Bioorganic Chemistry **27,** 119–134 (1999)
Article ID bioo.1998.1117, available online at http://www.idealibrary.com on IDEAL®

Enzyme-Catalyzed Chemoselective Transesterification Reactions on Hydroxymethylated Phenolic Compounds

Virinder S. Parmar,*,[1] Ashok K. Prasad,* Hari N. Pati,* Rajesh Kumar,* Abul Azim,* Sucharita Roy,* and William Errington†

**Department of Chemistry, University of Delhi, Delhi-110 007, India; and*
†Department of Chemistry, University of Warwick, Coventry CV4 7AL, England, United Kingdom

Received October 30, 1998

The chemoselective capabilities of porcine pancreatic lipase (PPL) in tetrahydrofuran and *Candida rugosa* lipase (CRL) in diisopropyl ether have been investigated for selective acetylation and deacetylation of hydroxymethylated phenols and hydroxyaryl alkyl ketones and their peracetylated derivatives. Both PPL and CRL exhibited exclusive selectivity for the acetylation of alcoholic hydroxyl group over the phenolic hydroxyl group(s) of the hydroxymethylated phenols **1–5** and aryl alkyl ketones **6–9**, and for the deacetylation of ester group involving the phenolic hydroxyl group over the ester group involving alcoholic hydroxyl of the peracetates **19–24**. The preliminary results indicate that this strategy of chemoselective acetylation can also be used in the enantiomeric resolution of racemic ketones **6–9**. Single crystal X-ray diffraction studies have confirmed the structures of compounds **4, 15**, and **17**.

INTRODUCTION

In recent years, chemoenzymatic synthetic strategies have become standard techniques for the preparation of a variety of chiral and nonchiral precursors and target molecules (*1*,*2*). Among the different enzymatic methodologies, lipase-catalyzed acylations and deacylations represent an important class of enzymatic transformations in organic synthesis (*3*), wider applications of lipases are attributed to their low-cost and tolerance toward a variety of organic molecules (*4*). In recent years, we have successfully used lipases from porcine pancreas (PPL) and *Candida rugosa* (CRL) for the regioselective deacetylation of peracetates of different classes of polyphenolics (*5*) and for the regioselective and enantioselective esterifications of polyols (*6*). It has been observed that the change of enzyme may lead to complementary result. For example, use of lipases from *Aspergillus* species for the deacetylation of 2,4-diacetoxyphenyl alkyl ketones led to the formation of 2-hydroxy-4-acetoxy ketones (*7*), which is complementary to the result of deacetylation catalyzed by PPL or CRL (*5*).

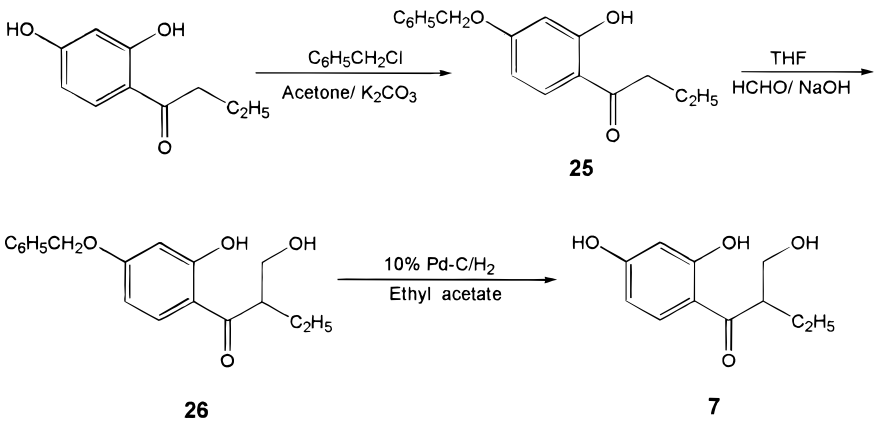
[1] To whom correspondence and reprint requests should be addressed. Fax: 91-11-725 7206. E-mail: vparmar.duchem@axcess.net.in.

