Tetrahedron: *Asymmetry* xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Hydrolase-mediated resolution of the hemiacetal in 2-chromanols: The impact of remote substitution

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ARTICLE INFO

Article history:
Received 15 March 2017
Revised 27 March 2017
Accepted 28 March 2017
Available online xxxx

ABSTRACT

Hydrolase-catalysed dynamic kinetic resolutions of chroman-2-ol and 3-methyl chroman-2-ol can be effected with up to 88% conversion and 92% *ee* through the use of organic solvents. Extension to the resolution of the tolterodine precursor **1** proved more challenging. The presence of the remote phenyl substituent had a significant impact on the resolution and it was not possible to achieve high enantioselectivity together with efficient conversion from the focussed panel of enzymes screened.

1. Introduction

The use of enzymes as catalysts in organic synthesis is associated with several economic and environmental benefits. Biocatalytic processes typically operate at ambient temperature, neutral pH and without the need for functional group protection. Moreover, enzymatic catalysis can in some cases replace classical resolutions or more traditional synthetic methods which often require metal catalysts with associated cost and toxicity implications. Hydrolases have found particularly widespread use in the pharmaceutical, fine chemicals and textiles industries, amongst others.[1] This family of enzymes boasts several characteristics, which make them excellent catalysts for asymmetric synthesis; they do not require external cofactors and often display broad substrate specificity, stability and excellent enantioselectivity, even in organic solvents.[2]

Hydrolases are widely used for kinetic resolutions, one of the most reliable methods for the enzyme-mediated production of enantiopure compounds,[3,4] despite the limitation of a maximum yield of 50%. A dynamic kinetic resolution is a more attractive approach since quantitative yields of the desired (enantiopure) stereoisomer are attainable in compounds bearing a single racemisable stereocentre.[5,6]

Lipases, a sub-group of hydrolases whose natural function is the hydrolysis of lipids, have been extensively utilised as biocatalysts, most notably in the resolution of alcohols, amines and carboxylic acids.[7] Dynamic kinetic resolutions have also been achieved with lipases, principally through racemisation of a stereocentre featuring an alcohol/amine functionality.[5,8] The racemisation is often induced via a compatible transition metal catalyst[8] or, in some cases, it may even occur spontaneously.[9]

The anomeric centre in the hemiacetal functional group has been previously resolved using lipase-mediated (*trans*) esterification. Feringa et al. have reported the dynamic resolution of 5-acyloxy-2(5*H*)-furanones and pyrrolidinones and 6-acyloxy-2*H*-pyran-3(6*H*)-one.[10,11] Deoxysugars bearing a second stereocentre have also been enzymatically acetylated, furnishing a mixture of the *cis*- and *trans*-stereoisomers and in some cases the ring-opened aldehyde product.[12] Unsurprisingly, due to the dynamic nature of the hemiacetal functional group, enzymatic enantiodiscrimination can be difficult to achieve.

Lactol **1** (Fig. 1) is of interest to our group as it is an intermediate in the preparation of tolterodine and fesoterodine, antimuscarinic drugs.[13] Currently these drugs are synthesised via a classical resolution from a mix of stereoisomers; therefore, a biocatalytic approach is attractive to enable a greener, more sustainable synthetic route. The lactol exists in dynamic equilibrium whereby the hemiacetal interconverts with the corresponding ring-opened hydroxyaldehyde (Fig. 2). Therefore, in approaching the resolution of lactol **1**, there are two distinct processes to be considered; the first involves the classical resolution of the remote stereogenic centre, while the second involves the dynamic resolution of the hemiacetal centre (Figure 2). From a synthetic perspec-

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http://dx.doi.org/10.1016/j.tetasy.2017.04.001
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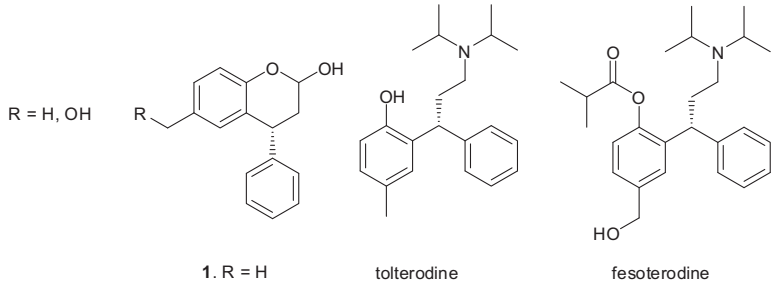


Figure 1.

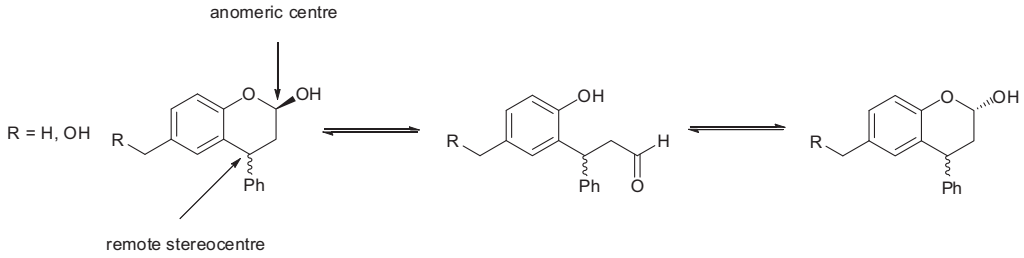


Figure 2.

tive, it is the resolution of the remote stereocentre which is important, since the hemiacetal centre is labile and not retained in tolterodine and fesoterodine.

Therefore, theoretically a single enantiomer (from the 4 possible lactol stereoisomers) can be garnered from a lipase-mediated acetylation, giving a possible conversion of 50%. Building on previous work in our group on substrates featuring a remote stereocentre,[14] we aimed to achieve a highly selective resolution of the remote benzylic stereocentre of lactol **1**. For the purposes of this work stereocontrol at the anomeric centre is not critical. A resolution of this type would therefore require either: (1) an enzyme which is non-selective at the anomeric centre but selective at the remote stereocentre or; (2) dynamic kinetic resolution involving enzymatic acylation of only one of the 4 possible lactol enantiomers, i.e. a kinetic resolution of the remote stereocentre combined with a dynamic resolution of the anomeric centre. A resolution of either type could enable a maximum possible yield of 50% with enzymatic discrimination of the remote stereocentre.

2. Results and discussion

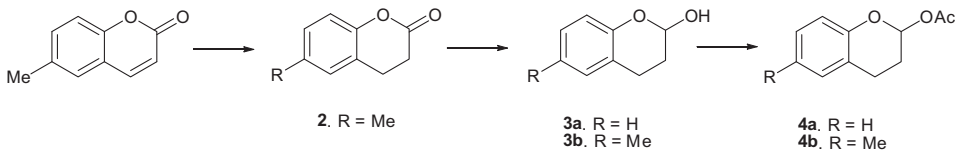
2.1. Model substrates

As limited examples of dynamic resolutions of hemiacetals have been reported only in monocyclic systems,[11] model systems **3a** and **3b** were first explored to ensure the fused aromatic ring system was compatible with this methodology. Initial enzyme screening focused on the model compounds **3a** and **3b.** Firstly, the aim was to establish if a dynamic kinetic resolution at the anomeric centre was feasible to clarify the reactivity of the hemiacetal functional group with the hydrolases. Two potential sites of esterification were possible; the hydroxyl group of the closed hemiacetal functionality and also the phenolic position of the ring-opened tautomer (Fig. 2). Chroman-2-ol **3a** and its 6-methyl analogue **3b** were prepared from commercially available chroman-2-ones via known methods[15] and then acetylated to give the racemic products **4a** and **4b** (Scheme 1).

