Special Collection

Lipase-Catalysed Enzymatic Kinetic Resolution of Aromatic Morita-Baylis-Hillman Derivatives by Hydrolysis and Transesterification

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Acylated Morita-Baylis-Hillman (MBH) adducts were synthesised and subjected to enzymatic kinetic resolution (EKR) by hydrolysis employing various lipase enzymes: from *P. fluorescens, P. cepacia* (PCL), *C. antarctica* A (CAL–A), *C. antarctica* B (CAL–B) and Novozyme 435. In a number of instances enantiopure Morita-Baylis-Hillman acetates or butyrates and their corresponding hydrolysed MBH adducts were obtained with *ee* values of >90%, at ca. 50% conversion, corresponding to enantiomeric ratio (E) values of >200. Enantioselective transesterification reactions on MBH adducts was achieved using acyl anhydrides in THF or the greener organic solvent 2-MeTHF in the presence of CAL–A. This is the first report of successful lipase-catalysed EKR of aromatic MBH adducts by transesterification in organic medium.

Introduction

The Morita-Baylis-Hillman (MBH) reaction has great synthetic potential with its atom-economical formation of a carbon–carbon bond. This reaction was first reported in 1972 by Baylis and Hillman[1] using a tertiary amine catalyst and in 1968 by Morita *et al.*[2] using a phosphine catalyst. Reaction of an aldehyde **1** and activated alkene **2** in the presence of a tertiary amine or phosphine **3** gives a highly functionalised α-methylene-β-hydroxy compound **4**, with DABCO being the most widely used catalyst (Scheme 1). The MBH reaction has become an important reaction in synthetic chemistry as evidenced by the vast number of published reviews and research papers written on the subject.[3]

MBH reactions have been widely used for the synthesis of compounds which display various biological activities for potential medicinal uses. This is evident in the bioactivities displayed by aromatic MBH adducts, for example activity against *Plasmodium falciparum* which is a malaria-causing parasite,[4] and anti-cancer activity where these compounds have been reported to exhibit positive results in *in vitro* studies on human tumour cell lines.[5] In addition, antibacterial,[6] antifungal, herbicidal and anti-leishmanial activity have also been reported. Compound **4b**, which showed good activity against the *Leishmania amazonenesis* species, is considered to be a lead compound in the development of anti-leishmanial drugs due to its enhanced potency and minimal toxicity when compared to the commercially used drug, pentostam.[7]

EWG = COOR' ,$CONEt_2$, CN, COR"
R, R' = Alkyl or Aryl
PR_3/NR_3= DABCO, Indolizine, Quinuclidine, Ph_3P

Scheme 1. Morita-Baylis-Hillman reaction.

The use of racemic compounds for biological testing limits the development of efficient drugs[8] as the opposite enantiomer can have undesirable biological properties. The preparation of enantiopure Morita-Baylis-Hillman adducts remains a major challenge in organic chemistry, but fortunately enzymatic kinetic resolution (EKR) can offer a viable solution to the problem. EKR is one of the most common methods employed in industry for the separation of enantiomers, and its use of less toxic solvents and enzymes (lipases) presents a greener approach to obtaining optically active compounds.

In general terms, EKR of MBH adducts can be achieved via an enzyme-catalysed esterification of *sec*-alcohols in an organic solvent or the alternative approach of using enzymes to hydrolyse esters in an aqueous environment.[9] Various groups have reported success using the latter approach for acylated MBH adducts derived from aromatic aldehydes and acrylonitrile.[10] For example, in early work Basavaiah *et al.*[11] reported the hydrolysis of MBH acetates using pig liver acetone powder (PLAP) which resulted in low to moderate *ee* values of

