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Enzymatic esterification of bicyclic *meso*-diols derived from 1,4-bis(hydroxymethyl)furan. An enantioselective Diels–Alder reaction equivalent

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Abstract

meso-Diols derived from the Diels–Alder adduct 1,4-bis(hydroxymethyl)furan/dimethyl acetylenedicarboxylate can be enantioselectively monoacetylated under CRL or PSL catalysis with very good yields and moderate to excellent ees. (+)-Monoacetates are always preferentially formed in the reactions catalyzed by CRL, and their (−)-enantiomers are the main products in the acetylations under PSL catalysis. Absolute configurations have been determined by X-ray analysis of a single crystal of an (*R*)-α-methoxyphenylacetyl derivative.

1. Introduction

The combination of the diastereoselectivity of cycloaddition reactions with the enantioselectivity of many enzymatic processes has developed into a powerful tool with a considerable potential for the synthesis of chiral organic products. The desymmetrization of *meso*-diesters and *meso*-diols by means of commercially available esterases and lipases represents an interesting, and often cheaper, alternative to asymmetric cycloadditions with chiral auxiliaries.[1–5] Esterification reactions are particularly interesting for synthetic work. In contrast with enzymatic hydrolyses, they may be performed in organic solvents, which obviates the solubility problems associated with the presence of water in the reaction medium.[6–9] The sequential cycloaddition–enzymatic desymmetrization procedure has revealed a simple and useful route for the preparation of chiral bicyclic compounds, which are in turn valuable precursors for the

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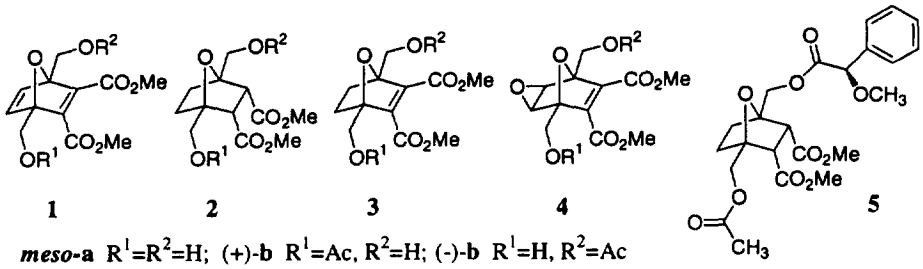

Fig. 1.

synthesis of cyclic and acyclic compounds with multiple stereocenters.[10–15] As an example of this, we have previously shown[16,17] that bicyclic Diels–Alder adducts of furan may be efficiently desymmetrized with the aid of inexpensive, commercially available lipases. The chiral intermediates derived thereof should be useful for the preparation of various substituted cyclohexanes and tetrahydrofurans.

In connection with the last point, several reports have appeared dealing with enzyme-mediated acylation of *cis*- and *trans*-2,5-bis(hydroxymethyl)tetrahydrofuran derivatives and hydrolysis of the corresponding esters.[18–21] However, no studies have been performed so far on the enzymatic desymmetrization of *meso*-Diels–Alder adducts of the parent 2,5-bis(hydroxymethyl)furan. This could be a particularly interesting aspect, due to the fact that up to four stereogenic centers may be generated in such a process. As part of our interest in the use of enzymes in organic solvents for the desymmetrization of bicyclic *meso*-diols,[16,17] we here report a study on the lipase-catalyzed monoacetylation of *meso*-cycloadducts **1a–4a**.

2. Results and discussion

The starting materials were prepared through the Diels–Alder reaction between 2,5-bis(hydroxymethyl)furan and dimethyl acetylenedicarboxylate to yield **1**, followed by either hydrogenation **2**, **3**, or epoxidation **4** (Fig. 1). The oxidation was carried out in dichloromethane solution[22] instead of water[23] due to the high solubility of the epoxide in this last solvent.† The acylations were catalyzed by commercially available lipases (see below).

