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On the kinetic resolution of sterically hindered *myo*-inositol derivatives in organic media by lipases

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ABSTRACT

Sterically hindered *myo*-inositol derivatives were assayed against different commercial lipases. It was found that DL-1,3,6-tri-*O*-benzyl-*myo*-inositol undergoes efficient kinetic resolutions mediated by *Pseudomonas* sp. lipases (PS-C, PS-IM) and CaLB (Novozym 435). Under the best conditions, the O-acylated L-enantiomorph was obtained in up to >99% ee with conversions of up to >49%. Differences in the immobilization support of the *Pseudomonas* sp. lipases had a marked effect on their resolution performance.

1. Introduction

Lipases are now a part of synthetic chemists' toolbox, in great part due to the versatility that such enzymes show.[1,2] In reactions that they mediate, high chemo-, regio- and stereoselectivities are attained under mild conditions and, notably, in organic solvents.[3]

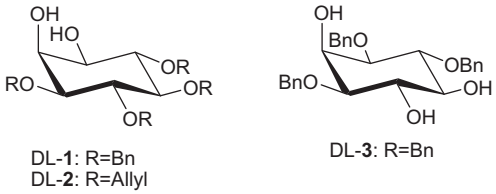
In the present work, we discuss our results on the kinetic resolution (KR) of selectively *O*-polyalkylated *myo*-inositols by lipases, aiming at the efficient chemoenzymatic syntheses of precursors of chiral *myo*-inositol derivatives. Knowledge on the roles of *myo*-inositol phosphates in intricate cellular signaling pathways continue to evolve.[4] Thus, novel derivatives of these compounds are needed for bioassays, which may require new methodologies or improvement of the existing routes.[5,6] Moreover, more practical routes for known inositol derivatives are welcome as they increase their availability for biological studies. In this sense, the use of O-alkylated (i.e., O-benzylated) substrates combined with lipase-catalyzed KRs offer alternative routes to these target molecules that might prove rewarding. Benzyl groups (Bn = $C_6H_5CH_2$) are particularly important for the protection of hydroxyl groups owing to their stability and mode of removal, allowing strategies based on the so-called orthogonal lability.[7]

The use of lipases in the syntheses of chiral inositols has been limited, notwithstanding the clear advantages offered by such biocatalysts.[8–10] Mostly, the known routes for such bioactive substances employ *myo*-inositol itself as a precursor and chiral partially-protected derivatives thereof are subjected to resolutions by derivatization. Recently, we showed that *Candida antarctica* lipase A (Novozym 435) was very effective in the kinetic resolution of DL-1,2-*O*-isopropylidene-3,6-di-*O*-benzyl-*myo*-inositol, a bulky key precursor of different *myo*-inositol phosphates bearing two benzyl groups.[10]

2. Results and discussion

2.1. Screening of lipases and substrates

We reinvestigated the resolution of tetraether DL-**1** (Fig. 1). This is an important precursor of naturally occurring inositol derivatives.[11] Ling and Ozaki[9b] reported that they did not succeed in enzymatically acylating this compound or in hydrolyzing the corresponding acetate. However, they did not mention the enzymes that were tried. Thus, we screened 13 commercial lipases against DL-**1** employing vinyl acetate as the acylating agent in solvent-free media. In no case was conversion to an acetylated product observed. Thus, we investigated the resolution of tetraallylated *myo*-inositol DL-**2**[12] (Fig. 1) (allyl = CH_2CHCH_2) in the hope that a significantly less sterically hindered substrate would yield to



DL-**1**: R=Bn
DL-**2**: R=Allyl

DL-**3**: R=Bn

Figure 1. *myo*-Inositol derivatives assayed in this study.