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the enantiopure MBH adduct. In more recent work, Xavier *et al.*[7] reported a successful EKR of the acetates of *p*-nitrobenzaldehyde and *m*-nitrobenzaldehyde-derived Morita-Baylis-Hillman (MBH) adducts using *Candida antarctica* B (CAL–B) lipase. This resulted in the resolution of these MBH adducts with excellent *ee* values of >99%. Xia *et al.*[12] also used CAL–B lipase in a successful chemo-enzymatic dynamic kinetic resolution of similar substituted benzaldehyde-derived MBH acetates using triethylamine to achieve substrate racemisation. Our group succeeded in resolving aromatic MBH substrates and their related rigid or more flexible homologues by hydrolysis in order to map the ideal substrate profile for various lipases.[13] Although enzymatic kinetic resolution was successful on all three of the substrates tested, cinnamaldehyde-derived adducts gave the best results with a lipase from *Pseudomonas fluorescens* and Amano AK lipase was the most selective on the benzaldehyde derivatives. In contrast to the success achieved in the lipase-catalysed EKR by hydrolysis of MBH adducts derived from aromatic aldehydes, there are not, to the best of our knowledge, any reports to date of successful transesterification reactions for these adducts, although they have been attempted. For example, Bornscheuer *et al.* reported only 9% conversion after 35 days for the transesterification reaction of benzaldehyde-derived MBH adducts.[14] Interestingly, there are literature reports on the EKR of MBH adducts derived from aliphatic aldehydes by transesterification.[8,15] The earlier success we achieved in the EKR of MBH acetates by hydrolysis prompted us to expand the substrate scope for the EKR reactions using different substrates, acyl groups and enzymes and we were also motivated to try and achieve EKR using transesterification on aromatic MBH substrates for the first time. In this paper we discuss the lipase-catalysed hydrolysis of various *para*-substituted MBH acetates and their corresponding novel butyrate substrates to obtain enantiopure MBH adducts in excellent *ee*. In addition, we report here for the first time the successful transesterification reactions of aromatic MBH adducts, giving moderate to good *ee* values of the acyl products in reasonable reaction times using CAL–A.

Results and Discussion

Synthesis

Racemic MBH adducts **4a–d** were efficiently synthesised through a one-pot solvent-free reaction between aldehydes **1a–d** and acrylonitrile **2a** with DABCO **3a** at room temperature (Scheme 1). The rate of reaction and yields of the MBH adducts were very sensitive to the nature of the benzaldehyde substituent. Electron-withdrawing groups (EWGs) on the benzaldehyde starting material usually result in more rapid reactions and higher yield as opposed to electron-donating groups (EDGs). This is presumably due to the EWGs reducing the electron density of the benzaldehyde carbonyl group, increasing the electrophilicity of the electrophilic carbon atom which then results in rapid nucleophilic addition of the zwitterionic enolate formed between DABCO and acrylonitrile to the benzaldehyde (rate determining step). For fluoro-derivative **4a** the electron-withdrawing effect of F is mitigated by the efficient overlap of the F 2p orbital with the carbon 2p orbital, allowing electron donation to the aromatic ring through resonance, thus making F a weaker EWG overall and possibly resulting in the lower overall yield observed for synthesis of this adduct (Table 1). The MBH adducts **4a–d** were subsequently acylated with acetic anhydride or butyric anhydride (Scheme 2) to form the esters **5a–d** and **6a–d**, respectively.

The MBH acetates were obtained in good yields, whilst the MBH butyrates were obtained in moderate yields (Table 1). This lower yield is due, in part, to the difficulty encountered in separating the unreacted butyric anhydride from the ester products. NMR spectroscopic data for known compounds **4a–d** and **5a–d** was in agreement with that previously reported.[12,16–18]

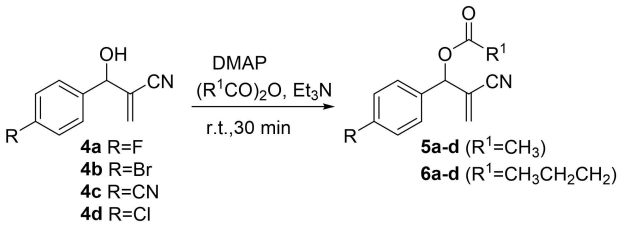
Enzymatic kinetic resolution by hydrolysis

