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Highly Selective Biocatalytic Transesterification Reactions on Aryl 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoates

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Abstract Acid anhydrides have been used to carry out the regioselective acylation of primary hydroxyl group in benzyl 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate and 4-fluorobenzyl 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate and deacylation of their diesters in the presence of Lipozyme® TL IM in diisopropyl ether. Amongst different acid anhydrides used, butanoic anhydride was found to be the best acylating agent as compared to others. Both acylation and deacylation reactions were highly selective and efficient yielding exclusively the monoacylated products in 70–88 % yields.

Keywords Aryl 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoates · Lipase · Lipozyme® TL IM · Acylation · Deacylation · Regioselectivity

Dedicated with affection to our Friend and Collaborator, Professor Kalle Levon (NYU Polytechnic School of Engineering, Brooklyn, New York, USA) in commemoration of the completion of his 26 years in active research.

1 Introduction

Enzymes have been established as valuable catalysts in organic synthesis for performing chemo-, regio- and stereoselective chemical transformations during the last three decades, lipases in particular are known for their low cost and wide tolerance towards their substrates. Lipase-catalyzed regioselective esterifications offer an alternative to the poor selectivity in chemical syntheses, which suffers from many shortcomings like high reaction temperatures causing polymerization of the products and hence low yields, coloration of the final products and dehydration or cyclization side reactions leading to formation of many undesirable side products. Furthermore, generally regioselective chemical synthesis can only be achieved by protection/deprotection strategies, which are highly cumbersome and not suitable for industrial processes. These shortcomings can be overcome by the use of enzymatic catalysis because of the high degree of regioselectivity exhibited by enzymes and mild reaction conditions under which they work.

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In the past, in our laboratory we have used a battery of lipases, viz. porcine pancreatic Lipase (PPL), *Candida rugosa* Lipase (CRL), Amano PS, *Candida antarctica* Lipase (CAL), *Theremomyces lanuginous* (Lipozyme® TL IM) and from *Pseudomonas* sp. for carrying out selective manipulations of hydroxyl groups in different classes of compounds [1–8], viz. aryl alkyl ketones [1, 2], hydroxymethylated phenolic compounds [3], amides and esters [4], sugar derivatives [5], benzoxazines [6], dihydrocoumarins [7], chromanones [8], etc. In addition to carrying out selective manipulations of similar functional groups in the small molecules, we have used these lipases extensively for the development of novel amphiphilic co-polymers useful in drug encapsulation and drug delivery applications. More specifically, we have exploited *C. antarctica* lipase-B (CAL-B) to co-polymerize polyethylene glycol (PEG), with different aromatic diesters, aliphatic diesters, naturally occurring dibasic amino acid diesters and hydroxy diesters under solvent-free conditions, thus providing a *greener* route to the synthesis of these polymers [9–12].

The desymmetrization reactions on prochiraldiols, e.g. serinol analogues have been reported earlier [13–16]. Herein we are reporting a novel strategy to carry out regioselective acylation reactions on aryl 1,3-dihydroxy-2-methylpropanoates **1** and **2** using acid anhydrides as acylating agents. Of the six lipases screened in this study, we found Lipozyme® TL IM to be the best catalyst. We have also successfully demonstrated the efficacy of Lipozyme® TL IM in regioselective deacylation of the corresponding diacyl derivatives **5a–5e** and **6a–6e**. Diacyl derivatives were synthesized from **1** and **2** chemically by treating them with the anhydrides of acetic, propanoic, butanoic, pentanoic and hexanoic acids as acylating agents and 4-*N*,*N*-dimethylaminopyridine (DMAP) as catalyst in anhydrous dichloromethane. Four different lipases were screened, viz. *C. antarctica* lipase-B (Novozyme-435), porcine pancreatic lipase (PPL), *C. rugosa* lipase (CRL) and Lipozyme® TL IM for selective deacylation of the diacyl compounds **5a–5e** and **6a–6e** in different organic solvents in the presence of *n*-butanol as the acyl group scavenger. Lipozyme® TL IM in diisopropyl ether was found to be the most efficient biocatalyst for the regioselective deacylation of compounds **5a–5e** and **6a–6e** (Scheme 2; Table 2). No clear selectivity or conversion was observed with Novozyme-435, PPL and CRL. The monoacylated products **3a–3e** and **4a–4e** formed, by either lipase catalyzed acylation of the diols **1** and **2**, or by the deacylation of their corresponding diacyl derivatives **5a–5e** and **6a–6e**,were fully characterized from their spectral data and all twenty compounds are novel and are being reported here for the first time. These compounds may find applications in the synthesis of copolymers for drug encapsulation and targeted drug delivery.

2 Experimental

2.1 General

The IR spectra were recorded either on a Perkin–Elmer model 2000 FT-IR or RXI FT-IR spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC-300 Avance spectrometer at 300 and at 75.5 MHz, respectively using TMS as internal standard. The chemical shift values are on δ scale and the coupling constant (J) are in Hz. The FAB-HRMS spectra of all the compounds were recorded on a micro TOF-Q instrument from Bruker Daltonics, Bremen high resolution mass spectrometer in positive mode using the matrix HEDS (bishydroxyethylsulfide) doped with sodium acetate. *Candida antarctica* lipase B and Lipozyme® TL IM immobilized on silica were gifted by Novozymes Inc., Copenhagen. The enzymes, *C. rugosa* lipase (CRL, Type VII) and porcine pancreatic lipase (PPL, Type II) were purchased from Sigma Chemical Co. (USA). All enzymes were used after storing under vacuum over P_2O_5 for 24 h. Diisopropyl ether (DIPE) was distilled over activated molecular sieves (4 Å) prior to use. Analytical TLCs were performed on pre-coated Merck silica gel $60F_{254}$ plates; the spots were detected under UV light. Silica gel (100–200 mesh) was used for column chromatography.

2.2 Synthesis of 4-Fluorobenzyl 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate (**2**)

2,2-Bis(hydroxymethyl)propanoic acid (5 g, 37.28 mmol) and KOH (3.13 g, 55.91 mmol) were taken in DMF (25 mL), 4-fluorobenzyl bromide (11.21 g, 59.64 mmol) was then added. After 15 h of stirring at 100 °C, DMF was evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (100 mL) and extracted with water (2 × 50 mL). The crude product was recrystallized from hexane/CH_2Cl_2 to give 4-fluorobenzyl 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate as viscous oil (6.5 g, 82 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.06 (3H, *s*, C-2 methyl), 2.97 (2 *brs* × OH), 3.72 (2H, *d*, $J = 11.4$ Hz, C-1′H_a and C-3H_a), 3.92 (2H, *d*, $J = 11.4$ Hz C-1′H_b and C-3H_b) 5.16 (2H, *s*, C-1″H), 7.02-7.08 (2H, *m*, C-3‴ and C-5‴), 7.31–7.36 (2H, *m*, C-2‴ and C-6‴); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 17.08 (C-2 *C*H_3), 49.25 (C-2) 65.97 (C-1″), 68.14 (C-1′ and C-3), 115.44 & 115.72 (C-3‴ & C-5‴), 129.84 & 129.95 (C-2‴ & C-6‴), 131.50 & 131.54 (C-1‴), 161.03 & 164.30 (C-4‴), 175.65 (C=O); IR (KBr): 3234 (OH), 1729 (C=O), 1513, 1224, 1047 and 831 cm^{-1}; FAB-HRMS: m/z 265.0848 ($[M+Na]^+$, $C_{12}H_{15}FO_4Na$ calcd 265.0847).