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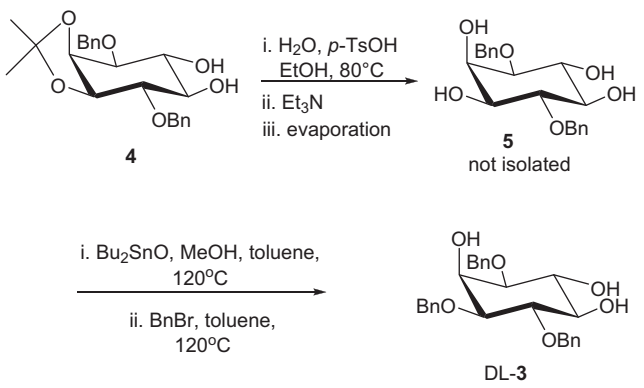
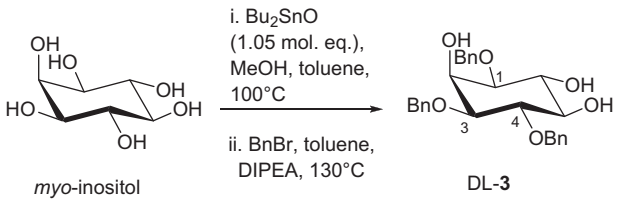
biocatalysis. This compound was assayed against the same set of lipases. We did observe (TLC) what appeared to be monoacylated product in the reactions with PS-IM, PS-C amano, Novozym 435, PS amano, RM-IM, A amano 12, AK amano, 34P. However, as the degree of conversion was very low, we did not pursue these reactions further. This lack of reactivity may result from the combination of stereochemical (reaction on a *cis* 1,2-diol) and steric issues (the additional hydroxyl groups are protected). In few reports in the literature wherein lipase-catalyzed reaction occurs at C_1-OH in *myo*-inositol derivatives, the 1,2,3-triol moiety was free and the remaining hydroxyl groups were only partially protected.[8b,9c]

Next, we proceeded to investigate the resolution of triether DL-**3** (Fig. 1). The enzymatic resolution of tri-*O*-benzylated *myo*-inositol derivatives or congeners had not been previously reported, to the best of our knowledge. We wondered if such a bulky *myo*-inositol would be resolved by lipases. The enantiomorphs D-(+)-**3** and/or L-(−)-**3** had been employed in the synthesis of inositol phosphates and had been prepared either by resolution via the formation of diastereomeric mixtures[13] or by lipase-catalyzed resolution of a conduritol B derivative.[14] We prepared DL-**3** from diether DL-**4**[15] via a simplified procedure (Scheme 1).[16] We had previously shown that selective mono-O-benzylation of intermediate tetrol **5** at C-3 via stannylene acetal technology leads to DL-**3**.[17] Thus, we anticipated the possibility of a one-pot preparation of DL-**3** (from DL-**4**). Hydrolysis of DL-**4** in MeOH in the presence of a controlled amount of H_2O led to **5**. Interestingly, in the absence of H_2O, no reaction took place when run at the same temperature. Such a simple hydrolysis procedure allowed us to perform the following transformation in the same reaction vessel. Indeed, we found that crude DL-**5** successfully undergoes selective *O*-monobenzylation to substance DL-**3** via stannylene chemistry (67% for the two steps). The presence of triethylammonium sulfonate in crude DL-**5** did not interfere with either stannylene acetal formation or its reaction with BnBr. Recently, we reported on the development of the direct controlled protection of multiple hydroxyl groups via iterative regeneration of stannylene acetals (use of only 1.00–1.05 mol equiv of Bu_2SnO). By means of this methodology, triether DL-**3** could be obtained in reasonable yield from *myo*-inositol in a single step (Scheme 2).[15a] As we had disclosed in that report, each individual step in the dynamic iterative O-alkylations follows the regioselectivity expected for the classical stannylene acetal protocol. Thus, in this case, *cis* 1,2-diol moieties react first (selectively at C-1 and C-3 equatorial OH groups). Next, a third 1,2-diol moiety is mono-O-alkylated, mostly at the OH group at C-4 (or C-6). This provided a practical alternative access to substrate DL-**3**. The main advantage of the first route is that it enables differentiation of the protecting groups at C-1, C-4 from the one at C-3.



Scheme 1. One-pot synthesis of triether DL-**3** from diether DL-**4**.



Scheme 2. Alternative synthesis of DL-**3** from *myo*-inositol.

Table 1
Screening of different enzymes in the enzymatic resolution of DL-**3**

Lípase	Time (h)	Conversion[a] (%)	ee_s[b]	ee_p[c]	*E*
CaLB	112	43.5	77	98	>200 (220)
Lipase PS-C Amano II	96	47.7	88	99	>200 (645)
Lipase Amano PS-IM	48	48.9	91	99	>200 (646)
Lipase Amano PS	112	2.5	1	n.d.	0
Lipozyme RM IM	48	1.2	n.d.	n.d.	—
A 'Amano' 12	48	2.1	n.d.	n.d.	—
AK 'amano'	48	2.1	n.d.	n.d.	—
Lipomod 34P	48	2.3	n.d.	n.d.	—
Lipozyme TL IM	48	—	—	—	—
F 'Amano' AP15	48	—	—	—	—
Lipase D Amano II	48	—	—	—	—
G 'Amano'	48	—	—	—	—
AY 'Amano' 30	48	—	—	—	—

n.d.: not determined.
[a] Determined by HPLC.
[b] Enantiomeric excess (*ee*) of unreacted substrate DL-**3** determined by HPLC.
[c] *ee* of monoacetate formed (after methanolysis) determined by HPLC.