Five lipase enzyme preparations: *Pseudomonas fluorescens* lipase (**I**), *Pseudomonas cepacea* lipase (PCL) (**II**), *Candida antarctica* lipase A (CAL–A) (**III**), *Candida antarctica* lipase B (CAL–B) (**IV**) and Novozyme 435 (**V**) were chosen for the hydrolysis of the MBH acetates **5a–d** and MBH butyrates **6a–d** (Scheme 3). The hydrolysis reactions were run on a small scale using substrate and enzyme in a mixture of phosphate buffer (pH 7) and

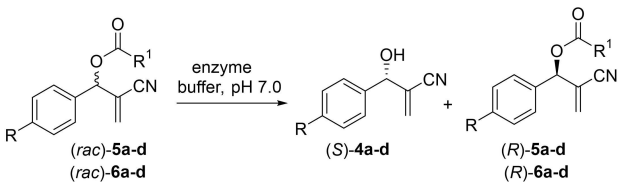
Table 1. Yields of products **4–6**.

Entry	MBH adduct yield [%][a]	MBH acetate yield [%]	MBH butyrate yield [%]
1	**4a** 71 (18 h)	**5a** 83	**6a** 61
2	**4b** 88 (20 h)	**5b** 74	**6b** 68
3	**4c** 86 (3 h)	**5c** 80	**6c** 49
4	**4d** 89 (1.5 h)	**5d** 81	**6d** 64

[a] Reaction time in hours is shown in parentheses.



Scheme 2. Synthesis of MBH esters.



Scheme 3. Enzymatic kinetic resolution of **5** and **6**.

acetone as a co-solvent (95:5) at 25–30 °C until hydrolysis was observed by TLC and then reactions were further analysed by chiral HPLC. Results for these enzymatic hydrolysis reactions are shown in Table 2 where the selectivity of the reaction is expressed as the Enantiomeric ratio (E), a value that is consistent throughout the reaction and independent of the extent of conversion.

All of the MBH acetate substrates were successfully resolved through enzymatic kinetic resolution (Table 2, entries 1–20). Data from Table 2 shows that compound **5a** was the least readily resolved substrate, with only *P. fluorescens* and *P. cepacia* lipases showing reasonable selectivity. PCL gave the best results for this substrate, with an ee_p of 92% and E ratio of 53 (entry 2). Compound **5b** was the most readily resolved substrate, as all 5 enzymes tested were able to selectively convert the ester to the corresponding alcohol product with *ee* values of 85%-98% (entries 6–10). Novozyme 435 was the enzyme best able to resolve substrate **5b**, with an E ratio of 147 (entry 10). Compound **5c** was also readily resolved, with all of the enzymes showing significant selectivity towards this substrate (entries 11–15). Compound **5d** gave similar results to **5a**, with only *P. fluorescens* lipase and PCL giving good selectivity. These enzymatic screening results show that *P. fluorescens* and *P. cepacia* lipases are the best performing enzymes overall in resolution of MBH acetates, with excellent alcohol *ee* values being obtained within reasonable reaction times. Novozyme 435, an immobilised version of CAL–B, which is expected to be more stable in the presence of solvents and temperature conditions,[19] displayed slightly superior selectivity to CAL–B, but the results observed were similar. It is interesting to note that the two substrates most readily resolved by all the enzymes tested were **5b** and **5c**, bearing the largest substituents in the *para*-position: a bromine atom and nitrile group, respectively. On the other hand, the smaller fluorine atom and chlorine atom-bearing substrates were readily resolved by only *Pseudomonas fluorescens* lipase (**I**) and *Pseudomonas cepacea* lipase (PCL) (**II**). This could possibly be rationalised on the basis of **5b** and **5c** being the compounds with the biggest size difference between the two substituents of the secondary alcohol. It is also possible that the aromatic rings bearing the larger substituents such as Br and CN fill the enzyme binding pockets more effectively, restricting the number of possible conformations of the tetrahedral intermediate formed during ester hydrolysis. It may be that the unreactive enantiomer (*R* in this case) fits in a non-productive manner,[20] leading to slow reaction, resulting in excellent enantioselectivity favouring the *S* product. The best selectivity in an individual hydrolysis reaction for an acetate substrate was seen for compound **5b**, which was resolved by Novozyme 435 with an E value of 147.