2.3 General Procedure of Lipozyme® TL IM Lipase Catalyzed Acylation of Benzyl 3-Hydroxy-2-(hydroxymethyl)-2-methylpropanoate (**1**) and 4-Fluorobenzyl 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate (**2**) with Acid Anhydrides

To a mixture of **1**or **2** (1 mmol) in dry diisopropyl ether (40 mL), appropriate acid anhydride (1.2 mmol) was added, followed by Lipozyme® TL IM lipase (350 mg). The reaction mixture was stirred at 37–42 °C in an incubator shaker and progress of the reaction was monitored periodically by TLC. On completion, the reaction was stopped by filtering off the enzyme and solvent evaporated to dryness under vacuum. The crude products **3a**–**3e** and **4a**–**4e** thus obtained were purified by column chromatography on silica gel using a mixture of petroleum ether-ethyl acetate for elution.

*2.3.1 Benzyl 2-(acetoxymethyl)-3-hydroxy-2-methylpropanoate (**3a**)*

It was obtained as a viscous oil (635 mg, 77 %).^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22 (3H, *s*, C-2 methyl), 1.98 (3H, *s*, OCOC*H*$_3$), 3.66 (1H, *d*, $J = 12.3$ Hz, C-3H$_a$), 3.72 (1H, *d*, $J = 12.3$ Hz, C-3H$_b$), 4.22 (1H, *d*, $J = 12$ Hz, C-1′H$_a$), 4.32 (1H, *d*, $J = 12$ Hz, C-1′H$_b$), 5.17 (2H, *s*, C-1″H), 7.34 (5H, *s*, aromatic protons); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 17.81 (C-2 *C*H$_3$), 21.01(OCO*CH*$_3$), 48.57 (C-2), 65.32, 66.25 & 67.02 (C-1″, C-1′ & C-3), 128.33 (C-3‴ & C-5‴), 128.67 (C-2‴ & C-6‴), 128.94 (C-4‴), 135.98 (C-1‴), 171.47 & 174.57 (2× C=O); IR (KBr): 3502 (OH), 2946, 1732 (C=O), 1456, 1243, 1042 and 698 cm^{-1}; FAB-HRMS: m/z 289.1054 ([M+Na]$^+$, $C_{14}H_{18}O_5Na$ calcd 289.1046).

*2.3.2 Benzyl 2-(propanoyloxymethyl)-3-hydroxy-2-methylpropanoate (**3b**)*

It was obtained as a viscous oil (720 mg, 83 %).^{1}H NMR (300 MHz, $CDCl_3$): δ 1.09 (3H, *t*, $J = 7.5$ Hz, OCOCH$_2$-C*H*$_3$), 1.23 (3H, *s*, C-2 methyl), 2.24–2.31 (2H, *q*, $J = 7.2$ Hz, OCOC*H*$_2$CH$_3$), 3.66 (1H, *d*, $J = 12.0$ Hz, C-3H$_a$), 3.72 (1H, *d*, $J = 12.0$ Hz, C-3H$_b$), 4.22 (1H, *d*, $J = 11.1$ Hz, C-1′H$_a$) 4.36 (1H, *d*, $J = 11.1$ Hz, C-1′H$_b$), 5.17 (2H, *s*, C-1″H), 7.34 (5H, *s*, aromatic protons); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 9.0 (OCOCH$_2$*C*H$_3$), 17.46 (C-2 *C*H$_3$), 20.20 (OCO*C*H$_2$CH$_3$), 48.27 (C-2), 64.97, 65.69 & 66.64 (C-1″, C-3 & C-1′), 127.95 (C-3‴ & C-5‴), 128.29 (C-2‴ & C-6‴), 128.56 (C-4‴), 135.59 (C-1‴), 174.22 & 174.50 (2× C=O); IR (KBr): 3504 (OH), 2981, 1735 (C=O), 1459, 1192, 1052 and 698 cm^{-1}; FAB-HRMS: m/z 303.1198 ([M+Na]$^+$, $C_{15}H_{20}O_5Na$ calcd 303.1203).

*2.3.3 Benzyl 2-(butanoyloxymethyl)-3-hydroxy-2-ethylpropanoate (**3c**)*

It was obtained as a viscous oil (812 mg, 88 %).^{1}H NMR (300 MHz, $CDCl_3$): δ 0.91 (3H, *t*, $J = 7.5$ Hz, OCO(CH$_2$)$_2$C*H*$_3$), 1.25 (3H, *s*, C-2 methyl), 1.55–1.65 (2H, *m*, OCOCH$_2$C*H*$_2$CH$_3$), 2.23 (2H, *t*, $J = 7.5$ Hz, OCOC*H*$_2$CH$_2$CH$_3$), 3.65 (1H, *d*, $J = 12$ Hz, C-3H$_a$), 3.72 (1H, *d*, $J = 12$ Hz, C-3H$_b$), 4.21 (1H, *d*, $J = 11.1$ Hz, C-1′H$_a$), 4.35 (1H, *d*, $J = 11.1$ Hz, C-1′H$_b$), 5.17 (2H, *s*, C-1″H), 7.34 (5H, *s*, aromatic protons); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 13.62 (OCO(CH$_2$)$_2$*C*H$_3$), 17.51 (C-2 *C*H$_3$), 18.35 (OCOCH$_2$*C*H$_2$CH$_3$), 35.99 (OCO*C*H$_2$CH$_2$-CH$_3$), 48.30 (C-2), 65.00, 65.66 & 66.68 (C-1″, C-3 & C-1′), 127.99 (C-3‴ & C-5‴), 128.33 (C-2‴ & C-6‴), 128.60 (C-4‴), 135.62 (C-1‴), 173.77 & 174.27 (2× C=O); IR (KBr): 3502 (OH), 2966, 1732 (C=O), 1457, 1178, 1049 and 698 cm^{-1}; FAB-HRMS: m/z 317.1353 ([M+Na]$^+$, $C_{16}H_{22}O_5Na$ calcd 317.1359).

*2.3.4 Benzyl 2-(pentanoyloxymethyl)-3-hydroxy-2-methylpropanoate (**3d**)*

It was obtained as a viscous oil (780 mg, 81 %).^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (3H, *t*, $J = 7.2$ Hz, OCO(CH$_2$)$_3$C*H*$_3$), 1.22 (3H, *s*, C-2 methyl), 1.24–1.29 (2H, *m*, OCO(CH$_2$)$_2$C*H*$_2$CH$_3$), 1.52-1.61 (2H, *m*, OCOCH$_2$-C*H*$_2$CH$_2$CH$_3$), 2.21 (2H, *t*, $J = 7.8$ Hz, OCOC*H*$_2$(CH$_2$)$_2$-CH$_3$), 3.65 (1H, *d*, $J = 11.7$ Hz, C-3H$_a$), 3.72 (1H, *d*, $J = 11.7$ Hz, C-3H$_b$), 4.22 (1H, *d*, $J = 11.1$ Hz, C-1′H$_a$), 4.33 (1H, *d*, $J = 11.1$ Hz, C-1′H$_b$), 5.17 (2H, *s*, C-1″H), 7.34 (5H, *s*, aromatic protons); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 13.64 (OCO(CH$_2$)$_3$*C*H$_3$), 17.49 (C-2 *C*H$_3$), 22.19 (OCO(CH$_2$)$_2$*C*H$_2$CH$_3$), 26.90 (OCOCH$_2$*C*H$_2$CH$_2$-CH$_3$), 33.82 (OCO*C*H$_2$(CH$_2$)$_2$CH$_3$), 48.31 (C-2), 65.00, 65.69 & 66.67 (C-1″, C-1′ & C-3), 127.97 (C-3‴ & C-5‴), 128.31 (C-2‴ & C-6‴), 128.59 (C-4‴), 135.64 (C-1‴), 173.93 & 174.25 (2× C=O); IR (KBr): 3504 (OH), 2960, 1737 (C=O), 1457, 1173, 1052 and 697 cm^{-1}; FAB-HRMS: m/z 331.1509 ([M+Na]$^+$, $C_{17}H_{24}O_5Na$ calcd 331.1516).

