnamic acids **2**–**4** with corresponding phenols **5**–**7** for the synthesis of coumarins **11**–**18** in the presence of concentrated HCl–HCl gas were not successful. Further, condensation of cinnamic acid/substituted cinnamic acids with orcinol may lead to the formation of two isomeric dihydrocoumarins, (±)-5-hydroxy-7-methyl-4-aryl-3,4-dihydrocoumarins and (±)-7-hydroxy-5-methyl-4-aryl-3,4-dihydrocoumarins; however, the reaction of orcinol with cinnamic acid (**1**) or its derivatives **2** or **3** under the above conditions has led to the isolation of only 5-hydroxy-7-methyl-4-aryldihydrocoumarins **10**, **13** and **16**; this has been established unambiguously by NOE experiments in the ^1H NMR spectral recordings of these three coumarins. Thus, the irradiation of –OH group signals resonating at δ 8.70, 8.58 and 8.61 Hz in the ^1H NMR spectra of dihydrocoumarins **10**, **13** and **16** gives 48.4, 29.0 and 34.6% enhancement in one of the signals of C-3 protons resonating at δ 2.90, 3.15 and 3.03, respectively. Similarly, irradiation of the particular signal of C-3 protons of **10**, **13** and **16** gave significant NOE enhancement on the –OH group signals. These NOE results indicate that the –OH group is closer to the C-3 position in the dihydrocoumarins **10**, **13** and **16**, that is at position C-5 rather than C-7. This observation was again substantiated by NOESY experiments performed on compounds **10**, **13** and **16**, which showed cross peaks due to the spatial coupling between the protons of C-5 OH group and the one at the C-3 position. Further, the physical/spectral data of known compounds **10** and **13** matched well with the reported values in the literature.[19,21]



	5 & 17	6 & 18	28	29
R_1	OH	H	OAc	H
R_2	H	OH	H	OAc

Scheme 2.



Scheme 3.

The 5/6/7-hydroxydihydrocoumarins **8**–**18** were acetylated with acetic anhydride–pyridine mixture in the presence of catalytic amount of 4-*N,N*-dimethylaminopyridine to afford the corresponding acetates, (±)-7-acetoxy-4-phenyl-3,4-dihydrocoumarin (**19**), (±)-6-acetoxy-4-phenyl-3,4-dihydrocoumarin (**20**), (±)-5-acetoxy-7-methyl-4-phenyl-3,4-dihydrocoumarin (**21**), (±)-7-acetoxy-4-(4′-methoxyphenyl)-3,4-dihydrocoumarin (**22**), (±)-6-acetoxy-4-(4′-methoxyphenyl)-3,4-dihydrocoumarin (**23**), (±)-5-acetoxy-7-methyl-4-(4′-methoxyphenyl)-3,4-dihydrocoumarin (**24**), (±)-7-acetoxy-4-(3′,4′,5′-trimethoxyphenyl)-3,4-dihydrocoumarin (**25**), (±)-6-acetoxy-4-(3′,4′,5′-trimethoxyphenyl)-3,4-dihydrocoumarin (**26**), (±)-5-acetoxy-7-methyl-4-(3′,4′,5′-trimethoxyphenyl)-3,4-dihydrocoumarin (**27**), (±)-7-acetoxy-4-(2′,5′-dimethoxyphenyl)-3,4-dihydrocoumarin (**28**) and (±)-6-acetoxy-4-(2′,5′-dimethoxyphenyl)-3,4-dihydrocoumarin (**29**) in 81–96% yields (Schemes 1 and 2). All the 5/6/7-hydroxydihydrocoumarins **8**–**18** and their corresponding acetates **19**–**29** were identified on the basis of their spectral data. The mps of known compounds **8**–**13**[19–22] and **19**–**21**[19] and the spectral data of compounds **11**[20] and **13**[21] were quite comparable with the corresponding reported values. We have, for the first time, reported the complete spectral data of known compounds **8**–**10**, **12** and **19**–**21** (cf. Experimental).

Our initial attempts at screening of porcine pancreatic lipase (PPL) in tetrahydrofuran (THF), *Candida rugosa* lipase (CRL) in diisopropyl ether (DIPE) and *Candida antarctica* lipase (CAL) in dioxane for the deacetylation of 5/6/7-acetoxy-4-aryl-3,4-dihydrocoumarins **19**–**29** revealed that these coumarins are not the substrates for PPL in THF and the deacetylation catalysed by CRL in DIPE was too slow to be of any practical significance; however, deacetylation of acetoxydihydrocoumarins **19**–**27** and **29** catalysed by CAL in dioxane proceeds satisfactorily. *Candida antarctica* lipase does not accept the (±)-dihydrocoumarin **28** as a substrate; no deacetylation was observed in this case even after 120 h

of incubation. In order to investigate the capability of CAL for resolution of these compounds, racemic dihydrocoumarins **19–21** were first incubated with CAL in dioxane and the reaction was stopped by filtering off the enzyme after about 50% conversion of the starting acetate to a slow moving product on TLC, that is hydroxydihydrocoumarins **8–10** (Scheme 3). The deacetylated 5/6/7-hydroxydihydrocoumarins **8–10** and recovered, unreacted 5/6/7-acetoxydihydrocoumarins **19–21** were separated by column chromatography on silica gel with a gradient solvent system of chloroform–acetone and their optical rotations were measured. The results revealed that *Candida antarctica* lipase in dioxane does not discriminate between the two enantiomers of (±)-dihydrocoumarins **19** and **20** and leads to the formation of racemic hydroxydihydrocoumarins **8** and **9** and racemic acetoxydihydrocoumarins **19** and **20**, respectively (Table 1). However, deacetylation of (±)-dihydrocoumarin **21** was enantioselective leading to the isolation of (+)-5-hydroxy-7-methyl-4-phenyl-3,4-dihydrocoumarin (**10**) and (−)-5-acetoxy-7-methyl-4-phenyl-3,4-dihydrocoumarin (**21**) in 73 and 94% yields, respectively. Encouraged by this result, (±)-acetoxydihydrocoumarins **22–27** and **29** were incubated with CAL in dioxane until about 50% conversion and the deacetylated hydroxydihydrocoumarins **11–16** and **18**, and unreacted acetoxydihydrocoumarins **22–27** and **29**, were separated by column chromatography (Scheme 3). Measurements of the optical rotation values of the deacetylated hydroxydihydrocoumarins and recovered, unreacted acetoxydihydrocoumarins revealed that CAL catalyses the deacetylation of (±)-**22–25** and (±)-**29** in an enantioselective fashion leading to the formation of optically active (+)-hydroxydihydrocoumarins **11–14** and **18** and (−)-acetoxydihydrocoumarins **22–25** and **29** in 68–85 and 67–88% yields, respectively (Table 1). The enzyme failed to discriminate between the two enantiomers of racemic acetoxydihydrocoumarins **26** and **27** and afforded racemic hydroxy- and acetoxydihydrocoumarins **15–16** and **26–27**, respectively. The yields of optically enriched products (+)-**10–14** and (+)-**18**, and (−)-**21–25** and (−)-**29** were calculated by assuming corresponding single enantiomer as 100% in the starting (±)-**21–25** and (±)-**29**.