MBH butyrate substrates were also subjected to enzymatic studies as shown in Table 2 (entries 21–40). What is immediately evident from these results is the fact that substrate **6a** was not readily resolved by any of the enzymes. Moderate selectivity for this substrate was displayed by Novozyme 435, with an E value of 20 (entry 25). An E value of 15 or above is required to allow for practical separation of enantiomers.[21] The other substrates **6b**–**d** were all effectively resolved, with Novozyme 435 displaying the best selectivity of all the enzymes tested. This is in stark contrast to the corresponding acetates, where Novozyme 435 was completely ineffective against substrates **5a** and **5d**. Thus, this enzyme displays a clear preference for the longer-chain ester which is expected for a lipase. All of the enzymes tested successfully resolved **6b**, giving moderate to good E values of 16–79 (entries 26–30).

Compound **6c** was not resolvable using *P. fluorescens* lipase, or PCL, both of which were able to effectively resolve the corresponding acetate derivative **5c**. Outstanding selectivity was observed for **6c** using Novozyme 435 (E > 3000) and CAL–B lipase (E > 400) (entries 34–35). This butyrate derivate was, in fact, the compound for which the best selectivity overall was demonstrated. Compound **6d** gave satisfactory results as the 5 enzymes tested were able to resolve the substrate. Another interesting observation for the butyrate substrates was how fast

Table 2. Enzymatic kinetic resolution results for hydrolysis of MBH acetates (**5**) and butyrates (**6**).

Entry	Substrate	Enzyme	Time [h]	ee_p [%][a]	ee_s [%][b]	Conv.[c] [%]	E[d]
1	**5a**	I	3	89	22	20	21
2	**5a**	II	31.5	92	74	45	53
3	**5a**	III	3	54	54	50	6
4	**5a**	IV	5	no selectivity observed			0
5	**5a**	V	5	no selectivity observed			0
6	**5b**	I	68	95	48	34	63
7	**5b**	II	8.5	96	39	29	72
8	**5b**	III	4	85	67	44	25
9	**5b**	IV	74	97	37	28	94
10	**5b**	V	42	98	40	29	147
11	**5c**	I	57	89	66	43	34
12	**5c**	II	10.5	81	82	50	24
13	**5c**	III	5	85	82	49	31
14	**5c**	IV	47	93	45	33	47
15	**5c**	V	38	93	93	50	94
16	**5d**	I	25.5	91	66	42	42
17	**5d**	II	26	95	70	42	82
18	**5d**	III	101	82	20	20	12
19	**5d**	IV	10	17	8	32	2
20	**5d**	V	10	36	17	32	2
21	**6a**	I	5	no selectivity observed			0
22	**6a**	II	5	no selectivity observed			0
23	**6a**	III	0.08	67	62	48	9
24	**6a**	IV	7	58	34	37	5
25	**6a**	V	99	84	54	39	20
26	**6b**	I	58	91	60	40	39
27	**6b**	II	50	77	55	42	16
28	**6b**	III	0.7	90	92	51	62
29	**6b**	IV	25	88	45	34	24
30	**6b**	V	32	95	67	41	79
31	**6c**	I	16	73	41	36	10
32	**6c**	II	16	76	43	36	11
33	**6c**	III	0.08	93	85	48	75
34	**6c**	IV	46	> 99	53	35	424
35	**6c**	V	25	> 99	94	48	3583
36	**6d**	I	76	89	73	45	36
37	**6d**	II	76	92	73	44	53
38	**6d**	III	0.08	85	63	43	23
39	**6d**	IV	24	82	52	39	17
40	**6d**	V	28	96	53	36	83