A focused panel of diverse commercial hydrolases was tested for activity against the chosen substrate; the widely used vinyl acetate was selected as both the acyl source and the solvent for the initial enzyme screening (Table 1, selected results shown). As summarised in Table 1**,** from a panel of over 50 biocatalysts, several enzymes were active against substrates **3a** and **3b**, generating the acylated products **4a** and **4b**. Indeed a number of hydrolases gave high enantioselectivity, albeit with modest efficiency. For compound **3a**, of the 50 enzymes tested, 10 gave a conversion greater than background acetylation (≈1%). In particular, lipases from the *Alcaligenes* family were relatively selective. Immobilised CAL-B furnished the highest *ee* (95%) for product **4a** of all the enzymes tested albeit with low conversion in 24 h. Extending the reaction time to 120 h gave the acetylated product **4a** with excellent *ee* and 20% yield (Table 1, entry 6).

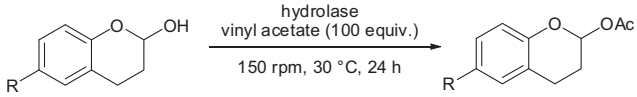
2.2. Use of organic solvents

The use of organic solvents in conjunction with an acyl source is a well-established approach for improving yield and/or enantioselectivity in resolutions involving hydrolase biocatalysts.[16] Accord-



Scheme 1. Synthesis of model lactols **3a** and **3b** and their acetylated analogues, **4a** and **4b**. Reagents and conditions: (i) Pd/C, H_2, EtOAc; (ii) DIBAL, MePh, −78 °C; (iii) Ac_2O, DMAP, pyridine, DCM.

Table 1
Enzyme screen against substrates **3a** and **3b**



3a. R = H
3b. R = Me

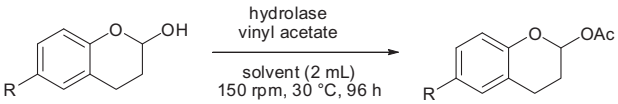
4a. R = H
4b. R = Me

Entry	R	Hydrolase	Conversion[a] (%)	Product	Product (% *ee*)[b]
1	H	Lipase A from *Candida rugosa*	25	**4a**	73 (*R*)
2	H	Lipase from *Pseudomonas cepacia*	29	**4a**	58 (*R*)
3	H	Lipase B from *Alcaligenes* sp.	14	**4a**	80 (*R*)
4	H	Lipase D from *Alcaligenes* sp.	28	**4a**	78 (*R*)
5	H	*Candida antarctica* lipase B (immob.)	8	**4a**	94 (*R*)
6	H	*Candida antarctica* lipase B (immob.)*	20	**4a**	95 (*R*)
7	H	Lipase E from *Alcaligenes* sp.	47	**4a**	77 (*R*)
8	H	Lipase from *Pseudomonas stutzeri*	13	**4a**	33 (*R*)
9	Me	Lipase A from *Candida rugosa*	26	**4b**	64 (+)
10	Me	Lipase from *Candida cylindracea*	28	**4b**	65 (+)
11	Me	Lipase E from *Alcaligenes* sp.	30	**4b**	70 (+)
12	Me	Lipase from *Pseudomonas stutzeri*	19	**4b**	33 (+)
13	Me	Lipase from *Thermomyces lanuginosus*	12	**4b**	97 (+)
14	Me	Lipase B from *Candida rugosa*	20	**4b**	59 (+)
15	Me	*Candida antarctica* lipase A	25	**4b**	8 (+)

Selected results. Lactol *ee* not determined due to spontaneous racemisation.
* Reaction time 120 h.
[a] Determined by ^{1}H NMR.
[b] Determined by chiral HPLC analysis. **4a** [Phenomenex Cellulose 4, hexane/*i*-PrOH = 99:1, flow rate 1 mL/min, 25 °C, λ = 209.8 nm]. **4b** [Phenomenex Cellulose 4, hexane/*i*-PrOH = 95:5, flow rate 1 mL/min, 25 °C, λ = 209.8 nm].

ingly, the lead enzymes from the previous screening were further screened against the substrates with vinyl acetate as the acyl source, with variation of the reaction medium. Selected results are shown in Table 2. As can be seen from this table, the use of organic solvents was found to have a profound effect on both conversion and enantioselectivity. Initially, 4.2 equiv of vinyl acetate were used (entries 1–7; R = H, substrate **3a**). The *Alcaligenes* family of lipases were especially active and enantioselective with this