Table 1. Enantioselective deacetylation of (±)-5/6/7-acetoxy-4-aryl-3,4-dihydrocoumarins **19–29** catalysed by CAL in dioxane containing *n*-butanol as the acyl trap at 40–42 °C[a,b]

Entry	Substrate	Reaction time (h)	Products (*% yield*)
1	(±)-**19**	4.5	(±)-**8** (*42*[d]) *and* (±)-**19** (*43*[d])
2	(±)-**20**	2.0	(±)-**9** (*43*[d]) *and* (±)-**20** (*45*[d])
3	(±)-**21**	8.5	(+)-**10** (*73*[c]) *and* (−)-**21** (*94*[c])
4	(±)-**22**	29.0	(+)-**11** (*77*[c]) *and* (−)-**22** (*80*[c])
5	(±)-**23**	5.0	(+)-**12** (*72*[c]) *and* (−)-**23** (*68*[c])
6	(±)-**24**	43.0	(+)-**13** (*82*[c]) *and* (−)-**24** (*84*[c])
7	(±)-**25**	74.0	(+)-**14** (*85*[c]) *and* (−)-**25** (*88*[c])
8	(±)-**26**	43.0	(±)-**15** (*39*[d]) *and* (±)-**26** (*38*[d])
9	(±)-**27**	94.0	(±)-**16** (*40*[d]) *and* (±)-**27**(*42*[d])
10	(±)-**28**	—	No reaction
11	(±)-**29**	0.5	(+)-**18** (*68*[c]) *and* (−)-**29** (*67*[c])

[a]All these reactions, when performed under identical conditions, but without adding *Candida antarctica* lipase, did not yield any product.
[b]All deacetylation reactions were stopped by filtering off the enzyme after about 50% conversion of the starting material into the product.
[c]Yields of optically enriched products are calculated by assuming corresponding single enantiomer as 100% in the starting (±)-4-aryl-3,4-dihydrocoumarins **21–25** and **29**.
[d]No resolution was observed in the case of deacetylation of dihydrocoumarins **19**, **20**, **26** and **27**; in these cases yields of deacetylated racemic dihydrocoumarins **8**, **9**, **15** and **16** and recovered, unreacted racemic acetates **19**, **20**, **26** and **27** were calculated on the basis of the amounts of (±)-4-aryl-3,4-dihydrocoumarins taken for the biocatalytic reactions.

In order to determine the enantiomeric excess values of enzymatically deacetylated hydroxydihydrocoumarins (+)-**10–14** and (+)-**18** and recovered, unreacted acetoxydihydrocoumarins (−)-**21–25** and (−)-**29**, the separation of two enantiomers of racemic hydroxydihydrocoumarins **10–14** and **18** and racemic acetoxydihydrocoumarins **21–25** and **29** was attempted on HPLC using Chiracel OJ and Chiracel OD columns. However, separation of enantiomers was not observed in either racemic hydroxy- or in racemic acetoxydihydrocoumarins. Further, the synthesis of racemic *O*-acetylmandelates (100% conversion on TLC) were achieved by the reaction of (±)-5/6/7-hydroxydihydrocoumarins **10–14** and **18** with D-(−)-*O*-acetylmandelic acid in dichloromethane according to the procedure of Whitesell and Reynolds[23] and the ^{1}H NMR spectra of the diastereomeric mandelates were analysed to find out the splitting in chemical shift values of diastereomeric protons. Unfortunately no separation of the signals in the ^{1}H NMR spectra of diastereomeric mandelates was observed. Thus, enantiomeric excess values of enzymatically deacetylated (+)-hydroxydihydrocoumarins **10–14** and **18**, or recovered, unreacted (−)-acetoxydihydrocoumarins **21–25** and **29** could not be determined.

However, to show that the lipase exhibits enantioselectivity and yields optically enriched (+)-hydroxydihydrocoumarins **10–14** and **18**, and (−)-acetoxydihydrocoumarins **21–25** and **29**, the optically active acetates were deacetylated by stirring with methanolic HCl at 25–28 °C (Scheme 3). The comparison of optical rotation values of (+)-hydroxydihydrocoumarins **10–14** and **18** obtained by enzymatic deacetylation of (±)-**21–25** and **29**, and (−)-hydroxydihydrocoumarins **10–14** and **18** obtained by chemical deacetylation of recovered, unreacted (−)-**21–25** and **29** revealed that they are quite comparable in three cases (within practical limits) and had opposite signs of rotation (Table 2). This indicates that there is optical enrichment during CAL-catalysed deacetylation of (±)-**21–25** and **29**. Further, to strengthen the observation that there is optical enrichment during CAL-catalysed deacetylation of (±)-**21–25** and **29**, the enzymatically deacetylated (+)-hydroxydihydrocoumarins **10–14** and **18** were chemically acetylated by standard Ac_2O–pyridine method, which led to the formation of acetates (+)-**21–25** and (+)-**29** (Scheme 3). The optical rotation values of these acetoxydihydrocoumarins (+)-**21–25** and (+)-**29** and recovered, unreacted dihydrocoumarins (−)-**21–25** and **29** are quite comparable in all the six cases (Table 2), which confirms that *Candida antarctica* lipase catalyses the deacetylation of racemic acetylated

Table 2. Optical rotation values of (+)-4-aryl-5/6/7-hydroxy-3,4-dihydrocoumarins **10**–**14** and **18** obtained by enzymatic deacetylation of (±)-5/6/7-acetoxy-4-aryl-3,4-dihydrocoumarins **21**–**25** and **29**, the acetates (+)-**21**–**25** and **29** obtained by chemical acetylation of (+)-**10**–**14** and **18**, the recovered, unreacted (−)-5/6/7-acetoxy-4-aryl-3,4-dihydrocoumarins **21**–**25** and **29** and (−)-4-aryl-5/6/7-hydroxy-3,4-dihydrocoumarins **10**–**14** and **18** obtained by chemical deacetylation of recovered, unreacted (−)-**21**–**25** and **29**

$[\alpha]_D^{25}$			
(+)-**10**–**14** and **18**	(−)-**10**–**14** and **18**	(+)-**21**–**25** and **29**	(−)-**21**–**25** and **29**
(+)-**10**: +10.0	(−)-**10**: −7.6	(+)-**21**: +3.0	(−)-**21**: −2.2
(+)-**11**: +25.9	(−)-**11**: −10.4	(+)-**22**: +15.1	(−)-**22**: −10.0
(+)-**12**: +2.6	(−)-**12**: −1.5	(+)-**23**: +6.0	(−)-**23**: −10.0
(+)-**13**: +4.8	(−)-**13**: −11.6	(+)-**24**: +3.6	(−)-**24**: −2.5
(+)-**14**: +24.2	(−)-**14**: −2.1	(+)-**25**: +8.0	(−)-**25**: −6.6
(+)-**18**: +4.8	(−)-**18**: −3.3	(+)-**29**: +3.0	(−)-**29**: −4.8

dihydrocoumarins **21**–**25** and **29** in an enantioselective fashion. All the hydroxy- and acetoxydihydrocoumarins obtained by enzymatic deacetylation of racemic acetoxydihydrocoumarins and by chemical acetylation and deacetylation of (±)-dihydrocoumarins were identified on the basis of their spectral data, which were found to be identical with the data of corresponding racemic compounds prepared chemically. All these enzymatic reactions, when performed under identical conditions but without addition of the enzyme, did not yield any product.

It is revealed from the results of enzymatic deacetylation of different dihydrocoumarins that the rate of the reaction depends on the number of methoxy substituents on the C-4 aryl moiety and also on the position of the reactive acetoxy substituent on the benzenoid ring of the dihydrocoumarin nucleus. Thus, the rate of deacetylation of 4-phenyldihydrocoumarins **19**, **20** and **21** is about 6.5, 2.5 and 5.0 times faster than the rate of deacetylation of their corresponding 4-(4′-methoxyphenyl)dihydrocoumarins **22**, **23** and **24**, which in turn is faster than the rate of deacetylation of their corresponding 4-(3′,4′,5′-trimethoxyphenyl)dihydrocoumarins **25**, **26** and **27**. Among the two dihydrocoumarins **28** and **29** having 2,5-dimethoxyphenyl substituent at C-4 position, the former does not undergo deacetylation in the presence of CAL in dioxane, whereas the enzyme takes only 0.5 h for about 50% conversion of the acetate **29** to its corresponding hydroxy analogue. Further, the rate of deacetylation of 4-phenyldihydrocoumarins **19**, **20** and **21** depends on the position of the acetoxy group in the benzenoid ring; for example, the rate of deacetylation of 7-acetoxy-4-phenyldihydrocoumarin **19** is 2.25 times slower than the rate of deacetylation of 6-acetoxy-4-phenyldihydrocoumarin **20**, which in turn is 4.25 times faster than the rate of deacetylation of 5-acetoxy-4-phenyldihydrocoumarin **21**. A similar trend has been observed during the deacetylation of 4-(4′-methoxyphenyl)dihydrocoumarins **22**–**24** and 4-(3′,4′,5′-trimethoxyphenyl)dihydrocoumarins **25**–**27**. These results indicate that the increase of bulk on phenyl group at C-4 position of the dihydrocoumarins results in decrease in the rate of deacetylation of 4-aryldihydrocoumarins. Among the dihydrocoumarins having the same group at C-4 position, 6-acetoxydihydrocoumarins undergo deacetylation faster than 7-acetoxydihydrocoumarins, which in turn biotransform faster than 5-acetoxydihydrocoumarins.