Enzymatic reactions were performed in all cases by adding 400 mg of the commercial enzyme to a stirred solution of 200 mg of the corresponding *meso*-diol in 10 mL of solvent. Reaction time and temperature are indicated in the Table 1. Since only one enantiomerically pure compound is formed, the optimum reaction time corresponds to the moment at which the optical rotation reaches its maximum value. So, the course of the reaction was followed by monitoring the optical rotation. When the maximum value was reached the reactions were stopped by enzyme filtration. After solvent removal *in vacuo*, the monoacetylated derivatives were purified by column chromatography on silica gel, except in the case of compounds **4**, where alumina had to be used in order to avoid the acid-promoted epoxide opening. The enantiomeric excess was determined by means of ^{1}H NMR spectroscopy in the presence of 0.1–0.2 equiv. of the chiral shift reagent (+)-Eu(hfc)$_3$. Esterification with Mosher's acid chloride was not possible in any case, probably because of steric hindrance. Chemical yields and enantiomeric excesses (ee %) of the monoacetates are summarized in Table 1.

† The *exo*-configuration of **4a** has been ascertained by X-ray analysis of the bis(methoxymethyl) analog.

Table 1
Lipase-mediated acetylation of bicyclic *meso*-diols **1a–4a**

Substrate	Enzyme	Solvent[a]	Time	Product	Yield(%)	$[\alpha]_D^{20}$ [b]	Ee (%)[c]
1a	CRL[d]	EA:VA (5:1)	4h	(+)-**1b**	80	+13.3	80
1a	PSL[d]	VA	7h	(−)-**1b**	88	-14.5	86
2a	CRL[d]	EA:VA (5:1)	3h15m	(+)-**2b**	95	+13.2	95
2a	PSL[d]	VA	24h	(−)-**2b**	85	-8.9	64
3a	CRL[d]	EA:VA (5:1)	7h30m	(+)-**3b**	70	+12.6	80
3a	PSL[d]	VA	15h	(−)-**3b**	84	-11.9	76
4a	CRL[d]	EA:VA (5:1)	15h	(+)-**4b**	90	+11.9	82
4a	PSL[e]	VA	19h	(−)-**4b**	91	-11.5	80

[a] EA: ethyl acetate; VA: vinyl acetate.
[b] ($CHCl_3$, *c* 1.0)
[c] Determined by ^{1}H NMR using (+)-Eu(hfc)$_3$ as a chiral shift reagent
[d] Reaction conducted at room temperature.
[e] Reaction conducted at 40°C.

Monoacetylation of compounds **1a–4a** was assayed with three commercially available lipases: porcine pancreatic lipase (PPL) and *Candida rugosa* lipase (CRL) from Sigma, and *Pseudomonas cepacia* lipase (PSL) from Amano.[‡] The reactions were performed in either ethyl acetate (EA) or vinyl acetate (VA), which acted both as solvents and acylating reagents. Only CRL and PSL yielded the desired products **1b–4b** with good chemical yields and enantioselectivities. Acylations with PPL were extremely slow, even in vinyl acetate (VA) at 40°C. For instance, the conversion of diols **1a** and **2a** into their monoacetylated derivatives **1b** and **2b** under these conditions was less than 50% after 40 h, the ee being about 10%. In contrast, the CRL-mediated acylation of **1a** and **2a** in vinyl acetate (VA) was very fast, significant amounts of the diacylated product being formed in only 60 min at room temperature. In order to get a better control over the monoacylation reaction, VA was diluted to 1:5 with the less reactive EA (the use of less polar solvents was problematic due to the low solubility of the starting diols). These conditions caused a slower reaction and, consequently, a more satisfactory ee. Reactions catalyzed with CRL proceeded faster than with PSL in all cases. The acylation of the epoxy adduct **4a** proved quite slow with both lipases and was thus performed in VA at 40°C when PSL was used as catalyst. Interestingly, CRL and PSL showed opposite stereopreferences in the monoacylation of **1a–4a**. In fact, while the use of CRL gave rise in all cases to the preferential formation of the (+)-enantiomer, its (−)-antipode was always the main product in monoacetylations catalyzed by PSL. Both enantiomers are thus readily available with good yields and ees, depending exclusively on which lipase was chosen for the acylation process.