[a] (ee_p) = % ee of alcohol product. [b] (ee_s) = % ee of substrate. [c] Conversion % (c) = $ee_s/(ee_s + ee_p) \times 100$; enantiomeric excess of the product. [d] E (enantiomeric ratio) = {ln [eep (1-ee_s)/ee_p + ee_s]/ln [ee_p (1 + ee_s)/ee_p + ee_s]. Values were obtained from chiral HPLC analysis. Enzymes: (**I**) *P. fluorescens* lipase, (**II**) PCL, (**III**) CAL–A, (**IV**) CAL–B, (**V**) Novozyme 435.

the CAL–A hydrolysis reactions occurred, although reasonable selectivity was still observed for substrates **6b–6d**. The larger acyl group is clearly preferred by CAL–A,[22] which has not previously been reported as a suitable enzyme for EKR of MBH derivatives. Various methods were employed to try and slow this reaction; for example using smaller amounts of enzyme and dilution of the reaction, but the reaction still proceeded extremely rapidly. Where selectivity was observed, all five enzymes hydrolysed the same enantiomer for each of the compounds tested.

Previously, we reported that for the unsubstituted MBH acetate **5** (R=H), enzymes such as PCL, *P. fluorescens* lipase and CAL–B selectively hydrolysed the (*S*)-enantiomer, leaving the (*R*)-acetate unreacted.[13] The hydrolysis reaction for resolution of compound **4b** was scaled sufficiently to isolate enantiopure product (+)-**4b** and enantiopure starting material (−)-**5b** (Table 3). Enantiopure (−)-**5b** was subsequently hydrolysed using a non-selective lipase to give enantiopure (−)-**4b**.

Enantiopure alcohol (+)-**4b** was reacted with Mosher esters (*S*)-MTPA and (*R*)-MTPA to give products **7** and **8**, respectively, while reaction of (−)-**4b** with (*S*)- and (*R*)-MTPA gave **9** and **10**, respectively (Figure 1). Thus, using Mosher's double derivatisation protocol[23] we were able to determine the absolute stereochemistry of (+)-**4b** as (*S*)-(+)-**4b** and that of (−)-**4b** as (*R*)-(−)-**4b**.

This matches the results we obtained previously for the unsubstituted MBH acetate. This enantiomer is also predicted to be the fast reacting enantiomer according to Kazlauskas' rule.[24] Selected ^{1}H NMR shift values for products **7**–**10** used in the determination of absolute configuration are shown in Table 4.

Table 3. Scaled-up reactions.

Entry	Resolved compound	Reaction time [h]	Conv. [%]	Ee [%]	$[\alpha]_D$[a]
1[b]	(+)-**4b** (*S*)	97	44	98	+50.5
2[c]	(−)-**5b** (*R*)	34	55	97	−28.0

[a] (c 0.5, MeOH); [b] **5b** (0.73 g), Novozyme 435 (0.73 g), buffer (21 mL), acetone (3 mL); [c] **5b** (0.30 g), PCL (0.30 g), buffer (18.5 mL), acetone (1.5 mL).

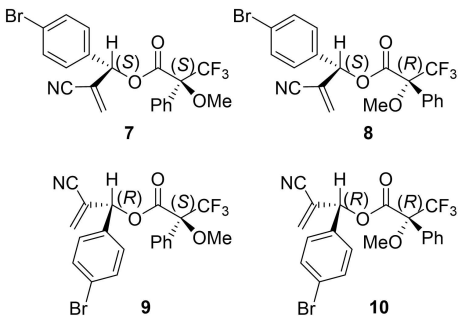


Figure 1. Mosher's ester derivatives of **4b**.

Table 4. Selected ^{1}H NMR shift values for compounds **7**–**10**.

