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Authors: Paweł Borowiecki, Marcin Poterała, and Maciej Dranka

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Chemoenzymatic preparation of enantiomerically enriched (*R*)-(–)-mandelic acid derivatives: application in the synthesis towards the active agent pemoline

Marcin Poterała,*[a]‡ Maciej Dranka,[a] and Paweł Borowiecki*[a]‡

Dedicated to Prof. Jan Plenkiewicz in honor of his scientific achievements within academic career.

Abstract: The enantioselective resolutions of a several racemic derivatives of mandelic acid methyl ester catalyzed by lipases from *Pseudomonas fluorescens* (Amano AK) or *Burkholderia cepacia* (Amano PS-C II and Amano PS-IM) are demonstrated. A gram-scale lipase-mediated kinetic resolution approach have been developed, allowing facile synthesis of the corresponding (*R*)-(–)-methyl mandelates with excellent enantiomeric excesses (up to >99% ee) and the reactions enantioselectivity (*E*-value up to >200). The dopaminergic agent pemoline – used in the treatment of attention-deficit hyperactivity disorder (ADHD) and narcolepsy – was synthesized with 98% ee in a straightforward route by condensing the prepared methyl (*R*)-(–)-mandelate with guanidine hydrochloride under basic conditions. The desired (*R*)-(+)-pemoline in an optically pure form (>99% ee) was obtained after two recrystallizations from ethanol. However, it was confirmed by chiral HPLC that optically active pemoline undergoes racemization in methanol solution.

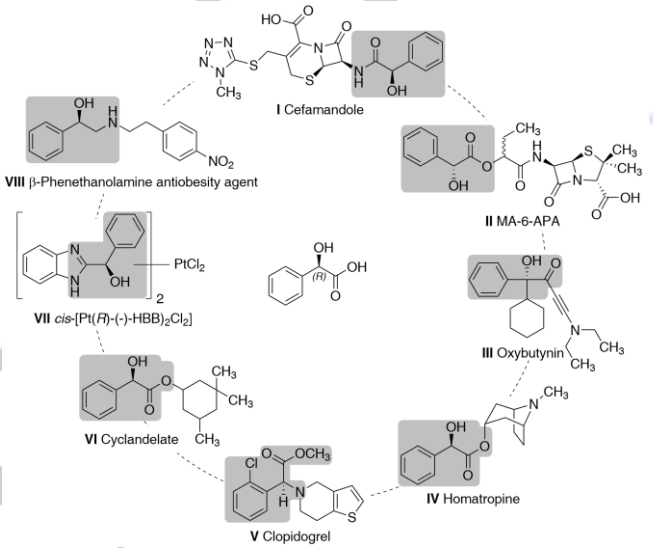

Figure 1. Examples of biologically active compounds obtainable *via* (*R*)-mandelic acid (RMA).

Introduction

The single enantiomers of mandelic acid (MA), mainly (*R*)-mandelic acid (RMA) and its analogous, constitute a valuable intermediates for the synthesis of biologically active compounds of high therapeutic importance and economic impact. Exemplary, the RMA structural motif is the core of many pharmacologically relevant compounds **I**–**VIII** (**Fig. 1**), such as semi-synthetic antibiotics including modified cephalosporins (cefamandole[1] **I**) and penicillins (MA-6-APA **II**)[2] as well as anti-cholinergic medications (oxybutynin[3] **III** and homatropine[4] **IV**). Moreover, derivatives of (*R*)-mandelic acid are also used as chiral synthons in the preparation of anti-platelet/anti-thrombotic (clopidogrel[5] **V**), vasodilator (cyclandelate[6] **VI**), anti-tumor {complex of *cis*-[Pt(2-*α*-hydroxybenzylbenzimidazole)$_2$Cl$_2$] **VII**}[7] and anti-obesity agents **VIII**.[8] Due to the importance of enantiomerically pure mandelic acid, tremendous efforts have been made to establish enantioselective routes for its production in the past few decades. In this purpose, a large number of asymmetric methods using the respective organic catalysts or metal-based complexes have been developed to obtain optically active MA. However, traditional synthetic routes towards mandelic acid single enantiomers require harsh reaction conditions including high pressure and temperature as well as costly, toxic and difficult to remove catalysts, and thus reliable and efficient '*purely-synthetic*' procedures remain still impaired. Therefore, simultaneously to those methods, a plethora of practical and environmentally friendly enzymatic approaches toward the preparation of enantiomerically pure MA have been developed. Among them noteworthy are: (i) stereoselective hydrolysis of mandelonitrile to RMA by means of various nitrilases from i.e. *Alcaligenes faecalis* ZJUTB10,[9] *Alcaligenes faecalis* ATCC 8750,[10] *Alcaligenes sp.* ECU0401,[11] *Alcaligenes sp.* MTCC 10675,[12] *Pseudomonas putida* MTCC 5110,[13] *Burkholderia cenocepacia* J2315,[14] *Microbacterium paraoxydans* and *Microbacterium liquefaciens*;[15] (ii) enantioselective microbial degradation of SMA using growing cells of i.e. *Alcaligenes bronchisepticus*,[16] *Pseudomonas polycolor*,[17] *Gibberella fujikuroi*,[18] and *Pseudomonas sp.* MA02[19] or the respective degradation of RMA by means of a bacterial strain of *Pseudomonas putida* ECU1009;[20] (iii) stereoselective bioreduction of benzoyl formate [phenylglyoxylic acid (PGA)] by *Saccharomyces cerevisiae* FD11b,[21] benzoylformate reductase

[a] Faculty of Chemistry, Warsaw University of Technology.
Address: Koszykowa St. 3, 00–664 Warsaw, Poland.
E-mail: mpotera@ch.pw.edu.pl (Dr. M. Poterała – Department of Organic Chemistry) and pawel_borowiecki@onet.eu or pborowiecki@ch.pw.edu.pl (Dr. P. Borowiecki – Department of Drugs Technology and Biotechnology).
Homepage: http://www.ch.pw.edu.pl.
[‡] These authors contributed equally to the work.

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extracted from cells of *Streptococcus faecalis* IFO 12964,[22] by a D-(−)-mandelic acid dehydrogenase from *Lactobacillus curvatus*[23] or reduction of PGA esters *via* actively fermenting *Saccharomyces cerevisiae* yeast,[24] NADPH-dependent benzil reductase (KRED1-Pglu) from the *Pichia glucozyma* CBS 5766 yeast,[25] NADP-dependent alcohol dehydrogenase from *Clostridium acetobutylicum* (CaADH),[26] and the resting cells of *Bacillus pumilus* Phe-C3;[27] and finally (iv) stereoselective *α*-hydroxylation of methyl 2-phenylacetate to (*S*)-methyl mandelate by the monooxygenase from *Helminthosporium sp.* CIOC3.3316[28] or with engineered cytochrome P450 BM-3,[29] respectively. Interestingly, enantiomerically pure (*S*)-mandelic acid was also synthesized from benzaldehyde by sequential hydrocyanation and hydrolysis in a bienzymatic conversion—combined (catalytic) procedure composed of a cross-linked-enzyme aggregate (combi-CLEA) of the (*S*)-selective oxynitrilase from *Manihot esculenta* and the non-selective nitrilase from *Pseudomonas fluorescens* EBC 191[30] as well as by a whole-cell recombinant *Escherichia coli* biocatalyst expressing simultaneously different variants of both those enzymes.[31] In turn, other bi-enzyme system, in which a FMN-dependent (*S*)-mandelate dehydrogenase (SMDH) from *Pseudomonas aeruginosa* and laccase from *Agaricus bisporus* were employed as biocatalysts, afforded enantiomerically pure (*R*)-mandelic acid[32] in high yield. Less studied in the literature, but also very promising in terms of efficiency of MA enantiomers preparation seems to be highly active nitrile hydratase/amidase enzyme system present in whole cells of *Rhodococcus erythropolis* NCIMB 11540.[33]

From the view point of the frequency of the usage in biotechnological processes toward asymmetric synthesis of MA enantiomers, the isolated enzymes constitute predominant source of the catalysts. Except few examples of utilization of carbonic anhydrase,[34] mandelate dehydrogenases,[35] esterases,[36] mandelate racemase,[37] and *D*-lactate oxidase (GO-LOX),[38] it is undoubtedly the lipases that focus the largest considerable interest on this field. In this context, many investigations have been performed over the years on the use of lipases as biocatalysts for MA enantiomers synthesis. So far, the most typical existing lipase-based protocol being predominant among these efforts is (i) enantioselective transesterification.[39] In reference to it, two various dynamic kinetic resolution (DKR) approaches based on tandem metal–enzyme protocol[40] and a lipase-mandelate racemase two-enzyme system in aqueous/organic two-phase system[41] or in sole ionic liquids[42] both applying racemization-esterification methodology are reported. Other approaches performed in enzymatic kinetic resolution (EKR) manner, such as (ii) enantioselective hydrolysis of MA esters,[43] (iii) asymmetric esterification of racemic MA with aliphatic alcohols,[44] (iv) direct enantioselective amidation of MA with ammonia,[45] and (v) aminolysis of racemic methyl mandelate with *n*-butylamine[46] have also been successfully used.

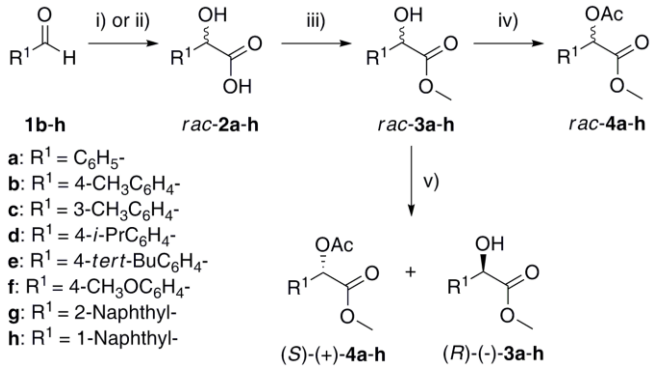
Although many approaches towards preparation of (*R*)-mandelic acid have been proposed, in our opinion satisfactory results are still demanding. Moreover, although optically active MA scaffold is a privileged structure in medicinal chemistry, and can be found in many biologically active molecules (**Fig. 1**), surprisingly, it has not been used toward synthesis of enantiomerically pure pemoline until now. Pemoline (2-amino-5-phenyl-4(5*H*)-oxazolone, 2-imino-5-phenyl-4-oxazolidinone, phenylisohydantoin; marketed under trade names: *Betanamin*; *Ceractiv*; *Cylert*; *Tradon*) is non-narcotic, mild and long-acting central nervous system (CNS) stimulant of dopaminergic activity, likely acting *via* inducing the release of dopamine and norepinephrine in brain tissue, being a surrogate for dopamine, although not affecting endogenous intracellular dopamine. In view of the above mentioned properties, this medication was administrated for decades to treat attention-deficit hyperactivity disorder (ADHD)[47] and narcolepsy.[48] Albeit pemoline is currently withdrawn from market as it is considered to be hepatotoxic,[49] it would be a great challenge to find if '*an old drug could not serve as a new again*' ? In this regard, it is strongly recommended to perform pharmacological evaluations on both enantiomers of pemoline in order to estimate, which of its antipode is responsible for positive biological response useful for management of behavioral disorders in children, and which cause unwanted fatal liver toxicity. Therefore, straightforward and preparative synthetic method of the preparation of both homochiral forms of pemoline is desirable. Based on the above considerations and to alleviate the desire of better recognition of the mechanism of pemoline toxic liver damage in future, we developed an integrated lipase-catalyzed bioprocess for the synthesis of the RMA and its application towards the active agent (*R*)-pemoline.

Results and Discussion

In this study, we aimed at developing a simple and efficient chemoenzymatic synthesis of (*R*)-(–)-mandelic acid derivatives, and at using one of them (RMA) as a chiral synthon for the preparation of active pharmaceutical ingredient – pemoline **5**. Our approach focuses exclusively on lipase-catalyzed enantioselective transesterification reactions of racemic methyl mandelates *rac*-**3a**-**h** carried out under kinetically–controlled conditions. In this context, the required for enzymatic transformations racemic methyl esters of MA *rac*-**3a**-**h** and their respective diesters *rac*-**4a**-**h** (used as an analytical standards for reaction's progress monitoring and enantiomeric excess measurements) were prepared according to **Scheme 1**.