In order to verify the values of enantiomeric purity deduced from the ^{1}H NMR spectra in the presence of (+)-Eu(hfc)$_3$, several transformations of the enzymatic acylation products have been carried out. Observed $[\alpha]_D$ and ees deduced from the ^{1}H NMR spectra for the products of these reactions are included in Table 2. The monoacetyl derivatives (+)-**1b** and (−)-**1b**, obtained with CRL and PSL from **1a** (80%

‡ *Candida rugosa* and *Pseudomonas cepacia* were previously classified as *Candida cylindracea* and *Pseudomonas fluorescens*, respectively. See Kaslauskas et al.[24] for additional details.

Table 2
Chemical correlations of enantiomeric monoacetates

Substrate	$[\alpha]_D^{20}$ [a]	Ee[b](%)	Product	$[\alpha]_D^{20}$ [a]	Ee[b](%)
(+)-**1b**	+13.3	80	(+)-**2b**	+11.2	80
			(+)-**3b**	+12.5	80
			(+)-**4b**	+11.5	80
(−)-**1b**	-14.5	86	(−)-**2b**	-12.1	86
			(−)--**3b**	-13.5	86
			(−)-**4b**	-12.5	86
(+)-**3b**	+12.6	80	(+)-**2b**	+11.2	80
(−)-**3b**	-11.9	76	(−)-**2b**	-10.6	76

[a] ($CHCl_3$, *c* 1.0)
[b] Determined by ^1H NMR using (+)-Eu(hfc)$_3$ as chiral shift reagent

and 86% ee, respectively), were submitted to catalytic hydrogenation in the presence of Pd(C) to give (+)-**2b** and (−)-**2b**, respectively. The ees obtained for these saturated bicyclic structures were in agreement with those of the precursors. Hydrogenation of (+)-**1b** and (−)-**1b** in the presence of Pd($CaCO_3$) gave the corresponding monohydrogenated analogs (+)-**3b** and (−)-**3b**, whose ee values were also identical to those of the starting monoacetates. The epoxidation of (+)-**1b** and (−)-**1b** under the same conditions as described for the corresponding *meso*-diol **1a** gave the corresponding epoxy monoacetyl esters (+)-**4b** and (−)-**4b** which showed, again, 80% and 86% ees, respectively. Moreover, monoacetyl esters (+)-**3b** and (−)-**3b** (80% and 76% ee, respectively), obtained by enzymatic acetylation of **3a**, were also submitted to hydrogenation in the presence of Pd(C). Compounds (+)-**2b** and (−)-**2b** obtained from them showed the same enantiomeric purity as their precursors. (See the experimental section for chemical yields and reaction conditions.)

The determination of the absolute configuration of the products resulting from the enzymatic acylation appeared to be rather difficult. The specific rotations of the monoacetyl esters or derivatives are not known, so we decided to establish the molecular structure by X-ray crystallography. (+)-**2b**, a solid monoacetate obtained with excellent enantiomeric excess from CRL, was chosen for the analysis. Unfortunately, all attempts at determining the absolute configuration of this compound based on the presence of the oxygen atoms as heavy atoms were unsuccessful. Several solid derivatives of (+)-**2b**, obtained by esterification of the free hydroxyl group with homochiral carboxylic acids, were prepared in order to obtain a single crystal suitable for X-ray analysis. Finally, the X-ray analysis of the ester **5** derived from (*R*)-α-methoxyphenylacetic acid allowed us to determine the absolute configuration of (+)-**2b** which was revealed as (1*S*,2*S*,3*R*,4*R*) (see Fig. 2). From this result, the absolute configuration of the remaining (+)-monoacetates obtained in the CRL catalyzed acylation could be established as follows: (1*S*,4*R*) for (+)-**1b**, (1*S*,4*R*) for the monohydrogenated analog (+)-**3b** and (1*S*,4*R*,5*R*,6*S*) for the epoxide (+)-**4b**. The opposite configurations could be assigned to the corresponding (−)-enantiomers, obtained preferentially under PSL catalysis.