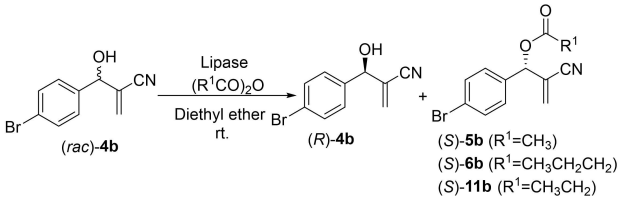
Compound	Aromatic ^{1}H NMR shift [ppm][a]	Alkene ^{1}H NMR shift [ppm][a]
7	7.49 (d), 7.10 (d)	6.15 (d), 5.99 (d)
8	7.56 (d), 7.26 (d)	6.08 (d), 5.95 (d)
9	7.56 (d), 7.26 (d)	6.08 (d), 5.95 (d)
10	7.49 (d), 7.10 (d)	6.15 (d), 5.99 (d)

[a] Peak multiplicity is shown in parentheses.

Enzymatic kinetic resolution by transesterification

After successful conclusion of the EKR by hydrolysis, we moved to our investigation of the transesterification reaction. Our previous attempts to conduct transesterification reactions on aromatic MBH adducts using typical acylating agents such as vinyl acetate, isopropenyl acetate and 4-chlorophenyl acetate that were successfully applied to aliphatic MBH derivatives,[15] met with failure, and no conversion was observed even after an extended period.[25] This motivated us to move onto testing anhydrides as acyl donors, in the hope that they would promote enantioselective conversion to the desired acyl products. Substrate **4b** was chosen for these reactions, based on the fact that all of the enzymes tested were able to successfully resolve the acetate derivative of this substrate, **5b**. Initial enzyme screening was conducted using substrate **4b** (Scheme 4, $R^1 = CH_3$), and the results are shown in Table 5, where transesterification reactions were performed in diethyl ether in the presence of acetic anhydride as acyl donor. It is clear from Table 5 that the best-performing enzyme was CAL–A (entry 3), with a conversion of 39% and a reasonable E value of 14 after 96 h. *P. fluorescens* lipase gave very poor conversion (entry 1), while PCL and Novozyme 435 resulted in very poor selectivity (entries 2 and 4).

Based on the screening reactions, CAL–A (entry 3) was chosen for further optimisation of the transesterification reactions and these were performed by varying the acyl donor



Scheme 4. EKR by transesterification reaction of **4b**.

Table 5. Transesterification screening reactions in Et_2O.

Entry	Enzyme	Reaction time [h]	Conv. [%]	ee_p [%]	ee_s	E
1	I	96	8	71	6	6
2	II	24	36	29	16	2
3	III	96	39	78	52	14
4	V	24	21	22	6	2

Reaction conditions: Acetic anhydride (15 mg), enzyme (3 mg), **4b** (12 mg), diethyl ether (1 mL); Enzymes: (**I**) *P. fluorescens* lipase, (**II**) PCL, (**III**) CAL–A, (**V**) Novozyme 435.

and solvent. Interestingly, Table 6 shows that selectivity was highly solvent dependent. For example, transesterification reactions with acetic anhydride as the acyl donor resulted in ee_p values of 93% with a conversion of 22% in THF (E = 36) (entry 2), whilst use of another ether, MTBE, as solvent led to better conversion of 38% but much poorer selectivity (E = 6) (entry 4). As might be expected, 2-MeTHF performed in a similar manner to THF in the reaction, giving an E value of 27 (entry 3). Use of propionic anhydride and butyric anhydride as the acyl donor generally resulted in lower conversions and E values when compared to acetic anhydride. The only satisfactory results obtained using propionic anhydride as an acyl donor were in THF and 2-MeTHF, with the former giving a good ee_p value of 92% at an E value of 29 and the latter an ee_p value of 88% and an E value of 19. Butyric anhydride performed poorly in all solvents tested. Using an ester as solvent proved to be a poor choice, with no selectivity being observed for reactions with propionic anhydride and butyric anhydride as acyl donor (entries 5 and 9). Overall, acetic anhydride was the best acyl donor and THF and 2-MeTHF were the best solvents in terms of conversion and selectivity.