Synthesis of the racemic starting materials

According to the literature, the most convenient method of mandelic acid and its derivatives preparation is the three-step procedure: synthesis of sodium benzaldehyde-bisulfate adduct, subsequent cyanohydrin formation and final acidic hydrolysis of the obtained mandelonitrile.[50] However, this standard procedure assumes usage of extremely poisonous and flammable hydrogen cyanide or at least highly toxic sodium/potassium cyanide, which upon standing hydrolyze to release a hydrocyanic acid as well.

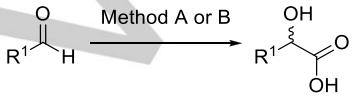


Scheme 1. Chemoenzymatic preparation of enantiomerically enriched mandelic acid derivatives (*R*)-(–)-**3a**-**h**. Reagents and conditions: (i) TEBACl (cat.), $CHCl_3$, 50% NaOH, 56±2 °C, then 5 h at RT, then 18% HCl (pH 1); (ii) $CHBr_3$ (1 equiv), LiCl (1.9 equiv), KOH (3.9 equiv), fine-crushed ice-cubes, 1,4-dioxane, 36 h at 0–5 °C, then 24 h at 35 °C; (iii) $SOCl_2$ (1.1 equiv), dry MeOH, at -30 °C over 30 min, then 4 h at RT; (iv) Ac_2O (1.0 equiv), dry Py (1.1 equiv), DMAP (cat.), dry CH_2Cl_2, 6 h at RT; (v) vinyl acetate, lipase (30% w/w), TBME, RT, 250 rpm (laboratory shaker).

Mandelic acid derivatives might also be obtained from the respective acetophenones, using chlorine in order to form dichloromethyl aryl ketones, which easily undergo hydrolysis to desired MAs in the presence of base.[51] Unfortunately, this two-steps procedure requires application of chlorine, which is highly corrosive and toxic reagent, and in addition the dichloroacetophenone formed in the first step is a harmful heavy lachrymatory oil. From the obvious reasons, we decided to use much safer approach based on the transformation of commercially available and cheap arylaldehydes **1b**-**h** under phase-transfer catalysis (PTC) conditions using the 50% NaOH/$CHCl_3$ liquid/liquid system in the presence of benzyltriethylammonium chloride (TEBACl) at elevated temperature (Method A)[52] or alternatively, the reactions of **1b**-**h** with $CHBr_3$ performed in the presence of KOH and LiCl in a cooled to 0–5 °C mixture of 1,4-dioxane/H_2O (Method B)[53] (**Table 1**). Both methods require additional basic hydrolysis of the formed intermediate adducts, which is rapidly afforded *in situ* under reaction conditions.

At first, to estimate the utility of these methods we decided to compare their efficiency toward three model compounds **1b**-**d** selected out of the set of the available substrates **1b**-**h**. After series of experiments it turned out that reactions conducted *via* Method B were superior over the Method A. Apart from the substantially simplified reaction procedure, Method B outclasses the PTC synthetic methodology in the fields of overall yield and mildness. The desired *rac*-**2b**-**d** prepared by means of Method B were obtained with yields in range of 69–75% (**Table 1**, entries 1, 3, and 5), whilst in the case of Method A the products *rac*-**2b**-**d** were isolated in 41–70% yields (**Table 1**, entries 2, 4, and 6). The differences in the yields are due to the fact that in Method A the temperature regime must be strictly controlled, otherwise complicated side reactions remain difficult to avoid. Exceeding even ±2–3 °C threatens drastic decline in efficiency of the reaction. Another serious drawback of Method A is it's limited accessibility towards aldehydes possessing benzene ring substituted by electron withdrawing groups. This electronic effect leads to a much complex mixtures formation, presumably because of increased incorporation of the trichloromethyl anion (CCl_3^-) or consecutively formed dichlorocarbene (:CCl_2) into an aromatic ring system instead of their addition to a carbonyl group by either direct attack of the nucleophilic CCl_3^- on the partially positive carbon atom or insertion of ambident :CCl_2 into a carbon-oxygen double bond. Since dihalocarbenes are generated strictly in the organic phase *via* the trihalomethyl anion, and more, the corresponding formation of dibromocarbene from bromoform especially in homogeneous systems where water is present is generally poor from bromoform, Method B seems to be particularly attractive procedure as the reactions with benzaldehydes proceed exclusively through the nucleophilic attack by the anion on the carbonyl group.

Table 1. Synthesis of the racemic mandelic acid derivatives *rac*-**2b**-**h**.



Entry	Product	R^1	Method[a]	Yield[b] (%)
1	*rac*-**2b**	4-$CH_3C_6H_4$-	A	70
2			B	75
3	*rac*-**2c**	3-$CH_3C_6H_4$-	A	54
4			B	71
5	*rac*-**2d**	4-*i*-PrC_6H_4-	A	41
6			B	69
7	*rac*-**2e**	4-*tert*-BuC_6H_4-	B	57
8	*rac*-**2f**	4-$CH_3OC_6H_4$-	B	73
9	*rac*-**2g**	2-Naphthyl-	B	75
10	*rac*-**2h**	1-Naphthyl-	B	68[c]

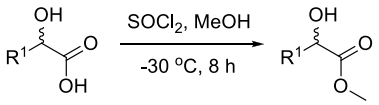
[a] Method A: **1b**-**d** (0.1 mol), TEBACl (1.23 g, 5 mmol), $CHCl_3$ 160 mL, 50% NaOH 25 mL, 56±2 °C, then 5 h at RT, then 18% HCl (pH 1); Method B: **1b**-**h** (0.13 mol), $CHBr_3$ (31.6 g, 0.13 mol), LiCl (10.6 g, 0.25 mol), KOH (28.1 g, 0.50 mol), fine-crushed ice-cubes 100 g, 1,4-dioxane 100 mL, 36 h at 0–5 °C, then 24 h at 35 °C. [b] Isolated yield after recrystallization from *n*-hexane/AcOEt or *n*-heptane/AcOEt mixtures, respectively. [c] Isolated as a crude product.

In turn, although Method B proved to be adequate toward the employed benzaldehydes **1e**-**h** affording products *rac*-**2e**-**h** in moderate 57% (**Table 1**, entry 7) to high 68–75% yields (**Table 1**, entries 8–10), certain modifications had to be performed. Worthy of mention is that during the optimization procedure (not shown herein) we found that decrease of the pH value below 12, drastically dropped the yield of *rac*-**2b**-**h**, probably due to suppression of the replacement of the bromide atoms with hydroxyl anions in an intermediate adducts. Therefore, maintaining the pH of the reaction medium above 12

by successive KOH addition was required. We also found that an addition of small portion of grounded NaOH during work-up has a beneficial influence on the extraction procedure since very stable emulsions were avoided. Simple addition of NaOH gave by far better results than various physical methods applied by us in the course of the experiments. In example, the formed emulsions were tedious to breakdown by convenient filtering of the crude multi-phasic extraction mixture over the Celite pad.

In the next step, mandelic acid methyl esters *rac*-**3a**-**h** were readily prepared starting from the respective acids *rac*-**2a**-**h** by their treatment with thionyl chloride used in 1.1 molar ratio in the presence of anhydrous methanol. The previously established protocol,[54] which was adopted from the literature[55] and slightly modified by us (the temperature was decreased from -10 °C to -30 °C), allowed the reaction to proceed smoothly, affording the expected pure products *rac*-**3a**-**h** in high 87–95% yields and excellent >99% purity according to gas chromatography (GC) indications (**Table 2**).

Table 2. Synthesis of the racemic mandelate methyl esters *rac*-**3a**-**h**.



rac-**2a-h** *rac*-**3a-h**

Entry	Product[a]	R^1	Yield[b] (%)
1	*rac*-**3a**	C_6H_4-	93
2	*rac*-**3b**	4-$CH_3C_6H_4$-	90
3	*rac*-**3c**	3-$CH_3C_6H_4$-	91
4	*rac*-**3d**	4-*i*-PrC_6H_4-	90
5	*rac*-**3e**	4-*tert*-BuC_6H_4-	95
6	*rac*-**3f**	4-$CH_3OC_6H_4$-	87
7	*rac*-**3g**	2-Naphthyl-	92
8	*rac*-**3h**	1-Naphthyl-	89

[a] *rac*-**2a**-**h** (0.1 mol), dry MeOH 180 mL, $SOCl_2$ (13.1 g, 0.11 mol, 8.1 mL) at 30 °C, then 4 h at RT. [b] Isolated yield after recrystallization from *n*-hexane/Et_2O or *n*-heptane/AcOEt, respectively.

In turn, mandelic acid diesters *rac*-**4a**-**h**, destined to be applied for the analytical purposes, were obtained in high 79-90% yields in convenient manner by using 1.0 equiv of acetic anhydride and 1.1 equiv of dry pyridine in the presence of 4-dimethylaminopyridine (DMAP) as the catalyst. The esterification reactions were carried out in dry dichloromethane at room temperature for 6 h. Yields of diesters *rac*-**4a**-**h** (79–90%) are collected in Experimental Section.

KR of *rac*-3a-h using lipase-catalyzed transesterification

Although biotransformations involving lipases from different organisms have become broadly investigated for the preparation of optically active mandelic acid derivatives, the extension of substrate scope in this group is still appealing. Moreover, the search for lipases that are capable to transform MAs with excellent enantioselectivities, which can work under harsh industrial conditions persists as well. The previously reported lipase-catalyzed enantioresolutions of racemic MA *via* kinetically-controlled transesterification approach gave the results of enantiomeric excess for the obtained RMA from poor (<80% ee),[39i] moderate (80–90% ee)[39m] to high (91–98% ee),[39a,39d,39j] and an excellent values (≥99% ee).[39c,39e,39l,40] Strikingly, the most efficient acetylative EKR protocol,[39k] which as the only one reached the theoretical boundary with a conversion yield of 50% and an ee-values of >99.9% for both enantiomeric forms of MA comprises the usage of an extracellular lipase isolated from solvent-tolerant bacterium *Burkholderia ambifaria* YCJ01. However, although this lipase demonstrated excellent enantioselective transesterification toward racemic MA, it's exploitability is rather limited as it requires cultivation broth composed of soil samples contaminated by unknown crude oil and undefined chemicals as well as thorough knowledge concerning isolation, purification, and cloning techniques in order to prepare it. Thus, it is highly desirable not only to extend the synthetic utilities of novel MA derivatives by exploring their EKR reactions, but also to prove that the chiral discrimination based on lipase-catalyzed acylation protocol can be significantly improved in terms of enantioselectivity by using biocatalysts that are easily available on the market.

On the basis of literature rapports on lipase-catalyzed transesterification of MA esters and our previous experience in the field of enzyme catalysis, we decided to exclude multivariate investigation of experimental factors including medium engineering, influence of the reaction medium temperature, different enzyme loading as well as effect of the acyl group donors on stereochemical outcome, and thus limit our studies toward comparing the efficiency of the most promising, according to literature, lipase catalysts [namely, *Pseudomonas fluorescens* lipase (PFL) and *Burkholderia cepacia* lipase (BCL)] In this regard, to determine suitable reaction conditions for the enantioselective transesterification of MA derivatives *rac*-**3a**-**h** under kinetically-controlled conditions, we initially investigated the reaction systems with vinyl acetate as the source of an acyl group in the presence of 30% weight/weight (w/w) loading of the respective lipase preparation with respect to the employed substrates *rac*-**3a**-**h** at room temperature (**Table 3**). Moreover, it is well-established that the highest enantioselectivity for methyl mandelates are observed in solvents with moderate to low polarity, such as diisopropyl ether (DIPE) and/or cyclohexane, respectively.

Table 3. Analytical-scale lipase-catalyzed KR of *rac*-**3a**-**h** with vinyl acetate in TBME.