Several empirical models have been recently suggested to predict which enantiomer of a racemic alcohol would be more reactive towards enzymatic acylation catalyzed by lipases. A simple rule, based on the size of the substituents at the stereocenter, has been proposed to explain the enantiopreference

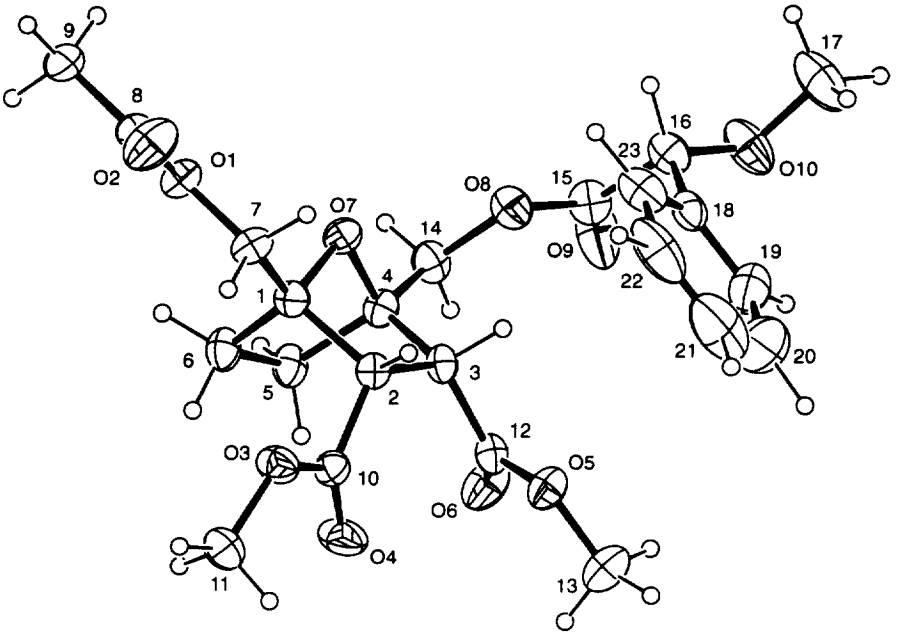

Fig. 2. Molecular structure of **5**. Thermal ellipsoids at 30%. Labels for carbon atoms only include the number used in the X-ray crystal analysis

of both *Candida rugosa* and *Pseudomonas cepacia* lipases in acylations of secondary alcohols.[24] This model correctly predicts the fast-reacting enantiomer of the alcohol in reactions catalyzed by lipase from *Pseudomonas cepacia*. Nevertheless, the enantioselectivity of CRL is only reliably predicted by this rule for cyclic secondary alcohols. Moreover, in the case of esterification of primary alcohols promoted by lipase from *Pseudomonas cepacia*, opposite enantiopreferences have been found depending on the presence or absence of oxygen substituents at the nearby stereocenter, which was tertiary in all the cases reported.[25,26] Consequently, the existing models do not allow us to convincingly predict which of the enantiotopic hydroxyl groups in *meso*-diols **1a–4a** would be preferentially acylated by CRL or PSL. The presence of the rigid oxygen bridge in the substrates assayed by us may possibly become a key factor to determine the interesting opposite enantiopreference observed.

3. Experimental

Melting points were determined on a Cambridge instrument and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC250 spectrometer with tetramethylsilane as internal reference (δ ppm) and $CDCl_3$ as solvent. IR spectra were run on a Perkin–Elmer 843 spectrometer. High resolution mass spectra (HRMS) were conducted on a VG AUTOSPEC instrument. Optical rotation measurements were determined on a Perkin–Elmer 241 polarimeter in chloroform (20 mg in 2 mL) at room temperature. Enantiomeric excess (ee) was determined by ^{1}H NMR with europium tris-[3-(heptafluoropropylhydroxymethylene)-(+)-camphorate] (+)-Eu(hfc)$_3$. All the solvents were used without further purification. Dichloromethane was dried with CaH_2 and was distilled prior to use. All the reagents were used as received except *m*-chloroperbenzoic acid, which was purified by stirring in a buffered solution (pH=7.5), followed by filtration and two successive crystallizations from diethyl

ether:hexane (3:1) at −20°C. *meso*-Diols were prepared according to literature methods described for similar transformations: **1**,[27] **2**,[17] **3**[28] and **4**.[22]