0045-2068/99 $30.00

So far, enzymes have seldom been employed for the chemoselective discrimination between the phenolic and alcoholic hydroxyl groups present in the same molecule, there is only one report in literature where such selection is achieved on 2- and 4-hydroxymethylphenols *via* enzymatic catalysis with *Aspergillus niger* lipase (*8*). We have employed PPL in tetrahydrofuran (THF) and CRL in diisopropyl ether (DIPE), relatively cheaper and more easily accessible enzymes than *Aspergillus niger* lipase for the selective acetylation of hydroxymethylated phenols and aryl alkyl ketones, novel precursors for the synthesis of phenyl alkyl carbinols (*9*), and 3-alkyl/aryl chromanones and their bioactive analogs (*10*). Further, the enzyme substrate ratio used in the present investigation is just half of that used during selective acetylation with *A. niger* lipase (*8*). The enzymatic methodology developed for chemoselective acetylation of hydroxymethylated phenols and aryl alkyl ketones has successfully been used for the enantioselective resolution of racemic hydroxymethylated aryl alkyl ketones **6–9**, which in turn can be used for the synthesis of optically pure isoflavanones and 3-alkylchromanones (*10*), which have not been synthesized in optically enriched/pure form thus far.

RESULTS AND DISCUSSION

The starting phenol, 2-hydroxymethylphenol (**1**) was procured from Aldrich Chemical Co. (U.S.A.) and other hydroxymethylated phenols; i.e., 4-hydroxymethylphenol (**2**), 4-hydroxymethyl-2-methoxyphenol (**3**), 5-hydroxymethyl-2-methoxyphenol (**4**), and 2-hydroxymethyl-6-methoxyphenol (**5**) were prepared by reduction of the corresponding aldehydes, i.e., 4-hydroxybenzaldehyde, 4-hydroxy-3-methoxybenzaldehyde, 3-hydroxy-4-methoxybenzaldehyde, and 2-hydroxy-3-methoxybenzaldehyde, respectively, with $NaBH_4$ in 80–90% yields. The diacetates of hydroxymethylated phenols **1–5**; i.e., 2-acetoxymethylphenyl acetate (**19**), 4-acetoxymethylphenyl acetate (**20**), 4-acetoxymethyl-2-methoxyphenyl acetate (**21**), 5-acetoxymethyl-2-methoxyphenyl acetate (**22**), and 2-acetoxymethyl-6-methoxyphenyl acetate (**23**) were prepared by



SCHEME 1. Preparation of 2-ethyl-3-hydroxy-1-(2′,4′-dihydroxyphenyl)propanone (**7**).

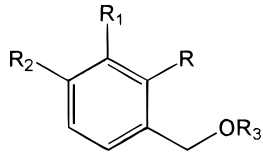
1. R=OH; R_1=R_2 =R_3= H

2. R=R_1=R_3= H; R_2=OH

3. R=R_3=H; R_1=OCH_3; R_2=OH

4. R=R_3= H; R_1= OH; R_2=OCH_3

5. R=OH; R_1=OCH_3; R_2=R_3=H



10. R=OH; R_1=R_2=H; R_3=$COCH_3$

11. R=R_1= H; R_2=OH; R_3=$COCH_3$

12. R= H; R_1=OCH_3; R_2=OH; R_3=$COCH_3$

13. R=H; R_1=OH; R_2=OCH_3; R_3=$COCH_3$

14. R=OH; R_1=OCH_3; R_2=H; R_3=$COCH_3$

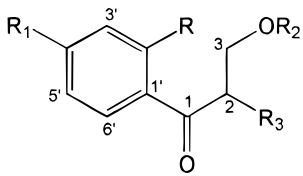
19. R=$OCOCH_3$; R_1=R_2=H; R_3=$COCH_3$