Entry	Substrate	Lipase[a]	t (*h*)	Conv.[b] (%)	ee_s[c] (%)	ee_p[c] (%)	*E*[d] (%)
1	*rac*-**3a**	Amano AK	147	51	89	84	34
2		Amano PS-C II	38	54	>99	92	126
3	*rac*-**3b**	Amano AK	144	53	>99	88	82
4		Amano PS-C II	23	48	95	89	117
5	*rac*-**3c**	Amano AK	190	45	74	89	38
6		Amano PS-C II	72	55	>99	81	49
7	*rac*-**3d**	Amano AK	168	49	85	90	51
8		Amano PS-C II	29	51	>99	94	170
9	*rac*-**3e**	Amano AK	192	52	79	85	23
10		Amano PS-C II	45	51	99	97	>200
11	*rac*-**3f**	Amano AK	240	49	71	73	13
12		Amano PS-IM	46	60	99	66	24
13	*rac*-***3g***	Amano AK	336	51	93	89	58
14		Amano PS-IM	96	54	>99	86	69
15	*rac*-**3h**	Amano AK	576	30	N.D.[e]	N.D.[e]	N.D.[e]
16		Amano PS-IM	456	63	90[f]	53[f]	9

[a] Conditions: *rac*-**3a**-**h** 100 mg, lipase 30 mg, vinyl acetate 0.2 mL, TBME 2 mL, RT, 250 rpm (laboratory shaker). [b] Based on GC, for confirmation the % conversion was calculated from the enantiomeric excess of the unreacted substrate (ee_s) and the product (ee_p) according to the formula conv. = $ee_s/(ee_s + ee_p)$. [c] Determined by chiral HPLC analysis by using a Chiralcel OD-H column. [d] Calculated according to Chen *et al*.,[56] using the equation: $E = \{\ln[(1 - conv.)(1 - ee_s)]\}/\{\ln[(1 - conv.)(1 + ee_s)]\}$. [e] Not determined as the enzyme activity was too low. [f] Determined by correlative method using polarimetry (calculated according to the formula: % ee = $|[\alpha_{obs}]|/|[\alpha]_D| \times 100\%$, where $[\alpha_{obs}]$ is an experimental value, and $[\alpha]_D$ is the literature value[57] given for the corresponding solution containing only a single pure enantiomer.

Unfortunately, both of the solvents are expensive and difficult to recover. In addition, diisopropyl ether easily forms explosive peroxides upon standing what drastically limits it's usage at industrial scale as the special safety precautions are required. Therefore, we decided to carry out all enzymatic reactions in *tert*-butyl methyl ether (TBME) as the co-solvent since it posses similar polarity to DIPE and constitutes also a safer alternative for it. Moreover, we were curious of the results with TBME since, surprisingly, although it is established as one of the most common media used in lipase-catalyzed biotransformations, until now the influence of this solvent on EKR of racemic mandelate esters *via* stereoselective acylation by means of PFL and BCL has not been examined.

In order to reduce the mechanical stress caused by the high-speed agitation and physical contact between lipase preparation and stirring element responsible for enzyme leaching from the carrier (in the case of immobilized biocatalysts), in all of our biotransformation experiments instead of magnetic stirrer, a laboratory shaker (agitation speed set at a moderate 250 rpm value) was used. Although this strategy generally results in the extension of reaction time as the mass transfer is hindered in heterogeneous biocatalytic reactions, it helps to maintain the activity of the enzyme in time, and thus expanding the number of cycles enzyme can be used before it requires replacing.

As a starting point, during the small-scale biotransformation studies, the influence of the acetylation rate on the reaction efficiency was investigated. In **Table 3** we have presented the results of reactions, which gave the best enantiomeric excess values for the RMA derivatives (*R*)-(–)-**3a**-**h** or for which the conversions were as close to 50% as possible. All of the enzymatic reactions were monitored by GC directly from the crude mixture, whereby the enantiomeric excesses for the resolved products were determined by enantioselective HPLC after isolation and chromatographic purification (HPLC analysis conditions for various compounds are collected in **Table S2** in supplementary document). This is due to difficulty associated with the simultaneous baseline resolution of the mandelates *rac*-**3a**-**h** (enzyme substrates) and its corresponding acetates *rac*-**4a**-**h** (products) in one run. Unfortunately, enantiomers of *rac*-**3h** were not separable on the available Chiralcel OD-H column, and thus the ee-values had to be determined by correlative method using polarimetry.

An initial screening indicated that both immobilized lipases isolated from *Burkholderia cepacia* (Amano PS-C II and Amano PS-IM) posses broader substrate specificity in the examined group of racemic mandelates *rac*-**3a**-**h** than native lipase from *Pseudomonas fluorescens* (Amano AK) as the higher enantioselectivity factors for Amano PS-C II (*E*-value from 117 up to >200) and for Amano PS-IM (*E*=9–69) were achieved for the corresponding substrates when compared with the results for Amano AK (*E*=13–82). Moreover, the rates of transesterification reactions catalyzed by Amano AK in the kinetic resolution of *rac*-**3a**-**h** were generally significantly lower (45–53% conversion after 144–336 h) than for those with immobilized preparations of BCL (48–60% conversion after 23–96 h). The stereochemical outcome of EKR of *rac*-**3h** was excluded from the above discussion since 1-naphthyl-substituted substrate turned out to be very resistant toward biocatalytic transformations carried out with the examined enzymatic systems, and also because the accuracy of the optical purity assessment is more questionable and less comparable with

Table 4. Preparative-scale lipase-catalyzed KR of *rac*-**3a**-**h** with vinyl acetate in TBME.

Entry	Substrate	Lipase[a]	t (*h*)	Conv.[b] (%)	ee_s[c] (%)/conf.[d]/Yield[e] (%)	ee_p[c] (%)/conf.[d]/Yield[e] (%)	*E*[f] (%)
1	*rac*-**3a**	Amano PS-C II	1.5	53	>99/(*R*)/40	87/(*S*)/51	75
2	*rac*-**3b**	Amano AK	6	53	>99/(*R*)/40	88/(*S*)/53	82
3	*rac*-**3c**	Amano PS-C II	3	57	>99/(*R*)/29	76/(*S*)/58	37
4	*rac*-**3d**	Amano PS-C II	1	52	>99/(*R*)/37	90/(*S*)/50	99
5	*rac*-**3e**	Amano PS-C II	2	50	99/(*R*)/33	98/(*S*)/48	>>200
6	*rac*-**3f**	Amano PS-IM	2	61	99/(*R*)/33	62/(*S*)/54	23
7	*rac*-***3g***	Amano PS-IM	4	54	98/(*R*)/31	85/(*S*)/48	56
8	*rac*-**3h**	Amano PS-IM	19	50	86[g]/(*R*)/31	86[g]/(*S*)/55	37

[a] Conditions: *rac*-**3a**-**h** 3.5 g, lipase 1.05 g, vinyl acetate 7 mL, TBME 70 mL, RT, 250 rpm (laboratory shaker). [b] Based on GC, for confirmation the % conversion was calculated from the enantiomeric excess of the unreacted substrate (ee_s) and the product (ee_p) according to the formula conv. = $ee_s/(ee_s + ee_p)$. [c] Determined by chiral HPLC analysis by using a Chiralcel OD-H column. [d] Assigned by comparison of the specific rotation sign values and/or HPLC retention times with the data reported in the literature; the absolute configurations of the non-reported compounds [(*R*)-(–)-**3d**-**e**, (*S*)-(+)-**4c**-**e**, and (*S*)-(+)-**4g**] have been assumed by analogy with the results of the other transformations. [e]Isolated yield after purification by silica-gel chromatography (*n*-hexane/AcOEt). [f] Calculated according to Chen *et al*.,[56] using the equation: $E = \{\ln[(1 - conv.)(1 - ee_s)]\}/\{\ln[(1 - conv.)(1 + ee_s)]\}$. [g] The % ee-value is calculated from the equation: % ee = $|[\alpha_{obs}]|/|[\alpha]_D| \times 100\%$, where $[\alpha_{obs}]$ is the experimental value of specific rotation, and $[\alpha]_D$ is the literature value of specific rotation given for a solution containing only a single pure enantiomer.[58]

those obtained from chiral HPLC for ee. In this case, we found that the reactions proceeded very sluggishly even catalyzed by Amano PS-IM lipase (63% conversion after 456 h), and with poor enantioselectivity (*E*=9) since slower reacting enantiomer (*R*)-(–)-**3h** was afforded with moderate 90% ee, and the formed optically active diester (*S*)-(+)-**4h** with 53% ee. Nevertheless, it is worth to mention that only Amano PS-IM could catalyze acetylation of *rac*-**3h** on reasonable time-scale. The results summarized in **Table 3** clearly indicate that the best analytical-scale lipase-catalyzed KRs of *rac*-**3a**-**h** in terms of enantioselectivity, optical purity of desired products (*R*)-(–)-**3a**-**h**, and the reaction rates were obtained by employing Amano PS-C II and/or Amano PS-IM as the catalyst. Among these attempts, the most enantioselective assay (*E*>200) was achieved by Amano PS-C II for MA derivative substituted with bulky *tert*-butyl group situated in *para*-position of benzene ring *rac*-**3e**. In this case, when the reaction was arrested at 51% conversion, the generated acetate (*S*)-(+)-**4e** and the remaining alcohol (*R*)-(–)-**3e** were both obtained with very high enantiomeric purity reaching 97% ee and 99% ee, respectively (**Table 3**, entry 10). Amano PS-C II lipase was also superior towards other substrates, including *rac*-**3a** and *rac*-**3c**-**d**, toward which kinetic resolution resulted in almost homochiral alcohols (*R*)-(–)-**3a** and (*R*)-(–)-**3c**-**d** (≥99% ee) if the proper conversion degrees were retained (**Table 3**, entries 2, 6, and 8). In turn, although PFL is less efficient catalyst for the tested group of racemates, the (Amano AK)-catalyzed KR of *rac*-**3b** resulted in 88% ee for (*S*)-(+)-**4b**, whereby the remaining enantiomer (*R*)-(–)-**3b** was recovered in enantiomerically pure form (>99% ee) when the reaction was carried out for at least 144 h until 53% conversion was achieved (**Table 3**, entry 3). Taking into account the results of analytical-scale studies, we decided to abandon further investigations with Amano AK due to its moderate selectivity and activity, however, with the exception of the substrate possessing methyl group in the *para*-position of benzene ring *rac*-**3b**, within which reaction proceeded with a industrially acceptable *E*-value larger than 80, and led to furnished enantiomerically pure (*R*)-(–)-**3b** (>99% ee).

The results of lipase-catalyzed kinetic resolutions of *para*-substituted mandelates *rac*-**3b** and *rac*-**3d**-**e**, with the exception of *para*-methoxyphenyl derivative *rac*-**3f**, showed an increase in enantioselectivity (*E*-value from 117 up to >200). In general, for comparable conversions BCL lipases display the highest activity with the less bulky *para*-substituted substrates *rac*-**3a**-**b** and *rac*-**3d**-**f**, intermediate activity with *meta*-substituted compound *rac*-**3c**, and low activity toward both naphthalene derivatives *rac*-**3g**-**h**. In addition, the great differences in reaction rates and enantioselectivity values observed for PFL and BCL lipases revealed that immobilized preparations of BCL were more adequate for the chosen set of the racemic mandelates *rac*-**3a**-**h** although literature data report that the efficiency of both should be more or less the same. This suggests that the reaction environment must have some impact on their catalytic activity, and that TBME strongly promotes transformations catalyzed by BCL preparations, and not by PFL.