3.1. Dimethyl 1,4-bis(hydroxymethyl)-7-oxabicyclo[2.2.1]hept-2,5-dien-2,3-dicarboxylate *1a*

2,5-Bis(hydroxymethyl)furan (5 g, 39 mmol) was dissolved in toluene (50 mL) and dimethyl acetylenedicarboxylate (4.78 mL, 39 mmol) was added. The solution was refluxed for 8 h until disappearance of the reagents by TLC. After reaching room temperature, the formation of an insoluble oil was observed. The toluene solution was decanted and the solvent evaporated under vacuum. The oily residue was purified by column chromatography on silica gel (hexane:ethyl acetate=1:3), affording the corresponding adduct (3.7 g, 35%). ^{1}H NMR: 3.30 (b.s, 2H), 3.80 (s, 6H), 4.25 (m, 4H), 7.05 (s, 2H); ^{13}C NMR: 52.55 (q), 59.64 (t), 97.31 (s), 144.07 (d), 153.26 (s), 164.15 (s); IR (CH_2Cl_2) ν: 3423, 1710, 1640, 1312 cm^{-1}; HRMS analysis (EI, M$^+$): 270.0748, calcd ($C_{12}H_{14}O_7$): 270.0739.

3.2. Dimethyl 1,4-bis(hydroxymethyl)-7-oxabicyclo[2.2.1]heptan-2,3-dicarboxylate *2a*

To a solution of **1a** (3 g, 11.1 mmol) in ethyl acetate (300 mL), 200 mg of Pd(C) were added. The mixture was stirred for 20 h in a hydrogen atmosphere at room temperature and atmospheric pressure. The solid was filtered off and the filtrate was concentrated to give the diol **2a** as a white solid (2.94 g, 98%). Mp 111–115°C; ^{1}H NMR: 1.59 (d, J=7 Hz, 2H), 2.05 (d, J=7 Hz, 2H), 3.27 (s, 6H), 3.41 (s, 2H), 3.83 (s, 4H); ^{13}C NMR: 28.03 (t), 48.21 (d), 51.79 (q), 62.52 (t), 89.52 (s), 171.03 (s); IR (CH_2Cl_2) ν: 3247, 2996, 2955, 2922, 1743, 1467, 1211 cm^{-1}; HRMS analysis (EI, M$^+$): 274.1130, calcd ($C_{12}H_{18}O_7$): 274.1131.

3.3. Dimethyl 1,4-bis(hydroxymethyl)-7-oxabicyclo[2.2.1]hept-2-en-2,3-dicarboxylate *3a*

To a solution of **1a** (3.1 g, 11.4 mmol) in ethyl acetate (290 mL), 150 mg of Pd($CaCO_3$) was added. The mixture was stirred for 4 h in a hydrogen atmosphere at room temperature and atmospheric pressure. The solid was filtered off and the filtrate was concentrated to give the diol **3a** as an oil (2.81 g, 90%), which was crystallized from hexane:ethyl acetate. Mp 97–100°C; ^{1}H NMR: 1.19 (dd, J=3.7 and 11.3 Hz, 2H), 1.59 (dd, J=3.7 and 11.3 Hz, 2H), 3.45 (b.s, 2OH), 3.77 (s, 6H), 3.98 (d, J=12.7 Hz, 2H), 4.20 (d, J=12.7 Hz, 2H); ^{13}C NMR: 28.15 (t), 52.17 (q), 61.17 (t), 92.23 (s), 143.84 (s), 163.63 (s); IR (CH_2Cl_2) ν: 3284, 2953, 1750, 1455, 1330, 1288, 1251, 1141 cm^{-1}; HRMS analysis (EI, M$^+$): 272.0886, calcd ($C_{12}H_{16}O_7$): 272.0896.

3.4. Dimethyl exo-5,6-epoxy-1,4-bis(hydroxymethyl)-7-oxabicyclo[2.2.1]hept-2-en-2,3-dicarboxylate *4a*

The procedure described in the literature was followed for the epoxidation of **1a**.[22] The obtained residue was purified by column chromatography on alumina (dichloromethane:methanol=98:2) to afford **4a** as an oil (38%). ^{1}H NMR: 3.70 (s, 2H), 3.75 (s, 6H), 4.11 (m, 4H); ^{13}C NMR: 52.66 (q), 57.28 (t), 58.70 (d), 91.20 (s), 148.49 (s), 163.19 (s); IR (CH_2Cl_2) ν: 3423, 1710, 1640, 1312 cm^{-1}; HRMS analysis (EI, M$^+$): 286.0770, calcd ($C_{12}H_{18}O_8$): 286.0767.