The present study has revealed the moderate enantioselective capability of *Candida antarctica* lipase for the deacetylation of (±)-5/6/7-acetoxy-4-aryl-3,4-dihydrocoumarins **21**–**25** and **29** in dioxane. The rate of deacetylation of 5/6/7-acetoxy-4-aryldihydrocoumarins depends on the bulk of the 4-aryl group and on the position of the acetoxy substituent in the benzenoid ring. Since dihydrocoumarins of this class possess useful biological activities, the methodology developed here may be useful for the preparation of optically enriched bioactive compounds having this skeleton. We may mention that this study is one of the rare examples of resolution using phenolic ester moiety as a remote handle towards chiral recognition by a lipase.

Experimental

Reactions were monitored by TLC on precoated Merck silica gel $60F_{254}$ aluminium plates. Flash column chromatography was carried out using silica gel (CDH, 100–200 mesh). The spots on TLC were visualised either under UV light (254 nm) or by charring with 5% alcoholic H_2SO_4 solution. Melting points were determined in a sulphuric acid bath and are uncorrected. Optical rotations were measured with a Bellingham Stanley ADP 220 polarimeter. The HPLC was performed using a Shimadzu LC-10AS instrument attached with SPD-10A UV–vis detector and Shimpack CLC-ODS (4.6 × 150 nm) reverse phase column; solvent system used was methanol–water (3:2.5) at a flow rate of 0.50 mL/min. The IR spectra were recorded either on Perkin–Elmer model RX/FT IR or on 2000 FT-IR spectrometers. The ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on a Bruker Advance 300 spectrometer at 300 and 75.5 MHz, respectively, using TMS as internal standard. The chemical shift values are on δ scale and the coupling constants (*J*) are in Hz. The EI mass spectra were recorded on Jeol JMA-DA 5000 and ESI and APCI mass spectra were recorded on Jeol AX-505W instruments at 70 eV. The *Candida antarctica* lipase (CAL) immobilised on accurel was a gift from Novo Nordisk Co. The chemicals, dioxane (G.R. grade) and $POCl_3$ were obtained from E. Merck, and BF_3–Et_2O and (*S*)-(+)-*O*-acetylmandelic acid (*ee* 99%) were obtained from Aldrich Chemical Company.

General method of synthesis of 4-phenyl-3,4-dihydrocoumarins 8, 9 and 10

A slow stream of hydrogen chloride gas was passed through a boiling mixture of cinnamic acid (5 mmol), phenol (**5**–**7**, 5 mmol) and concentrated hydrochloric acid (20 mL) until a clear solution was obtained (4–6 h), the solution was cooled and the solid that separated out was filtered, washed repeatedly with cold water, dried and purified by flash column chromatography using

acetone–chloroform (3:97) as eluent to afford pure 4-phenyldihydrocoumarins **8**, **9** and **10** in 89–91% yields. The structures of dihydrocoumarins **8**, **9** and **10** were unambiguously established on the basis of analysis of their spectral data (being reported here for the first time) and by comparison of their mps with those reported in the literature.[19]

(±)-7-Hydroxy-4-phenyl-3,4-dihydrocoumarin (8). It was obtained as a white solid (1.09 g) in 91% yield; mp 142–143 °C (lit.[19] mp 140 °C). R_f: 0.28 (chloroform–acetone, 19:1); EIMS, *m/z* (% rel. int.): 240 ($[M]^+$, 85), 212 (20), 197 (100), 163 (10), 115 (10), 77 (10) and 44 (40); ^{1}H NMR (300 MHz, CD_3COCD_3): δ 3.00 (1H, dd, J = 6.5 Hz and 15.8 Hz, C-3H$_\alpha$), 3.13 (1H, dd, J = 6.1 Hz and 15.8 Hz, C-3H$_\beta$), 4.39 (1H, br t, J = 6.2 Hz, C-4H), 6.61–6.65 (2H, m, C-6H and C-8H), 6.89 (1H, d, J = 8.0 Hz, C-5H), 7.20–7.37 (5H, m, C-2′H, 3′H, 4′H, 5′H and 6′H) and 8.78 (1H, br s, OH); ^{13}C NMR (75.5 MHz, CD_3COCD_3): δ 38.23 and 41.00 (C-3 and C-4), 104.84 (C-8), 112.50 (C-6), 118.12 (C-10), 127.18, 127.51, 129.80 and 130.43 (C-5, C-2′, C-3′, C-4′, C-5′ and C-6′), 143.29 (C-1′), 154.00 (C-9), 159.05 (C-7) and 168.34 (C-2); IR (KBr): 3459(OH), 1735(CO), 1624, 1455, 1234, 1142, 822 and 697 cm^{-1}.

(±)-6-Hydroxy-4-phenyl-3,4-dihydrocoumarin (9). It was obtained as a white solid (1.06 g) in 89% yield; mp 135–136 °C (lit.[19] mp 133 °C). R_f: 0.27 (chloroform–acetone, 19:1); ESIMS, *m/z* (% rel. int.): 263.2 ($[M+Na]^+$, 100) and 241 ($[M+H]^+$, 55); ^{1}H NMR (300 MHz, CD_3COCD_3): δ 2.86 (1H, dd, J = 7.0 Hz and 15.8 Hz, C-3H$_\alpha$), 2.94 (1H, dd, J = 6.1 Hz and 15.8 Hz, C-3H$_\beta$), 4.26 (1H, t, J = 6.5 Hz, C-4H), 6.35 (1H, d, J = 2.8 Hz, C-5H), 6.66 (1H, dd, J = 2.8 and 8.7 Hz, C-7H), 6.75 (1H, d, J = 8.7 Hz, C-8H), 7.07–7.24 (5H, m, C-2′H, C-3′H, C-4′H, C-5′H and C-6′H) and 8.26 (1H, br s, OH); ^{13}C NMR (75.5 MHz, CD_3COCD_3): δ 36.93 and 40.91 (C-3 and C-4), 114.99, 115.53 and 117.93 (C-5, C-7, C-8 and C-10), 127.74, 128.04 and 129.34 (C-2′, C-3′, C-4′, C-5′ and C-6′), 141.76 (C-1′), 145.49 (C-9), 154.44 (C-6) and 167.75 (C-2); IR (Nujol): 3327 (OH), 1741 (CO), 1597, 1492, 1463, 1282, 1196, 1142, 820 and 703 cm^{-1}.

(±)-5-Hydroxy-7-methyl-4-phenyl-3,4-dihydrocoumarin (10). It was obtained as a white solid (1.14 g) in 90% yield; mp 220–221 °C (lit.[19] mp 218 °C). R_f: 0.24 (chloroform–acetone, 19:1); ESIMS, *m/z* (% rel. int.): 277.2 ($[M+Na]^+$, 100) and 255 ($[M+H]^+$, 33); ^{1}H NMR (300 MHz, CD_3COCD_3): δ 2.11 (3H, s, CH_3), 2.90 (1H, dd, J = 1.8 Hz and 15.7 Hz, C-3H$_\alpha$), 3.20 (1H, dd, J = 6.9 Hz and 15.7 Hz, C-3H$_\beta$), 4.52 (1H, dd, J = 1.4 Hz and 6.9 Hz, C-4H), 6.49 and 6.58 (2H, 2d, 1H each, J = 2.0 Hz each, C-6H and C-8H), 7.10–7.33 (5H, m, C-2′H, C-3′H, C-4′H, C-5′H and C-6′H) and 8.70 (1H, br s, OH); ^{13}C NMR (75.5 MHz, CD_3COCD_3): δ 20.21 (CH_3), 39.57 and 39.81 (C-3 and C-4), 103.62 (C-8), 115.56 (C-6), 116.69 (C-10), 129.10, 129.23 and 131.04 (C-2′, C-3′, C-4′, C-5′ and C-6′), 140.08 and 143.93 (C-7 and C-1′), 155.43 (C-9), 159.65 (C-5) and 168.94 (C-2); IR (Nujol): 3350 (OH), 1732 (CO), 1633, 1589, 1454, 1320, 1280, 1152, 1137, 967 and 842 cm^{-1}.