Furthermore, the usefulness of developed enzymatic protocol was demonstrated by the investigation into the 3.5 g-scale enantioselective acetylation of racemic mandelates *rac*-**3a**-**h** (**Table 4**). Under kinetically-controlled conditions the reaction times were adjusted for each substrate independently. However,

over-reaction (>50% of conversion) was mandatory to ensure the desired optical purity (≥98% ee) for slower-reacting enantiomers (*R*)-(–)-**3a**-**h**. Extending by 35-fold the KRs scale gave very similar results when compared to (100 mg)-scale biotransformations in terms of reaction rates and enantioselectivity. However, except for the substrates *rac*-**3b**, *rac*-**3f** and *rac*-**3g**, for which selectivity remained unaffected, some insignificant differences can be noticed as in the case of the reactions performed with *rac*-**3a**, *rac*-**3c**, and *rac*-**3d** the *E*-values slightly dropped, whereas in case of the compounds possessing naphthalene ring *rac*-**3g**-**h** enantioselectivity was improved. In turn, a remarkable increase of the enantiomer selectivity was obtained for *rac*-**3e** in the presence of Amano PS-C II as the catalyst. These have some visible consequences, and it is worth noting that whilst the (Amano AK)- and (Amano PS-IM)-mediated kinetic resolution gave moderate (**Table 4**, entries 6–8, *E*=23–56) to good (**Table 4**, entry 2, *E*=82) results with respect to enantioselectivity, the acetylation catalyzed by Amano PS-C II proved to be superior, especially in the reaction with *para*-substituted *tert*-butyl phenyl derivative *rac*-**3e** (**Table 4**, entry 5, *E*>>200). The results of optical purity assessment from the conducted experiments clearly indicate that in almost all cases, except 1-substituted naphthalene derivative *rac*-**3h**, the ee-values of (*R*)-mandelic acid methyl esters were above 98%. In addition, the yields of the enantioselective transesterifications shown in **Table 4** were in 29-40% range for slower-reacting enantiomers (*R*)-(–)-**3a**-**h**, and in 48-58% range for faster-reacting isomers (*S*)-(+)-**4a**-**h**. However, it should be noted that the above-mentioned values indicate isolated yields after column chromatography, and were calculated on the basis of the theoretical number of moles, relative to theoretical amount arising from conversion rate established by HPLC. This means, that i.e. when 50% conversion is reached, up to the half of the acetate could be obtained.

As it was already stated above, the enantiorecognition of methyl mandelates by means of lipase-catalyzed enantioselective acylation in traditional media are reported with already good-to-excellent enantioselectivity, hence, there was not much margin for improvement. Nonetheless, the enantiomeric ratio of some of these resolutions improved considerably when the reaction was performed with immobilized lipases from *Burkholderia cepacia* suspended in TBME. Moreover, the preparation of novel RMAs in almost enantiomerically pure form and in multi-gram quantities in easily accessible manner showed feasibility of our protocol for large-scale production of potentially valuable building blocks.

Assignment of the stereochemistry of the EKRs' products

During the course of the present study, the absolute configurations of the resolution products were assigned mainly on the basis of optical rotation measurements and HPLC analysis. The signs of the optical rotation as well as the retention times of the respective picks of HPLC analyses collected from experimental data were compared with those reported in the literature for the respective solutions (in the case of polarimetry), and the employed chiral column (for HPLC).[59,69] The detailed data for optical properties evaluations, including literature references, are provided in Experimental Section. From the stereochemical course of the performed reactions it was obvious that in transesterification catalyzed by PFL and BCL the (*S*)-form of the methyl mandelates reacted preferentially over (*R*)-counterpart. However, six out of sixteen of the prepared by us optically active alcohols (*R*)-(–)-**3a**-**h** and acetates (*S*)-(+)-**4a**-**h**, including (*R*)-(–)-**3d**-**e** and (*S*)-(+)-**4c**-**e**, (*S*)-(+)-**4g**, have not been reported, thereby their absolute configuration of the chiral center could be deduced only on the basis of the optical rotation signs with the assumption that they structurally belong to the same homological series, and so the chemical correlation can be applied accordingly to an empirical Tschúgaeff's rule.[60] Although the above enunciated empirical rule of configuration assignment is '*stereochemically adequate*' and sufficiently reliable within compounds possessing single asymmetric atom, however, we aimed to unambiguously confirm this hypothesis by derivatization of methyl (2*R*)-(4-*tert*-butylphenyl)(hydroxy)ethanoate (*R*)-(–)-**3e** toward product, which efficient crystallization of proper single crystal would be feasible, and thus XRD analysis could be performed. In this regard, we decided to transform (*R*)-(–)-**3e** into corresponding pyridinium hexafluorophosphate salt (*R*)-(–)-**11** which X-ray crystal structure is presented in **Fig. 2**.

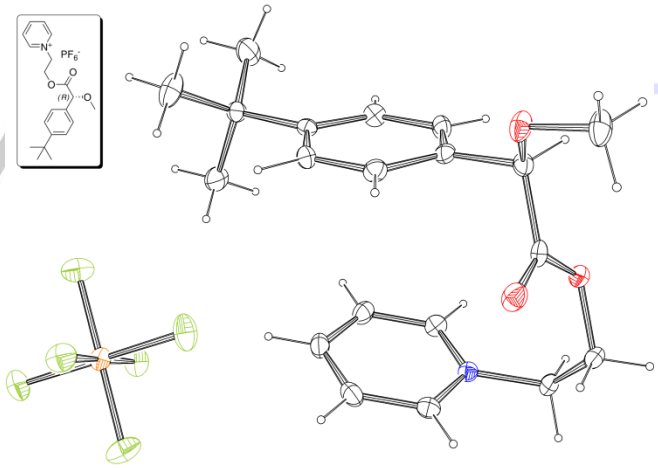
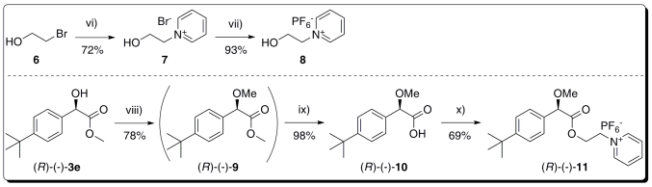


Figure 2. An ORTEP plot representing molecular structure of optically active (*R*)-(–)-**11**. Thermal ORTEP-ellipsoids were drawn at 50% probability level (C black, H gray, N blue, O red, F green, P orange). The following crystal structure has been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC-1527174.

This task was accomplished by using straightforward approach consisting of five-step reaction sequence as shown in **Scheme 2**.

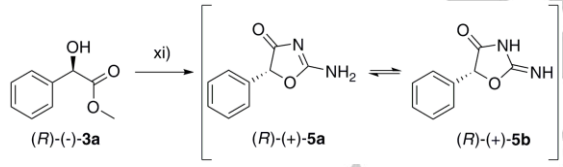


Scheme 2. Synthesis of optically active (*R*)-(-)-**11**. Reagents and conditions: (vi) dry pyridine (1 equiv), 72 h at 50 ºC, low-light conditions; (vii) KPF_6 (1.9 equiv), dry CH_3CN, 24 h at reflux; (viii) Ag_2O (1.18 equiv), roasted $CaSO_4$ (1.18 equiv), CH_3I (4.88 equiv), 36 h at reflux, low-light conditions; (ix) LiOH (4 equiv), MeOH, 24 h at RT, then 36% HCl at 0–5 °C; (x) **8** (0.9 equiv), DCC (0.9 equiv), DMAP (cat.), dry CH_3CN, 48 h at RT.

Synthesis of the optically active pemoline

At first, racemic imino-oxazoline CNS stimulant pemoline *rac*-**5** was obtained according to the method reported by Howell and co-workers.[61] In the original approach ethyl mandelate was condensed with guanidine free base (generated from the respective hydrochloride salt by addition of equimolar amounts of NaOH) used in a 2-fold molar excess under reflux for 1 h, thus affording desired API in high 68% yield. In our investigation we used racemic methyl ester *rac*-**3a** instead of ethyl ester and followed the procedure given by Howell. Surprisingly, only trace of the product *rac*-**5** was formed after indicated time period. At elongated to 60 h reaction time *rac*-**5** was obtained with moderate 35% yield. Moreover, after repeating the reaction with optically active precursor (*R*)-(–)-**3a** (>99% ee), the enantiomeric purity of (*R*)-(+)-**5** was very poor, barely reaching 13% ee (**Table 5**, entry 1).

Table 5. Synthesis of (*R*)-pemoline (*R*)-(+)-**5**.



(*R*)-(-)-**3a** (*R*)-(+)-**5a** (*R*)-(+)-**5b**

Entry	GuanidineHCl[a]	Temp.	ees[b] (%)	Yield[c] (%)	$[\alpha]_D$[d]
1	2 equiv	Reflux	13	35	N.D.[e]
2	2 equiv	RT	11	35	N.D.[e]
3	1 equiv	RT	98	40	+110 (*c* 0.20, 24.1°C)
4	1 equiv	RT	85[f]	38	+95 (*c* 0.20, 24.3°C)
5	2 equiv	RT	>99[g]	N.D.[e]	+108 (*c* 0.20, 27.6°C)

[a] (*R*)-(–)-**3a** (3.13 mmol, >99% ee), dry EtOH 7.5 mL, NaOH (125 mg, 3.13 mmol), 60 h. [b] Determined by chiral HPLC analysis by using a Chiralcel OD-H column. [c] Isolated yield. [d] Specific rotation, *c* solution in methanol. [e] Not determined. [f] The precipitated NaCl was filtered off the reaction mixture. [g] After additional two recrystallizations from EtOH.

To overcome this drawback, at first we have lowered the reaction temperature. Unfortunately, enantiomeric excess of (*R*)-(+)-**5** obtained in room temperature reaction mixture was even worst as it decreased to 11% (**Table 5**, entry 2). From this experiment it was clear that the elevated temperature was not responsible for deterioration in the ee-values. Therefore, the next step was to eliminate an excess of the employed guanidine since the presence of such a strong base could deprotonate the formed (*R*)-(+)-**5**, and thus promote deleterious for stereochemical outcome product racemization. This attempt gave successful result not only in term of optical purity of the obtained non-racemic pemoline (*R*)-(+)-**5** (98% ee), but also in increased isolated yield (40%) (**Table 5**, entry 3). Simultaneously, we decided to remove sodium chloride, since an inorganic salt formed during the liberation of guanidine by means of NaOH might have been responsible for the low yields. Albeit this procedure has not improved neither the yield (38%) nor enantiomeric purity of the isolated pemoline (*R*)-(+)-**5** (85% ee) (**Table 5**, entry 4). Finally, with a highly enantioenriched product (*R*)-(+)-**5** (98% ee) in hand, we turned our efforts to increase the optical purity of the titled API. This was achieved by two recrystallizations from ethanol, which yielded (*R*)-pemoline in homochiral form with >99% ee (**Table 5**, entry 5).

During the studies, a curious phenomenon was observed, namely, that the enantiomeric purity of optically pure (*R*)-pemoline (>99% ee) gradually declined accompanying the aging of it's methanolic solution. After one day of storage at ambient temperature, ee-value dropped to 96% ee, whereas after four days it decreased to 30% ee. Finally, an almost complete racemization of the analyzed sample of (*R*)-(+)-**5** (8% ee) occurred after 8 days. This is a crucial information, not only for designing an appropriate conditions for crystal growth, but especially from the view-point of pharmaceutical and other industries, which need to know how to store analytical reference standards of the drug samples. In addition, awareness of racemization progress of non-racemic pemoline in a solution in time may also be helpful for the investigations of biological activity, as this API may also be prone to racemization in the body fluids. However, the evaluation of this issue in other solutions was not our main task and exceed the frame of this article, thus was deliberately abandoned.