Different lipases were screened against DL-**3** in reactions in vinyl acetate (Table 1). This acylating reagent is known to lead to more efficient lipase-catalyzed resolutions.[10b,18] Lipases PS 'Amano' (lyophilized), Lipozyme RM-IM, A 'Amano' 12, AK 'Amano' e Lipomod 34P were active but led to low conversions (~2.0%). Conversely, lipases CaLB (Novozym 435, 10 U/g), PS-C (836 U/g) e PS-IM (17.763 U/g) mediated high conversions, with the reactions of the latter two being interrupted earlier, as conversions approached 50%. Moreover, we observed a sharp decrease in rates as conversions were close to 50%, what suggested high enantioselectivities. In all cases, HPLC analysis showed the formation of a single regioisomeric acetate.

It is noteworthy that, while immobilized forms of the lipase from *Pseudomonas* sp. (PS-C, PS-IM) performed very well, the lyophilized form (Amano PS) displayed low activity. Such results with a challenging and rather uncommon substrate DL-**3** highlight the importance of lipase immobilization. This may increase the activity and stability of enzymes by a number of processes.[19] For instance, inclusion inside the support pores prevent the contact of the enzyme with inhibitors, proteases from the lipase extract and even air bubbles. Aggregation or simple inactivation is thus avoided.

At this point, it is not clear whether DL-**3** is reactive toward CaLB due to diequatorial diol moiety, or to having one less benzyl group or to both features. These are not shared by DL-**1** and DL-**2** (vide supra). In a previous study,[10] we showed that the same biocatalyst is highly effective in resolving another challenging substrate, DL-1,2-*O*-isopropylidene-3,6-di-*O*-benzyl-*myo*-inositol as well. Actually, only CaLB was able to mediate this resolution. In that study, we employed computational tools to rationalize the interactions involved in this substrate with both CaLB and RM-IM lipase (RMl), which was inactive. The L-enantiomorph of that *myo*-inositol derivative fitted well CaLB's active site and formed a stable tetrahedral intermediate. Conversely, Trp88 in RMl's structure appears to hin-

der the substrate binding to this enzyme. As DL-**3** resembles this inositol, the same interactions with both enzymes may account for their performances in the reactions of DL-**3**.

2.2. Product analysis

After separation from the unreacted triol, the monoacylated product was subjected to methanolysis ($MeOH/K_2CO_3$). The resulting triol (enantioenriched **3**), as well as the unreacted substrate **3** were analyzed by HPLC for the determination of *ee* (Fig. 2). These data showed that the three lipases mediated successful resolutions (E >200 and high conversions), especially PS-IM (Table 1). A quantitative experiment of kinetic resolution of DL-**3** with CaLB was carried out. By comparing the specific rotation of unreacted **3** with the data in the literature,[13] this compound was determined to be the enriched D-(+)-**3** enantiomorph (Scheme 3), establishing the configuration of the acylated product as L. NMR-analysis (Table 5 in the Section 4) determined that the acylation of L-(−)-**3** took place at the C-5 hydroxyl group, leading to acetate L-(−)-**6**. The three successful catalysts displayed the same enantiospecificity, favoring the formation of L-(−)-**6**.

2.3. Effects of co-solvents and acylating agents

An investigation into the effect of solvents and acylating agent on the performance of the resolution of DL-**3** catalyzed by PS-C, PS-IM, and CaLB (Novozym 435) was carried out. It is known that the solvent affects both enzyme activity and selectivity in the complex mechanism involving interactions among the enzyme, the substrate and the reaction medium. Indeed, the literature has shown the strong solvent influence on enantioselectivity.[20] We chose to investigate the effects of hexane, TBME (*t*-butylmethyl ether), and EtOAc as solvents. Isopropenyl acetate and EtOAc as acylating agents were assayed as well.

2.3.1. PS-C and PS-IM

In the case of reactions with PS-C (Table 2), compared to the reaction in vinyl acetate, TBME and EtOAc led to better performances (conversions, ee and E). Conversion was kept high with hexane, but the enantioselectivities decreased, especially of the acylated product (ee_p). The reaction in isopropenyl acetate did not perform well.