*2.3.5 Benzyl 2-(hexanoyloxymethyl)-3-hydroxy-2-methylpropanoate (**3e**)*

It was obtained as a viscous oil (755 mg, 76 %).^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (3H, t, $J = 6.9$ Hz, OCO (CH$_2$)$_4$C*H*$_3$), 1.23 (3H, *s*, C-2 methyl), 1.25–1.31 (4H, *m*, OCO(CH$_2$)$_2$*CH*$_2$*CH*$_2$CH$_3$), 1.52–1.61 (2H, *m*, OCOCH$_2$-C*H*$_2$(CH$_2$)$_2$CH$_3$), 2.24 (2H, *t*, $J = 7.8$ Hz, OCOC*H*$_2$(-CH$_2$)$_3$CH$_3$), 3.65 (1H, *d*, $J = 11.4$ Hz, C-3H$_a$), 3.72 (1H, *d*, $J = 11.4$ Hz, C-3H$_b$), 4.22 (1H, *d*, $J = 11.1$ Hz, C-1′H$_a$),

4.33 (1H, *d*, $J = 11.1$ Hz, C-1′H_b), 5.17 (2H, *s*, C-1″H), 7.34 (5H, *s*, aromatic protons); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 13.88 ($OCO(CH_2)_4CH_3$), 17.51 (C-2 CH_3), 22.26 ($OCO(CH_2)_3CH_2CH_3$), 24.53 ($OCO(CH_2)_2CH_2CH_2CH_3$), 31.23 ($OCOCH_2CH_2(CH_2)_2CH_3$), 34.06 ($OCOCH_2(CH_2)_2CH_3$), 48.31 (C-2), 64.99, 66.68 & 65.67 (C-1″, C-1′ & C-3), 127.97 (C-3‴ & C-5‴), 128.32 (C-2‴ & C-6‴), 128.60 (C-4‴), 135.62 (C-1‴), 173.98 & 174.27 (2× C=O); IR (KBr): 3505 (OH), 2957, 1736 (C=O), 1458, 1166, 1050 and 697 cm^{-1}; FAB-HRMS: m/z 345.1662 ($[M+Na]^+$, $C_{18}H_{26}O_5Na$ calcd 345.1672).

2.3.6 4-Fluorobenzyl 2-(acetoxymethyl)-3-hydroxy-2-methylpropanoate (**4a**)

It was obtained as a viscous oil (625 mg, 76 %).^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21 (3H, *s*, C-2 methyl), 1.99 (3H, *s*, $OCOCH_3$), 3.65 (1H, *d*, $J = 11.4$ Hz, C-3H_a), 3.71 (1H, *d*, $J = 11.4$ Hz, C-3H_b), 4.21 (1H, *d*, $J = 11.4$ Hz, C-1′H_a), 4.37 (1H, *d*, $J = 11.4$ Hz, C-1′H_b), 5.14 (2H, *s*, C-1″H), 7.01-7.08 (2H, *m*, C-2‴H and C-6‴H), 7.30–7.35 (2H, *m*, C-3‴H and C-5‴H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 17.44 (C-2 CH_3), 20.64 ($OCOCH_3$), 48.23 (C-2), 64.97, 65.81 & 65.97 (C-1″, C-1′ & C-3),115.40 & 115.6912 (C-2‴ & C-6‴), 130.01 & 130 (C-3‴ & C-5‴), 131.47 & 131.51 (C-1‴), 161.04 & 164.32 (C-4‴), 171.08 & 174.14 (2× C=O); IR (KBr) 3502 (OH), 2948, 1732 (C=O), 1531, 1226, 1042 and 829 cm^{-1}; FAB-HRMS: m/z 307.0942 ($[M+Na]^+$, $C_{14}H_{17}FO_5Na$ calcd 307.0952).

2.3.7 4-Fluorobenzyl 2-(propanoyloxymethyl)-3-hydroxy-2-methylpropanoate (**4b**)

It was obtained as a viscous oil (630 mg, 74 %).^{1}H NMR (300 MHz, $CDCl_3$): δ 1.09 (3H, *t*, $J = 7.8$ Hz, $OCOCH_2CH_3$), 1.21 (3H, *s*, C-2 methyl), 2.23–2.31 (2H, *q*, $J = 7.5$ Hz, $OCOCH_2CH_3$), 3.65 (1H, *d*, $J = 11.7$ Hz, C-3H_a), 3.71 (1H, *d*, $J = 11.7$ Hz, C-3H_b), 4.22 (1H, *d*, $J = 11.4$ Hz, C-1′H_a), 4.33 (1H, *d*, $J = 11.4$ Hz, C-1′H_b), 5.13 (2H, *s*, C-1″H), 7.01-7.07 (2H, *m*, C-2‴H and C-6‴H), 7.30-7.34 (2H, *m*, C-3‴H and C-5‴H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 9.01 ($OCOCH_2CH_3$), 17.45 (C-2 CH_3), 27.39 ($OCOCH_2CH_3$), 48.31 (C-2), 64.97, 65.64 & 65.95 (C-1″, C-1′ & C-3), 115.39 & 115.67 (C-3‴ & C-5‴), 130.0 & 130.11 (C-2‴ & C-6‴), 131.48 & 131.52 (C-1‴), 161.04 & 164.32 (C-4‴), 174.17 & 174.49 (2× C=O); IR (KBr): 3508 (OH), 2945, 1732 (C=O), 1513, 1225, 1051 and 828 cm^{-1}; FAB-HRMS: m/z 321.1098 ($[M+Na]^+$, $C_{15}H_{19}FO_5Na$ calcd 321.1109).

2.3.8 4-Fluorobenzyl 2-(butanoyloxymethyl)-3-hydroxy-2-methylpropanoate (**4c**)

It was obtained as a viscous oil (725 mg, 80 %).^{1}H NMR (300 MHz, $CDCl_3$): δ 0.91 (3H, *t*, $J = 7.5$ Hz, $OCO(CH_2)_2CH_3$), 1.21 (3H, *s*, C-2 methyl), 1.52–1.65 (2H, *m*, $OCOCH_2CH_2CH_3$), 2.23 (2H, *t*, $J = 7.5$ Hz, $OCOCH_2CH_2CH_3$), 3.64 (1H, *d*, $J = 11.4$ Hz, C-3H_a), 3.70 (1H, *d*, $J = 11.4$ Hz, C-3H_b), 4.21 (1H, *d*, $J = 11.1$ Hz, C-1′H_a), 4.33 (1H, *d*, $J = 11.1$ Hz, C-1′H_b), 5.13 (2H, *s*, C-1″H), 7.07-7.01 (2H, *m*, C-2‴H and C-6‴H), 7.30–7.35 (2H, *m,* C-3‴H and C-5‴H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 13.59 ($OCO(CH_2)_2CH_3$), 17.47 (C-2 CH_3), 18.34 ($OCOCH_2CH_2CH_3$), 35.98 ($OCOCH_2CH_2CH_3$), 48.30 (C-2), 64.97, 65.59 & 65.96 (C-1″, C-1′ & C-3), 115.39 & 115.68 (C-3‴ & C-5‴), 130.03 & 130.14 (C-2‴ & C-6‴), 131.47 & 131.51 (C-1‴), 161.04 & 164.32 (C-4‴), 173.73 & 174.18 (2× C=O); IR (KBr) 3505 (OH), 2967, 1732 (C=O), 1513, 1225, 1049 and 828 cm^{-1}; FAB-HRMS: m/z 335.1257 ($[M+Na]^+$, $C_{16}H_{21}FO_5Na$ calcd 335.1265).