Table 2
Solvent screen with active enzymes against substrates **3a** and **3b**



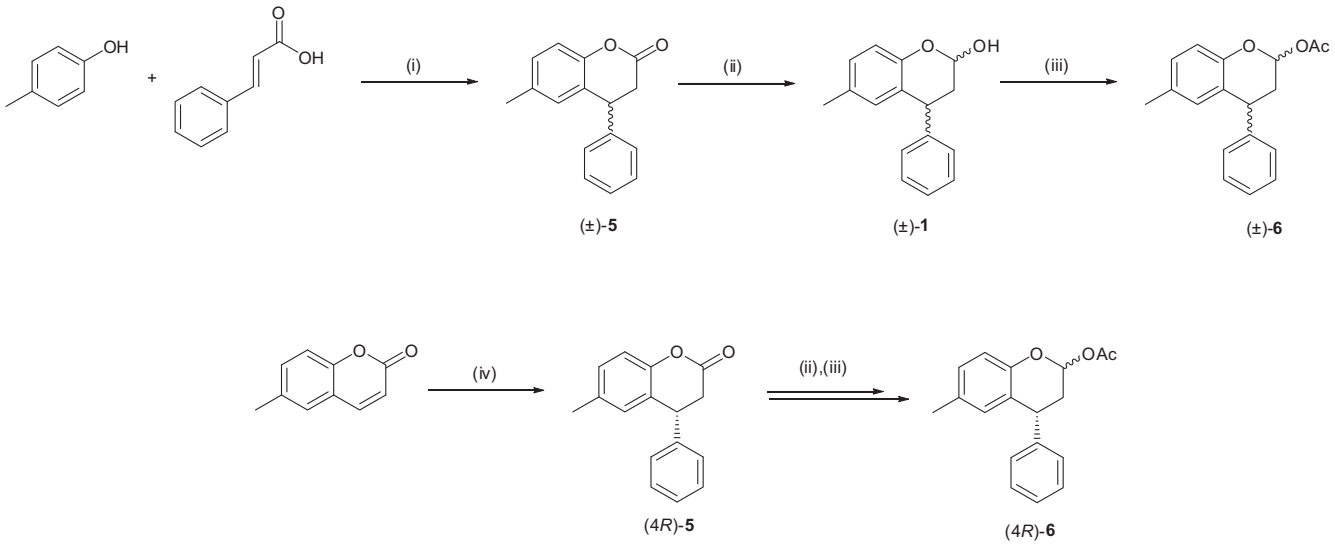
3a. R = H
3b. R = Me

4a. R = H
4b. R = Me

Entry	R	Hydrolase	Solvent	Equivalents vinyl acetate	Conversion[a] (%)	Product	Product (% *ee*)[b]
1	H	*Candida Antarctica* lipase B (immob.)	Hexane	4.2	13	**4a**	91 (*R*)
2	H	Lipase B from *Alcaligenes* sp.	Hexane	4.2	40	**4a**	70 (*R*)
3	H	Lipase B from *Alcaligenes* sp.	Toluene	4.2	76	**4a**	77 (*R*)
4	H	Lipase D from *Alcaligenes* sp.	Toluene	4.2	35	**4a**	73 (*R*)
5	H	Lipase D from *Alcaligenes* sp.	Diethyl ether	4.2	67	**4a**	75 (*R*)
6	H	Lipase E from *Alcaligenes* sp.	Hexane	4.2	81	**4a**	56 (*R*)
7	H	Lipase E from *Alcaligenes* sp.	Toluene	4.2	26	**4a**	77 (*R*)
8	H	Lipase A from *Candida rugosa*	Heptane	4.2	50	**4a**	59 (*R*)
9	Me	Lipase E from *Alcaligenes* sp.	Hexane	100	93	**4b**	44 (+)
10	Me	Lipase E from *Alcaligenes* sp.	Heptane	100	58	**4b**	58 (+)
11	Me	Lipase E from *Alcaligenes* sp.	Toluene	100	53	**4b**	62 (+)
12	Me	Lipase A from *Candida rugosa*	Hexane	100	48	**4b**	67 (+)
13	Me	Lipase A from *Candida rugosa*	Toluene	100	26	**4b**	69 (+)
14	Me	Lipase from *Candida cylindracea*	Hexane	100	63	**4b**	66 (+)
15	Me	Lipase from *Candida cylindracea*	Heptane	100	41	**4b**	63 (+)
16	Me	Lipase from *Candida cylindracea*	Toluene	100	30	**4b**	73 (+)
17	Me	Lipase from *Thermomyces lanuginosus*	Hexane	100	88	**4b**	92 (+)
18	Me	Lipase from *Thermomyces lanuginosus*	Heptane	100	38	**4b**	95 (+)
19	Me	Lipase from *Thermomyces lanuginosus*	Toluene	100	55	**4b**	94 (+)
20	Me	Lipase from *Thermomyces lanuginosus*	Diisopropyl ether	100	27	**4b**	96 (+)

Selected results. Lactol *ee* not determined due to spontaneous racemisation.
[a] Determined by ^{1}H NMR.
[b] Determined by chiral HPLC analysis. **4a** [Phenomenex Cellulose 4, hexane/*i*-PrOH = 99:1, flow rate 1 mL/min, 25 °C, λ = 209.8 nm]. **4b** [Phenomenex Cellulose 4, hexane/*i*-PrOH = 95:5, flow rate 1 mL/min, 25 °C, λ = 209.8 nm].

Please cite this article in press as: Gavin, D. P.; et al. *Tetrahedron: Asymmetry* (2017), http://dx.doi.org/10.1016/j.tetasy.2017.04.001



Scheme 2. Synthesis of enantiopure and racemic lactone **5**, lactol **1** and acetylated analogue **6**. Reagents and conditions: (i) I_2, 120–130 °C, neat, 70%; (ii) DIBAL, MePh, −78 °C, (±)-**1**: 66%; (4*R*)-**1**: 65%; (iii) Ac_2O, DMAP, Pyridine, CH_2Cl_2; (iv) $Rh(acac)_2$, (*R*)-Josiphos, $PhB(OH)_2$, 1,4-dioxane/H_2O, 60 °C, 39%.

substrate in a range of organic solvents. For example, Lipase B from *Alcaligenes sp* furnished the product with 77% *ee* with 76% conversion in toluene (Table 2, entry 3), indicating that a resolution involving a dynamic process was occurring.

Due to the low conversion of **3b** at a similar loading of vinyl acetate, 100 equivalents of the acyl source were used (Table 2, entries 9–20). There was no evidence of an increase in the rate of the background non-enzymatic acylation under these conditions.