Conclusions

In summary, a convenient approach towards highly enantiomerically enriched (*R*)-(–)-mandelic acid derivatives was developed. The enzyme-catalyzed kinetic resolution of the corresponding racemic mixtures was achieved by using a simple protocol that involved an enantioselective transesterification step in the presence of various lipase preparations as catalysts and vinyl acetate as an acyl group donor. An analytical-scale investigations gave the rates of enzymatic reactions and enantiomeric purity comparable to that obtained by the methods published previously. The best rates and enantioselectivity factors were predominantly obtained with immobilized lipases from *Burkholderia cepacia* (Amano PS-C II and Amano PS-IM) suspended in TBME. The scale-up of the enzymatic process was carried out to verify its potential in an industrial application. Eight compounds were resolved on a 3.5 gram-scale by using optimal reaction conditions. For the majority of examples

preparative EKR succeeded in obtaining the (*R*)-mandelates with excellent enantiomeric excess (≥99%) and acceptable isolated yields (29-58%). The enantiomeric ratio (*E*) reached up to >>200 for acylation of the *para*-substituted *tert*-butyl derivative of methyl mandelate (*R*)-(–)-**3e** catalyzed by Amano PS-C II. On the basis of the enzymatic studies toward group of the examined MA esters, it was obvious that stereochemical outcome of lipase-catalyzed reactions were profoundly influenced by the steric effect of the substituents rather than electronic properties of the aromatic ring system. For novel non-racemic MA derivative (*R*)-(–)-**3e** the absolute configuration was unambiguously determined by X-ray structure analysis of a single crystal of the prepared heavy-atom analog (*R*)-(-)-**11** possessing hexafluorophosphate ion. Homochiral methyl (*R*)-(–)-mandelate (>99% ee) was further utilized to obtain enantiomerically enriched (*R*)-pemoline (98% ee). Finally, single (*R*)-enantiomer of pemoline (>99% ee) was achieved upon additional double recrystallization procedure from ethanol. Thanks to development of this synthesis, both enantiomers of active agent pemoline can be throughout evaluated in terms to avoid life threatening hepatic failure in future. Furthermore, crucial analysis for non-racemic pemoline was performed for the first time, namely, evaluation of spontaneous racemization in a solution of methanol determined by chiral HPLC.

Experimental Section

General Remarks: Reagents (including racemic mandelic acid *rac*-**2a**) and solvents were purchased from various commercial sources (Sigma Aldrich, Alfa Aesar, POCH) and were used without further purification, except aromatic aldehydes which were distilled or recrystallized (solid reagents) before use. High-performance liquid chromatography (HPLC)-grade solvents were purchased from POCH (Poland). Methylene chloride, acetonitrile, and ethanol were dried by allowing them to stand over activated (oven-roasted in high-vacuum) 3Å molecular sieves [20% mass/volume (m/v) loading of the desiccant] at least for 48 h before use;[62] methanol was dried by refluxing it in the presence of magnesium powder for 2–3 h, and subsequent fractional distillation in damp-protected apparatus under nitrogen. All non-aqueous reactions were carried out under oxygen-free argon-protective conditions using flame-dried glassware. Lipase from *Burkholderia cepacia* (formerly *Pseudomonas cepacia*) [Amano PS-IM – immobilized on diatomite, specified activity: 500 U/g, Amano PS-C II – immobilized on ceramic, purchased from Amano Pharmaceutical Co., Ltd.], lipase from *Pseudomonas fluorescens* [Amano AK – native lipase, specified activity: >20.000 U/g, purchased from Amano Pharmaceutical Co., Ltd.]. Melting points, uncorrected, were determined with a commercial apparatus on samples contained in rotating glass capillary tubes open on one side (1.35 mm inner diam. and 80 mm length). Analytical thin-layer chromatography was carried on TLC aluminum plates (Merck) covered with silica gel of 0.2 mm thickness film containing a fluorescence indicator green 254 nm (F_{254}), and using UV light as a visualizing agent. Preparative separations were carried out by column chromatography using thick-walled glass columns and silica gel (230–400 mesh) with grain size 40–63 μm or activated charcoal as stationary phase, respectively. The chromatographic analyses (GC) were performed with a Agilent Technologies 6890N instrument equipped with a flame ionization detector (FID) and fitted with HP-50+ (30 m) semipolar column (50 % phenyl–50 % methylpolysiloxane); Helium (2 mL/min) was used as carrier gas; retention times (t_R) are given in minutes under these conditions. The enantiomeric excesses (% ee) of kinetic resolution products were determined by HPLC analysis performed on Shimadzu CTO-10ASV chromatograph equipped with STD-20A UV detector and Chiralcel OD-H (Diacel) chiral column using mixtures of *n*-hexane/*i*-PrOH as mobile phase in the appropriate ratios given in further paragraphs of Experimental Section [the wavelength of UV detection and both the mobile phase composition as well as the flow rate were fine tuned for each analysis (see **Table S2** placed in supplementary document)]; the HPLC analyses were executed in an isocratic and isothermal (30 °C) manner. UV spectra were measured with Cary 3 spectrometer; samples were dissolved in absolute EtOH. Optical rotations ([*α*]) were measured with a PolAAr 32 polarimeter in a 2 dm long cuvette using the sodium D line (λ=589 nm); the units of the specific rotation are: (deg×mL)/(g×dm). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Varian Mercury 400 MHz spectrometer and ^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra were recorded on a Varian NMR System 500 MHz spectrometer; ^{1}H and ^{13}C chemical shifts (δ) are reported in parts per million (ppm) relative to the solvent signals {i.e. $CDCl_3$, δ_H (residual $CHCl_3$) 7.26 ppm, δ_C 77.16 ppm; or acetone-d_6, δ_H (residual acetone-d_6) 2.09 ppm, δ_C 205.87 ppm); or DMSO-d_6 δ_H [residual $(CD_3)_2SO$) 2.49 ppm with HDO at 3.30 ppm, δ_C 40.45 ppm]; or acetone-d_6, δ_H (residual acetone-d_6) 2.09 ppm, δ_C 205.87 ppm); or diaminophenyl sulfone (DDS, for spectra in D_2O)}. Chemical shifts are quoted as s (singlet), d (doublet) dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), and br s (broad singlet); coupling constants (*J*) are reported in Hertz. Infrared (IR) spectra of samples prepared in nujol were taken on a Carl Zeiss Specord M80 instrument; Fourier transform infrared (FTIR) spectra of neat samples were recorded on a Perkin Elmer System 2000 FTIR Spectrometer equipped with a Pike Technologies GladiATR attenuated total reflectance (ATR) accessory with a monolithic diamond crystal stage and a pressure clamp; resolution was 2 cm^{-1}; absorption maxima (ν_{max}) are given in cm^{-1}. Elemental analyses were performed on a Elementar Analysensysteme GmbH-VARIO EL III (Element Analyzer: CHNS).

Preparation of the racemic mandelic acid derivatives *rac*-2a-h (*Method A*): To a solution of the appropriate aromatic aldehyde **1b-d** (0.10 mol) and benzyltriethylammonium chloride (TEBACl, 1.23 g, 5 mmol) in $CHCl_3$ (160 mL), a 50% aqueous NaOH solution (25 mL) was added drop by drop (1-2 drops per minute) at 56 °C under vigorous stirring using a mechanical stirrer. The reaction temperature was controlled between 54 and 58 °C during the addition of the NaOH solution. After addition, the reaction mixture was stirred at room temperature for a further 5 h. Next, a sufficient amount of H_2O was added in order to dissolved the precipitate formed. Water phase was separated and acidified to pH 1 with 36% HCl solution, and subsequently extracted with Et_2O in Soxhlet apparatus for 10 h. The ethereal layer was dried over anhydrous $MgSO_4$, the solvent was evaporated, and the resulting crude acid *rac*-**2b-d** (41–70%) was purified by recrystallization from *n*-hexane/AcOEt mixture.

Preparation of the racemic mandelic acid derivatives *rac*-2a-h (*Method B*): At first, in the Erlenmeyer flask a mixture of LiCl (10.6 g, 0.25 mol), KOH (28.1 g, 0.50 mol), fine-crushed ice-cubes (100 g), 1,4-dioxane (100 mL), the appropriate aromatic aldehyde **1b-h** (0.13 mol), $CHBr_3$ (31.6 g, 0.13 mol) was prepared. The content of the flask was stirred with ice-cooling at 0–5 °C for 36 h. The pH value of the solution was periodically controlled, and when its value dropped below 12, grounded KOH (3.37 g, 60 mmol) was added. Next, the flask was placed in a laboratory thermoshaker and mixed at 35 °C for a further 24 h. After this time, the reaction mixture was diluted with a 0.5 M aqueous KOH solution (300 mL). Thereafter, the contents of the flask were extracted with Et_2O (3 × 50 mL), and the water phase was acidified to pH 1 with concentrated 36% HCl solution, and again extracted with Et_2O (4 × 80 mL). The combined ether extracts were dried over anhydrous $MgSO_4$, the

drying agent was filtered off, and the solvent was evaporated under reduced pressure to yield the appropriate crude acid *rac*-**2b**-**h**, which was further purified by recrystallization from the mixtures of *n*-hexane/AcOEt, $PhCH_3$/AcOEt or *n*-heptane/AcOEt, respectively.

Hydroxy(phenyl)acetic acid (*rac*-2a): purchased from commercial supplier.

Hydroxy(4-methylphenyl)acetic acid (*rac*-2b): Yield 70% (according to Method A); white solid; mp 145 °C (*n*-hexane/AcOEt); Yield 75% (Method B); mp 143–144 °C (*n*-hexane/AcOEt) [lit.[63] 145 °C ($PhCH_3$)]; ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 2.35 (s, 3H), 5.21 (s, 1H), 7.18–7.20 (m, 2H), 7.31–7.33 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 21.4, 72.6, 126.7, 129.6, 134.7, 139.0, 177.0; IR (nujol): ν_{max} = 3406 (br s), 1705, 1290, 1055, 815, 724, 700.

Hydroxy(3-methylphenyl)acetic acid (*rac*-2c): Yield 54% (according to Method A); white solid; mp 91–93 °C (*n*-hexane/AcOEt), Yield 71% (according to Method B); mp 91.5–92.5 °C (*n*-hexane/AcOEt) [lit.[64] 93–94 °C (benzene)]; ^{1}H NMR [500 MHz, $(CD_3)_2SO$]: *δ* 2.30 (s, 3H), 4.99 (s, 1H), 5.78 (br s, 1H), 7.09–7.11 (m, 1H), 7.20–7.24 (m, 3H), 12.56 (br s, 1H); ^{13}C NMR [126 MHz, $(CD_3)_2SO$]: *δ* 21.1, 72.5, 123.8, 127.3, 128.1, 128.3, 137.2, 140.2, 174.2; IR (nujol): ν_{max} = 3460, 3170 (br s), 2520 (br s), 1715, 1705, 1670, 1530, 1340, 1328, 1310, 1268, 1250, 1235, 1210, 1150, 1079, 871, 810, 772, 710, 690, 650.

Hydroxy[4-(propan-2-yl)phenyl]acetic acid (*rac*-2d): Yield 41% (according to Method A); white solid; mp 156.5–158 °C (*n*-hexane/AcOEt), Yield 69% (according to Method B); mp 156–157 °C (*n*-hexane/AcOEt) [lit.[65] 159.2–160 °C (petroleum ether)]; ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 1.24 (d, *J* = 6.8 Hz, 6H), 2.91 (sept, *J* = 6.8 Hz, 1H), 5.23 (s, 1H), 7.24–7.26 (m, 2H), 7.35–7.37 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 23.9, 33.9, 72.5, 126.6, 126.9, 134.9, 149.7, 175.8; IR (nujol): ν_{max} = 3405 (br s), 1710, 1290, 1219, 1187, 1071, 815, 691.

(4-*Tert*-butylphenyl)(hydroxy)acetic acid (*rac*-2e): Yield 57% (according to Method B); white solid; mp 147–149 °C (*n*-hexane/AcOEt) [lit.[65] 149.5–150 °C (benzene)]; ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 1.31 (s, 9H), 5.23 (s, 1H), 7.35–7.42 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 31.4, 34.8, 72.6, 125.9, 126.4, 134.6, 152.1, 177.7; IR (nujol): ν_{max} = 3430 (br s), 1711, 1262, 1148, 1080, 1055, 814, 681.

Hydroxy(4-methoxyphenyl)acetic acid (*rac*-2f): Yield 73% (according to Method B); white solid; mp 104–106 °C (*n*-hexane/AcOEt) [lit.[66] 108 °C (no data)]; ^{1}H NMR [500 MHz, $(CD_3)_2SO$]: *δ* 3.73 (s, 3H), 4.95 (s, 1H), 6.89–6.91 (m, 2H), 7.31–7.34 (m, 2H); ^{13}C NMR [126 MHz, $(CD_3)_2SO$] *δ*: 55.1, 71.9, 113.5, 127.9, 132.3, 158.8, 174.4.