20. R=R_1=H; R_2=$OCOCH_3$; R_3=$COCH_3$

21. R=H; R_1=OCH_3; R_2=$OCOCH_3$; R_3=$COCH_3$

22. R=H; R_1=$OCOCH_3$; R_2=OCH_3; R_3=$COCH_3$

23. R=$OCOCH_3$; R_1=OCH_3; R_2=H; R_3=$COCH_3$



6. R=R_2= H; R_1= OH; R_3=CH_3

7. R=R_1=OH; R_2= H; R_3=CH_2CH_3

8. R=OH; R_1=OCH_3; R_2= H; R_3= C_6H_5

9. R=OH; R_1=R_2 = H; R_3=$CH_2C_6H_4(p)OCH_3$

15. R=H; R_1=OH; R_2=$COCH_3$; R_3=CH_3

16. R=R_1=OH; R_2=$COCH_3$; R_3=CH_2CH_3

17. R=OH; R_1=OCH_3; R_2=$COCH_3$; R_3=C_6H_5

18. R=OH; R_1=H; R_2=$COCH_3$; R_3= $CH_2C_6H_4(p)OCH_3$

24. R=H; R_1=$OCOCH_3$; R_2=$COCH_3$; R_3= CH_3

SCHEME 2

the acetic anhydride-pyridine method in the presence of catalytic amounts of dimethylaminopyridine (DMAP) in quantitative yields. The hitherto unknown diacetates **19** and **23** were identified on the basis of their spectral analysis, whereas the known hydroxymethylated phenols **2–5** and the known diacetates **20–22** were characterized by analysis of their spectral data and by comparison of their spectral and/or physical

data with those reported in literature (*11–13*). The structure of 5-hydroxymethyl-2-methoxyphenol (**4**) was also confirmed by its single crystal X-ray diffraction studies (Table 5). Though compound **22** has been synthesized earlier (*13*), its spectral data has not been reported and we herein report its complete data (*cf.* Experimental). The compound 3-hydroxy-1-(4′-hydroxyphenyl)-2-methylpropanone (**6**) was prepared by stirring a suspension of 4-hydroxypropiophenone in 37% formaldehyde and 0.5 N sodium hydroxide solution in a molar ratio of 1.0:1.1:1.1 at 28–30°C for 15 h (*14*). Further, 2-ethyl-3-hydroxy-1-(2′,4′-dihydroxyphenyl)propanone (**7**) was prepared by hydroxymethylation of 4-benzyloxy-2-hydroxybutyrophenone (**25**) (*15*) as above, followed by debenzylation of the hydroxymethylated ketone, 1-(4′-benzyloxy-2′-hydroxyphenyl)-2-ethyl-3-hydroxypropanone (**26**) by catalytic hydrogenation (Scheme 1). The hydroxypropanones, 3-hydroxy-1-(2′-hydroxy-4′-methoxyphenyl)-2-phenylpropanone (**8**) and 3-hydroxy-1-(2′-hydroxyphenyl)-2-(4″-methoxybenzyl)propanone (**9**) were prepared by reacting 2-hydroxy-4-methoxydesoxybenzoin (*16*) and 2′-hydroxy-4-methoxydihydrochalcone (*17*) with ethoxymethyl chloride in the presence of dry potassium carbonate in dry acetone in 65 and 70% yields, respectively. The diacetate, 3-acetoxy-1-(4′-acetoxyphenyl)-2-methylpropanone (**24**) was prepared as in the case of hydroxymethylated phenols in 98% yield. Attempts to prepare di/triacylated derivatives of **7, 8**, and **9** resulted in the enolization of ketonic carbonyl group and subsequent formation of the corresponding enolic peracetates as per our earlier observation in similar compounds (*18*). The hitherto unknown compounds **7, 9, 24**, and **26** were characterized on the basis of their spectral analysis and the known compounds **6** and **8** were characterized by their spectral analysis and comparison with the data reported in the literature (*14,16*).