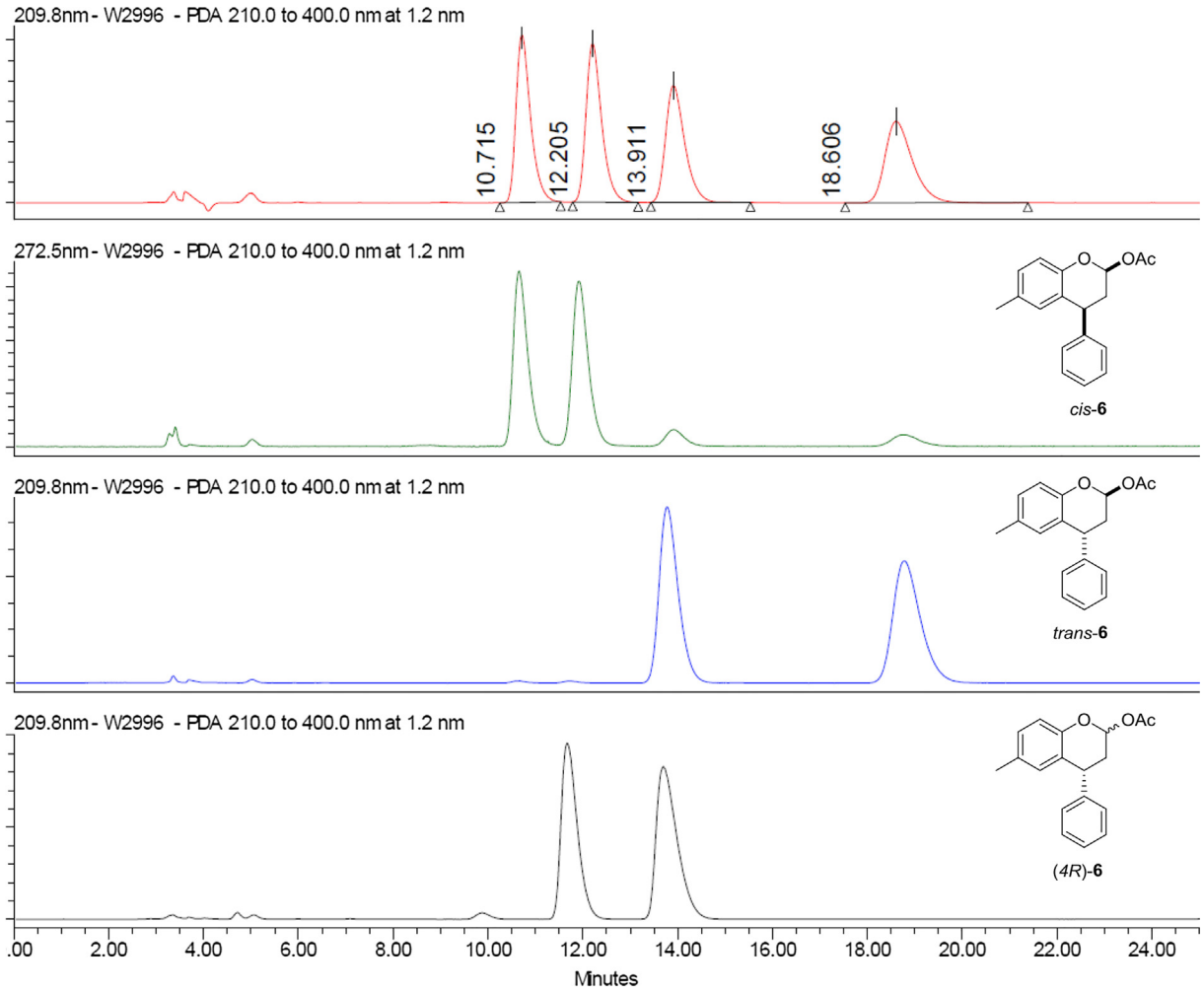


Figure 3. Stacked HPLC chromatograms which allowed identification of each stereoisomer. Shown: Mixture of *cis*-**6** and *trans*-**6** *cis*-**6** (slightly impure with *trans*-**6**) *trans*-**6** enantiopure (4*R*)-**6**. Experiments performed using Daicel Chiralcel OD-H column, hexane/*i*-PrOH = 98:2, flow rate 1 mL/min, 25 °C, λ = 209.8 nm.

Lipase from *Thermomyces lanuginosus* was particularly selective for substrate **3b** in a range or solvents. In hexane the conversion was 88% with 92% *ee* (Table 2, entry 17). Other hydrocarbon media such as heptane and toluene were also effective media for this asymmetric transformation, giving enantioselectivities of 95% *ee* and 94% *ee* respectively, albeit at a lower conversion (Table 2, entries 18 and 19). Despite the structural similarity of substrates **3a** and **3b**, there was not a large crossover of activity amongst the enzymes with these substrates, although CAL-B and members of the *Alcaligenes* family were active against both compounds.

2.3. Tolterodine intermediate

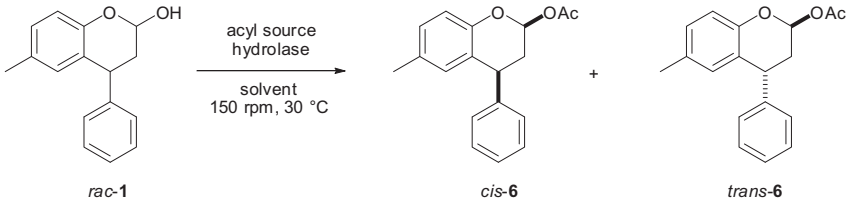
Having established that a dynamic resolution was possible and that exclusive acetylation of the hemiacetal functionality in **3a** and **3b** could furnish an enantioenriched product in high conversion, we turned our attention to the tolterodine intermediate **1**. Condensation of cinnamic acid with *p*-cresol furnished chroman-2-one **5**,[17] which was reduced to the corresponding lactol **1** at low temperature. The hemiacetal group was then acetylated to give compound **6** (Scheme 2) in a 1:1.5 *cis*:*trans* mixture. The 4 stereoisomers of acetylated product **6** were resolved on a single HPLC trace along with lactol **1** to facilitate analysis of the enzyme-catalysed acetylations. Compound *trans*-**6** was preferentially crystallised from a crude mixture of the two diastereomers in ethanol and a crystal of this diastereomer was subsequently grown. The tolterodine precursor (4*R*)-**5** (Scheme 2) was also synthesised *via* a known method.[18] Subsequent reduction gave (4*R*)-**1** and acetylation gave (4*R*)-**6**, which allowed for the identification of each stereoisomer on the HPLC trace (Fig. 3).

With the analytics for the reaction in hand, we then undertook the biocatalyst screening (Table 3). Again, vinyl acetate was both the solvent and acyl source for the initial screen. Although over 50 hydrolases were tested, the vast majority returned starting material only. Evidently, overall acetylation of hemiacetal **1** is more challenging than that of the model systems **3a** and **3b**, with lower conversions than in the absence of the aryl ring at the 4-position of the lactol (see Table 1). By far the most active enzyme was the lipase from *Pseudomonas stutzeri*, which exclusively furnished the *cis*-acetate **6** in 39% conversion in 4 h. This enzyme also displayed some enantiodiscrimination, giving *cis*-**6** in a 3:1 ratio of enantiomers (Table 3, entry 6). Interestingly, this biocatalyst was considerably less active with model substrates **3a** and **3b**, with poor conversion and enantioselection for both (Table 1, entries 8 and 12). Lipase from *Pseudomonas stutzeri* uniquely provided a high conversion, and, most significantly, only the *cis*-isomer was acetylated indicating that the efficient enzymatic acetylation of the hemiacetal is sensitive to the remote stereogenic centre, auguring well for our overall objective of resolution of the remote stereocentre. Encouraged by the high activity exhibited by this enzyme against our chosen substrate, we then screened organic solvents as we had already seen a large effect in our studies with the model substrate. Indeed, the effect of organic solvents on this transformation was exhaustively investigated. Disappointingly, the lipase from *Pseudomonas stutzeri* did not yield the acylated product with high *ee* in any of a wide range of solvents, and indeed addition of organic solvents resulted in decreased enantioselectivity relative to the transformations in neat vinyl acetate. The use of hydrocarbons hexane and heptane dramatically reduced the enantioselectivity of the process (Table 4, entries 4 and 5), in contrast to their effect on the resolution of products **4a** and **4b**. Decreasing the amount of vinyl acetate to 5 equiv in these solvents, with the aim of decreasing the rate of the reaction and potentially allowing increased enantioselection, also resulted in poor *ee* (Table 4, entries 9 and 10).

Another factor which can influence the selectivity and conversion is variation of the acyl source. For example, the use of enol esters such as vinyl acetate and isopropenyl acetate, help to overcome the challenge of reversibility in enzyme-mediated (*trans*)esterifications. The choice of acyl source can have a dramatic impact on the enzymatic resolution and many other acyl sources have been investigated, including carboxylic acids, methoxyacetates and anhydrides.[19] Herein we found that the less reactive esters ethyl acetate and isopropyl acetate resulted in little or no conversion to product (Table 4, entries 2 and 3) when the lipase from *Pseudomonas stutzeri* was used to promote the reaction, which is in contrast to the outcome in vinyl acetate with the same biocatalyst (reaching 39% conversion in only 4 h). Whilst isopropenyl acetate facilitated conversion to product, it led to less enantioselectivity and considerably less activity than its vinyl counterpart, taking 24 h to reach 53% conversion with 26% *ee* (Table 4, entry 1). Therefore, in a broad range of reaction conditions, despite the consistent, excellent selectivity for the *cis*-product rather than the *trans*-, this biocatalyst did not exhibit sufficient enantiodiscrimination to deliver the acetylated lactol **6** with high *ee*.