Hydroxy(naphthalen-2-yl)acetic acid (*rac*-2g): Yield 75% (according to Method B); white solid; mp 156–157.5 ºC (*n*-heptane/AcOEt) [lit.[67] 162–163.5 °C (chloroform/ethanol)]; ^{1}H NMR (400 MHz, CD_3COCD_3): *δ* 5.40 (s, 1H), 7.47–7.54 (m, 2H), 7.64 (dd, *J* = 1.6 Hz, *J* = 8.5 Hz, 1H), 7.87–7.95 (m, 3H), 8.02 (s, 1H); ^{13}C NMR (100 MHz, CD_3COCD_3): *δ* 73.6, 125.5, 126.6, 126.9, 127.1, 128.5, 128.8, 134.1, 134.2, 138.3, 174.4; IR (nujol): ν_{max} = 3380 (br s), 3260, 1720, 1690, 1295, 1220, 1075, 1050, 823, 810, 735, 696.

Hydroxy(naphthalen-1-yl)acetic acid (*rac*-2h): Yield 68% (according to Method B); white solid; mp 123–125 ºC ($PhCH_3$/AcOEt) [lit.[68] 124–125 °C (benzene)]; ^{1}H NMR [500 MHz, $(CD_3)_2SO$]: *δ* 5.67 (s, 1H), 7.45–7.56 (m, 3H), 7.87–7.94 (m, 1H), 8.27–8.29 (m, 1H); ^{13}C NMR [126 MHz, $(CD_3)_2SO$]: *δ* 71.0, 124.6, 125.3, 125.7, 125.8, 126.0, 128.3, 128.4, 130.7, 133.5, 136.3, 174.4; Complete spectroscopic analysis was performed for its methyl ester *rac*-**3h**.

Preparation of the racemic mandelate esters *rac*-3a-h: To a stirred and cooled to –30 °C solution of the appropriate *α*-hydroxy-*α*-arylacetic acid *rac*-**2a-h** (0.1 mol) in dry MeOH (180 mL), thionyl chloride (13.1 g, 0.11 mol, 8.1 mL) was added dropwise at a rate that the temperature of the reaction mixture did not rise above –30 °C. When the addition was complete, the solution was stirred at –30 °C for further 10 min, and then left for 4 h at room temperature. Afterwards, the content of the flask was poured into a mixture of fine-crushed ice (75 g), saturated aqueous Na_2CO_3 solution and Et_2O (250 mL). Thus prepared mixture was vigorously shaken in a separating funnel, and the combined organic layer was washed with brine, and dried over anhydrous $MgSO_4$. After removal of the drying agent and evaporation of the solvent in vacuum, the resulting appropriate crude methyl ester *rac*-**3a-h** was purified by recrystallization from *n*-hexane/Et_2O or *n*-heptane/AcOEt mixture, respectively.

Methyl hydroxy(phenyl)acetate (*rac*-3a): Yield 93%; white solid; mp 54–55 °C (*n*-hexane/Et_2O) [lit.[69] 54–55 °C (*n*-heptane)]; ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 3.51 (d, *J* = 5.6 Hz, 1H), 3.76 (s, 3H), 5.18 (d, *J* = 5.6 Hz, 1H), 7.32–7.44 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 72.8, 126.6, 128.5, 128.6, 138.2, 174.1 (The spectral data are fully consistent with those reported previously in lit.[70]); IR (nujol): ν_{max} = 3430, 1740, 1050, 1065; HPLC [*n*-hexane-*i*-PrOH (90:10, v/v); f=0.8 mL/min; λ=254 nm]: t_R=8.939 (*S*-isomer) and 14.612 min (*R*-isomer).

Methyl hydroxy(4-methylphenyl)acetate (*rac*-3b): Yield 90%; white solid; mp 49–50 °C (*n*-hexane/Et_2O) [lit.[71] 49 °C (benzene/ligroin)]; ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 2.35 (s, 3H), 3.39 (d, *J* = 5.6 Hz, 1H), 3.76 (s, 3H), 5.14 (d, *J* = 5.6 Hz, 1H) 7.17–7.19 (m, 2H), 7.29–7.31 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 21.3, 53.2, 72.9, 126.7, 129.5, 135.3, 138.5, 174.4 (The spectral data are fully consistent with those reported previously in lit.[70]); IR (nujol): ν_{max} = 3318, 1732, 1245, 1210, 1083, 995, 975, 780, 728; HPLC [*n*-hexane-*i*-PrOH (90:10, v/v); f=0.8 mL/min; λ=254 nm]: t_R=9.185 (*S*-isomer) and 12.946 min (*R*-isomer).

Methyl hydroxy(3-methylphenyl)acetate (*rac*-3c): Yield 91%; white solid; mp 52–53 °C (*n*-hexane/Et_2O) [lit.[72] 52 °C (*n*-hexane)]; ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 2.36 (s, 3H), 3.42 (br s, 1H), 3.76 (s, 3H), 5.14 (s, 1H), 7.14–7.16 (m, 1H), 7.19–7.28 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 21.6, 53.2, 73.1, 123.9, 127.4, 128.7, 129.5, 138.3, 138.6, 174.4; IR (nujol): ν_{max} = 3470 (br s), 2980, 1730, 1600, 1480, 1435, 1200, 1148, 1100, 1070, 970, 775, 732, 695; HPLC [*n*-hexane-*i*-PrOH (90:10, v/v); f=0.8 mL/min; λ=254 nm]: t_R=8.939 (*S*-isomer) and 14.612 min (*R*-isomer).

Methyl hydroxy[4-(propan-2-yl)phenyl]acetate (*rac*-3d): Yield 90%; white solid; mp 78–79 °C (*n*-heptane/AcOEt) [lit.[73] 81–82 °C (no data)]; ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 1.24 (d, *J* = 6.9 Hz, 6H), 2.91 (sept, *J* = 6.9 Hz, 1H), 3.36 (d, *J* = 5.8 Hz, 1H), 3.77 (s, 3H), 5.15 (d, *J* = 5.8 Hz, 1H), 7.23–7.25 (m, 2H), 7.31–7.35 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 24.1, 34.0, 53.1, 72.9, 126.7, 126.9, 135.8, 149.4, 174.4; IR (nujol): ν_{max} = 3240–3540, 1737, 1188, 1080, 980, 902, 828, 779, 715; HPLC [*n*-hexane-*i*-PrOH (95:5, v/v); f=0.8 mL/min; λ=254 nm]: t_R=11.062 (*S*-isomer) and 13.837 min (*R*-isomer).

Methyl (4-*tert*-butylphenyl)(hydroxy)acetate (*rac*-3e): Yield 95%; white solid; mp 52–54 °C (*n*-hexane/Et_2O); ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 1.31 (s, 9H), 3.36 (d, *J* = 5.8 Hz, 1H), 3.77 (s, 3H), 5.16 (d, *J* = 5.8 Hz, 1H), 7.32–7.35 (m, 2H), 7.38–7.41 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 31.4, 34.8, 53.2, 72.8, 125.8, 126.4, 135.4, 151.7, 174.4; IR (nujol): ν_{max}

= 3450 (br s), 1730, 1255, 1190, 1083, 972, 898, 825, 769, 698; HPLC [*n*-hexane-*i*-PrOH (95:5, v/v); f=0.8 mL/min; λ=254 nm]: t_R=10.437 (*S*-isomer) and 12.668 min (*R*-isomer).

Methyl hydroxy(4-methoxyphenyl)acetate (*rac*-3f): Yield 87%; white solid; mp 38–39 °C (*n*-heptane/AcOEt) [lit.[74] 37–38 °C (H_2O)]; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.75 (s, 3H), 3.80 (s, 3H), 5.12 (s, 1H), 6.87–6.90 (m, 2H) 7.30–7.34 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 53.1, 55.4, 72.6, 114.2, 128.0, 130.6, 159.9, 174.4 (The spectral data are fully consistent with those reported previously in lit.[70]); IR (neat): ν_{max} = 3429, 3008, 2968, 2917, 2842, 1726, 1608, 1583, 1510, 1441, 1449, 1384, 1328, 1301, 1248, 1213, 1182, 1169, 1112, 1078, 1027, 977, 908, 834, 818, 796, 750, 712, 573, 526, 429; HPLC [*n*-hexane-*i*-PrOH (90:10, v/v); f=0.7 mL/min; λ=254 nm]: t_R=14.707 (*S*-isomer) and 24.420 min (*R*-isomer).

Methyl hydroxy(naphthalen-2-yl)acetate (*rac*-3g): Yield 92%; white solid; mp 73–74 °C (*n*-hexane/Et_2O) [lit.[75] 72.8–73 °C (*n*-hexane/AcOEt)]; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.63 (br s, 1H), 3.76 (s, 3H), 5.36 (s, 1H), 7.45–7.55 (m, 3H), 7.81–7.87 (m, 3H), 7.91 (br s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 53.2, 73.2, 124.2, 126.1, 126.5, 127.8, 128.6, 128.62, 133.3, 133.4, 135.7, 174.3 (The spectral data are fully consistent with those reported previously in lit.[75]); FTIR (neat): ν_{max} = 3470, 3055, 2965, 1725, 1510, 1440, 1390, 1365, 1305, 1270, 1255, 1220, 1170, 1145, 1085, 985, 945, 925, 905, 870, 860, 830, 775, 750, 735, 665; HPLC [*n*-hexane-*i*-PrOH (90:10, v/v); f=0.8 mL/min; λ=254 nm]: t_R=14.588 (*S*-isomer) and 17.379 min (*R*-isomer).

Methyl hydroxy(naphthalen-1-yl)acetate (*rac*-3h): Yield 89%; white solid; mp 78–80 °C (*n*-heptane/AcOEt); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.53 (d, *J* = 4.8 Hz, 1H), 3.74 (s, 3H), 5.82 (d, *J* = 4.8 Hz, 1H), 7.45–7.58 (m, 4H), 7.85–7.90 (m, 2H), 8.14–8.16 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 53.1, 71.4, 123.6, 125.2, 125.9, 126.0, 126.6, 128.8, 129.5, 131.0, 133.9, 134.0, 174.7 (The spectral data are fully consistent with those reported previously in lit.[76]); FTIR (neat): ν_{max} = 3470, 3055, 2965, 1725, 1510, 1440, 1390, 1365, 1305, 1270, 1255, 1220, 1170, 1145, 1085, 985, 945, 925, 905, 870, 860, 830, 775, 750, 735, 665; HPLC: compound is indivisible on available Chiralcel OD-H column.

Preparation of the racemic mandelate diesters *rac*-4a-h: The mixture of the appropriate racemic methyl mandelate *rac*-**3a-h** (0.1 mmol), DMAP (1 mg), Ac_2O (1.0 equiv), and dry pyridine (1.1 equiv) was dissolved in dry CH_2Cl_2 (0.5 mL) and stirred for 6 h at room temperature. After completion of the reaction (according to TLC indications), content of the flask was diluted with CH_2Cl_2 (0.5 mL) and quenched with 2M HCl (2 × 0.5 mL), the water phase was extracted with Et_2O (3 × 0.5 mL). The combined organic phases were washed with brine (0.5 mL) and saturated solution of Na_2CO_3 (0.5 mL), dried over anhydrous Na_2SO_4. After filtration of drying agent and concentration to dryness, the crude product was purified by column chromatography on silica gel using gradient *n*-hexane/AcOEt (70:10 and 60:10 v/v) as an eluent, yielded corresponding mandelic diester *rac*-**4a**-**h** as colorless oil.

Methyl (acetyloxy)(phenyl)acetate (*rac*-4a): Yield 90%; colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.20 (s, 3H), 3.72 (s, 3H), 5.94 (s, 1H), 7.38–7.41 (m, 3H), 7.45–7.48 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 20.8, 52.7, 74.6, 127.8, 128.9, 129.4, 133.9, 169.4, 170.4 (The spectral data are fully consistent with those reported previously in lit.[77]); IR (nujol): ν_{max} = 1752, 1735, 1230, 1200, 1160, 1050, 805, 778, 740; HPLC [*n*-hexane-*i*-PrOH (99:1, v/v); f=0.4 mL/min; λ=254 nm]: t_R=22.997 (*S*-isomer) and 24.383 min (*R*-isomer).