General method of synthesis of 4-aryl-3,4-dihydrocoumarins 11–18

To a mixture of $POCl_3$ (10 mmol) and BF_3–Et_2O (20 mmol) at 0 °C, substituted cinnamic acid (**2**–**4**, 5 mmol) was added and the reaction mixture stirred for 15 min at 0 °C. Phenol (**5**–**7**, 5 mmol) was added to the above reaction mixture in small portions and stirring continued at 25–28 °C for 4–12 h. The reaction mixture was poured on to ice-water, sodium acetate (1 g) was added and the mixture was warmed on a water bath for 2 min. It was cooled, extracted with ethyl acetate (2 × 150 mL), washed with water (150 mL), dried and solvent removed under reduced pressure to obtain the crude product, which was purified by column chromatography using acetone–chloroform as eluent to afford pure 4-aryldihydrocoumarins **11**–**18** in 54–75% yields. The structures of compounds **11**–**18** were unambiguously established on the basis of their spectral data. The spectral data of known compounds **11** and **13** and mps of compounds **11**, **12** and **13** were quite comparable with the data reported in the literature.[20–22] We have, for the first time, reported the spectral data of the known compound **12**.

(±)-6-Hydroxy-4-(4′-methoxyphenyl)-3,4-dihydrocoumarin (12). It was obtained as a brown solid (958 mg) in 71% yield; mp 172–173 °C (lit.[22] mp 176 °C). R_f: 0.24 (chloroform–acetone, 19:1); EIMS, *m/z* (% rel. int.): 270 ($[M]^+$, 100), 252 (9), 242 (6), 227 (85), 213 (26), 197 (95), 184 (10), 77 (10), 58 (24) and 43 (65); ^{1}H NMR (300 MHz, CD_3COCD_3): δ 2.94–3.10 (2H, m, C-3H), 3.78 (3H, s, OCH_3), 4.34 (1H, br t, J = 6.6 Hz, C-4H), 6.48 (1H, d, J = 2.8 Hz, C-5H), 6.78 (1H, dd, J = 2.8 Hz and 8.7 Hz, C-7H), 6.90–6.95 (3H, m, C-3′H, C-5′H and C-8H) and 7.15 (2H, d, J = 8.6 Hz, C-2′H and C-6′H); ^{13}C NMR (75.5 MHz, CD_3COCD_3): δ 37.10 and 40.08 (C-3 and C-4), 55.09 (OCH_3), 114.52 (C-3′ and C-5′), 114.79, 115.27 and 117.70 (C-5, C-7 and C-8), 127.42 (C-10), 127.92 (C-2′ and C-6′), 133.16 (C-1′), 145.22 (C-9), 154.22 (C-6), 159.29 (C-4′) and 167.81 (C-2); IR (Nujol): 3331 (OH), 1732 (CO), 1614, 1507, 1458, 1375, 1150, 1040 and 845 cm^{-1}.

(±)-7-Hydroxy-4-(3′,4′,5′-trimethoxyphenyl)-3,4-dihydrocoumarin (14). It was obtained as a yellow solid (891 mg) in 54% yield; mp 160–161 °C. R_f: 0.28 (chloroform–acetone, 19:1); HRMS, $C_{18}H_{18}O_6$ (M^+ 330.1081, calcd 330.1103); ESIMS, *m/z* (% rel. int.): 353.3 ($[M+Na]^+$, 82) and 331 ($[M+H]^+$, 48); ^{1}H NMR (300 MHz, CD_3COCD_3): δ 3.06 (2H, br d, J = 6.0 Hz, C-3H), 3.70 and 3.76 (9H, 2s, 3H and 6H each, 3 × OCH_3), 4.32 (1H, t, J = 6.0 Hz, C-4H), 6.54 (2H, s, C-2′H and C-6′H), 6.56 (1H, d, J = 2.4 Hz, C-8H), 6.61 (1H, dd, J = 2.4 and 8.3 Hz, C-6H), 6.89 (1H, d, J = 8.3 Hz, C-5H) and 8.75 (1H, br s, OH); ^{13}C NMR (75.5 MHz, CD_3COCD_3): δ 37.73 and 40.83 (C-3 and C-4), 56.39 and 60.48 (3 × OCH_3), 104.31 (C-8), 105.85 (C-2′ and C-6′), 112.46 (C-6), 117.83 (C-10), 129.98 (C-5), 138.28 (C-1′), 153.46, 154.59 and 158.62 (C-3′, C-4′, C-5′, C-7 and C-9) and 168.11 (C-2); IR (Nujol): 3389 (OH), 1757 (CO), 1624, 1596, 1510, 1453, 1353, 1272, 1229, 1156, 1120, 999 and 839 cm^{-1}.

(±)-6-Hydroxy-4-(3′,4′,5′-trimethoxyphenyl)-3,4-dihydrocoumarin (15). It was obtained as an oil (1.05 g) in 64%

yield. R_f: 0.26 (chloroform–acetone, 19:1); HRMS, $C_{18}H_{18}O_6$ (M^+ 330.1081, calcd 330.1103); ESIMS, *m/z* (% rel. int.): 353.3 ([M + Na],$^+$ 100); ^{1}H NMR (300 MHz, CD_3COCD_3): δ 2.94 (1H, dd, *J* = 8.1 Hz and 15.8 Hz, C-3H$_\alpha$), 3.05 (1H, dd, *J* = 6.8 Hz and 15.8 Hz, C-3H$_\beta$), 3.76 and 3.80 (9H, 2s, 6H and 3H each, 3 × OCH_3), 4.17 (1H, br t, *J* = 7.2 Hz, C-4H), 6.35 (2H, s, C-2′H and C-6′H), 6.48 (1H, d, *J* = 2.4 Hz, C-5H), 6.76 (1H, dd, *J* = 2.4 Hz and 8.6 Hz, C-7H), 6.94 (1H, d, *J* = 8.6 Hz, C-8H) and 7.28 (1H, br s, OH); ^{13}C NMR (75.5 MHz, CD_3COCD_3): δ 38.23 and 41.31 (C-3 and C-4), 56.91 and 61.05 (3 × OCH_3), 104.77 (C-5), 106.26 (C-2′ and C-6′), 112.88 (C-8), 118.16 (C-10), 130.34 (C-7), 138.59 (C-1′), 153.83, 154.95 and 158.96 (C-3′, C-4′, C-5′, C-6 and C-9) and 168.51 (C-2); IR (Nujol): 3386 (OH), 1758 (CO), 1626, 1596, 1456, 1377, 1272, 1231, 1158, 1119, 971, 839 and 715 cm^{-1}.

(±)-5-Hydroxy-7-methyl-4-(3′,4′,5′-trimethoxyphenyl)-3,4-dihydrocoumarin (16). It was obtained as a white solid (1.13 g) in 66% yield; mp 171–172 °C. R_f: 0.25 (chloroform–acetone, 19:1); ESIMS, *m/z* (% rel. int): 367.3 ([M + Na]$^+$, 90); APCI, *m/z* (% rel. int.): 345 ([M + H]$^+$, 100); ^{1}H NMR (300 MHz, CD_3COCD_3): δ 2.23 (3H, s, CH_3), 3.03 (1H, dd, *J* = 1.8 Hz and 15.7 Hz, C-3H$_\alpha$), 3.25 (1H, dd, *J* = 6.9 Hz and 15.7 Hz, C-3H$_\beta$), 3.77 and 3.82 (9H, 2s, 3H and 6H each, 3 × OCH_3), 4.55 (1H, dd, *J* = 1.8 Hz and 6.9 Hz, C-4H), 6.51 (2H, s, C-2′H and C-6′H), 6.56 and 6.66 (2H, 2d, 1H each, *J* = 2.2 Hz each, C-6H and C-8H) and 8.61 (1H, br s, OH); ^{13}C NMR (75.5 MHz, CD_3COCD_3): δ 19.25 (CH_3), 33.25 and 36.68 (C-3 and C-4), 56.00 and 56.68 (3 × OCH_3), 102.58 (C-6), 113.00, 114.65 and 115.89 (C-2′, C-6′ and C-8), 115.28 (C-10), 131.57 (C-1′), 139.14 (C-7), 152.29, 154.99 and 158.74 (C-3′, C-4′, C-5′, C-5 and C-9) and 168.25 (C-2); IR (Nujol): 3420 (OH), 1773 (CO), 1609, 1493, 1464, 1377, 1282, 1241, 1212, 1143, 1108, 1056, 803 and 732 cm^{-1}.