The most selective enzyme tested in the initial screen was acylase from *Aspergillus* sp. (Table 3, entry 8), however this transformation suffered from very poor conversion (6%). Despite the low activity of the enzyme under these conditions, the exclusive conversion to a single *cis*-stereoisomer [>98% *ee*, (2*S*,4*S*)] of product

Table 3
Enzyme screen against substrate **1**



rac-**1** *cis*-**6** *trans*-**6**

Entry	Hydrolase	Time (h)	Solvent	Acyl source (equiv)	*cis*-**6**		*trans*-**6**	
					Conversion (%)	*ee* (%)	Conversion (%)	*ee* (%)
1	Lipase A from *Candida rugosa*	24	–	Vinyl acetate (100)	8	77 (4*R*)	6	>98 (4*S*)
2	Lipase B from *Candida rugosa*	24	–	Vinyl acetate (100)	5	82 (4*R*)	5	>98 (4*S*)
3	Lipase A from *Alcaligenes* sp.	24	–	Vinyl acetate (100)	7	11 (4*S*)	<1	–
4	Lipase F from *Alcaligenes* sp.	24	–	Vinyl acetate (100)	14	12 (4*S*)	<1	–
5	Lipase from *Pseudomonas stutzeri*	12	–	Vinyl acetate (100)	57	39 (4*S*)	<1	–
6	Lipase from *Pseudomonas stutzeri*	4	–	Vinyl acetate (100)	39	50 (4*S*)	<1	–
7	Lipase from *Candida cylindracea*	24	–	Vinyl acetate (100)	7	78 (4*R*)	5	>98 (4*S*)
8	Acylase from *Aspergillus* sp.	24	–	Vinyl acetate (100)	6	>98 (4*S*)	<1	–

Table 4
Screening of conditions for enzymes active against substrate **1**

Entry	Hydrolase	Time (h)	Solvent	Acyl source (equiv)	*cis*-**6**		*trans*-**6**	
					Conversion[a] (%)	*ee*[b] (%)	Conversion[a] (%)	*ee*[b] (%)
1	Lipase from *Pseudomonas stutzeri*	24	—	Isopropenyl acetate (100)	53	26 (4*S*)	<1	—
2	Lipase from *Pseudomonas stutzeri*	24	—	Ethyl acetate (100)	—	—	—	—
3	Lipase from *Pseudomonas stutzeri*	24	—	Isopropyl acetate (100)	—	—	—	—
4	Lipase from *Pseudomonas stutzeri*	24	Hexane	Vinyl acetate (100)	80	14 (4*S*)	<1	—
5	Lipase from *Pseudomonas stutzeri*	24	Heptane	Vinyl acetate (100)	72	18 (4*S*)	<1	—
6	Lipase from *Pseudomonas stutzeri*	24	Cyclo-hexane	Vinyl acetate (100)	51	29 (4*S*)	<1	—
7	Lipase from *Pseudomonas stutzeri*	24	Toluene	Vinyl acetate (100)	50	24 (4*S*)	<1	—
8	Lipase from *Pseudomonas stutzeri*	24	MTBE	Vinyl acetate (100)	42	21 (4*S*)	2	66 (4*S*)
9	Lipase from *Pseudomonas stutzeri*	16	Toluene	Vinyl acetate (5)	52	25 (4*S*)	<1	—
10	Lipase from *Pseudomonas stutzeri*	16	Hexane	Vinyl acetate (5)	74	2	2	54 (4*S*)
11	Acylase from *Aspergillus* sp.	36	—	Vinyl acetate (100)	6	98 (4*S*)	—	—
12	Acylase from *Aspergillus* sp.	36	Hexane	Vinyl acetate (100)	17	97 (4*S*)	<1	—
13	Acylase from *Aspergillus* sp.	72	Hexane	Vinyl acetate* (100)	20	94 (4*S*)	—	—
14	Acylase from *Aspergillus* sp.	36	Heptane	Vinyl acetate (100)	13	97 (4*S*)	—	—
15	Acylase from *Aspergillus* sp.	36	Toluene	Vinyl acetate (100)	6	97 (4*S*)	—	—
16	Acylase from *Aspergillus* sp.	36	Toluene	Vinyl acetate* (4)	13	94 (4*S*)	<1	—

Lactol *ee* not determined due to spontaneous racemisation.
[a] Determined by ^{1}H NMR.
[b] Determined by chiral HPLC analysis. [Daicel Chiralcel OD-H, hexane/*i*-PrOH = 98:2, flow rate 1 mL/min, 25 °C, λ = 209.8 nm].
* Reaction performed at 37 °C, 250 rpm.

6 was observed and accordingly this catalyst was pursued as a potential candidate for the DKR of product **6**. A wide-ranging screening was conducted with the goal of increasing the conversion of this enzymatic transformation. Although the conversion improved, particularly with hexane as the solvent (Table 4, entry 12), the overall efficiency of the reaction was poor. The reaction time and temperature were also increased, but this resulted in a modest increase in enzyme activity (Table 4, entry 13). All of the conditions tested furnished almost exclusively the *cis*-stereoisomer with excellent enantiopurity (up to 98% *ee*), however, the conversion to acylated product **6** was never above 20%, even after 72 h. Other acyl sources, such as isopropenyl acetate and ethyl acetate, led to complete inactivity for the acylation of **1** with this enzyme.

3. Conclusion

In conclusion, we have demonstrated that dynamic kinetic resolution is possible upon treatment of model hemiacetals **3a** (up to 76% conversion with 77% *ee*) and **3b** (up to 88% conversion with 92% *ee*) with hydrolases. Extension to include the resolution of a remote stereocentre in the tolterodine intermediate **1** proved to be more challenging in terms of efficiency, while retaining high enantioselectivity (up to >98% *ee*).

4. Experimental

4.1. General

Dry solvents were distilled prior to use as follows: dichloromethane was distilled from phosphorus pentoxide and ethyl acetate was distilled from potassium carbonate; tetrahydrofuran and toluene were distilled from sodium and benzophenone. Molecular sieves were activated by heating at 150 °C overnight. Organic phases were dried using anhydrous magnesium sulphate. Infrared spectra were measured using a Perkin Elmer FTIR UATR2 spectrometer. ^{1}H (300 MHz) and ^{13}C (75.5 MHz) NMR spectra were recorded on a Bruker Avance 300 MHz NMR spectrometer. ^{1}H (400 MHz) NMR spectra were recorded on a Bruker Avance 400 MHz NMR spectrometer. All spectra used tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ_H and δ_C) are reported in parts per million (ppm) relative to TMS and coupling constants are expressed in Hertz (Hz). Low resolution mass spectra were recorded on a Waters Quattro Micro triple quadrupole spectrometer in electrospray ionization (ESI) mode using 50% water/acetonitrile containing 0.1% formic acid as eluent; samples were made up in acetonitrile. High resolution mass spectra (HRMS) were recorded on a Waters LCT premier Time of Flight spectrometer in electrospray ionization (ESI) mode using 50% water/acetonitrile containing 0.1% formic acid as eluent; samples were made up in acetonitrile. Elemental analysis was performed by the Microanalysis Laboratory, National University of Ireland, Cork, using Perkin–Elmer 240 and Exeter Analytical CE440 elemental analysers Melting points were carried out on a uni-melt Thomas Hoover Capillary melting point apparatus and are uncorrected. Wet flash chromatography was performed using Kieselgel Silica Gel 60, 0.040–0.063 mm (Merck). Thin layer chromatography (TLC) was carried out on precoated silica gel plates (Merck 60 PF254). Visualisation was achieved by UV (254 nm) light detection and $KMnO_4$ staining. Optical rotations were measured on a Perkin–Elmer 141 polarimeter at 589 nm in a 1 cm cell; concentrations (c) are expressed in g/100 mL. Enzymes were supplied by Almac Sciences/purchased from Sigma–Aldrich chemical company (see Supporting Information). All reagents are analytical grade and purchased from Sigma–Aldrich chemical company. All enzymatic reactions were performed on a VWR Incubating Mini Shaker 4450. The enantiomeric purity of products **4a** and **4b** were determined by chiral HPLC analysis on a Phenomenex Cellulose 4 column (250 × 4.6 mm); the enantiomeric purity of dihydrocoumarin **5** was determined on a Phenomenex Amylose 2 column, both purchased from Phenomenex Inc., UK. Enantioselectivities for Compound **6** were determined on a Chiralcel OD-H column (250 × 4.6 mm), purchased from Daicel Chemical Industries, Japan. Mobile phase, flow rate, detection wavelength and temperature are stated in the appropriate Tables 1–4. HPLC analysis was performed on a Waters alliance 2690 separations module with a PDA detector. All solvents employed were of HPLC grade.