3.5. *General procedure for the enzymatic acetylation of* meso-*diols* **1a–4a**

Reactions with CRL were always conducted in a mixture of ethyl acetate:vinyl acetate (5:1). For the reactions with PSL, pure vinyl acetate was employed as solvent and acylating reagent. To a solution of the diol in the prescribed solvent (0.05 mL/mg diol), the corresponding enzyme (2 mg/mg diol) was added. The reaction mixture was vigorously stirred for the time and at the temperature given for each case in Table 1. The reaction was stopped by enzyme filtration. Evaporation of the solvents and column chromatography of the residue gave the purified monoacetates. Chemical yields and enantiomeric excess for each monoacetate are also included in Table 1.

3.5.1. *Enzymatic acetylation of* **1a**

Following the general procedure described above, the CRL catalyzed acetylation of **1a** gave, after purification of the crude mixture by column chromatography on silica gel and a mixture of hexane:ethyl acetate (1:1) as eluent, dimethyl (1*S*,4*R*)-1-acetoxymethyl-4-hydroxymethyl-7-oxabicyclo[2.2.1]hept-2,5-dien-2,3-dicarboxylate (+)-**1b** (80% yield). The same procedure applied to the reaction with PSL afforded the (1*R*,4*S*) enantiomer (−)-**1b** (88% yield). ^{1}H NMR: 2.08 (s, 3H), 3.81 (s, 3H), 3.82 (s, 3H), 4.29 (b.s, 2H), 4.74 (d, J=12.8 Hz, 1H), 4.83 (d, J=12.8 Hz, 1H), 7.04 (d, J=5.1 Hz, 1H), 7.12 (d, J=5.1 Hz, 1H); ^{13}C NMR: 20.55 (q), 52.64 (q), 53.40 (q), 59.83 (t), 60.70 (t), 94.45 (s), 97.24 (s), 143.63 (d), 144.60 (d), 152.10 (s), 153.93 (s), 163.59 (s), 163. 94 (s), 170.42 (s); IR (CH_2Cl_2) ν: 3510, 2952, 1709, 1637, 1437, 1235, 1045 cm^{-1}; HRMS analysis (CI, MH$^+$): 313.0923, calcd ($C_{14}H_{17}O_8$): 313.0923.

3.5.2. *Enzymatic acetylation of* **2a**

The CRL catalyzed acetylation of **2a** following the general procedure indicated above gave, after purification of the crude mixture by silica gel chromatography (hexane:ethyl acetate=1:2), dimethyl (1*S*,2*S*,3*R*,4*R*)-1-acetoxymethyl-4-hydroxymethyl-7-oxabicyclo[2.2.1]heptan-2,3-dicarboxylate (+)-**2b** in 95% yield. When PSL was used as a catalyst for the acetylation of **2a** following the same procedure, the corrresponding (1*R*,2*R*,3*S*,4*S*) enantiomer (−)-**2b** was obtained in 85% yield. ^{1}H NMR: 1.61 (m, 4H), 2.04 (s, 3H), 3.25 (d, J=12 Hz, 1H), 3.40 (d, J=12 Hz, 1H), 3.611 (s, 6H), 3.83 (s, 2H), 4.29 (d, J=12.6 Hz, 1H), 4.36 (d, J=12.5 Hz, 1H); ^{13}C NMR: 20.71 (q), 27.81 (t), 28.56 (t), 48.28 (d), 48.32 (d), 51.82 (q), 51.85 (q), 62.57 (t), 63.41 (t), 89.39 (s), 89.51 (s), 170.62 (s), 170.62 (s), 170.88 (s); IR (CH_2Cl_2) ν: 3488, 2950, 1734, 1206, 1042 cm^{-1}; HRMS analysis (EI, M$^+$): 316.1166, calcd ($C_{14}H_{20}O_8$): 316.1158.