(±)-7-Hydroxy-4-(2′,5′-dimethoxyphenyl)-3,4-dihydrocoumarin (17). It was obtained as a yellow solid (1.12 g) in 75% yield; mp 170–171 °C. R_f: 0.26 (chloroform–acetone, 19:1); HRMS, $C_{17}H_{16}O_5$ (M^+ 300.0988, calcd 300.0998); ESIMS, *m/z* (% rel. int.): 323.3 ([M + Na]$^+$, 91); ^{1}H NMR (300 MHz, CD_3COCD_3): δ 2.99 (1H, dd, *J* = 10.6 Hz and 15.4 Hz, C-3H$_\alpha$), 3.06 (1H, dd, *J* = 6.4 Hz and 15.4 Hz, C-3H$_\beta$), 3.64 and 3.79 (6H, 2s, 3H each, 2 × OCH_3), 4.58 (1H, t, *J* = 5.7 Hz, C-4H), 6.43 (1H, d, *J* = 2.9 Hz, C-8H), 6.58–6.63 (2H, m, C-4′H and C-6′H), 6.79 (1H, dd, *J* = 2.9 and 8.8 Hz, C-6H), 6.90–6.95 (2H, m, C-3′H and 5H) and 8.74 (1H, br s, OH); ^{13}C NMR (75.5 MHz, CD_3COCD_3): δ 35.71 and 35.97 (C-3 and C-4), 55.70 and 56.10 (2 × OCH_3), 104.26 (C-8), 112.54, 112.70 and 112.72 (C-3′, C-4′ and C-6′), 115.76 (C-6), 116.58 (C-10), 130.08 (C-5), 133.43 (C-1′), 151.95, 154.18, 154.64, 158.60 (C-2′, C-5′, C-7 and C-9) and 167.98 (C-2); IR (Nujol): 3351 (OH), 1743 (CO), 1627, 1598, 1502, 1461, 1365, 1281, 1216, 1152, 1103, 1048,1024, 986, 852, 814 and 761 cm^{-1}.

(±)-6-Hydroxy-4-(2′,5′-dimethoxyphenyl)-3,4-dihydrocoumarin (18). It was obtained as a yellow solid (1.12 g) in 75% yield; mp 165–166 °C. R_f: 0.27 (chloroform–acetone, 19:1); HRMS, $C_{17}H_{16}O_5$ (M^+ 300.1010, calcd 300.0998); ESIMS, *m/z* (% rel. int.): 323.1 ([M + Na]$^+$, 100); ^{1}H NMR (300 MHz, CD_3COCD_3): δ 2.93–3.01 (2H, m, C-3H), 3.66 and 3.80 (6H, 2s, 3H each, 2 × OCH_3), 4.61 (1H, t, *J* = 6.1 Hz, C-4H), 6.48 and 6.53 (2H, 2d, 1H each, *J* = 2.7 Hz and 2.9 Hz, respectively C-5H and C-6′H), 6.76–6.84 (2H, m, C-7H and C-8H), 6.92–6.98 (2H, m, C-3′H and C-4′H) and 8.32 (1H, br s, OH); ^{13}C NMR (75.5 MHz, CD_3COCD_3): δ 36.01 and 36.71 (C-3 and C-4), 56.16 and 56.62 (2 × OCH_3), 113.28, 113.34, 115.71, 116.21, 116.30 and 118.58 (C-5, C-7, C-8, C-3′, C-4′ and C-6′), 127.59 and 131.61 (C-1′ and C-10), 146.64, 152.46, 155.14 and 155.22 (C-2′, C-5′, C-5 and C-9) and 168.62 (C-2); IR (Nujol): 3322 (OH), 1715 (CO), 1598, 1491, 1417, 1355, 1281, 1218, 1194, 1165, 1145, 1105, 1053, 1023, 989, 916, 873, 804 and 686 cm^{-1}.

General procedure of acetylation of (±)-4-aryl-5/6/7-hydroxy-3,4-dihydrocoumarins 8–18: preparation of acetylated dihydrocoumarins 19–29

To a solution of (±)-4-aryl-5/6/7-hydroxy-3,4-dihydrocoumarin (**8–18**, 3 mmol) in acetic anhydride (1.1 equiv) and pyridine (2 equiv) was added a catalytic amount of 4-*N*,*N*-dimethylaminopyridine and the reaction mixture stirred at 25–28 °C for 12 h. The reaction was worked up by addition of ice-cold water and the aqueous reaction mixture extracted with ethyl acetate (3 × 20 mL). The combined ethyl acetate layers were washed with aqueous sodium bicarbonate solution (50 mL) and concentrated to afford the corresponding acetoxy compounds **19–29** in 81–96% yields. The structures of acetylated dihydrocoumarins **19–29** were unambiguously established on the basis of their spectral data; the mps of known compounds **19–21** were quite comparable with the data reported in the literature.[19] We have, for the first time, reported the spectral data for compounds **19–21**.

(±)-7-Acetoxy-4-phenyl-3,4-dihydrocoumarin (19). It was obtained as a white solid (702 mg) in 83% yield; mp 85–86 °C (lit.[19] mp 89 °C). R_f: 0.39 (chloroform–acetone, 19:1); ESIMS, *m/z* (% rel. int.): 305.2 ([M + Na]$^+$, 78) and 283 ([M + H]$^+$, 71); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.30 (3H, s, $COCH_3$), 2.99 (1H, dd, *J* = 8.2 Hz and 15.9 Hz, C-3H$_\alpha$), 3.10 (1H, dd, *J* = 6.1 Hz and 15.9 Hz, C-3H$_\beta$), 4.32 (1H, t, *J* = 7.2 Hz, C-4H), 6.82 (1H, dd, *J* = 2.2 Hz and 8.3 Hz, C-6H), 6.90 (1H, d, *J* = 2.2 Hz, C-8H), 6.95 (1H, d, *J* = 8.3 Hz, C-5H), 7.15–7.17 (2H, m, C-3′H and C-5′H) and 7.26–7.38 (3H, m, C-2′H, C-4′H and C-6′H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 21.07 ($CO\mathit{C}H_3$), 36.84 and 40.40 (C-3 and C-4), 110.00 (C-8), 117.89 (C-6), 125.91 (C-10), 127.62, 127.84, 128.89 and 129.24 (C-2′, C-3′, C-4′, C-5′, C-6′ and C-5), 139.97 (C-1′), 150.63 and 152.07 (C-7 and C-9), 167.07 and 169.14 (2 × CO); IR (Nujol): 1775 and 1756 (2 × CO), 1615, 1462, 1377, 1253, 1205, 1129, 979 and 911 cm^{-1}.