4.2. Synthesis of model substrates and derivatives

4.2.1. 6-Methylchroman-2-one 2[20]

A solution of 6-methyl coumarin (2.771 g, 16.67 mmol) and Pd/C (10 wt%, 0.566 g, 0.53 mmol, 5 mol%) in ethyl acetate (17.5 mL) was stirred under an atmosphere of hydrogen until TLC showed the dis-

appearance of the starting material (48 h). The solution was filtered through a bed of Celite® using ethyl acetate (10 mL) and concentrated to give a yellow oil, which solidified on cooling. The solid residue was dissolved in ethyl acetate (10 mL), filtered again through Celite®, and concentrated to give a pale yellow solid. The product was dried overnight under high vacuum to give a white waxy solid (2.704 g, 97%). The product did not require purification mp 77–78 °C. ν_{max}/cm^{-1} (ATR): 3476 (C—H, Ar), 2924 (C—H), 1740 (C=O), 1500 (C—C), 1209 (C—O). ^1H NMR (300 MHz, $CDCl_3$): δ 7.10–6.90 (m, 3H, ArH), 3.0–2.90 (m, 2H, CH_2), 2.81–2.72 (m, 2H, CH_2) 2.31 (s, 3H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 168.8 (C=O), 150.0, 134.0, 128.7, 128.5, 122.3, 116.7 (6 × ArC), 29.4 (CH_2), 23.8 (CH_3), 20.7 (CH_2). MS (ES^+): found 163.3. $C_9H_9O_2$ requires 163.2.

4.2.2. (±)-Chroman-2-ol (±)-3a[18]

To a three-neck round-bottom flask equipped with nitrogen bubbler and dropping funnel were added dihydrocoumarin (5 g, 33.7 mmol) and dry toluene (100 mL). The solution was cooled to −78 °C and DIBAL [1 M solution in hexanes, (37 mL, 37.0 mmol, 1.1 equiv)] was added dropwise over a period of 30 min. The reaction was stirred for 2 h, allowed to warm to room temperature and then quenched with H_2O (30 mL). The resulting white suspension was filtered over Celite® and washed through with diethyl ether (100 mL). The phases were separated and the aqueous phase was extracted with diethyl ether (200 mL). The combined organic phases were washed with H_2O (200 mL) and brine (200 mL). The combined organic layers were dried, filtered and the solvent was removed under vacuum to give the product (4.83 g, 95%) as a colourless oil which was used without further purification. ν_{max}/cm^{-1} (ATR): 3401 (OH), 3040 (C—H, Ar), 2939 (C—H), 1220 (C—O). ^1H NMR (300 MHz, $CDCl_3$): δ 7.14–7.04 (m, 2H, ArH), 6.92–6.80 (m, 2H, ArH), 5.62 (dd, J = 4 Hz, J = 2.6 Hz, 1H, OC*H*OH), 3.05–2.99 (m, 2H, CH_2 and OH), 2.76–2.66 (m, 1H, CH_2), 2.11–1.93 (m, 2H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 152.1, 129.4, 127.6, 122.2, 121.0, 117.0 (6 × ArC), 92.3 (OCHOH), 27.2, 20.4 (2 × CH_2). HRMS (ES^-): found 149.0608. $C_9H_9O_2$ requires 149.0603.

4.2.3. (±)-6-Methylchroman-2-ol (±)-3b[21]

This was prepared using the procedure for **3a** from 6-methylchroman-2-one **2** (1.557 g, 9.6 mmol) and DIBAL solution (1 M in hexanes, 11.2 mL, 11.2 mmol, 1.16 equiv) in dry toluene (30 mL). The crude product was purified by column chromatography on silica gel using hexane/ethyl acetate (85:15) to give the pure product as a colourless oil (0.93 g, 59%). ν_{max}/cm^{-1} (ATR): 3401 (OH), 2939 (C—H), 1498, 1206 (C—O). ^1H NMR (300 MHz, $CDCl_3$): δ 7.00–6.78 (m, 2H, ArH), 6.71 (d, J = 8.1 Hz, 1H, ArH),5.58 (br s, 1H, C*H*OH), 3.25 (s, 1H, OH), 3.04–2.82 (m, 1H, CH_2), 2.66 (dt, J = 16.4 Hz, J = 5.3 Hz, 1H, CH_2), 2.25 (s, 3H, CH_3), 2.11–1.85 (m, 2H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 149.7, 130.1, 129.6, 128.0, 121.7, 116.6 (6 × ArC), 92.1 (CHOH), 27.1 (CH_2), 20.5 (CH_3), 20.3 (CH_2). HRMS$^+$: found: 147.0808 $[M-H_2O]^+$. $C_{10}H_{11}O$ requires 147.0810.

4.2.4. (±) Chroman-2-yl acetate (±)-4a[22]

To a solution of lactol (±)-**3a** (0.097 g, 0.648 mmol) in dichloromethane (10 mL) were added acetic anhydride (0.48 mL, 5 mmol, 7.7 equiv), DMAP (5 mg) and pyridine (1 mL). The resulting solution was stirred for 4 h. A saturated solution of $NaHCO_3$ (10 mL) was added and the reaction was stirred vigorously until effervescence ceased (approximately 30 min). The layers were separated and the aqueous phase was washed with dichloromethane (2 × 10 mL). The organic layers were combined and washed with saturated $CuSO_4$ (30 mL), saturated $NaHCO_3$ solution (30 mL), water (20 mL) and brine (30 mL). The organic layer was dried, filtered and the solvent was removed under vacuum to give 0.12 g (97%) of the pure product as a colourless oil. ν_{max}/cm^{-1} (ATR): 2938 (C—H), 1748 (C=O), 1490, 1201, 1199 (C—O). ^1H NMR (300 MHz, $CDCl_3$): δ 7.05–7.17 (m, 2H, ArH), 6.84–6.96 (m, 2H, ArH), 6.52 (app. t, 1H, OC*H*OCH$_3$), 2.92–3.05 (m, 1H, CH_2), 2.65–2.76 (m, 1H, CH_2), 1.97–2.17 (m, 5H, CH_2 and CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 170 (C=O), 151.7, 129.4, 127.8, 121.8, 121.5, 117.2 (6 × ArC), 90.4 (*C*HOCH$_3$), 25.2 (CH_2), 21.3 (CH_3), 19.8 (CH_2). HRMS: MH^+, found 193.0867. $C_{11}H_{13}O_3$ requires 193.0865. Enantiomers separated using Phenomenex Cellulose 4 column (250 × 4.6 mm), conditions: *n*-hexane/*i*-PrOH = 99:1, flow rate 1 mL/min, 25 °C, λ = 209.8 nm; t_1 = 7.6, t_2 = 8.3.