To examine the chemoselective capabilities of PPL and CRL for the selective acetylation of two different types of hydroxyl groups, hydroxymethylated phenols **1–5** were incubated with vinyl acetate and PPL or CRL in THF and DIPE, respectively. Both the enzymes exhibited exclusive selectivity for the acetylation of aliphatic hydroxyl function over the phenolic hydroxyl, though the rate of acetylation catalyzed by CRL in DIPE was too slow with respect to PPL in THF. Thus, PPL-catalyzed acetylation of the hydroxymethylated phenols **1–5** afforded the monoacetates 2-acetoxymethylphenol (**10**), 4-acetoxymethylphenol (**11**), 4-acetoxymethyl-2-methoxyphenol (**12**), 5-acetoxymethyl-2-methoxyphenol (**13**), and 2-acetoxymethyl-6-methoxyphenol (**14**) exclusively in 95, 80, 90, 95, and 85% yields, respectively (Table 1). The results indicate that the turn over in PPL-catalyzed acetylation of hydroxymethylated phenols is almost quantitative. In addition to the selective acetylation studies on hydroxymethylated phenols, chemoselective capabilities of PPL and CRL were also investigated for the selective acetylation of hydroxymethylated aryl alkyl ketones. As in the case of acetylation of hydroxymethylated phenols, PPL and CRL exhibited 100% selectivity for the acetylation of aliphatic hydroxyl group with respect to phenolic hydroxyl group in hydroxymethylated aryl alkyl ketones. Thus, incubation of compounds **6** and **7** with PPL in THF afforded the monoacetoxy products, 3-acetoxy-1-(4′-hydroxyphenyl)-2-methylpropanone (**15**) and 3-acetoxy-2-ethyl-1-(2′,4′-dihydroxyphenyl)propanone (**16**) in 65 and 55% yields in 12 and 38 h (Table 1), respectively. It has been observed that PPL in THF catalyzes the acetylation of aliphatic hydroxyl group of **6** and **7** faster than CRL in DIPE, the rate of selective

TABLE 1

Selective Acetylation of Hydroxymethylated Phenols **1–5** and Aryl alkyl Ketones **6–9** Mediated by PPL in THF (A) and/or CRL in DIPE (B) at 42–45°C[a]

Substrate	Reaction condition	Time (h)	Product	Percentage yield
2-Hydroxymethylphenol (**1**)	A	120	2-Acetoxymethylphenol (**10**)(*8*)	95
4-Hydroxymethylphenol (**2**)(*11a*)	A	48	4-Acetoxymethylphenol (**11**)(*12*)	80
4-Hydroxymethyl-2-methoxyphenol (**3**)(*11b*)	A	24	4-Acetoxymethyl-2-methoxy-phenol (**12**)(*12*)	90
5-Hydroxymethyl-2-methoxyphenol (**4**)(*11b*)	A	96	5-Acetoxymethyl-2-methoxy-phenol (**13**)(*19*)	95
2-Hydroxymethyl-6-methoxyphenol (**5**)(*11c*)	A	120	2-Acetoxymethyl-6-methoxy-phenol (**14**)	85
3-Hydroxy-1-(4′-hydroxyphenyl)-2-methylpropanone (**6**)(*14*)	A/B	12/30	3-Acetoxy-1-(4′-hydroxyphenyl)-2-methylpropanone (**15**)	65
2-Ethyl-3-hydroxy-1-(2′,4′-dihydroxyphenyl)propanone (**7**)	A/B	38/72	3-Acetoxy-2-ethyl-1-(2′,4′-dihydroxyphenyl)propanone (**16**)	55
3-Hydroxy-1-(2′-hydroxy-4′-methoxyphenyl)-2-phenylpropanone (**8**)(*16*)	B	8	3-Acetoxy-1-(2′-hydroxy-4′-methoxyphenyl)-2-phenylpropanone (**17**)(*16*)	70
3-Hydroxy-1-(2′-hydroxyphenyl)-2-(4″-methoxybenzyl)propanone (**9**)	B	8	3-Acetoxy-1-(2′-hydroxyphenyl)-2-(4″-methoxybenzyl)propanone (**18**)	75

[a] All these reactions, when performed under identical conditions but without adding the lipase, did not yield any product.