In terms of the preferred enantiomer for reaction, the (*S*)-enantiomer of **4b** was selectively acylated by CAL–A to give **5b**.

This is a significant result because the opportunity now exists for testing DKR of aromatic MBH substrates in organic solvents, in the presence of a lipase together with a redox catalyst, that can be used for racemisation of the unwanted alcohol enantiomer.[26]

Experimental Section

General procedure for the preparation of Morita-Baylis-Hillman adducts 4a–d: The MBH adducts were prepared by dissolving DABCO (1 eq.) and the relevant aldehyde (1–1.5 eq.) in excess acrylonitrile (10–12 mL) in a 50 mL round-bottomed flask and stirring at room temperature. After reaction completion, as determined by TLC, ethyl acetate (30 mL) and water (30 mL) were added to the reaction mixture. The organic layer was separated from the aqueous layer, and the aqueous layer was extracted twice more with ethyl acetate. The organic medium was dried over anhydrous $MgSO_4$ and purified by column chromatography (30% ethyl acetate/hexane) using normal silica gel (150 g). The reaction products were then concentrated under reduced pressure to afford the desired product.

General procedure for the preparation of Morita-Baylis-Hillman acetates 5a–d: The MBH acetates were prepared by initially stirring the corresponding MBH adduct (**4a–d**) (1 eq.) in 2-methyltetrahydrofuran (20–25 mL) for 5 min, followed by the addition of trimethylamine (1.1–1.25 eq.), acetic anhydride (1.1–1.25 eq.) and DMAP (1–2 mol%) to the solution. The resulting mixture was stirred at room temperature for 30 min–1 h. The organic layer was washed with an aqueous saturated solution of $NaHCO_3$ (2×25 mL) and then dried over anhydrous $MgSO_4$, concentrated *in vacuo* and purified by column chromatography (30% ethyl acetate/hexane) to afford the desired products.

General procedure for the preparation of Morita-Baylis-Hillman butyrates 6a–d: MBH adducts **4a–d** were dissolved in 2-methyltetrahydrofuran (10–20 mL) and trimethylamine (1–1.6 eq.), butyric anhydride (1–1.3 eq.) and DMAP (2–4 mol%) were added to these stirring solutions. The resulting mixture was stirred at room temperature for 40 min–1 h. The organic layer was washed with aqueous saturated $NaHCO_3$ (2×25 mL) and then dried over anhydrous $MgSO_4$. After removal of the solvent, the product was purified by silica gel column chromatography (30% ethyl acetate/hexane).

General procedure for the enzymatic kinetic resolution reactions by hydrolysis: To a mixture of enzyme (8 mg) in phosphate buffer (0.1 M, pH = 7, 950 μl) in an Eppendorf tube was added substrate (8 mg) dissolved in acetone (50 μl). Reactions were incubated at 25–30 °C and were monitored using TLC then analysed by chiral HPLC when hydrolysis was observed.

Scale-up of enzymatic kinetic resolution reaction by hydrolysis

Isolation of the (+)-4b enantiomer: A pH 7 phosphate buffer solution (21 mL) containing Novozyme 435 (0.730 g) at pH 7 was added to a stirred solution of acetone (3 mL) and **5b** (0.73 g) at room temperature. The mixture was left to stir for 97 h, after which the product was extracted using ethyl acetate. Further purification by column chromatography (30% ethyl acetate/hexane) afforded the products as light yellow oils: (+)-**4b** [253 mg, c = 44%, ee = 98%; $[\alpha]_D = +50.5$ (c 0.5, MeOH)] as a single enantiomer and scalemic (−)-**5b** (296 mg, c = 44%, ee = 77%).