2.3.9 4-Fluorobenzyl 2-(pentanoyloxymethyl)-3-hydroxy-2-methylpropanoate (**4d**)

It was obtained as a viscous oil (660 mg, 70 %).^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (3H, *t*, $J = 7.2$ Hz, $OCO(CH_2)_3CH_3$), 1.21 (3H, *s*, C-2 methyl), 1.25–1.37 (2H, *m*, $OCO(CH_2)_2CH_2CH_3$), 1.49–1.59 (2H, *m*, $OCOCH_2CH_2CH_2CH_3$), 2.24 (2H, *t*, $J = 7.5$ Hz, $OCOCH_2(CH_2)_2CH_3$), 3.64 (1H, *d*, $J = 11.4$ Hz, C-3H_a), 3.70 (1H, *d*, $J = 11.4$ Hz, C-3H_b), 4.21 (1H, *d*, $J = 11.4$ Hz, C-1′H_a), 4.32 (1H, *d*, $J = 11.4$ Hz, C-1′H_b), 5.13 (2H, *s*, C-1″H), 7.01-7.07 (2H, m, C-2‴H & C-6‴H), 7.30–7.34 (2H, m, C-3‴H & C-5‴H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 13.62 ($OCO(CH_2)_3CH_3$), 17.46 (C-2 CH_3), 22.18 ($OCO(CH_2)_2CH_2CH_3$), 26.90 ($OCOCH_2CH_2CH_2CH_3$), 33.81 ($OCOCH_2(CH_2)_2CH_3$), 48.30 (C-2), 64.96, 65.59 & 65.95 (C-1″, C-1′ & C-3), 115.38 & 115.67 (C-3‴ & C-5‴), 130.00 & 130.10 (C-2‴ & C-6‴), 131.48 & 131.52 (C-1‴), 161.04 & 164.31 (C-4‴), 173.90 & 174.17 (2× C=O); IR (KBr): 3502 (OH), 2961, 1732 (C=O), 1514, 1225, 1052 and 827 cm^{-1}; FAB-HRMS: m/z 349.1418 ($[M+Na]^+$, $C_{17}H_{23}FO_5Na$ calcd 349.1422).

2.3.10 4-Fluorobenzyl 2-(hexanoyloxymethyl)-3-hydroxy-2-methylpropanoate (**4e**)

It was obtained as a viscous oil (730 mg, 73 %).^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (3H, *t*, $J = 6.9$ Hz, $OCO(CH_2)_4CH_3$), 1.21 (3H, *s*, C-2 methyl), 1.24–1.34 (4H, *m*, $OCO(CH_2)_2CH_2CH_2CH_3$), 1.51–1.66 (2H, *m*, $OCOCH_2$

$CH_2(CH_2)_2CH_3$), 2.24 (2H, *t*, $J = 7.5$ Hz, $OCOCH_2$(-$CH_2)_3CH_3$), 3.64 (1H, d, $J = 11.4$ Hz, C-3H_a), 3.70 (1H, *d*, $J = 11.4$ Hz, C-3H_b), 4.21 (1H, *d*, $J = 11.1$ Hz, C-1′H_a), 4.32 (1H, *d*, $J = 11.1$ Hz, C-1′H_b), 5.13 (2H, *s*, C-1″H), 7.01–7.07 (2H, *m*, C-2‴H & C-6‴H), 7.30–7.34 (2H, *m*, C-3‴H & C-5‴H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 13.84 ($OCO(CH_2)_4CH_3$), 17.46 (C-2 CH_3), 22.24 ($OCO(CH_2)_3$-CH_2CH_3), 24.52 ($OCO(CH_2)_2CH_2CH_2CH_3$), 31.21 ($OCOCH_2CH_2(CH_2)_2CH_3$), 34.06 ($OCOCH_2(CH_2)_2CH_3$), 48.30 (C-2), 64.97, 65.59 & 65.95 (C-1″, C-3 & C-1′), 115.39 & 115.68 (C-3‴ & C-5‴), 129.99 & 130.10 (C-2‴ & C-6‴), 131.47 & 131.51 (C-1‴), 161.04 & 164.32 (C-4‴), 173.93 & 174.18 (2× C=O); IR (KBr): 3501 (OH), 2958, 1738 (C=O), 1514, 1226, 1050 and 827 cm^{-1}; FAB-HRMS: m/z 363.1569 ($[M+Na]^+$, $C_{18}H_{25}FO_5Na$ calcd 363.1578).

2.4 General Procedure of Chemical Diacylation of Benzyl 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate (**1**) and 4-Fluorobenzyl 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate (**2**) with Acid Anhydrides

To a mixture of **1** or **2** (1 mmol) in dry dichloromethane (40 mL), appropriate acid anhydride (2 mmol) and catalytic amount of 4-*N*,*N*-dimethylaminopyridine (DMAP) were added and the reaction mixture was stirred at 25–28 °C for 12 h. The reaction was worked up by the 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 (2 × 50 mL), followed by water (2 × 50 mL) and concentrated to afford the corresponding diacyloxy compounds **5a**–**5e** and **6a**–**6e** in 81–96 % yields; the structures of all these novel compounds were unambiguously established on the basis of their spectral data.

2.4.1 *Benzyl 3-acetoxy-2-(acetoxymethyl)-2-methylpropanoate (**5a**)*

It was obtained as a viscous oil (870 mg, 91 %).^{1}H NMR (300 MHz, $CDCl_3$): δ 1.17 (3H, *s*, C-2 methyl), 1.88 (6H, *s*, 2× $OCOCH_3$), 4.10–4.15 (4H, *d* = 11.0 Hz, C-1′H and C-3H), 5.08 (2H, *s*, C-1″H), 7.25 (5H, *s*, aromatic protons); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 18.13 (C-2 CH_3), 20.99 (2× $OCOCH_3$), 46.64 (C-2) 65.85 (C-1′ & C-3), 67.15 (C-1″), 128.51 (C-3‴ & C-5‴), 128.71 (C-2‴ & C-6‴), 128.94 (C-4‴), 136.01 (C-1‴), 170.85 (2× C=O), 172.95 (C-1); IR (KBr): 1744 (C=O), 1457, 1232, 1043 and 699 cm^{-1}; FAB-HRMS: m/z 331.1147 ($[M+Na]^+$, C_{16}-$H_{20}O_6Na$ calcd 331.1152).

2.4.2 *Benzyl 3-propanoyloxy-2-(propanoyloxymethyl)-2-methylpropanoate (**5b**)*

It was obtained as a viscous oil (890 mg, 86 %).^{1}H NMR (300 MHz, $CDCl_3$): δ 1.08 (6H, *t*, $J = 7.5$ Hz, 2× $OCOCH_2CH_3$), 1.26 (3H, *s*, C-2 methyl), 2.21–2.29 (4H, *q*, $J = 7.5$ Hz, 2× $OCOCH_2CH_3$), 4.20–4.28 (4H, *d* = 11.2 Hz, C-1′H & C-3H), 5.16 (2H, *s*, C-1″H), 7.37 (5H, *s*, aromatic protons); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 8.98 (2× $OCOCH_2CH_3$), 17.78 (C-2 CH_3), 27.34 (2× $OCOCH_2CH_3$), 46.43 (C-2), 65.33 (C-1′ & C-3), 66.75 (C-1″), 128.09 (C-3‴ & C-5‴), 128.31 (C-2‴ & C-6‴), 128.54 (C-4‴), 135.63 (C-1‴), 172.64 (2× C=O), 173.85 (C-1); IR (KBr): 1739 (C=O), 1463, 1175, 1084 and 698 cm^{-1}; FAB-HRMS: m/z 359.1462 ($[M+Na]^+$, C_{18}-$H_{24}O_6Na$ calcd 359.1465).