4.2.5. (±)-6-Methylchroman-2-yl acetate (±)-4b

This was prepared using the procedure for **4a** from compound **3a** to give a colourless oil (0.093 g, 70%) which used without further purification. ν_{max}/cm^{-1} (ATR): 2933, 1748 (C=O), 1498, 1199. ^1H NMR (300 MHz, $CDCl_3$): δ 6.84–6.98 (m, 2H, ArH), 6.76 (d, J = 8.2, 1H, ArH), 6.50 (t, J = 2.6, 1H, OC*H*OCH$_3$), 2.86–3.03 (m, 1H CH_2), 2.57–2.74 (m, 1H, CH_2), 2.26 (s, 3H, CH_3), 1.90–2.18 (m, 5H, CH_2 & CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 169.9, 149.3, 130.7, 129.6, 128.2, 121.3, 116.8, 90.3, 25.2, 21.2, 20.5, 19. HRMS$^+$: found: 147.0816 $[M-OAc]^+$. $C_{10}H_{11}O$ requires 147.0816. Enantiomers separated using Phenomenex Cellulose 4 column (250 × 4.6 mm), conditions: *n*–hexane/*i*-PrOH = 95:5, flow rate 1 mL/min, 25 °C, λ = 209.8 nm; t_1 = 6.0, t_2 = 6.5.

4.3. Synthesis of tolterodine lactol and derivatives

4.3.1. (±)-6-Methyl-4-phenylchromanone (±)-5[17]

To a solution of *trans*-cinnamic acid (10.22 g, 69 mmol) in *p*-cresol (7.2 mL, 69 mmol, 1 equiv) was added I_2 (3.5 g, 13.8 mmol, 20 mol %). The solution was stirred at 130 °C for 3 h. It was then allowed to cool to room temperature, dissolved in ethyl acetate (300 mL) and washed with saturated aqueous sodium thiosulfate solution (2 × 100 mL), H_2O (100 mL) and brine (200 mL). The organic layer was then passed through a silica plug and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel with hexane/diethyl ether (5:1) to afford the pure product (±)-**5** (11.06 g, 70%) as a white solid (mp 76–78 °C). ν_{max}/cm^{-1} (ATR): 3028 (C—H), 1763 (C=O), 1493, 1126 (C—O). ^1H NMR (300 MHz, $CDCl_3$): δ 7.38–7.27 (m, 3H, ArH), 7.18–7.13 (m 3H, ArH), 7.09 (dd, J = 8.3 Hz, J = 1.8 Hz, 1H, ArH), 6.78 (s, 1H, ArH), 4.29 (app t, J = 6.9 Hz, CH), 3.10–2.93 (m, 2H, CH_2), 2.25 (s, 3H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 168.0 (C=O), 149.8, 140.7, 134.5, 129.5, 129.3, 128.8, 127.8, 127.7, 125.5, 117.0 (10 × ArC), 40.9 (CH), 37.3 (CH_2), 20.9 (CH_3).

4.3.2. (4*R*)-6-Methyl-4-phenylchromanone (4*R*)-5[18]

To a solution of Rh(acac)(C_2H_4)$_2$ (4.6 mg, 18 μmol), (*R*)-Josiphos (12.6 mg, 9.9 μmol) and 6-methylcoumarin (96 mg, 0.60 mmol) in a deoxygenated mixture of 1,4-dioxane (2 mL) and H_2O (0.20 mL) was added PhB(OH)$_2$ (0.732 g, 6 mmol). The mixture was stirred at 65 °C for 8 h and then passed through a short pad of silica gel with Et_2O as eluent, and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel with hexane/diethyl ether (5/1) as eluent to afford the pure product (4*R*)-**5** (28 mg, 39%) as a white solid (with identical spectroscopic properties as those above) in >99% *ee*.%. The *ee* was determined on a Phenomenex Amylose 2 column with 90:10 hexane/isopropanol, flow = 1 mL/min, wavelength = 209.8 nm. Retention time: 14 min [(*S*)-enantiomer], 16 min [(*R*)-enantiomer]. $[\alpha]_D^{20} = -9$ (*c* 0.2, $CHCl_3$). Absolute configuration confirmed by comparison with literature value.[18]

4.3.3. (±)-6-Methyl-4-phenylchroman-2-ol 1[18]

This was prepared using the procedure for **3a** from (±)-6-methyl-4-phenylchromanone **5** (5.81 g, 24.38 mmol) and DIBAL

solution (1 M in hexanes, 26.8 mL, 26.8 mmol, 1.1 equiv) in dry toluene (80 mL). The crude product was purified by column chromatography on silica gel using 100% DCM to give the pure product as a colourless oil which solidified overnight to give a white waxy solid (3.85 g, 66%) in a 4:1 mix of diastereomers. Mp 86–88 °C. ν_{max}/cm^{-1} (ATR): 3433 (O—H), 3031 (Ar C—H), 2968 (C—H), 1493, 1202, 1012 (C—O). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.38–7.16 (m,5H, ArH), 6.94 (m, 1H, ArH), 6.81–6.76 (m, 1H, ArH), 6.58 (s, 0.79H, ArH), 6.54 (s, 0.21H, ArH), 5.63 (br s, 0.79H, *CH*OH), 5.48 (m, 0.21H, *CH*OH), 4.30 (dd, J = 10.8 Hz, J = 5.9 Hz, 0.79H, CHPh), 4.19 (dd, J = 10.9 Hz, J = 6.0 Hz, 0.21H, CHPh), 3.17–3.04 (m, 1H, OH), 2.49–2.40 (m, 2H, CH_2), 2.30–2.22 (m, 0.79H, CH_2), 2.19–2.06 (m, 4H, CH_2 and CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 151.1, 149.6, 144.0, 130.4, 130.3, 129.9, 129.7, 128.8, 128.7, 128.7, 128.6, 128.5, 129.6, 128.5, 126.9, 126.7, 124.9, 124.8, 116.7, 116.7 (20 × ArC), 94.3, 91.3 (2 × CHOH), 41.3 (CH), 38.9 (CH_2), 37.0 (CH), 36.4 (CH_2), 20.6 (CH_3). Anal. Calcd for C_8H_8O: C, 79.97; H, 6.71. Found: C, 80.00; H, 6.71.