Isolation of the (−)-5b enantiomer: A phosphate buffer solution (18.5 mL) containing *Pseudomonas cepacia* lipase (0.295 g) at pH 7 was added to a stirred solution of acetone (1.5 mL) and **5b** (0.295 g) at room temperature. The mixture was left to stir for 34 h, after which the product was extracted using ethyl acetate. Further purification by column chromatography (30% ethyl acetate/hexane) afforded the products as light yellow oils: (−)-**5b** as an enantiopure product [168 mg, c = 55%, ee = 97%; $[\alpha]_D = -28.0$ (c 0.5, MeOH)] and scalemic (+)-**4b** (trace amount isolated, c = 55%, ee = 78%).

Isolation of the (−)-4b enantiomer: A phosphate buffer solution (10 mL) containing a non-selective lipase (117 mg) at pH 7 was added to a stirred solution of acetone (1 mL) and (−)-**5b** (117 mg) at room temperature. The mixture was left to stir for 26 h, after which the product was extracted using ethyl acetate. Further purification by column chromatography (30% ethyl acetate/hexane) afforded the product (−)-**4b** (46 mg, c = 49%, ee = 95%) and recovered starting material (−)-**5b** (52 mg, c = 49%, ee = 93%) as single enantiomers.

Table 6. Transesterification reactions using CAL–A.

Entry	Acyl donor R[1]	Solvent	Reaction time [h]	Conv. [%]	ee_p [%]	ee_s [%]	E
1	Me	butyl acrylate	96	23	82	24	13
2	Me	THF	96	22	93	26	36
3	Me	2-MeTHF	96	32	89	46	27
4	Me	MTBE	96	38	62	38	6
5	Et	butyl acrylate	120	0	–	–	0
6	Et	Et_2O	120	30	82	35	14
7	Et	2-MeTHF	120	17	88	18	19
8	Et	THF	72	18	92	20	29
9	Pr	butyl acrylate	120	0	–	–	0
10	Pr	Et_2O	120	9	81	8	10
11	Pr	2-MeTHF	120	6	46	3	3
12	Pr	THF	22	13	80	12	10

General procedure for the preparation of Mosher's ester derivatives: A mixture of DCC (15.7 mg, 2 eq.), DMAP (0.2 eq.), (+) or (−) alcohol (1 eq.) and (*R*)- or (*S*)-MPTA (24 mg, 2.6 eq.) in DCM (5 mL) was stirred at room temperature for 6 h. DCM (2×5 mL) and water (2×5 mL) were added to the reaction mixture and shaken. The DCM layer was separated and dried over anhydrous magnesium sulphate and purified using silica gel column chromatography (elution with 20–30% ethyl acetate/hexane).

General procedure for the enzymatic kinetic resolution reaction by transesterification: Acetic, butyric or propionic anhydride (15 mg) and enzyme (3 mg) were added to a solution of 4b (12 mg, 0.25 mmol) in the chosen solvent (1 mL) in a 2.5 mL Eppendorf tube and stirred at 30 °C. Reactions were monitored by TLC and then analysed by chiral HPLC once the reaction was observed.

Conclusions

All eight of the synthesised substrates were successfully resolved employing Enzymatic Kinetic Resolution (EKR), with instances where ee values of more than 99% and E ratio > 400 were obtained. The best selectivity overall was demonstrated for a butyrate ester substrate, **6c**, bearing a *p*-cyano substituent on the phenyl ring. The enzymes tested all preferentially hydrolysed the (*S*)-enantiomer of the acetyl and butyl derivatives, as demonstrated for **5b** using the Mosher's double derivatisation protocol. EKR thus provides a "greener" procedure for obtaining enantiopure MBH adducts which are key intermediates in the synthesis of compounds of biological importance. EKR of aromatic MBH adducts by transesterification was demonstrated here for the first time. The enzyme used to achieve this was CAL–A, which has not previously been demonstrated to effect the EKR of aromatic MBH adducts. Using acetic anhydride in either THF or **2**-MeTHF gave the best E values within a reasonable time of 96 h. This result opens the door for the use of redox catalysts, together with lipases, in the DKR of aromatic MBH adducts.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: enzymatic kinetic resolution · lipases · molecular modelling · Morita-Baylis-Hillman · Mosher derivatives · PCL

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