(±)-6-Acetoxy-4-phenyl-3,4-dihydrocoumarin (20). It was obtained as a white solid (726 mg) in 86% yield; mp 90–91 °C (lit.[19] mp 93 °C). R_f: 0.38 (chloroform–acetone, 19:1); ESIMS, *m/z* (% rel. int.): 305.2 ([M + Na]$^+$,

100) and 283 ([M + H]$^+$, 58); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.21 (3H, s, $COCH_3$), 2.97 (1H, dd, J = 7.5 Hz and 15.9 Hz, C-3H$_\alpha$), 3.07 (1H, dd, J = 6.1 Hz and 15.9 Hz, C-3H$_\beta$), 4.31 (1H, t, J = 8.0 Hz, C-4H), 6.68 (1H, d, J = 2.5 Hz, C-5H), 7.01 (1H, dd, J = 2.5 Hz and 8.6 Hz, C-7H), 7.11–7.20 (3H, m, C-3′H, C-5′H and C-8H) and 7.26–7.38 (3H, m, C-2′H, C-4′H and C-6′H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.94 ($CO\mathit{C}H_3$), 36.60 and 40.66 (C-3 and C-4), 117.94, 121.32 and 121.99 (C-5, C-7 and C-8), 127.00 (C-10), 127.61, 127.85 and 128.95 (C-2′, C-3′, C-4′, C-5′ and C-6′), 139.22 (C-1′), 146.94 and 149.18 (C-6 and C-9), and 167.19 and 169.35 (2 × CO); IR (Nujol): 1766 and 1747 (2 × CO), 1462, 1376, 1219, 1179, 1127, 972 and 725 cm^{-1}.

(±)-5-Acetoxy-7-methyl-4-phenyl-3,4-dihydrocoumarin (21). It was obtained as a brown solid (720 mg) in 81% yield; mp 163–164 °C (lit.[19] mp 160 °C). R_f: 0.37 (chloroform–acetone, 19:1); ESIMS, m/z (% rel. int.): 319.1 ([M + Na]$^+$, 100) and 297 ([M + H]$^+$, 81); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.17 and 2.30 (6H, 2s, 3H each, CH_3 and $COCH_3$), 2.99 (1H, dd, J = 2.7 Hz and 15.7 Hz, C-3H$_\alpha$), 3.10 (1H, dd, J = 6.1 Hz and 15.7 Hz, C-3H$_\beta$), 4.37 (1H, dd, J = 2.6 Hz and 6.1 Hz, C-4H), 6.76 and 6.80 (2H, 2d, 1H each, J = 2.0 Hz each, C-6H and C-8H), 7.03–7.05 (2H, m, C-3′H and C-5′H) and 7.20–7.31 (3H, m, C-2′H, C-4′H and C-6′H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.68 and 21.82 (CH_3 and $CO\mathit{C}H_3$), 38.23 and 38.82 (C-3 and C-4), 109.54 (C-8), 120.34 (C-6), 121.59 (C-10), 127.68, 128.37 and 129.96 (C-2′, C-3′, C-4′, C-5′ and C-6′), 138.86 and 140.53 (C-1′ and C-7), 151.07 and 153.30 (C-5 and C-9), and 167.43 and 169.96 (2 × CO); IR (Nujol): 1768 and 1770 (2 × CO), 1612, 1513, 1488, 1429, 1370, 1179, 1137, 1031, 918 and 830 cm^{-1}.

(±)-7-Acetoxy-4-(4′-methoxyphenyl)-3,4-dihydrocoumarin (22). It was obtained as a light yellow solid (786 mg) in 84% yield; mp 90–91 °C. R_f: 0.37 (chloroform–acetone, 19:1); HRMS, $C_{18}H_{16}O_5$ (M$^+$ 312.1006, calcd 312.0998); ESIMS, m/z (% rel. int.): 335.1 ([M + Na]$^+$, 12); APCI, m/z (% rel. int.): 313.2 ([M + H]$^+$, 100); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.30 (3H, s, $COCH_3$), 2.99 (1H, dd, J = 6.7 Hz and 15.7 Hz, C-3H$_\alpha$), 3.08 (1H, dd, J = 5.9 Hz and 15.7 Hz, C-3H$_\beta$), 3.77 (3H, s, OCH_3), 4.28 (1H, br t, J = 6.8 Hz, C-4H), 6.57 (1H, d, J = 2.4 Hz, C-8H), 6.61 (1H, dd, J = 2.4 and 8.2 Hz, C-7H), 6.86–6.92 (3H, m, C-3′H, C-5′H and C-5H) and 7.10–7.17 (2H, m, C-2′H and C-6′H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 21.00 ($CO\mathit{C}H_3$), 36.93 and 39.22 (C-3 and C-4), 55.25 (OCH_3), 110.78, 114.49 and 117.78 (C-3′, C-5′, C-6 and C-8), 123.82 (C-10), 128.61 and 128.77 (C-2′, C-6′ and C-5), 131.76 (C-1′), 150.43 and 151.88 (C-4′ and C-9), 163.07 (C-7), and 167.22 and 169.14 (2 × CO); IR (KBr): 1775 and 1777 (2 × CO), 1614, 1511, 1427, 1368, 1254, 1205, 1126, 1011 and 906 cm^{-1}.

(±)-6-Acetoxy-4-(4′-methoxyphenyl)-3,4-dihydrocoumarin (23). It was obtained as an oil (796 mg) in 85% yield. R_f: 0.38 (chloroform–acetone, 19:1); HRMS, $C_{18}H_{16}O_5$ (M$^+$ 312.0984, calcd 312.0998); ESIMS, m/z (% rel. int.): 335.3 ([M + Na]$^+$, 100) and 313 ([M + H]$^+$, 22); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.22 (3H, s, $COCH_3$), 2.99 (1H, dd, J = 8.7 Hz and 15.8 Hz, C-3H$_\alpha$), 3.05 (1H, dd, J = 5.9 Hz and 15.8 Hz, C-3H$_\beta$), 3.79 (3H, s, OCH_3), 4.28 (1H, dd, J = 5.9 Hz and 8.6 Hz, C-4H), 6.67 (1H, d, J = 2.4 Hz, C-5H), 6.86 (2H, d, J = 8.7 Hz, C-3′H and C-5′H), 7.00 (1H, dd, J = 2.4 Hz and 8.7 Hz, C-7H) and 7.07–7.13 (3H, m, C-2′H, C-6′H and C-8H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 21.73 ($CO\mathit{C}H_3$), 37.57 and 40.49 (C-3 and C-4), 56.07 (OCH_3), 115.39 (C-3′ and C-5′), 118.68, 122.05 and 122.66 (C-5, C-7 and C-8), 128.28 (C-10), 129.31 (C-2′ and C-6′), 132.26 (C-1′), 147.72 and 149.91 (C-4′ and C-9), 159.92 (C-6), and 168.14 and 170.16 (2 × CO); IR (Nujol): 1773 and 1775 (2 × CO), 1597, 1489, 1417, 1208, 1125, 1050, 1014, 909 and 701 cm^{-1}.

(±)-5-Acetoxy-7-methyl-4-(4′-methoxyphenyl)-3,4-dihydrocoumarin (24). It was obtained as a white solid (861 mg) in 88% yield; mp 163–164 °C. R_f = 0.37 (chloroform–acetone, 19:1); HRMS, $C_{19}H_{18}O_5$ (M$^+$ 326.1145, calcd 326.1209); ESIMS, m/z (% rel. int.): 349.3 ([M + Na]$^+$, 82) and 327 ([M + H]$^+$, 100); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.17 and 2.30 (6H, 2s, 3H each, CH_3 and $COCH_3$), 3.01 (2H, m, C-3H), 3.74 (3H, s, OCH_3), 4.33 (1H, br d, J = 3.3 Hz, C-4H), 6.76–6.81 (4H, m, C-2′H, C-3′H, C-5′H and C-6′H) and 6.94–6.97 (2H, m, C-6H and C-8H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 18.82 and 21.02 (CH_3 and $CO\mathit{C}H_3$), 36.07 and 36.83 (C-3 and C-4), 55.19 (OCH_3), 101.99 (C-8), 114.48 (C-3′ and C-5′), 119.07 (C-6), 121.15 (C-10), 127.95 (C-2′ and C-6′), 131.64 (C-1′), 138.00 (C-7), 150.13 and 152.34 (C-5 and C-9), 158.86 (C-4′), and 166.88 and 169.22 (2 × CO); IR (KBr): 1776 and 1778 (2 × CO), 1613, 1592, 1512, 1420, 1374, 1342, 1301, 1247, 1214, 1123, 829 and 758 cm^{-1}.