4.3.4. (4*R*)-6-methyl-4-phenylchroman-2-ol (4*R*)-1

This was prepared using the procedure for **3a** above from (4*R*)-6-methyl-4-phenylchromane **5** (0.020 g, 0.084 mmol) and DIBAL solution (1 M in hexanes, 0.1 mL, 0.1 mmol, 1.19 equiv) in dry toluene (1 mL). The crude product was purified by column chromatography on silica gel using hexane/ethyl acetate (85:15) to give the pure product as a colourless oil (0.013 g, 65%) with identical spectroscopic characteristics to (±)-**1** above.

4.3.5. (±)-6-Methyl-4-phenylchroman-2-yl acetate 6

This was prepared using the procedure for **4a** from compound (±)-**1** to give (±)-**6** as a 1:1.5 *cis:trans*-mix of diastereomers. *trans*-**6** was preferentially crystallised from the mixture in ethanol.

trans-**6**: White solid. Mp 110–112 °C. ν_{max}/cm^{-1} (ATR) 3029 (Ar C—H), 2928 (C—H), 1748 (C=O), 1494, 1203, 1184 (C—O). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39–7.21 (m, 5H, ArH), 6.95 (dd, J = 2.1 Hz, J = 8.3 Hz, 1H, ArH), 6.82 (d, J = 8.3 Hz, 1H, ArH), 6.55–6.52 (m, 2H, ArH and CHOAc), 4.23 (dd, J = 10.8 Hz, J = 7 Hz, 1H, CHPh), 2.28–2.16 (m, 2H, CH_2), 2.14 (s, 3H, CH_3), 2.10 (s, 3H, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 169.9 (C=O), 149.2, 143.5, 130.5, 129.5, 128.8, 128.8, 128.7, 127.0, 124.9, 116.8 (10 × ArC), 90.0 (CHOAc), 36.8 (CHPh), 34.6 (CH_2), 21.3, 20.6 (2 × CH_3). Anal. Calcd for C_6H_6O: C, 76.57; H, 6.43. Found: C, 76.61; H, 6.44. Enantiomers separated using Daicel Chiralcel OD-H column (250 × 4.6 mm), conditions: *n*-hexane/*i*-PrOH = 98:2, flow rate 0.5 mL/min, 25 °C, λ = 209.8 nm; t_1 = 14.0 min, t_2 = 18.6 min.

cis-**6**: Characterised from a mixture containing 15% *trans*-**6**. Colourless liquid. ν_{max}/cm^{-1} (ATR) 3027 (Ar C—H), 2926 (C—H), 1752 (C=O), 1493, 1199, 1799 (C—O). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39–7.15 (m, 5H, ArH), 6.99 (dd, J = 8.4 Hz, J = 2.1 Hz, 1H, ArH), 6.86 (d, J = 8.2 Hz, 1H, ArH), 6.68 (br s, 1H, ArH), 6.44 (dd, J = 5.9 Hz, J = 3 Hz, 1H, CHOAc), 4.27–4.19 (m, 1H, CHPh), 2.51–2.20 (m, 2H, CH_2), 2.20 (s, 3H, CH_3), 1.80 (s, 3H, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 169.7 (C=O), 150.3, 144.5, 131.1, 130.4, 129.1, 128.6, 128.5, 126.6, 123.6, 117.1 (10 × ArC), 91.9 (CHOAc), 38.8, (CHPh), 34.7 (CH_2), 20.9, 20.7 (2 × CH_3). HRMS (ES^+): MH^+, found 283.1343. $C_{18}H_{19}O_3$ requires 283.1334. Enantiomers separated using Daicel Chiralcel OD-H column (250 × 4.6 mm), conditions: *n*–hexane/*i*-PrOH = 98:2, flow rate 0.5 mL/min, 25 °C, λ = 209.8 nm; t_1 = 10.7 min, t_2 = 12.3 min.

4.3.6. (4*R*)-6-Methyl-4-phenylchroman-2-yl acetate 6

This was prepared using the procedure for compound **4a** (0.013 g, 0.054 mmol), acetic anhydride (0.04 mL), DMAP (1 mg) and pyridine (0.02 mL) to give product (4*R*)-**6** in quantitative yield (15 mg). Product was isolated in a 45:55 *cis:trans* ratio, corroborated via a HPLC chromatogram (5% *ee*).

4.4. Enzymatic resolutions

General procedure for enzymatic acylation screens:

The substrate (10 mg) was added to a small test tube. The acyl source and solvent (2 mL, if applicable) were then added along with a spatula tip of enzyme. The reaction was incubated in a mini-shaker at 30 °C. When the stipulated time period had elapsed, the solution was passed through a Pasteur pipette containing a layer each of Celite® and $MgSO_4$, using diethyl ether as eluent. The solvent was removed under reduced pressure and the resulting crude mixture was analysed using ^{1}H NMR spectroscopy for conversion data and chiral HPLC for enantioselectivity.

General procedure for preparative scale enzymatic resolutions:

The substrate (40 mg) was added to a small test tube. Vinyl acetate (50 equiv) and solvent (4 mL, if applicable) were added along with a spatula tip of enzyme. The small test tube was sealed and the reaction was incubated in a mini-shaker at 30 °C. When the stipulated time period had elapsed, the solution was passed through a Pasteur pipette containing a layer each of Celite® and $MgSO_4$, using diethyl ether as eluent. The solvent was removed under reduced pressure and the resulting crude mixture was purified using column chromatography. The purified product was analyzed using ^{1}H NMR spectroscopy for conversion data and chiral HPLC for enantioselectivity.

4.4.1. (+)-(*R*)-Chroman-2-yl acetate 4a

This was synthesised from chromanol **3a**. The sample was incubated for 120 h in toluene with immobilised CAL-B as the biocatalyst. ^{1}H NMR analysis of the crude mixture indicated a 54% conversion. The mixture was purified with hexane/dichloromethane (1/1) as eluent to give pure **4a** in 41% yield and 94% *ee*. $[\alpha]_D^{20}$ = +64.5 (c 0.2, $CHCl_3$).

4.4.2. (+)-6-Methylchroman-2-yl acetate 4b

This was synthesised from 6-methylchromanol **3b**. The sample was incubated for 96 h with *Thermomyces lanuginosus* as the biocatalyst. ^{1}H NMR analysis of the crude mixture indicated a 65% conversion. The mixture was purified with hexane/ethyl acetate (5/1) as eluent to give pure **4b** in 32% yield and 94% *ee*. $[\alpha]_D^{20}$ = +26.7 (c 0.2, $CHCl_3$).

4.4.3. (2*S*,4*S*)-6-Methyl-4-phenylchroman-2-yl acetate 6

This was synthesised from (±)-**1**. The sample was incubated for 6 h in hexane with lipase from *Pseudomonas stutzeri* as the biocatalyst. ^{1}H NMR analysis of the crude mixture indicated 51% conversion. The mixture was purified with hexane/dichloromethane (1:1) as eluent. Yield: 19 mg (47%). *ee*: 50%. $[\alpha]_D^{20}$ = −53 (c 0.2, $CHCl_3$).

Acknowledgements

This publication has emanated from research conducted with the financial support of the Synthesis and Solid State Pharmaceutical Centre, funded by SFI under Grant Number 12/RC/2275, as well as academic support from UCC. X-ray crystallography was made possible through SFI funding (05/PICA/B802/EC07).

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetasy.2017.04.001.

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