2.4.3 *Benzyl 3-butanoyloxy-2-(butanoyloxymethyl)-2-methylpropanoate (**5c**)*

It was obtained as a viscous oil (970 mg, 87 %).^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (6H, *t*, $J = 7.5$ Hz, 2× $OCO(CH_2)_2CH_3$), 1.26 (3H, *s*, C-2 methyl), 1.52–1.64 (4H, *m*, 2× $OCOCH_2CH_2CH_3$), 2.21 (4H, *t*, $J = 6.3$ Hz, 2× $OCOCH_2CH_2CH_3$), 4.20–4.28 (4H, *d* = 11.0 Hz, C-1′H and C-3H), 5.16 (2H, *s*, C-1″H), 7.33 (5H, *s*, aromatic protons); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 13.60 (2× $OCO(CH_2)_2CH_3$), 17.81 (C-2 CH_3), 18.32 (2× $OCOCH_2$-CH_2CH_3), 35.94 (2× $OCOCH_2CH_2CH_3$), 46.36 (C-2), 65.28 (C-1′ & C-3), 66.77 (C-1″), 128.11 (C-3‴ & C-5‴), 128.32 (C-2‴ & C-6‴), 128.55 (C-4‴), 135.60 (C-1‴), 172.66 (2 x C=O), 173.09 (C-1); IR (KBr) 1742 (C=O), 1458, 1170, 1007 and 698 cm^{-1}; FAB-HRMS: m/z 387.1776 ($[M+Na]^+$, $C_{20}H_{28}O_6Na$ calcd 387.1778).

2.4.4 *Benzyl 3-pentanoyloxy-2-(pentanoyloxymethyl)-2-methylpropanoate (**5d**)*

It was obtained as a viscous oil (1.02 gm, 83 %).^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (6H, *t*, $J = 6.9$ Hz, 2x$OCO(CH_2)_3CH_3$), 1.26 (3H, *s*, C-2 methyl), 1.29–1.34 (4H, *m*, 2x$OCO(CH_2)_2CH_2CH_3$), 1.49–1.56 (4H, m, 2 x $OCOCH_2CH_2CH_2CH_3$), 2.23 (4H, t, $J = 7.5$ Hz, 2× $OCOCH_2(CH_2)_2CH_3$), 4.16-4.24 (4H, *d* = 11.0 Hz, C-1′H and C-3H), 5.16 (2H, *s*, C-1″H), 7.35 (5H, *s*, aromatic protons); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 13.98 (2× $OCO(CH_2)_3CH_3$), 18.14 (C-2 CH_3), 22.51 (2× $OCO(CH_2)_2CH_2CH_3$), 27.20 (2× $OCOCH_2CH_2CH_2CH_3$), 34.11 (2× $OCOCH_2(CH_2)_2CH_3$) 46.69 (C-2), 65.65 (C-1′ &C-3), 67.09 (C-1″), 128.40 (C-3‴ & C-5‴), 128.64 (C-2‴ & C-6‴), 128.88 (C-4‴), 135.62 (C-1‴), 172.99 (2× C=O), 173.57 (C-1); IR (KBr): 1742 (C=O), 1467, 1164,

1022 and 697 cm^{-1}; FAB-HRMS: m/z 415.2085 ($[M+Na]^+$, $C_{22}H_{32}O_6Na$ calcd 415.2091).

2.4.5 Benzyl 3-hexanoyloxy-2-(hexanoyloxymethyl)-2-methylpropanoate (**5e**)

It was obtained as a viscous oil (1.05 gm, 80 %).^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (6H, *t*, $J = 3.9$ Hz, 2 x $OCO(CH_2)_4CH_3$), 1.26–1.33 (11H, *m*, 2× $OCO(CH_2)_2$-$CH_2CH_2CH_3$ and C-2 methyl), 1.51–1.63 (4H, *m*, 2× $OCOCH_2CH_2(CH_2)_2CH_3$), 2.22 (4H, *t*, $J = 7.5$ Hz, 2× $OCOCH_2(CH_2)_3CH_3$), 4.19–4.27 (4H, $d = 11.0$ Hz, C-1′H and C-3H), 5.16 (2H, *s*, C-1″H), 7.33 (5H, *s*, aromatic protons); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 13.86 (2× $OCO(CH_2)_4CH_3$), 17.80 (C-2 CH_3), 22.25 (2× $OCO(CH_2)_3CH_2CH_3$), 24.49 (2× $OCO(CH_2)_2$-$CH_2CH_2CH_3$), 31.22 (2× $OCOCH_2CH_2(CH_2)_2CH_3$), 34.02 (2× $OCOCH_2(CH_2)_2CH_3$) 46.38 (C-2), 65.32 (C-1′ and C-3), 66.75 (C-1″), 128.06 (C-3‴ & C-5‴), 128.30 (C-2‴ & C-6‴), 128.55 (C-4‴), 135.62 (C-1‴), 172.65 (2× C=O), 173.24 (C-1); IR (KBr): 1743 (C=O), 1466, 1161, 1015 and 698 cm^{-1}; FAB-HRMS: m/z 443.2407 ($[M+Na]^+$, $C_{24}H_{36}O_6Na$ calcd. 443.2404).

2.4.6 4-Fluorobenzyl 3-acetoxy-2-(acetoxymethyl)-2-methylpropanoate (**6a**)

It was obtained as a viscous oil (790 mg, 84 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (3H, *s*, C-2 methyl) 1.98 (6H, *s*, 2× $OCOCH_3$), 4.18–4.26 (4H, $d = 11.0$ Hz, C-1′H & C-3H), 5.13 (2H, *s*, C-1″H), 7.01-7.07 (2H, *m*, C-2 ‴H & C-6‴H), 7.30–7.35 (2H, *m*, C-3‴H & C-5‴H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 17.71 (C-2 CH_3), 20.61(2× $OCOCH_3$), 46.24 (C-2) 65.40 (C-1′ &C-3), 66.07 (C-1″), 115.35 & 115.64 (C-3‴ & C-5‴), 130.17 & 130.28 (C-2‴ & C-6‴), 131.45 & 131.49 (C-1‴), 161.04 & 164.32 (C-4‴), 170.45 (2× C=O), 172.53 (C-1); IR (KBr): 1743 (C=O), 1513, 1222, 1041 and 828 cm^{-1}; FAB-HRMS: m/z 349.1051 ($[M+Na]^+$, $C_{16}H_{19}FO_6Na$ calcd 349.1058).

2.4.7 4-Fluorobenzyl 3-propanoyloxy-2-(propanoyloxymethyl)-2-methylpropanoate (**6b**)

It was obtained as a viscous oil (875 mg, 86 %).^{1}H NMR (300 MHz, $CDCl_3$): δ 1.08 (6H, *t*, $J = 7.8$ Hz, 2× $OCOCH_2CH_3$), 1.25 (3H, *s*, C-2 methyl), 2.22–2.29 (4H, *q*, $J = 7.5$ Hz, 2× $OCOCH_2CH_3$), 4.19-4.27 (4H, $d = 11.0$ Hz, C-1′H and C-3H), 5.12 (2H, *s*, C-1″H), 7.01 × 7.06 (2H, *m*, C-2‴H and C-6‴H), 7.27 × 7.34 (2H, *m*, C-3‴H and C-5‴H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 8.96 (2× $OCOCH_2CH_3$), 17.73 (C-2 CH_3), 27.32 (2× $OCOCH_2CH_3$), 46.42 (C-2), 65.27 (C-1′ &C-3), 66.04 (C-1″), 115.34 & 115.62 (C-3‴ & C-5‴), 130.15 & 130.26 (C-2‴, C-6‴), 131.46 & 131.51 (C-1‴), 161.05 & 164.33 (C-4‴), 172.59 (2× C=O), 173.81 (C-1); IR (KBr): 1742 (C=O), 1513, 1225, 1025 and 829 cm^{-1}; FAB-HRMS: m/z 377.1357 ($[M+Na]^+$, $C_{18}H_{23}FO_6Na$ calcd 377.1371).