(±)-7-Acetoxy-4-(3′,4′,5′-trimethoxyphenyl)-3,4-dihydrocoumarin (25). It was obtained as a white solid (1.03 g) in 92% yield; mp 143–144 °C. R_f: 0.38 (chloroform–acetone, 19:1); HRMS, $C_{20}H_{20}O_7$ (M$^+$ 372.1184, calcd 372.1209); ESIMS, m/z (% rel. int.): 395.3 ([M + Na]$^+$, 100) and 373 ([M + H]$^+$, 6); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.31 (3H, s, $COCH_3$), 2.98 (1H, dd, J = 8.5 Hz and 15.8 Hz, C-3H$_\alpha$), 3.10 (1H, dd, J = 6.1 Hz and 15.8 Hz, C-3H$_\beta$), 3.81 and 3.84 (9H, 2s, 6H and 3H each, 3 × OCH_3), 4.25 (1H, br t, J = 8.5 Hz, C-4H), 6.36 (2H, s, C-2′H and C-6′H), 6.84 (1H, dd, J = 2.1 Hz and 8.3 Hz, C-6H), 6.90 (1H, d, J = 2.1 Hz, C-8H) and 7.00 (1H, d, J = 8.3 Hz, C-5H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 25.37 ($CO\mathit{C}H_3$), 41.17 and 45.03 (C-3 and C-4), 60.51 and 65.17 (3 × OCH_3), 108.98 (C-2′ and C-6′), 115.22 (C-8), 122.25 (C-6), 127.69 (C-10), 133.17 (C-5), 139.84 (C-1′), 154.96, 156.25 and 158.08 (C-3′, C-4′, C-5′, C-7 and C-9), and 171.37 and 173.46 (2 × CO); IR (Nujol): 1766 and 1738 (2 × CO), 1617, 1591, 1463, 1377, 1216, 1145, 1126, 1112, 984 and 849 cm^{-1}.

(±)-6-Acetoxy-4-(3′,4′,5′-trimethoxyphenyl)-3,4-dihydrocoumarin (26). It was obtained as an oil (993 mg) in 89% yield. R_f: 0.35 (chloroform–acetone, 19:1); HRMS, $C_{20}H_{20}O_7$ (M$^+$ 372.1163, calcd 372.1209); ESIMS, m/z (% rel. int.): 395.3 ([M + Na],$^+$ 100) and 373 ([M + H]$^+$, 22); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.26 (3H, s,

$COCH_3$), 3.00 (1H, dd, $J=6.5$ Hz and 15.8 Hz, C-3H$_\alpha$), 3.06 (1H, dd, $J=5.9$ Hz and 15.8 Hz, C-3H$_\beta$), 3.81 and 3.84 (9H, 2s, 6H and 3H each, 3 × OCH_3), 4.26 (1H, br t, $J=2.8$ Hz, C-4H), 6.38 (2H, s, C-2′H and C-6′H), 6.72 (1H, d, $J=2.5$ Hz, C-5H), 7.02 (1H, dd, $J=2.5$ Hz and 8.7 Hz, C-7H) and 7.13 (1H, d, $J=8.7$ Hz, C-8H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 21.03 ($COCH_3$), 36.60 and 40.88 (C-3 and C-4), 56.06 and 60.77 (3 × OCH_3), 104.50 (C-2′ and C-6′), 117.90, 121.40 and 121.96 (C-5, C-7 and C-8), 126.86 (C-10), 135.08 (C-1′), 146.84, 149.01 and 153.63 (C-3′, C-4′, C-5′, C-6 and C-9), and 167.26 and 169.46 (2 × CO); IR (KBr): 1762 and 1764 (2 × CO),1592, 1508, 1461, 1426, 1370, 1181, 1124, 1008 and 920 cm^{-1}.

(±)-5-Acetoxy-7-methyl-4-(3′,4′,5′-trimethoxyphenyl)-3,4-dihydrocoumarin (27). It was obtained as a white solid (1.07 g) in 92% yield; mp 159–160 °C. R_f: 0.38 (chloroform–acetone, 19:1); ESIMS, *m*/*z* (% rel. int.): 409.3 ($[M+Na]^+$, 100) and 387 ($[M+H]^+$, 10); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.20 and 2.30 (6H, 2s, 3H each, CH_3 and $COCH_3$), 3.04 (2H, m, C-3H), 3.74 and 3.79 (9H, 2s, 6H and 3H each, 3 × OCH_3), 4.32 (1H, br d, $J=3.3$ Hz, C-4H), 6.24 (2H, s, C-2′H and C-6′H), 6.79–6.80 (2H, br s, C-6H and C-8H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.91 and 21.28 (CH_3 and $COCH_3$), 37.53 and 38.29 (C-3 and C-4), 56.11 and 60.73 (3 × OCH_3), 104.10 (C-2′ and C-6′), 108.20 (C-6), 119.67 (C-8), 120.77 (C-10), 135.68 and 139.94 (C-1′ and C-7), 150.39, 152.44 and 153.59 (C-3′, C-4′, C-5′, C-5 and C-9), and 166.81 and 168.61 (2 × CO); IR (Nujol): 1746 and 1748 (2 × CO), 1595, 1500, 1450, 1345, 1206, 913, 883 and 824 cm^{-1}.

(±)-7-Acetoxy-4-(2′,5′-dimethoxyphenyl)-3,4-dihydrocoumarin (28). It was obtained as a white solid (985 mg) in 96% yield; mp 135–136 °C. R_f: 0.40 (chloroform–acetone, 19:1); HRMS, $C_{19}H_{18}O_6$ (M^+ 342.1111, calcd 342.1103); ESIMS, *m*/*z* (% rel. int.): 365.3 ($[M+Na]^+$, 100) and 343 ($[M+H]^+$, 38); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.30 (3H, s, $COCH_3$), 2.99 (1H, dd, $J=6.7$ Hz and 16.0 Hz, C-3H$_\alpha$), 3.07 (1H, dd, $J=6.1$ Hz and 16.0 Hz, C-3H$_\beta$), 3.69 and 3.76 (6H, 2s, 2 × OCH_3), 4.61 (1H, t, $J=6.3$ Hz, C-4H), 6.46 (1H, d, $J=2.7$ Hz, C-8H), 6.74–6.87 (3H, m, C-3′H, C-4′H and C-6H), 6.88 (1H, d, $J=2.0$ Hz, C-6′H) and 7.01 (1H, d, $J=8.3$ Hz, C-5H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 21.46 ($COCH_3$), 35.31 and 35.77 (C-3 and C-4), 55.99 and 56.06 (2 × OCH_3), 111.08, 112.07, 112.94, 115.56 and 118.13 (C-3′, C-4′, C-6′, C-6 and C-8), 122.70 (C-10), 129.36 (C-5), 130.03 (C-1′), 150.81, 151.48, 152.69 and 154.11 (C-2′, C-5′, C-7 and C-9), and 167.78 and 169.53 (2 × CO); IR (Nujol): 1763 (broad band, 2 × CO), 1618, 1596, 1494, 1462, 1376, 1280, 1239, 1205, 1139, 1102, 1052, 1024, 928, 917, 800 and 722 cm^{-1}.

(±)-6-Acetoxy-4-(2′,5′-dimethoxyphenyl)-3,4-dihydrocoumarin (29). It was obtained as a white solid (913 mg) in 89% yield; mp 105–106 °C. R_f: 0.35 (chloroform–acetone, 19:1); HRMS, $C_{19}H_{18}O_6$ (M^+ 342.1096, calcd 342.1103); ESIMS, *m*/*z* (% rel. int.): 365 ($[M+Na]^+$, 100) and 343 ($[M+H]^+$, 13); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.22 (3H, s, $COCH_3$), 2.97 (1H, dd, $J=6.7$ Hz and 16.1 Hz, C-3H$_\alpha$), 3.07 (1H, dd, $J=6.5$ Hz and 16.1 Hz, C-3H$_\beta$), 3.70 and 3.76 (6H, 2s, 3H each, 2 × OCH_3), 4.61 (1H, t, $J=6.5$ Hz, C-4H), 6.48 (1H, d, $J=2.8$ Hz, C-6′H), 6.75–6.85 (3H, m, C-3′H, C-4′H and C-5H), 7.00 (1H, dd, $J=2.6$ Hz and 8.7 Hz, C-7H) and 7.12 (1H, d, $J=8.7$ Hz, C-8H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 21.76 ($COCH_3$), 35.51 and 36.61 (C-3 and C-4), 56.41 and 56.43 (2 × OCH_3), 112.58, 113.54, 115.92, 118.58, 122.10 and 122.47 (C-3′, C-4′, C-6′, C-5, C-7 and C-8), 126.70 (C-10), 130.03 (C-1′), 147.66, 150.23, 151.88 and 154.51 (C-2′, C-5′, C-6 and C-9), and 168.35 and 170.13 (2 × CO); IR (KBr): 1779 (CO), 1747 (CO), 1507, 1465, 1370, 1225, 1193, 1146, 1043, 923 and 893 cm^{-1}.