As for the resolutions of DL-**3** (Table 3) catalyzed by PS-IM, these reactions remained rapid when run in hexane and EtOAc, with the latter solvent still affording high E-values. It is worth noting that TBME made the conversions drop sharply. In fact, the use of co-solvents did not improve the resolutions compared to the reaction with vinyl acetate as solvent.

Such results further illustrate the impact of the mode of immobilization on lipases reactivity. While PS-IM is immobilized on diatomite, PS-C is on ceramic. Kanerva et al. reported that the immobilization nature of two commercial PS lipases had a marked effect on their performance in the kinetic resolution of 1,2,3,4-tetrahydroisoquinoline-1-acetic acid esters with water in DIPE.[2m]

2.3.2. Novozym 435

In the resolutions by CaLB with vinyl acetate, the use of hexane and TBME improved conversions and selectivities, particularly ee_s. (Table 4). Differently from the PS-IM assays, the reactions in EtOAc (also employed as solvent and acylating agent) led to acetate L-(−)-**6** in good *ee*, albeit at low conversion. Even isopropenyl acetate (solvent and acylation agent) afforded L-(−)-**6** in high *ee*. In general, CalB proved to be very consistent concerning the achieved ee of acetate L-(−)-**6**. In general, less polar solvents led to better performances.

Overall, our results show that hexane was more consistent regarding conversions (Fig. 3). With the exception of the case of PS-IM, TBME stood out as well as it provided both high conversions and selectivities. Conversely, isopropenyl acetate proved not to be suitable for these resolutions.

3. Conclusion

We have established that sterically hindered *myo*-inositol derivative DL-**3** could be resolved by lipases. This compound is a known precursor of bioactive inositol phosphates (and a potential one for other biphosphates and triphosphates). To the best of our knowledge, tri-*O*-alkylated *myo*-inositols have not been reported to successfully undergo such transformation.

As we noted, the non-reactivity of DL-**1** and DL-**2** may be due to the additional alkyl group and/or the relative configuration of the 1,2-diol moiety (*cis*).

The differences in reactivity shown by PS-C, PS-IM, and their lyophilized counterpart illustrate the importance of lipase immobilization for their catalytic performance.

The efficient protocols for the resolution of DL-**3** advanced in our study make the synthesis of both enantiomorphs (and derivatives) very practical. We believe that our study demonstrates that the

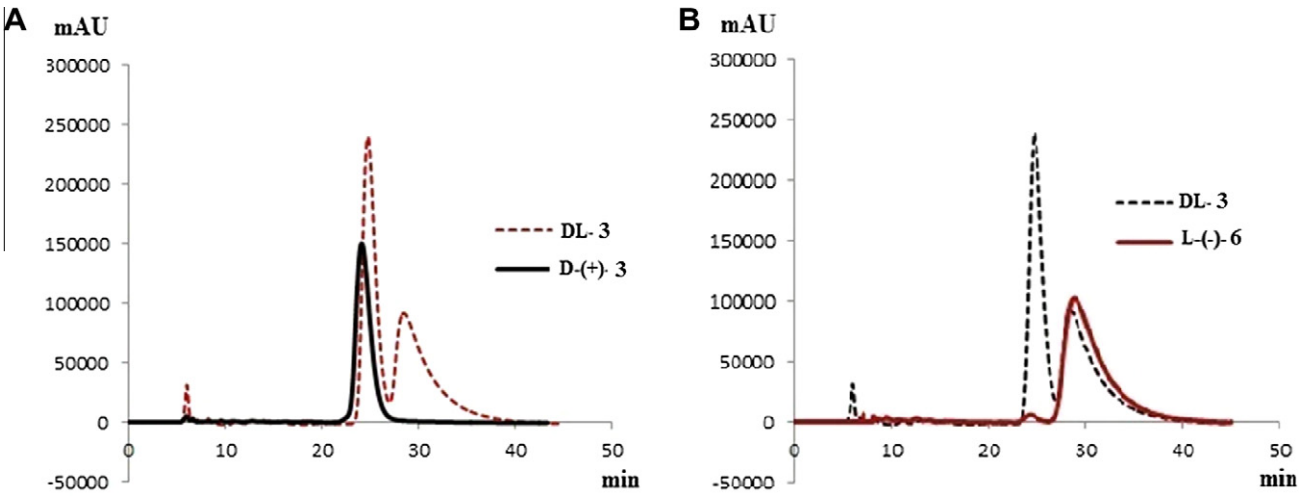
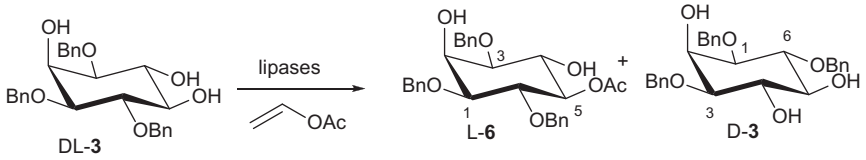


Figure 2. Determination of *ee* for resolution of DL-**3**. (a) Unreacted triol D-(+)-**3**. (b) L-(−)-**3** obtained by methanolysis of acetate L-(−)-**6**.