2.4.8 4-Fluorobenzyl 3-butanoyloxy-2-(butanoyloxymethyl)-2-methylpropanoate (**6c**)

It was obtained as a viscous oil (860 mg, 78 %).^{1}H NMR (300 MHz, $CDCl_3$): δ 0.94 (6H, *t*, $J = 7.5$ Hz, 2× $OCO(CH_2)_2CH_3$), 1.21 (3H, *s*, C-2 Methyl), 1.52–1.65 (4H, *m*, 2× $OCOCH_2CH_2CH_3$), 2.23 (4H, *t*, $J = 7.5$ Hz, 2× $OCOCH_2CH_2CH_3$), 4.18–4.26 (4H, $d = 11.0$ Hz, C-3H & C-1′H), 5.13 (2H, *s*, C-1″H), 7.01–7.07 (2H, *m*, C-2‴H & C-6‴H), 7.30–7.35 (2H, *m*, C-3‴H & C-5‴H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 13.92 (2× $OCO(CH_2)_2CH_3$), 18.12 (C-2 CH_3), 18.67 (2× $OCOCH_2CH_2CH_3$), 36.31 (2× $OCOCH_2CH_2CH_3$), 46.375 (C-2), 65.62 (C-1′ and C-3), 66.42 (C-1″), 115.71 & 116.00 (C-3‴ & C-5‴), 130.54 & 130.65 (C-2‴, C-6‴), 131.64 & 131.68 (C-1‴), 160.79 & 164.51 (C-4‴), 172.97 (2 x C=O), 173.38 (C-1); IR (KBr): 1739 (C=O), 1514, 1226, 1010 and 829 cm^{-1}; FAB-HRMS: m/z 405.1679 ($[M+Na]^+$, $C_{20}H_{27}FO_6Na$ calcd. 405.1684).

2.4.9 4-Fluorobenzyl 3-pentanoyloxy-2-(pentanoyloxymethyl)-2-methylpropanoate (**6d**)

It was obtained as a viscous oil (980 mg, 83 %).^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (6H, *t*, $J = 7.2$ Hz, 2× $OCO(CH_2)_3CH_3$), 1.25–1.37 (7H, *m*, 2× $OCO(CH_2)_2$-CH_2CH_3 and C-2 methyl), 1.49–1.59 (4H, *m*, 2× $OCOCH_2CH_2CH_2CH_3$), 2.24 (4H, *t*, $J = 7.8$ Hz, 2× $OCOCH_2(CH_2)_2CH_3$), 4.19–4.27 (4H, $d = 11.0$ Hz, C-1′H and C-3H), 5.12 (2H, *s*, C-1″H), 7.01–7.07 (2H, *m*, C-2‴H and C-6‴H), 7.30–7.35 (2H, *m*, C-3‴H & C-5‴H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 13.79 (2× $OCO(CH_2)_3CH_3$), 17.94 (C-2 CH_3), 22.35 (2× $OCO(CH_2)_2CH_2CH_3$), 27.04 (2× $OCOCH_2CH_2CH_2CH_3$), 33.95 (2× $OCOCH_2(CH_2)_2$-CH_3) 46.54 (C-2), 65.45 (C-1′ &C-3), 66.22 (C-1″), 115.53 & 115.81 (C-3‴ & C-5‴), 130.31 & 130.42 (C-2‴ & C-6‴), 131.62 & 131.67 (C-1‴), 161.2 & 164.50 (C-4‴), 172.78 (2× C=O), 173.39 (C-1); IR (KBr): 1742 (C=O), 1513, 1226, 1015 and 828 cm^{-1}; FAB-HRMS: m/z 433.1993 ($[M+Na]^+$, $C_{22}H_{31}FO_6Na$ calcd. 433.1997).

2.4.10 4-Fluorobenzyl 3-hexanoyloxy-2-(hexanoyloxymethyl)-2-methylpropanoate (**6e**)

It was obtained as a viscous oil (978 mg, 77 %).^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (6H, *t*, $J = 6.6$ Hz, OCO $(CH_2)_4CH_3$), 1.24–1.28 (11H, *m*, 2× $OCO(CH_2)_2$

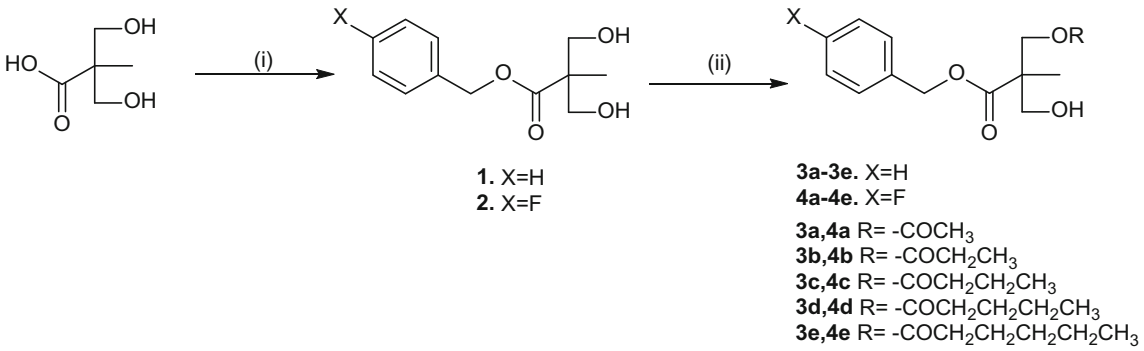
$CH_2CH_2CH_3$ & C-2 methyl), 1.53-1.58 (4H, *m*, 2× $OCOCH_2CH_2(CH_2)_2CH_3$), 2.22 (4H, *t*, $J = 7.5$ Hz, 2× $OCOCH_2(CH_2)_3CH_3$), 4.18–4.26 (4H, $d = 11.0$ Hz, C-1′H and C-3H), 5.11 (2H, *s*, C-1″H), 7.01–7.06 (2H, *m*, C-2‴H and C-6‴H), 7.29–7.34 (2H, *m*, C-3‴H & C-5‴H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 13.83 (2× $OCO(CH_2)_4CH_3$), 17.74 (C-2 CH_3), 22.23 (2× $OCO(CH_2)_3CH_2CH_3$), 24.47 (2× $OCO(CH_2)_2CH_2CH_2CH_3$), 31.19 (2× $OCOCH_2CH_2(CH_2)_2CH_3$), 33.99 (2× $OCOCH_2(CH_2)_2CH_3$), 46.34 (C-2), 65.24 (C-3 & C-1′), 66.02 (C-1″), 115.33 & 115.62 (C-2‴ & C-6‴), 130.11 & 130.22 (C-3‴ & C-5‴), 131.43 & 131.47 (C-1‴), 161.02 & 164.29 (C-4‴), 172.59 (2× C=O), 173.22 (C-1); IR (KBr): 1744 (C=O), 1513, 1226, 1016 and 828 cm^{-1}; FAB-HRMS: m/z 461.2303 ($[M+Na]^+$, $C_{24}H_{35}FO_6Na$ calcd. 461.2310).

2.5 General Procedure of Lipozyme® TL IM Catalyzed Deacylation of the Diacylated Derivatives **5a–5e** and **6a–6e**

In a typical experiment, a mixture of the diacylated derivative (**5a–5e** and **6a–6e**, 1.0 mmol), dry diisopropyl ether (35 mL), *n*-butanol (1.1 mmol) and Lipozyme® TL IM (350 mg) was stirred at 30–35 °C in an incubator shaker. The progress of the reaction was monitored periodically on TLC. On completion, the reaction was stopped by filtering off the enzyme and solvent evaporated to dryness under vacuum. The crude products **3a–3e** and **4a–4e** thus obtained from **5a–5e and 6a–6e**, respectively were purified by column chromatography on silica gel using a mixture of petroleum ether-ethyl acetate for elution. All these compounds were fully characterized from their different spectral data and melting points which were identical to the data of the corresponding compounds **3a–3e** and **4a–4e** obtained by the Lipozyme® TL IM catalyzed selective acylation reactions on the diols **1** and **2**, respectively.