General procedure of enzymatic deacetylation of (±)-5/6/7-acetoxy-4-aryl-3,4-dihydrocoumarins 19–29

To a solution of (±)-4-aryl-3,4-dihydrocoumarin (**19**–**29**, 1.5 mmol) in anhydrous dioxane (30 mL), *n*-butanol (2–3 equiv) was added, followed by the addition of *Candida antarctica* lipase (200 mg). The suspension was stirred at 40–42 °C in a shaker incubator and progress of the reaction was monitored periodically by HPLC and/or TLC. After about 45–50% conversion of the starting material into product, the reaction was quenched by filtering off the enzyme and solvent evaporated to dryness in vacuo to afford the crude product, which was purified by flash column chromatography on silica gel using gradient solvent system of chloroform–acetone to afford racemic 4-aryl-5/6/7-hydroxy-3,4-dihydrocoumarins **8**, **9**, **15** and **16** in 39–43% yields and optically enriched (+)-4-aryl-5/6/7-hydroxy-3,4-dihydrocoumarins **10**–**14** and **18** in 68–85% yields, and racemic 5/6/7-acetoxy-4-aryl-3,4-dihydrocoumarins **19**, **20**, **26** and **27** in 38–45% yields and optically enriched (−)-5/6/7-acetoxy-4-aryl-3,4-dihydrocoumarins **21**–**25** and **29** in 67–94% yields. (±)-7-Acetoxy-4-(2′,5′-dimethoxyphenyl)-3,4-dihydrocoumarin (**28**) is not a substrate for *Candida antarctica* lipase as the enzyme failed to catalyse the deacetylation of acetoxy function of compound **28**. The enzymatically deacetylated (±)- and (+)-hydroxy-3,4-dihydrocoumarins **8**–**16** and **18** and recovered, unreacted (±)- and (−)-acetoxy-3,4-dihydrocoumarins **19**–**27** and **29** were unambiguously identified on the basis of their spectral data, which were found to be identical with the corresponding data of chemically synthesised racemic hydroxydihydrocoumarins **8**–**16** and **18** and acetoxydihydrocoumarins **19**–**27** and **29** as reported above.

General procedure for chemical deacetylation of optically enriched unreacted, recovered acetates (−)-21–25 and 29

The (−)-acetoxydihydrocoumarin (**21**–**25** and **29**, 60 mg) was dissolved in MeOH (5 mL) containing 2–3 drops of hydrochloric acid. The reaction mixture was stirred for 5–6 h at 25–28 °C and quenched by the addition of ice-cold water (5 mL). The reaction mixture was extracted with ethyl acetate (2 × 10 mL), combined ethyl acetate layer was washed with brine, dried over anhydrous Na_2SO_4 and evaporated to dryness at reduced pressure

to afford the (−)-hydroxydihydrocoumarins **10–14** and **18** in quantitative yields, which were identified on the basis of their spectroscopic data, which were found to be identical with the spectroscopic data of corresponding (+)-hydroxydihydrocoumarins obtained by enzymatic deacetylation of the corresponding acetates or with the data of racemic hydroxydihydrocoumarins synthesised chemically.

General procedure for chemical acetylation of enzymatically deacetylated (+)-5/6/7 hydroxy-3,4-dihydrocoumarins 10–14 and 18

The enzymatically deacetylated dihydrocoumarins (+)-**10–14** and **18** were acetylated following the same procedure as applied for acetylation of racemic **10–14** and **18**, discussed earlier. The acetylated dihydrocoumarins (+)-**21–25** and **29** were obtained in 82–90% yields and identified on the basis of their spectral data, which were found to be identical with the spectral data of corresponding racemic acetoxydihydrocoumarins **21–25** and **29**.

Acknowledgements

The authors thank the Council of Scientific and Industrial Research (CSIR, New Delhi) and Danish International Development Agency (DANIDA, Denmark) for generous financial support.

References and Notes

1. Iinuma, M.; Tanaka, T.; Asai, F. *Phytochemistry* **1994**, *36*, 941.
2. Iinuma, M.; Tanaka, T.; Mizuno, M.; Katsuzaki, T.; Ogawa, H. *Chem. Pharm. Bull.* **1989**, *37*, 1813.
3. Hsu, F.-L.; Nonaka, G.-I.; Nishioka, I. *Chem. Pharm. Bull.* **1985**, *33*, 3142.
4. Nonaka, G.-I.; Kawahara, O.; Nishioka, I. *Chem. Pharm. Bull.* **1982**, *30*, 4277.
5. Polya, G. M.; Foo, L. Y. *Phytochemistry* **1994**, *35*, 1399.
6. Takechi, M.; Tanaka, Y.; Takehara, M.; Nonaka, G.-I.; Nishioka, I. *Phytochemistry* **1985**, *24*, 2245.
7. Waldmann, H.; Sebastian, D. *Chem. Rev.* **1994**, *94*, 911.
8. Roberts, S. M. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1.
9. Parmar, V. S.; Kumar, A.; Bisht, K. S.; Mukherjee, S.; Prasad, A. K.; Sharma, S. K.; Wengel, J.; Olsen, C. E. *Tetrahedron* **1997**, *53*, 2163.
10. Parmar, V. S.; Kumar, A.; Poonam; Pati, H. N.; Saxena, R. K.; Davidson, W. S.; Gupta, R. *Biochim. Biophys. Acta* **1998**, *1387*, 325.
11. Parmar, V. S.; Prasad, A. K.; Pati, H. N.; Kumar, R.; Azim, A.; Roy, S.; Errington, W. *Bioorg. Chem.* **1999**, *27*, 119.
12. Poonam; Prasad, A. K.; Azim, A.; Kumar, R.; Olsen, C. E.; Errington, W.; Jain, S. C.; Parmar, V. S. *Tetrahedron* **2001**, *57*, 7395.
13. Prasad, A. K.; Sorensen, M. D.; Parmar, V. S.; Wengel, J. *Tetrahedron Lett.* **1995**, *36*, 6163.
14. Sharma, S. K.; Roy, S.; Kumar, R.; Parmar, V. S. *Tetrahedron Lett.* **1999**, *40*, 9145.
15. Raunak; Prasad, A. K.; Shakil, N. A.; Himanshu; Parmar, V. S. *Pure Appl. Chem.* **2001**, *73*, 167.
16. Roy, S.; Kumar, R.; Wengel, J.; Olsen, C. E.; Parmar, V. S.; Prasad, A. K. *Tetrahedron 59*, in press.
17. Bisht, K. S.; Parmar, V. S.; Crout, D. H. G. *Tetrahedron: Asymmetry* **1993**, *4*, 957.
18. Parmar, V. S.; Sinha, R.; Bisht, K. S.; Gupta, S.; Prasad, A. K.; Taneja, P. *Tetrahedron* **1993**, *49*, 4107.
19. Simpson, J. D.; Stephen, H. *J. Chem. Soc.* **1956**, 1382.
20. Jain, N.; Krishnamurty, H. G. *Indian J. Chem.* **1999**, *38B*, 1237.
21. Speranza, G.; Meo, A. D.; Zanzola, S.; Fontana, G.; Manitto, P. *Synthesis* **1997**, 931.
22. Lespagnol, A.; Schmidt, J.; Brunand, P. *Bull. Soc. Chem. Fr.* **1951**, 82.
23. Whitesell, J. K.; Reynolds, D. *J. Org. Chem.* **1983**, *48*, 3548.