Scheme 3. Kinetic resolution of DL-**3**.

Table 2
Conversions, enantiomeric excess values and E-values obtained from kinetic resolution DL-**3** catalyzed by PS-C[a]

Acyl donor	Solvents	% C_{HPLC}	ee_s	ee_p	*E*
Vinyl acetate	Vinyl acetate	47.7	88	>99	>200
	Hexane	47.9	89	92	71
	TBME	49.9	97	>99	>200
	Ethyl acetate	46.0	97	>99	>200
Isopropenyl acetate	Isopropenyl acetate	23.0	20	66	5.9

[a] 96 h-Reactions.

Table 3
Conversions, enantiomeric excess values and E-values obtained from the kinetic resolution of DL-**3** catalyzed by PS-IM[a]

Acyl donor	Solvents	% C_{HPLC}	ee_s	ee_p	*E*
Vinyl acetate	Vinyl acetate	48.9	91	99	>200 (646)
	Hexane	48.0	88	92	56
	TBME	34.0	49	95	63
	Ethyl acetate	45.0	80	98	>200 (245)
Ethyl acetate	Ethyl acetate	2.3	1	45	2.7
Isopropenyl acetate	Isopropenyl acetate	11.6	28	70	6.2

[a] 48 h-Reactions.

Table 4
Conversions, enantiomeric excess values and *E*-values obtained from the kinetic resolution DL-**3** catalyzed by CaLB[a]

Acyl donor	Solvents	% C_{HPLC}	ee_s	ee_p	*E*
Vinyl acetate	Vinyl acetate	43.5	77	98	>200
	Hexane	48.3	88	97	>200
	TBME	46.7	99	96	>200
	Ethyl acetate	29.4	43	93	39
Ethyl acetate	Ethyl acetate	12.2	26	92	27
Isopropenyl acetate	Isopropenyl acetate	38.9	61	90	33

[a] 112 h-Reactions.

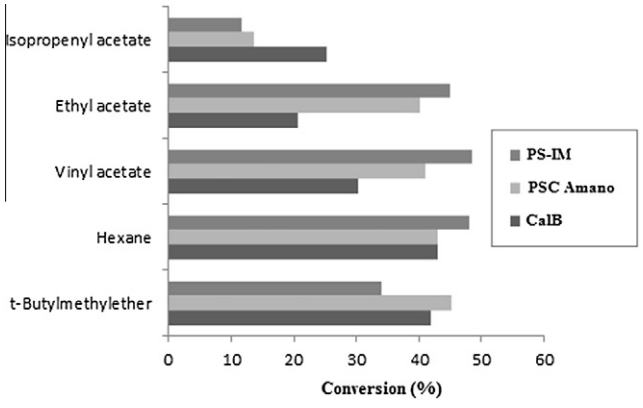


Figure 3. Effect of the solvent on lipase-catalyzed kinetic resolutions of DL-**3** after 48 h with vinyl acetate as acyl donor.

combination of selective multiple O-alkylations and lipase-catalyzed resolutions could be considered in the synthesis of chiral inositols. The use of selectively-acylated product, L-**6**, is particularly attractive.

4. Experimental

4.1. Materials

Enzymes: Lipozyme TL IM (*Thermomyces lanuginosus*), Lipozyme RM IM (*Rhizomucor miehei*) e Novozym 435 (*Candida antarctica* B, CaLB) were supplied by Novo Nordisk. The lipases PS-C (*Pseudomonas species*), PS-IM (*Pseudomonas species*), PS amano (*Burkholderia cepacia*) F-AP15 (*Rhizopus javonicus*), A-12 (*Aspergillus niger*), AK (*Pseudomonas fluorescens*), AY-30 (*Candida rugosa*), lipase G 'amano' (Amano) and D (*Rhizopus oryzae*) were supplied by Amano. Lipomod 34P was supplied by Biocatalysts. *myo*-Inositol derivatives DL-1,4,5,6-tetra-*O*-benzyl-*myo*-inositol DL-**1**[20] and DL-1,2-*O*-isopropylidene-3,6-di-*O*-benzyl-*myo*-inositol DL-**4**[15a] were prepared according to literature procedures. DL-1,4,5,6-Tetra-*O*-allyl-*myo*-inositol (DL-**2**) was prepared by a procedure similar to the one used in the preparation of DL-**1**. HPLC grade acetonitrile, n-hexane and 2-propanol were purchased from Tedia. Vinyl acetate was purchased from Fluka.