Methyl (acetyloxy)(4-methylphenyl)acetate (*rac*-4b): Yield 83%; colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.19 (s, 3H), 2.36 (s, 3H), 3.72 (s, 3H), 5.89 (s, 1H), 7.19–7.21 (m, 2H), 7.34–7.36 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.7, 21.2, 52.6, 74.3, 127.6, 129.5, 130.8, 139.3, 169.5, 170.4 (The spectral data are fully consistent with those reported previously in lit.[78]); IR (nujol): ν_{max} = 1755, 1735, 1230, 1200, 1160, 1050, 805, 780, 745; HPLC [*n*-hexane-*i*-PrOH (99:1, v/v); f=0.25 mL/min; λ=254 nm]: t_R=34.677 (*S*-isomer) and 36.610 min (*R*-isomer).

Methyl (acetyloxy)(3-methylphenyl)acetate (*rac*-4c): Yield 80%; colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.20 (s, 3H), 2.37 (s, 3H), 3.73 (s, 3H), 5.89 (s, 1H), 7.19–7.21 (m, 1H), 7.23–7.30 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.9, 21.5, 52.8, 74.6, 124.9, 128.4, 128.9, 130.2, 133.7, 138.8, 169.6, 170.5; FTIR (neat): ν_{max} = 2960, 2922, 2850, 1750, 1607, 1432, 1370, 1223, 1151, 1052, 780, 745, 696; HPLC [*n*-hexane-*i*-PrOH (99:1, v/v); f=0.25 mL/min; λ=254 nm]: t_R=21.782 (*S*-isomer) and 23.480 min (*R*-isomer).

Methyl (acetyloxy)[4-(propan-2-yl)phenyl]acetate (*rac*-4d): Yield 79%; colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25 (d, *J* = 6.9 Hz, 6H), 2.19 (s, 3H), 2.92 (sept, *J* = 6.9 Hz, 1H), 3.73 (s, 3H), 5.90 (s, 1H), 7.22–7.28 (m, 2H), 7.36–7.40 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.9, 24.0, 34.1, 52.7, 74.5, 127.1, 127.8, 131.2, 150.3, 169.6, 170.6; FTIR (neat): ν_{max} = 2960, 2922, 1750, 1510, 1432, 1368, 1225, 1170, 1050, 1017, 975 928, 825, 775, 740, 720; HPLC [*n*-hexane-*i*-PrOH (99:1, v/v); f=0.4 mL/min; λ=254 nm]: t_R=18.176 (*S*-isomer) and 19.740 min (*R*-isomer).

Methyl (acetyloxy)(4-*tert*-butylphenyl)acetate (*rac*-4e): Yield 87%; colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.32 (s, 9H), 2.19 (s, 3H), 3.73 (s, 3H), 5.91 (s, 1H), 7.38–7.42 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 20.9, 31.4, 34.8, 52.7, 74.5, 125.9, 127.6, 130.8, 152.6, 169.6, 170.5; FTIR (neat): ν_{max} = 2960, 2863, 1750, 1570, 1512, 1430, 1367, 1262, 1225, 1170, 1102, 1050, 1015, 975, 928, 822, 770, 715; HPLC [*n*-hexane-*i*-PrOH (99:1, v/v); f=0.4 mL/min; λ=254 nm]: t_R=17.587 (*S*-isomer) and 19.371 min (*R*-isomer).

Methyl (acetyloxy)(4-methoxyphenyl)acetate (*rac*-4f): Yield 85%; colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.18 (s, 3H), 3.72 (s, 3H), 3.81 (s, 3H), 5.87 (s, 1H), 6.91 (m, 2H), 7.38 (m, 2H); ^{13}C NMR (100 MHz $CDCl_3$): δ 20.8, 52.6, 55.3, 74.1, 114.1, 125.7, 129.0, 160.2, 169.4, 170.2; FTIR (neat): ν_{max} = 1754, 1735, 1605, 1508, 1315, 1320, 1300, 1250, 1220, 1200, 1168, 1110, 1052, 1025, 1000, 970, 920, 860, 830, 812, 790, 755, 710, 645; HPLC [*n*-hexane-*i*-PrOH (96:4, v/v); f=0.4 mL/min; λ=254 nm]: t_R=22.440 (*S*-isomer) and 27.504 min (*R*-isomer).

Methyl (acetyloxy)(naphthalen-2-yl)acetate (*rac*-4g): Yield 83%; colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.24 (s, 3H), 3.74 (s, 3H), 6.12 (s, 1H), 7.50–7.54 (m, 2H), 7.56–7.58 (m, 1H), 7.84–7.89 (m, 3H), 7.95–7.96 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 20.9, 52.8, 74.7, 124.8 126.7, 126.9, 127.5, 127.9, 128.3, 128.9, 131.2, 133.2, 133.7, 169.5, 170.4; IR (neat): ν_{max} = 1750, 1730, 1335, 1265, 1230, 1196, 1040, 920, 860, 830, 810, 752; HPLC [*n*-hexane-*i*-PrOH (98:2, v/v); f=0.2 mL/min; λ=254 nm]: t_R=55.647 (*S*-isomer) and 61.512 min (*R*-isomer).

Methyl (acetyloxy)(naphthalen-1-yl)acetate (*rac*-4h): Yield 86%; colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.22 (s, 3H); 3.71 (s, 3H), 6.70 (s, 1H), 7.47–7.63 (m, 4H), 7.89–7.91 (m, 2H), 8.18–8.19 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 20.9, 52.9, 72.6, 123.8, 125.4, 126.2, 127.1, 127.6, 129.0, 130.1, 130.3, 131.2, 134.1, 169.8, 170.5 (The spectral data are fully consistent with those reported previously in lit.[57]); FTIR (neat): ν_{max} = 1756, 1744, 1729, 1434, 1369, 1218, 1203, 1167, 1087, 1051, 970, 927, 786, 773, 555, 496, 425, 415; HPLC: compound is indivisible on available Chiralcel OD-H column.

General procedure for the analytical-scale enzymatic KR of *rac*-3a-h: To a solution of the appropriate racemic methyl mandelate *rac*-**3a-h** (100 mg) in TBME (2 mL) the respective commercial lipase formulation [30 mg, 30% w/w (catalyst/substrate)] and vinyl acetate (0.2 mL) were added in one portion. The reaction mixture was shaken (250 rpm) at room temperature by using a laboratory rotatory shaker, and its aliquots were regularly analyzed by analytical chromatographic assays (GC and HPLC) after proper filtration of the enzyme residue. In the case of KR of *rac*-**3h**, for which direct HPLC analysis of % ee-values from reaction mixture failed, additional elaboration was performed as follows: the enzyme was filtered off and washed with TBME (5 × 2 mL), the solvent was evaporated and the crude obtained was purified by column chromatography eluting with gradient of *n*-hexane/AcOEt (80:10, 70:10, 60:10 v/v) mixture to afford (*R*)-(–)-**3h** and (*S*)-(+)-**4h** ready-to-analyze by correlative method using polarimetry. The results of enzymatic KR reactions carried out at analytical scale are collected in **Table 3**.

General procedure for the preparative-scale enzymatic KR of *rac*-3a-h: To a suspension of the appropriate racemic methyl mandelate *rac*-**3a-h** (3.5 g) and the lipase [1.05 g, 30% w/w (catalyst/substrate)] in TBME (70 mL), vinyl acetate (7 mL) was added. Thus composed enzymatic reaction system was shaken at room temperature and 250 rpm on a laboratory rotatory shaker until the appropriate conversion was reached (according to GC and HPLC analysis). Next, the reaction was terminated by filtering off the enzyme. After washing the enzyme with TBME (2 × 25 mL), the permeate was concentrated under reduced pressure, and the crude residue was purified by column chromatography on silica gel using gradient of *n*-hexane/AcOEt (80:10, 70:10, 60:10, 50:10 and 40:10 v/v) mixture as an eluent thus yielding the respective resolution products [(*R*)-(–)-**3a-h** and (*S*)-(+)-**4a-h**]. The detailed experimental conditions, yields and the results of enzymatic KR reactions (including enantiomeric excess data for the resolved products and values of enantioselectivity factor) are collected in **Table 4**. Physical, spectroscopic and analytical data are identical as for the corresponding racemic standard compounds *rac*-**3a-h** and *rac*-**4a-h**. The specific rotations for the enantiomerically enriched esters (*R*)-(–)-**3a-h** and diesters (*S*)-(+)-**4f-h** are as follows: (*R*)-(–)-**3a**: $[\alpha]_D^{26}$ = –202.40 (*c* 1.02, $CHCl_3$, >99% ee) {lit.[70] $[\alpha]_D^{30}$ = –108.70 (*c* 1.00, $CHCl_3$, 78% ee)}. (*R*)-(–)-**3b**: $[\alpha]_D^{25}$ = –161.70 (*c* 1.11, $CHCl_3$, >99% ee) {lit.[70] $[\alpha]_D^{30}$ = –82.60 (*c* 1.00, $CHCl_3$, 90% ee)}. (*R*)-(–)-**3c**: $[\alpha]_D^{24}$ = –147.20 (*c* 1.00, $CHCl_3$, >99% ee). (*R*)-(–)-**3d**: $[\alpha]_D^{22}$ = –147.50 (*c* 1.28, $CHCl_3$, >99% ee) {lit.[58] $[\alpha]_D^{21}$ = –127.20 (*c* 1.00, $CHCl_3$, 96% ee)}. (*R*)-(–)-**3e**: $[\alpha]_D^{24}$ = –136.80 (*c* 1.00, $CHCl_3$, 99% ee). (*R*)-(–)-**3f**: $[\alpha]_D^{26}$ = –112.68 (*c* 1.03, acetone, 99% ee) {lit.[70] $[\alpha]_D^{30}$ = –129.1 (*c* 1.00, $CHCl_3$, 90% ee) or lit.[76] $[\alpha]_D^{24}$ = +93.00 (*c* 1.08, acetone, 66% ee) for (*S*)-enantiomer}. (*R*)-(–)-**3g**: $[\alpha]_D^{23}$ = –168.29 (*c* 1.02, $CHCl_3$, 98% ee) {lit.[75] $[\alpha]_D^{28}$ = –164.00 (*c* 1.00, $CHCl_3$, >99.9% ee)}. (*R*)-(–)-**3h**: $[\alpha]_D^{24}$ = –157.90 (*c* 1.00, $CHCl_3$, 86% ee) {lit.[58] $[\alpha]_D^{25}$ = +184.60 (*c* 1.00, $CHCl_3$, >99% ee) for (*S*)-enantiomer or lit.[79] $[\alpha]_D^{28}$ = +157.70 (*c* 0.50, EtOH, >99.2% ee) for (*S*)-enantiomer}. (*S*)-(+)-**4f**: $[\alpha]_D^{26}$ = +118.94 (*c* 1.14, acetone, 62% ee) {lit.[39m] $[\alpha]_D^{25}$ = –7.05 (*c* 0.45, $CHCl_3$, 97.1% ee)}. (*S*)-(+)-**4g**: $[\alpha]_D^{23}$ = +164.67 (*c* 1.00, $CHCl_3$, 85% ee). (*S*)-(+)-**4h**: $[\alpha]_D^{25}$ = +194.10 (*c* 1.00, $CHCl_3$, 86% ee) {lit.[57] $[\alpha]_D^{27}$ = +226.40 (*c* 1.00, $CHCl_3$, >99% ee)}.