acetylation of aliphatic hydroxyl function by the former is about 2.5 and 2 times faster, respectively, than the latter (Table 1). The hydroxymethylated aryl alkyl ketones **8** and **9** were poor substrates for PPL and no appreciable acetylation was observed even upon incubation for several days. However, CRL in DIPE catalyses the acetylation of compounds **8** and **9** to afford the monoacetates involving the aliphatic hydroxyl group, i.e., 3-acetoxy-1-(2′-hydroxy-4′-methoxyphenyl)-2-phenylpropanone (**17**) and 3-acetoxy-1-(2′-hydroxyphenyl)-2-(4″-methoxybenzyl)propanone (**18**) in 70 and 75% yields, respectively (Table 1). This result indicates that the substituent at C-2 position in compounds **6–9** plays an important role during their binding with PPL and CRL.

The hitherto unknown monoacetates **14, 15, 16**, and **18** were identified on the basis of their spectral analysis, while the known monoacetates **10–13** and **17** were identified on the basis of spectral analysis and their comparison with those reported in the literature (*8,12,16,19*). The formation of monoacetates involving aliphatic hydroxyl group during enzyme-catalyzed acetylation reactions has been well established as the carbinol protons in all the monoacetates (except in **16**) are deshielded (by $\Delta\delta$ 0.35–0.68 ppm) with respect to the corresponding protons in the respective hydroxylated compounds (Table 2). The presence of chelated and free phenolic hydroxyl groups at δ 12.87 and 9.99, respectively, and the absence of any resonance due to aliphatic hydroxyl group in the ^{1}H NMR spectrum of **16** in deuterated chloroform establishes the acetylation of aliphatic hydroxyl group in this compound as well. The compounds **16, 17**, and **18** gave dark brown Fe^{3+} coloration when their TLC spots were sprayed

TABLE 2

Comparison of Chemical Shift Values of Methylene Protons of the Starting Carbinols and in Their Corresponding Acetates

Carbinol	δ CH_2OH	Acetate	δ CH_2OCOCH_3	Deshielding effect (ppm)
1	4.78	**10**	5.20	0.42
2	4.54	**11**	5.08	0.54
3	4.51	**12**	5.00	0.49
4	4.49	**13**	5.05	0.56
5	4.70	**14**	5.18	0.48
6	3.59 and 3.66	**15**	4.10 and 4.34	0.51 and 0.68
7	4.33	**16**	4.23 and 4.32	
8	3.74 and 4.20	**17**	4.35 and 4.75	0.61 and 0.55
9	3.88	**18**	4.23 and 4.38	0.35 and 0.50

with 3% alcoholic $FeCl_3$ solution, thus indicating that the *ortho* (phenolic) hydroxy function to the nuclear carbonyl group is not acetylated in any of them. This is further proved by the presence of chelated hydroxyl group at δ 12.87, 12.68, and 12.31 in the ^{1}H NMR spectra of **16, 17**, and **18**, respectively. The X-ray crystallographic studies on the monoacetates **15** and **17** (Figs. 1 and 2) finally confirmed the involvement of the aliphatic hydroxyl group of **6** and **8** in ester formation during enzyme-catalyzed acetylation reaction.