3.5.3. *Enzymatic acetylation of* **3a**

The general procedure described above was also followed for the acetylation of **3a** with CRL. Dimethyl (1*S*,4*R*)-1-acetoxymethyl-4-hydroxymethyl-7-oxabicyclo[2.2.1]hept-2-en-2,3-dicarboxylate (+)-**3b** was obtained in 70% yield after purification by silica gel (hexane:ethyl acetate=1:1). The same procedure applied to the reaction in the presence of PSL gave the (1*R*,4*S*) enantiomer (−)-**3b** in 84% yield after purification. ^{1}H NMR: 1.59 (m, 2H), 1.89 (m, 2H), 2.06 (s, 3H), 2.50 (b.s, 1H), 3.80 (s, 3H), 3.82 (s, 3H), 4.05 (d, J=12.9 Hz, 1H), 4.19 (d, J=12.7 Hz, 1H), 4.47 (d, J=12.6 Hz, 1H), 4.77 (d, J=12.6 Hz, 1H); ^{13}C NMR: 20.45 (q), 28.00 (t), 28.79 (t), 52.19 (q), 52.41 (q), 61.30 (t), 61.97 (t), 89.08 (s), 92.08 (s), 143.03 (s), 143.84 (s), 163.10 (s), 163.38 (s), 170.38 (s); IR (CH_2Cl_2) ν: 3425, 1636, 1253 cm^{-1}; HRMS analysis (EI, M$^+$): 314.0994, calcd ($C_{14}H_{18}O_8$): 314.1001.

3.5.4. *Enzymatic acetylation of* **4a**

Following the general procedure, the acetylation of **4a** by CRL gave, after purification of the crude mixture by column chromatography on alumina (dichloromethane:methanol=98:2) dimethyl (1*S*,4*R*,5*R*,6*S*)-

exo-5,6-epoxy-1-acetoxymethyl-4-hydroxymethyl-7-oxabicyclo-[2.2.1]hept-2-en-2,3-dicarboxylate (+)-**4b** in 90% yield. The same procedure applied to the PSL catalyzed acetylation afforded the corresponding (1*R*,4*S*,5*S*,6*R*)-enantiomer (−)-**4b** in 91% yield after purification. ^{1}H NMR: 2.10 (s, 3H), 3.80 (s, 1H), 3.82 (s, 1H), 3.84 (s, 3H), 3.86 (s, 3H), 4,23 (s, 2H), 4.62 (d, J=12.5 Hz, 1H), 4.68 (d, J=12.5 Hz, 1H); ^{13}C NMR: 20.50 (q), 52.74 (q), 52,94 (q), 57.14 (d), 57.63 (d), 59.02 (t), 59.89 (t), 88.71 (s), 91.08 (s), 147.78 (s), 148.84 (s), 162.71 (s), 163.11 (s), 170.40 (s); IR (CH_2Cl_2) ν: 3486, 2953, 1720, 1436, 1242 cm^{-1}; HRMS analysis (CI, MH$^+$): 329.0872, calcd ($C_{14}H_{17}O_9$): 329.0872.

3.6. General procedure for the preparation of racemic monoacetyl esters (±)-1b–4b

To a stirred solution of the corresponding *meso*-diol (**1–4**) (0.3 mmol) in dichloromethane (10 mL), equimolar amounts of pyridine and acetic anhydride were added at room temperature. When the reaction was finished (TLC), the mixture was washed with 0.5 N HCl and 5% sodium bicarbonate. The organic layer was treated with anhydrous sodium sulfate and the solvent was evaporated. The residue was purified by column chromatography as above for the corresponding enzymatic acetylation products to afford (±)-**1b** (72% yield), (±)-**2b** (62% yield), (±)-**1b** (55% yield), and (±)-**1b** (60% yield).

3.7. Determination of the enantiomeric purity

The corresponding racemic acetyl ester (±)-**1b–4b** (8–10 mg) was dissolved in $CDCl_3$ in an NMR tube and the ^{1}H NMR spectrum was obtained. Solid (+)-Eu(hfc)$_3$ was added portionwise until baseline separation of the signals. The number of equivalents of shift reagent necessary to obtain baseline separation of peaks (0.1–0.2 equiv.) was then added to the sample of the corresponding enzymatic acetylation product for which the enantiomeric purity was to be determined.

3.8. Chemical correlations of the enantiomeric monoacetates

The same procedures described above applied to the *meso*-diol **1a** were followed for the hydrogenation and epoxidation of the monoacetates. The enantiomeric excesses of the purified products are given in Table 2. Chemical yields are included in the following sections.