3 Results and Discussion

The aryl 1,3-dihydroxy-2-methylpropanoates **1** and **2** were prepared in one step by reacting 2,2-bis(hydroxymethyl)propanoic acid with benzyl bromide and 4-fluorobenzyl bromide, respectively in approximately 75 % overall yield (Scheme 1). Both the compounds **1** [17] and **2** were fully characterized from their spectral data, the compound **2** is being reported for the first time. Six different lipases, i.e. Amano PS lipase, *C. antarctica* lipase-B immobilized on accurel [CAL-L (A)], *Candida antarctica* lipase-B immobilized on polyacrylate (Lewatit, CAL-B, also commonly known as Novozyme-435), *C. rugosa* lipase (CRL), porcine pancreatic lipase (PPL) and *Thermomyces lanuginosus* lipase immobilized on silica (Lipozyme® TL IM), were screened for the selective acylation of one of the two primary hydroxyl groups in the aryl 2-methylpropanoates **1** and **2** using propanoic anhydride as the acylating agent as the test case. DIPE was used as solvent in the case of Lipozyme® TL IM, PPL and CRL catalyzed reactions. No significant reaction was observed in the cases when PPL, CRL and Amano PS were used as lipases. However, both CAL-B and Lipozyme® TL IM were found to catalyze acylation of compounds **1** and **2** upon incubation with propanoic anhydride. The rate of transformation was found to be much slower in the reaction catalyzed by CAL-B as compared to the one with Lipozyme® TL IM. The transformation remained incomplete even after 72 h of stirring in the former case. On the basis of preliminary screening, Lipozyme® TL IM in DIPE was selected for acylation of compounds **1** and **2** using different acid anhydrides. In a typical reaction, a mixture of the 2-methylpropanoate **1**or **2** (1 mmol) and acid anhydride (1 mmol) in DIPE (50 mL) was stirred with Lipozyme® TL IM (350 mg) in an incubator shaker at 37–42 °C and the progress of the reaction was monitored by TLC (Scheme 1). On completion of the reaction, the enzyme was filtered off and solvent was removed under reduced pressure. The crude product thus obtained was purified by passing through a short column of silica gel, which afforded the monoacylated compounds in the pure form. It was observed that Lipozyme® TL IM in DIPE selectively acylates only one of the two primary hydroxyl groups in compounds **1** and **2** leading to the exclusive formation of the monoacyl derivatives. In order to find out the tolerance of Lipozyme® TL IM for different acid anhydrides as acylating agents and also to find out the optimum conditions for selective and efficient acylation, different acid anhydrides, viz. acetic, butanoic, pentanoic and hexanoic, together with propanoic anhydride were examined for the acylation of 2-methylpropanoates **1** and **2** (Scheme 1; Table 1). Lipozyme® TL IM in DIPE was found to accept all these acid anhydrides as acylating agents and transfer the acyl group exclusively at only one of the two primary hydroxyl groups in **1** and **2** leading to the formation of the monoacylated products **3a–3e** and **4a–4e** in 70–88 % yields (Table 1). Although Lipozyme® TL IM in DIPE accepts different aliphatic acid anhydrides as acylating agents, butanoic anhydride was found to be the most efficient acylating agent amongst all the five acid anhydrides studied. Out of two 2-methylpropanoates **1** and **2**, compound **1** appeared to be the better substrate for Lipozyme® TL IM mediated acylation reactions with all the five anhydrides, both in terms of the turnover of the reaction and the average reaction time. For example, the average turnover and the average reaction time in case of Lipozyme® TL IM catalyzed acylation of 2-methylpropanoate **1** are 81 % yield and 16 h, respectively, whereas the average turnover/reaction time in case of 2-methylpropanoate **2** are 74 % yield and 17 h, respectively.



Scheme 1 (*i*) KOH, DMF, benzyl bromide or 4-fluorobenzyl bromide; (*ii*) acid anhydride, Lipozyme® TL IM, DIPE, 37–42 °C

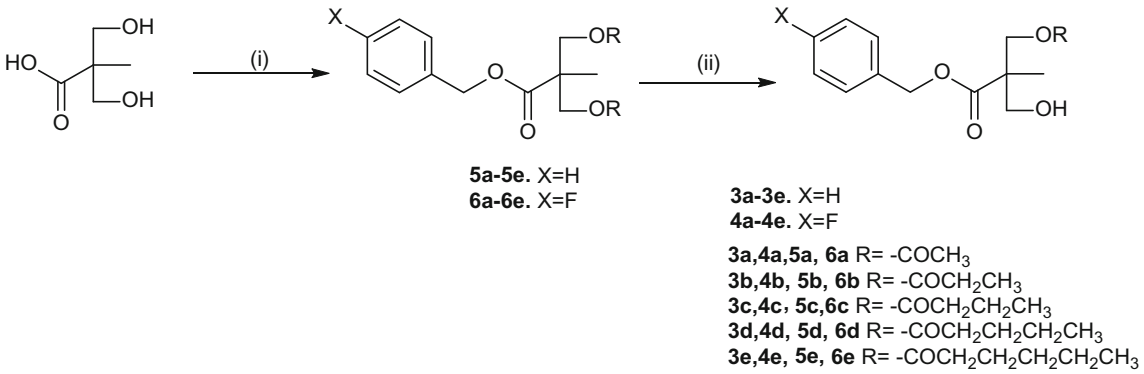
Table 1 Regioselective acylation of aryl 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoates **1** and **2** by Lipozyme® TL IM in DIPE at 37–42 °C using different acid anhydrides

Substrate	Acylating agent/reaction time	Product	Yield (%)
1	Acetic anhydride/22 h	Benzyl 2-(acetoxymethyl)-3-hydroxy-2-methylpropanoate (**3a**)	77
1	Vinyl acetate/13 h	Benzyl 2-(acetoxymethyl)-3-hydroxy-2-methylpropanoate (**3a**)	82
1	Propanoic anhydride/12 h	Benzyl 2-(propanoyloxymethyl)-3-hydroxy-2-methylpropanoate (**3b**)	83
1	Butanoic anhydride/8 h	Benzyl 2-(butanoyloxymethyl)-3-hydroxy-2-methylpropanoate (**3c**)	88
1	Pentanoic anhydride/16 h	Benzyl 2-(pentanoyloxymethyl)-3-hydroxy-2-methylpropanoate (**3d**)	81
1	Hexanoic anhydride/20 h	Benzyl 2-(hexanoyloxymethyl) -3-hydroxy-2-methylpropanoate (**3e**)	76
2	Acetic anhydride/26 h	4-Fluorobenzyl 2-(acetoxymethyl)-3-hydroxy-2-methylpropanoate (**4a**)	76
2	Vinyl acetate/16 h	4-Fluorobenzyl 2-(acetoxymethyl)-3-hydroxy-2-methylpropanoate (**4a**)	80
2	Propanoic anhydride/17 h	4-Fluorobenzyl 2-(propanoyloxymethyl)-3-hydroxy-2-methylpropanoate (**4b**)	74
2	Butanoic anhydride/11 h	4-Fluorobenzyl 2-(butanoyloxymethyl)-3-hydroxy-2-methylpropanoate (**4c**)	80
2	Pentanoic anhydride/22 h	4-Fluorobenzyl 2-(pentanoyloxymethyl)-3-hydroxy-2-methylpropanoate (**4d**)	70
2	Hexanoic anhydride/19 h	4-Fluorobenzyl 2-(hexanoyloxymethyl)-3-hydroxy-2-methylpropanoate (**4e**)	73

All these reactions, when performed under identical conditions but without adding the lipase yielded a complicated mixture of products with less than 10 % overall conversion

The Lipozyme® TL IM catalyzed acetylation of **1** and **2** was also tried using vinyl acetate, again exclusive regioselectivity was observed for the acetylation of only one primary hydroxyl group. Vinyl acetate was found to be a better acetylating agent as compared to acetic anhydride, as the yields with vinyl acetate were 80–82 % yield compared to the 76–77 % yields obtained with acetic anhydride (Table 1). The monoacylated 2-methylpropanoates **3a**–**3e** and **4a**–**4e** obtained in the present study are novel and have been synthesized for the first time, their structures were unambiguously established on the basis of their spectral analysis (IR, ^{1}H NMR, ^{13}C NMR and high resolution mass spectra, cf. Sect. 2).