In addition to the capabilities of PPL and CRL to catalyze the chemoselective

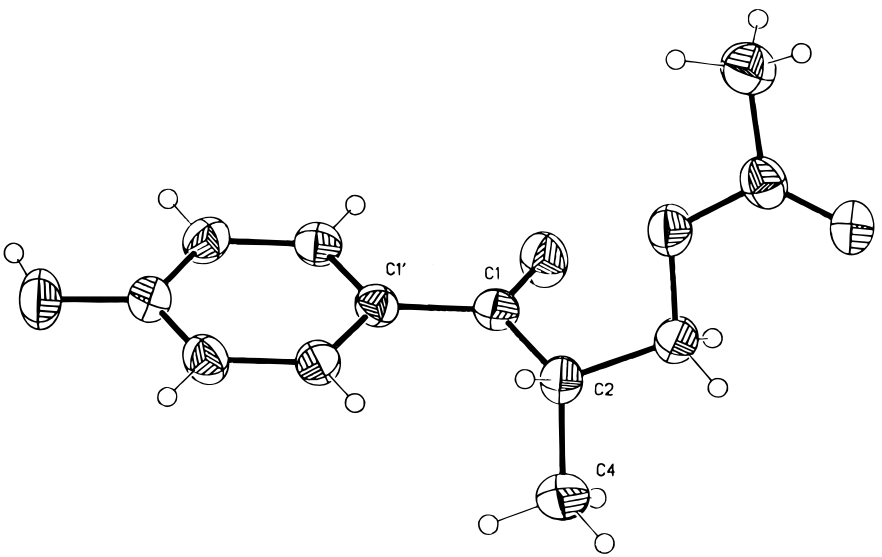


FIG. 1. Molecular structure of compound **15**. Displacement ellipsoids are shown at the 50% probability level.

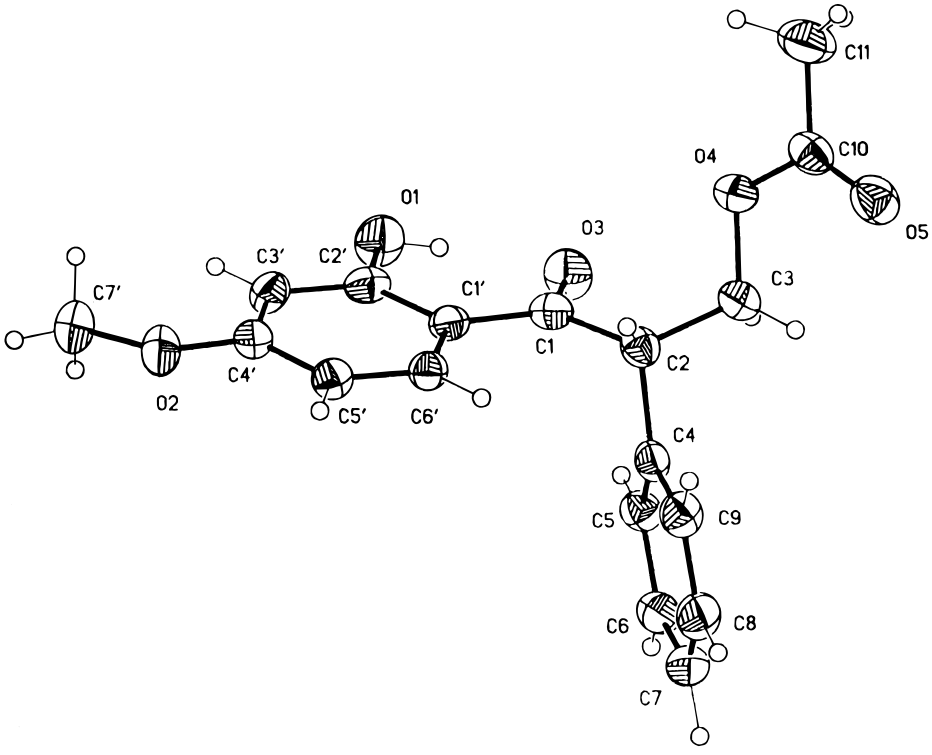


FIG. 2. Molecular structure of compound **17**. Displacement ellipsoids are shown at the 50% probability level.