Synthesis of optically active pemoline [(*R*)-(+)-5]: To a solution of guanidine hydrochloride (299 mg, 3.13 mmol) in dry EtOH (7.5 mL) NaOH (125 mg, 3.13 mmol) was added, and the whole was stirred at room temperature for 1 h until white precipitate was formed. Next, methyl (2*R*)-hydroxy(phenyl)ethanoate (*R*)-(–)-**3a** (520 mg, 3.13 mmol, >99% ee) was added, and thus composed reaction mixture was further stirred for 60 h at RT. After this time, the content of the flask was partially condensed to a 1/3 of the initial volume, and the ice-cold H_2O (5 mL) was added. The obtained suspension was neutralized by means of glacial acetic acid. Filtration gave white solid, which was rinsed with ice-cold H_2O (4 × 5 mL), and Et_2O (3 × 5 mL) to yield desired product (*R*)-(+)-**5** (221 mg, 40%, 98% ee). $[\alpha]_D^{24.1}$ = +110 (*c* 0.20, MeOH, 98% ee); $[\alpha]_D^{27.6}$ = +108 (*c* 0.20, MeOH, >99% ee); mp 240–240.5 °C (racemic pemoline, EtOH, decomp.) [reported for racemic pemoline: lit.[80] 242–250 °C (no data) or lit.[81] 256–257 °C (EtOH, decomp.)]; ^{1}H NMR [500 MHz, $(CD_3)_2SO$)]: δ 5.71 (s, 1H); 7.25–7.29 (m, 2H), 7.38–7.44 (m, 3H), 8.52 (br s, 1H), 8.74 (br s, 1H); ^{13}C NMR [126 MHz, $(CD_3)_2SO$)]: δ 82.3, 126.2, 128.6, 128.7, 134.6, 176.7, 186.4 [Attention: in DMSO-d_6 only imino form of pemoline, that is 2-imino-5-phenyl-1,3-oxazolidin-4-one (*R*)-(+)-**5b** was observed]; IR (nujol): ν_{max} = 3264, 1656, 1565, 1505, 1492, 1445, 1282, 1224, 1136, 1035, 1016, 969, 935, 765, 700, 656; HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_9H_9N_2O_2^+$ 177.0659, Found 177.0880; $[2M+H]^+$ $C_{18}H_{17}N_4O_4^+$ m/z: 353.1250, Found 353.1628; $[M-H]^-$ Calcd for $C_9H_7N_2O_2^-$ 175.0513, Found 175.0641; $[2M-H]^-$ Calcd for $C_{18}H_{15}N_4O_4^-$ m/z: 351.1099, Found 351.1701; HPLC [*n*-hexane-*i*-PrOH (90:10, v/v); f=0.8 mL/min; λ=215 nm]: t_R=32.816 and 40.669 min.

Absolute configuration assignment of the EKRs' products [derivatization of (*R*)-(–)-3e toward hexafluorophosphate salt (*R*)-(–)-11]

Preparation of 1-(2-hydroxyethyl)pyridinium bromide (7): In a three-neck round-bottom flask equipped with a thermometer, reflux condenser attached with drying tube filled with anhydrous $CaCl_2$, and argon gas inlet an equimolar amounts of freshly distilled pyridine (9.9 g, 0.13 mol) and bromoethanol **6** (15.6 g, 0.13 mol) were stirred for 72 h at 50 ºC under low-light conditions and a gentle argon flow. Next, the reaction mixture was cooled to room temperature, portion of dry Et_2O was added, and the precipitate formed was washed thoroughly with Et_2O (2 × 15 mL). Purification of the crude was performed by recrystallization from 2-PrOH to yield product **7** (19.1 g, 0.09 mol, 72%) as a white solid. Mp 96–98 °C (2-PrOH) [lit.[82] 99–103 °C (EtOH)]; ^{1}H NMR (400 MHz, D_2O): δ 4.07 (t, *J* = 4.8 Hz, 2H), 4.73 (t, *J* = 4.8 Hz, 2H), 8.10 (t, *J* = 7.0 Hz, 2H), 8.59 (t, *J* = 8.0 Hz, 1H), 8.87 (d, *J* = 6.8 Hz, 2H) (The spectral data are fully consistent with those reported previously in lit.[82]); ^{13}C NMR (100 MHz, D_2O): δ 61.0, 64.1, 128.8, 145.3, 146.6 (The spectral data are fully consistent with those reported previously in lit.[83]); Anal. Calcd for $C_7H_{10}BrNO$: C, 41.20; H, 4.94; N, 6.86. Found: C, 41.30; H, 5.04; N, 6.76

Preparation of 1-(2-hydroxyethyl)pyridin-1-ium hexafluorophosphate (8): A solution of **7** (0.5 g, 2.5 mmol) and KPF_6 (0.9 g, 4.80 mmol) in dry CH_3CN (4 mL) was stirred for 24 h at reflux temperature. Next, the reaction mixture was cooled to room temperature, the precipitate formed was filtered off, and the resulting permeate was evaporated to dryness. The remaining residue was dissolved in AcOEt (10 mL) and stirred for 1 h at room temperature. After this time, the precipitate formed was filtered off, the solvent was evaporated under reduced pressure, and AcOEt (8 mL) was added. The content of the flask was further stirred for 1 h at room temperature, and the precipitated KPF_6 was subsequently removed by filtration. The whole procedure was repeated twice by adding the respective portions of AcOEt (6 mL and 4 mL). After evaporation of the solvent residues, the desired product **8** (625 mg, 2.33 mmol, 93%) was afforded as white solid, which was further used without purification.

Preparation of methyl (2*R*)-2-(4-*tert*-butylphenyl)-2-methoxyacetate [(*R*)-(–)-9]: A mixture of (*R*)-(–)-**3e** (1.42 g, 6.02 mmol, 99% ee), freshly prepared powdered Ag_2O (1.7 g, 7.12 mmol), roasted $CaSO_4$ (2.3 g, 7.12 mmol), and methyl iodide (4.2 g, 29.36 mmol, 9.5 mL) was stirred for 36 h at reflux temperature under low-light conditions. After this time, the content of the flask was allowed to cool, the precipitate formed was filtered off, and washed with portions of Et_2O (3 × 5 mL). The permeate was evaporated to dryness, and the residual crude product was chromatographed on silica gel using gradient of *n*-hexane/AcOEt (90:10, 80:10, 70:10, and 60:10 v/v) mixture as an eluent to afford respective product (*R*)-(–)-**9** as an oil. The obtained product (*R*)-(–)-**9** was used as a

crude mixture in the next step. Caution: fresh Ag_2O was prepared by adding a 10% aqueous solution of NaOH to a 10% aqueous solution of $AgNO_3$ until precipitation stopped; next, the slurry of Ag_2O formed was filtered off, and the excess of water was evaporated under reduced pressure (all the procedure was performed under low-light conditions). In turn, $CaSO_4$ was oven-roasted at 270 °C, left to cool and used immediately.

Preparation of (2*R*)-2-(4-*tert*-butylphenyl)-2-methoxyacetic acid [(*R*)-(–)-10]: To a stirred solution of (*R*)-(–)-**9** (827 mg, 3.50 mmol) in MeOH (7 mL) 1M aqueous solution of LiOH monohydrate (14 mL) was slowly added. The reaction mixture was stirred for 24 h at RT. Afterwards, methanol was removed under vacuum, and residue was extracted with Et_2O (3 × 10 mL). Subsequently, the aqueous layer was cooled to 0–5 °C, acidified using concentrated 36% HCl solution until the pH 1, and back-extracted with Et_2O (3 × 10 mL). The combined organic layers were quenched with brine (2 × 5 mL), dried over anhydrous $MgSO_4$, and concentrated to give (*R*)-(–)-**10** (762 mg, 3.43 mmol, 98%) as a white solid. Mp 118–119.5 °C (Et_2O); $[\alpha]_D^{27}$ = –119.40 (*c* 0.65, $CHCl_3$, 99% ee); ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 1.31 (s, 9H), 3.41 (s, 3H), 4.76 (s, 1H), 7.34–7.37 (m, 2H), 7.37–7.40 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 31.3, 34.7, 57.3, 81.8, 125.6, 126.9, 132.0, 152.0, 175.1; FTIR (neat): ν_{max} = 3219 (br s), 2950, 2902, 2866, 2827, 1748, 1514, 1460, 1412, 1363, 1270, 1256, 1223, 1191, 1183, 1097, 989, 834, 772, 706, 679, 582, 542, 435; Anal. Calcd for C, 70.24; H, 8.16. Found: C, 70.27; H, 8.14.

1-(2-{[(2*R*)-2-(4-*tert*-butylphenyl)-2-methoxyacetyl]oxy}ethyl)pyridin-1-ium hexafluoro-λ5-phosphate [(*R*)-(–)-11]: A solution of **8** (299 mg, 1.11 mmol), (*R*)-(–)-**10** (273 mg, 1.23 mmol), and DMAP (10 mg) in dry CH_3CN (20 mL) was stirred for 48 h at RT. Next, the dicyclohexylurea (DCU) precipitate formed was filtered off, washed with portion of CH_3CN (5 mL), and the resulting filtrate was concentrated. The residue was rinsed with hot $PhCH_3$ (3 × 2.5 mL) and Et_2O (3 × 2.5 mL), respectively. The residual crude was chromatographed on activated charcoal using gradient of CH_3CN/AcOEt (50:10, 40:10, and 30:10 v/v) mixture as an eluent to afford corresponding salt, which was additionally recrystallized from mixture of CH_3CN/Et_2O to yield (*R*)-(–)-**11** (360 mg, 0.76 mmol, 69%) as a white solid. Mp 130–133 °C (CH_3CN/Et_2O); $[\alpha]_D^{22}$ = –25.50 (*c* 0.70, acetone, 99% ee); ^{1}H NMR (400 MHz, CD_3COCD_3): *δ* 1.31 (s, 9H), 3.29 (s, 3H), 4.75 (m, 2H), 4.84 (s, 1H), 5.12 (m, 2H), 7.26–7.28 (m, 2H), 7.40–7.42 (m, 2H), 8.16 (m, 2H), 8.71 (m, 1H), 8.96–8.98 (m, 2H); ^{13}C NMR (100 MHz, CD_3COCD_3): *δ* 31.5, 35.1, 57.4, 61.3, 63.3, 63.6, 82.4, 126.3, 127.8, 129.2, 134.5, 146.1, 147.3, 152.4, 170.7; Anal. Calcd for $C_{20}H_{26}F_6NO_3P$: C, 50.74; H, 5.54; N, 2.96. Found: C, 50.70; H, 5.50; N, 2.93.

X-ray crystallography

Crystal structure determination of (*R*)-(–)-**11**: Colorless single crystals, suitable for X-ray diffraction studies, were grown by slow diffusion of AcOEt (0.5 mL) into a concentrated solution of (*R*)-(–)-**11** (50 mg) in CH_3CN (1 mL) at 0-5 °C. Selected crystal of dimensions 0.32×0.15×0.1 mm^3 was mounted in inert oil and transferred to the cold gas stream of the diffractometer. Diffraction data were measured at 100.0(1) K with mirror monochromated Cu*K*α radiation on an Oxford Diffraction κ-CCD Gemini A Ultra diffractometer. Cell refinement and data collection as well as data reduction and analysis were performed with the crysalis^{pro} software.[84] The structure was solved by direct methods using the shelxs-97 structure solution program and refined by full-matrix least-squares against F^2 with shelxl-97[85] and olex2[86] programs. All non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms attached to carbon atoms were added to the structure model at geometrically idealized coordinates and refined as riding atoms. An absolute (*R*)-configuration for the compound molecule was successfully determined using anomalous dispersion effects. Flack parameter[87] calculated from 1544 selected quotients (Parsons' method)[88] equals −0.004(18). CCDC1527174 contains the supplementary crystallographic data for compound (*R*)-(–)-**11**. This can be obtained free of charge on application to CDC, 12 Union Road, Cambridge CB21EZ, UK (Fax: (+44)1223-336-033; email: deposit@ccdc.cam.ac.uk).

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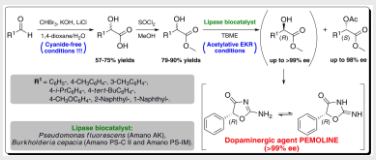

Chemoenzymatic synthesis of homochiral (*R*)-pemoline was developed. For this purpose, methyl (*R*)-(–)-mandelate was prepared in an environmentally benign route leading through cyanide-free synthesis of racemic mandelic acid, and acetylative kinetic resolution approach using lipases as biocatalysts. Enzymatic protocol can also be applied with excellent enantioselectivity toward novel MA derivatives.

Biocatalysis, Enantiopure drugs*

Marcin Poterała, Maciej Dranka, and Paweł Borowiecki**

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Chemoenzymatic preparation of enantiomerically enriched (*R*)-(–)-mandelic acid derivatives: application in the synthesis towards the active agent pemoline