Also we have screened the lipases for carrying out the regioselective deacylation of the diacyl derivatives of **1** and **2**. The diols **1** and **2** were converted into their diacyl derivatives **5a**–**5e** and **6a**–**6e**, by treating them with the acid anhydrides (acetic/propanoic/butanoic/pentanoic/hexanoic anhydrides) in dry dichloromethane containing catalytic amount of DMAP in 77–91 % yields (Scheme 2). The structures of all these novel compounds were confirmed from their spectral data. Based on our earlier experience of biocatalytic transacylation reactions on the peracylated furanose derivatives [18–21], we screened *C. antarctica* lipase-B, porcine pancreatic lipase (PPL), *C. rugosa* lipase (CRL) and Lipozyme® TL IM for selective deacylation of the diacylated derivatives **5a**–**5e** and **6a**–**6e** in different organic solvents in the presence of *n*-butanol as the acyl trapper. Lipozyme® TL IM in DIPE was found to act in the most efficient manner for the deacylation of compounds **5a**–**5e** and **6a**–**6e** (Scheme 2; Table 2). The other lipases, i.e. Novozyme-435, PPL and CRL did not



Scheme 2 (*i*) acid anhydride $(RCO)_2O$, DMAP, (*ii*) acid anhydride, Lipozyme® TL IM, DIPE, *n*-butanol 30–35 °C

Table 2 Regioselective deacylation of aryl 3-acyloxy-2-(acyloxymethyl)-2- methylpropanoates **5a-5e** and **6a-6e** by Lipozyme® TL IM in DIPE in the presence of *n*-butanol at 30-35 °C

Substrate	Product	Reaction time (h)	Yield (%)
5a	Benzyl 2-(acetoxymethyl)-3-hydroxy-2-methylpropanoate (**3a**)	8	77
5b	Benzyl 2-(propanoyloxymethyl)-3-hydroxy-2-methylpropanoate (**3b**)	11	74
5c	Benzyl 2-(butanoyloxymethyl)-3-hydroxy-2-methylpropanoate (**3c**)	5	80
5d	Benzyl 2-(pentanoyloxymethyl)-3-hydroxy-2-methylpropanoate (**3d**)	13	70
5e	Benzyl 2-(hexanoyloxymethyl) -3-hydroxy-2-methylpropanoate (**3e**)	9	77
6a	4-Fluorobenzyl 2-(acetoxymethyl)-3-hydroxy-2-methylpropanoate (**4a**)	10	75
6b	4-Fluorobenzyl 2-(propanoyloxymethyl)-3-hydroxy-2-methylpropanoate (**4b**)	13	70
6c	4-Fluorobenzyl 2-(butanoyloxymethyl)-3-hydroxy-2-methylpropanoate (**4c**)	7	83
6d	4-Fluorobenzyl 2-(pentanoyloxymethyl)-3-hydroxy-2-methylpropanoate (**4d**)	11	77
6e	4-Fluorobenzyl 2-(hexanoyloxymethyl)-3-hydroxy-2-methylpropanoate (**4e**)	14	72

All these reactions, when performed under identical conditions but without adding Lipozyme® TL IM did not yield any product

accept any of the ten diacylated aryl 2-methyl-propanoates **5a**–**5e** and **6a**–**6e** as substrate.

In all these acylation and deacylation reactions performed under identical conditions, but without adding lipase, either no reaction was observed or a mixture of products with less than 10 % conversion were formed. In the present work, we have successfully employed and demonstrated the use of Lipozyme® TL IM in the preparation of very useful compounds, **3a**–**3e** and **4a**–**4e**.

It has been demonstrated in the present study that Lipozyme® TL IM is very efficient in regioselective esterification and transesterification reactions with different acid anhydrides and vinyl acetate screened on the prochiral diols **1** and **2** and their prochiral diesters **5a**–**5e** and **6a**–**6e**, respectively. Although the monoacylated products **3a**–**3e** and **4a**–**4e** from both the series of biocatalytic reactions on prochiral diols and their corresponding diesters possess chirality, however surprisingly, no enantioselectivity was observed by us in any of these reactions as none of the ten products showed any optical rotation.

We and some other groups have reported chemo- and regioselective esterification/transesterification reactions on different classes of polyols and their peracyl derivatives [18–24]. However, very limited cases have been reported in the literature using Lipozyme® TL IM for enantioselective reactions on racemic compounds involving resolution protocols, where it has been successfully demonstrated that the enantioselectivity exhibited by Lipozyme® TL IM is due to its ability in picking one enantiomer over the other for hydrolytic or transesterification reactions on racemic compounds [25–28]. Guisan et al. have successfully demonstrated the enantioselectivity of *Thermomyces lanuginosus* (TLL) (previously *Humicola lanuginosa*) on prochiral dimethyl or diethyl phenylmalonate. They have also shown that different immobilized preparations of the same lipase, when acting under different experimental conditions, may exhibit a very different activity and enantioselectivity [29]. It has also been supported by other groups that its reactivity and properties can easily be altered by changing the way of its preparation and support type used for immobilization. It may be because immobilization of enzymes inside the porous structure of a

solid may permit to have the enzyme molecules fully dispersed and without the possibility of interacting with any external interface. Thus, this immobilization will stabilize the enzyme against interactions with molecules from the enzymatic extract, preventing aggregation, autolysis or proteolysis by proteases from the extract (that will be also dispersed and immobilized). Moreover, the immobilized enzyme molecules will not be in contact with any external hydrophobic interface, such as air bubbles originated by supplying some required gases or promoted by strong stirring, necessary to control pH. These gas bubbles sometimes produce enzyme inactivation of soluble proteins [30–33], but cannot inactivate the enzymes immobilized on a porous solid [34, 35]. It has been also well documented that experimental conditions like using different solvents, reaction time, temperature, pH value, buffer, etc. and nature of substrate also play significant role in its activity and selectivity [23, 36]. For example, immobilization may be very rapid, multipoint interaction between the non-complementary enzyme and support surfaces is a slow and time-dependent process: it requires the correct alignment of groups located in the already immobilized, and partially rigidified enzyme, and the rigid surface of the support. However, moderately high temperature may favor the vibration of enzyme and support, increasing the possibility of getting more enzyme-support linkages [36–38]. Our present study is also in accordance with these hypotheses. As documented earlier that when a prochiral dimethyl or ethyl phenylmalonate was subjected to TLL, same class of lipase derived from same source (*Thermomyces lanuginosus*) but immobilized on a different support (granulated silica via covalent adsorption) is showing enantioselectivity, whereas Lipozyme® TL IM was unable to show enantioselectivity when subjected to a prochiral molecule as its support (silicate via ionic adsorption) for immobilization is different.

4 Conclusion

This study has clearly demonstrated the specificity of Lipozyme® TL IM for the regioselective acylation of one of the two primary hydroxyl groups present in 2-methylpropanoates using acid anhydrides as acylating agents. This work further revealed that butanoic anhydride and vinyl acetate are the most suitable acylating agents for Lipozyme® TL IM in DIPE. The selective deacylation reaction was also found to be most efficient with Lipozyme® TL IM. The biocatalytic route developed in this study can find utility for the selective manipulation of prochiral diols and polyhydroxy compounds, and the partial esters obtained can prove to be useful monomers for making amphiphilic copolymers of utility in Health and Industrial sectors.

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