acetylation of aliphatic hydroxyl groups of hydroxymethylated phenols and aryl alkyl ketones, we tried to extend these studies to enantiomeric resolution of racemic ketones **6–9**. The racemic hydroxymethylated aryl alkyl ketones **6** and **7**, and **8** and **9** were incubated with PPL in THF and CRL in DIPE, respectively, and the reactions were stopped by filtering off the enzyme after about 45% conversion of the dihydroxy ketone to monoacetate. The monoacetates and the unreacted dihydroxy ketones were separated on silica gel column with a gradient solvent system of petrol:ethyl acetate and their optical rotations measured. The monoacetates and the unreacted dihydroxy compounds were found to be optically active in each case (Table 3). The monoacetates were deacetylated enzymatically and the rotations of the dihydroxy compounds thus obtained were measured, the rotations of these compounds were found to be of the same order and had opposite sign to the rotation of the corresponding unreacted dihydroxy compound (Table 3). These observation revealed that the chemoselective acetylation is also accompanied by enantiomeric resolution of the racemic ketones **6–9**. The evaluation of enantiomeric excess (*ee*) of the monoacetates in each case is in progress by ^{1}H NMR spectral studies using diamagnetic anthryl chiral shift reagent and on chiral HPLC columns.

Together with enzyme-catalyzed acetylation, deacetylation reactions of peracetates

TABLE 3

Optical Rotations of Products Obtained after Enzymatic Acetylation of Compounds **6–9**[a] and Deacetylation of the Monoacetoxy Products[b]

Substrate (*racemic*)	Enzyme/ solvent	$[\alpha]^{25}_{D}$ Values of the compounds obtained by incubation with enzymes: Monoacetoxy-propanones	Unreacted Dihydroxy-propanones	Dihydroxypropanones obtained by the CRL-catalyzed deacetylation of the monoacetoxypropanones in DIPE (containing *n*-butanol)[c]
6	PPL/THF	+23.7	−26.2	+29.9
7	PPL/THF	+3.7	+6.6	−10.8
8	CRL/DIPE	−46.0	+16.0	−12.7
9	CRL/DIPE	+30.8	−25.6	+29.5

[a] All the enzyme-assisted reactions carried out for enantiomeric resolution studies were stopped by filtering off the enzyme after about 45% conversion (as seen on HPLC) of the starting racemic substrate to the product.

[b] All these acetylation and deacetylation reactions, when performed under identical conditions but without adding the lipase, did not yield any product.

[c] The specific rotations of the unreacted dihydroxypropanones are less than those of the corresponding dihydroxypropanones obtained by enzymatic deacetylation of the monoacetates, except in the case of compound **8**. This may be due to partial racemization of the unreacted dihydroxypropanones taking place during their separation from the monoacetoxypropanones on silica gel column, the deacetylated products of monoacetates did not require purification as they were found to be sufficiently pure on TLC/HPLC examination. Such partial racemization on silica gel due to its acidic nature is known in literature (*24*).

of hydroxymethylated phenols **19–23** and hydroxymethylated aryl alkyl ketone **24** catalysed by PPL in THF and CRL in DIPE were also studied. It has been found that the incubation of compounds **19–23** with CRL in DIPE catalyzes the deacetylation of ester group involving the phenolic hydroxyl function and afforded the monoacetates **10, 11, 12, 13**, and **14** (Table 4), respectively. None of the diacetates was a substrate for PPL in THF. In general, the deacetylation reactions on peracetates **19–23** catalyzed by CRL in DIPE were slow than the acetylation reactions on corresponding dihydroxy compounds **1–5** (Tables 1 and 4). In contrast to the selective deacetylation reactions on **19–23** which were catalysed by CRL in DIPE, the deacetylation reaction on 3-acetoxy-1-(4′-acetoxyphenyl)-2-methylpropanone (**24**) was catalyzed by PPL in THF affording the monoacetate **15** in 60% yield due to the selective deacetylation of ester function involving phenolic hydroxyl group over alcoholic hydroxyl (Table 4). The incubation of compound **24** with CRL in DIPE led to the formation of an inseparable mixture.

The enzymatic method developed for the chemoselective acetylation of hydroxymethylated phenols and aryl alkyl ketones may find applications in the efficient synthesis of phenyl alkyl carbinols, known for their chloretic properties, and for the synthesis of bioactive analogs of naturally occurring 3-alkylchromanones and isoflavanones, respectively. It has been demonstrated that this strategy of selective esterification also leads to enantiomeric resolution of racemic aryl alkyl ketones which in turn can be used for the synthesis of optically pure isoflavanones. Further, in the