3.8.1. (+)-2b from (+)-1b and (−)-2b from (−)-1b

The procedure described for the transformation of **2a** from **1a** was applied to (+)-**1b** and (−)-**1b**. Chromatographic purification of the residues gave (+)-**2b** and (−)-**2b** in 64% yield.

3.8.2. (+)-3b from (+)-1b and (−)-3b from (−)-1b

The procedure described for the transformation of **3a** from **1a** was applied to (+)-**1b** and (−)-**1b**. (+)-**3b** and (−)-**3b** were obtained after purification (40% yield).

3.8.3. (+)-4b from (+)-1b and (−)-4b from (−)-1b

The epoxidation of (+)-**1b** and (−)-**1b** by the conditions described for **1a** gave the corresponding epoxy monoacetates (+)-**4b** and (−)-**4b** in 50% yield. Reaction time: 96 h.

3.8.4. (+)-2b from (+)-3b and (−)-2b from (−)-3b

Hydrogenation of the monoacetates (+)-**3b** and (−)-**3b** with Pd(C) by the conditions described for **1a** gave the saturated derivatives (+)-**2b** and (−)-**2b** in 60% yield.

3.9. *Dimethyl (1R,2R,3S,4S)-1-[(R)-α-methoxyphenylacetoxymethyl]-4-acetoxymethyl-7-oxa-bicyclo-[2.2.1]heptan-2,3-dicarboxylate* **5**

The procedure described for the synthesis of tert-butyl acetate[29] was followed for the esterification of (+)-**2a** with (*R*)-α-methoxyphenylacetyl chloride but pyridine was used instead of dimethylaniline. Compound **5** was obtained as a white solid in 78% yield. Mp (isobutanol): 111–112°C; $[\alpha]_D^{20}=-30$ ($CHCl_3$, *c* 1.0); ^{1}H NMR: 1.64 (m, 2H), 2.03 (m, 2H), 2.04 (s, 3H), 2.90 (d, J=12 Hz, 1H), 3.10 (d, J=12 Hz, 1H), 3.35 (s, 3H), 3.48 (s, 3H), 3.60 (s, 3H), 4,28 (s, 2H), 4.30 (d, J=12.5 Hz, 1H), 4.45 (d, J=12.5 Hz, 1H), 4.80 (s, 1H), 7.25–7.50 (m, 5H); ^{13}C NMR: 20.76 (q), 28.27 (2×t), 48.30 (d), 48.82 (d), 51.74 (q), 51,93 (q), 57.36 (q), 63.40 (t), 63.50 (t), 82.09 (s), 86.64 (2×s), 127.16 (2×d), 128.53 (2×d), 128.71 (d), 136.10 (s), 170.01 (2×s), 170.34 (s), 170.42 (s); IR (CH_2Cl_2) ν: 3486, 2953, 1720, 1436, 1242 cm^{-1}; HRMS analysis (CI, MH^+): 465.1759, calcd ($C_{23}H_{29}O_{10}$): 465.1760.

3.10. *Crystal structure determination for compound* **5**

Crystals were obtained by crystallization from isobutanol. Colorless prismatic crystal, orthorhombic $P2_12_12_1$ (no. 19), a=8.002(2), b=12.322(1), c=23.448(3) Å, V=2312.0(7) Å^3, Z=4, F(000)=984, MoKα radiation (λ=0.7107 Å), μ=1.05 cm^{-1}, D_c=1.33 g cm^{-3}, ENRAF-NONIUS CAD4 diffractometer. At 290 K, 2422 measured reflections in the range $1°<\theta<25°$, 2336 independent reflections, 1630 observed with $I>2\sigma(I)$. Solution by direct methods with the program SIR92,[30] non-hydrogen atoms anisotropically refined using the XRAY76 system,[31] hydrogen atoms placed at calculated positions and included as fixed contributors with a common isotropic temperature factor, 298 refined parameters. In the final stages, an empirical weighting scheme was chosen as to give no trends in $\langle w\Delta^2F\rangle$ vs $\langle F_o\rangle$ and vs $\langle \text{sen}\theta/\lambda\rangle$ using the program PESOS.[32] The final residuals were R=0.048, Rw=0.052, GOF=1.01 and a maximum peak in the final difmap of 0.22 e/Å^3. Geometrical calculations were performed with PARST.[33]

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