4.2. Enzyme activity assay

This assay was carried out by the absorbance increase (410 nm) over time produced by *p*-nitrophenol release in the 0.25 mL of 2.5 mM *p*NPL (*p*-nitrophenol laurate) hydrolysis in 2.2 mL of 25 mM sodium phosphate buffer at pH 7.0 carried out at 30 °C. The reaction was initialized by the addition of chosen amount of the crude enzyme. One international unit (IU) of the lipase was defined as the amount of enzyme necessary to hydrolyze 1 μmol of *p*NPL per minute under assay conditions.[21]

4.3. Screening of substrates and lipases

The screening was performed in sealed reactors kept at a constant, controlled temperature, and magnetically stirred. A mixture of 5.0 mg of substrate, 50 mg of enzyme (immobilized or lyophilized) and 2.5 mL vinyl acetate was kept at 30 °C under these conditions. The reaction was monitored by TLC on Merck silica gel 60 F254 plates eluted with a 7:3 hexanes/ethyl acetate mixture.

4.3.1. Synthesis of DL-3 via acetonide DL-4

A mixture of DL-**4** (0.4 g; 1.0 mmol), H_2O (36 μL; 2.0 mmol) and PTSA (8.6 mg; 0.05 mmol) in CH_3OH (2.0 mL) was heated to 80 °C for 1–1.5 h. The reaction mixture was then cooled to 40–50 °C and Et_3N (55 μL; 0.4 mmol) was added, after which the volatiles were evaporated under vacuum. Additional CH_3OH (2.0 mL) was added and subsequently removed under vacuum to afford DL-**5**. After that, a mixture of crude DL-**5** and Bu_2SnO (0.274 g, 1.1 mmol) in CH_3OH/toluene (1:1, 3 mL) was heated to 120 °C for 3 h. The solvents were evaporated, dry toluene (4 mL) was added to the residue and a second evaporation to dryness was effected, which is completed under high vacuum. Then, a mixture of the crude stannylene derivative, Bu_4NBr (0.0645 g, 0.2 mmol) and BnBr (0.343 g,

Table 5
2D NMR data of L-(−)-**6**

	HSQC		HMBC		COSY
	^{13}C	^{1}H	$^{2}J_{CH}$	$^{3}J_{CH}$	$^{2}J_{CH}$
CH-1	79.3	3.27 (dd, *J* = 9.6, 2.6 Hz, 1H)	H-2; H-6	—	H-2, H-6
CH-2	66.9	4.21 (t, *J* = 2.7 Hz, 1H)	H-1, H-3	H-4	H-1, H-3
CH-3	79.8	3.45 (dd, *J* = 9.5, 2.7 Hz, 1H)	H-2; H-4	H-7	H-2, H-4
CH-4	78.8	3.94 (t, *J* = 9.6 Hz, 1H)	H-3; H-5	H-2	H-3, H-5
CH-5	74.5	4.96 (t, *J* = 9.8 Hz, 1H)	H-4; H-6	—	H-4, H-6
AcO-5	170.8	—	Me/AcO-5	H-5	—
CH-6	70.5	4.03 (t, *J* = 9.7 Hz, 1H)	H-1; H-5	H-2	H-1, H-5
CH_2-7	75.5, 72.9, 72.4	4.87–4.61 (m)	—	H-1, H-3 e H-4	-
C-8	138.5, 137.7, 137.4	—	H-7	—	—
CH-8	128.6, 128.5, 128.3, 128.2, 127.99, 127.96, 127.91, 127.7, 127.6	7.28–7.41 (m)	H-8; H-7	—	—
Me/AcO-5	21.0	1.99 (s)	—	—	—

2.0 mmol) in dry toluene (4 mL) under argon was heated to 120 °C till the reaction went to completion (3 h). After solvent evaporation, the residue was purified by flash chromatography (silica gel) to yield triether DL-**3** (0.302 g, 67%).

4.3.1.1. DL-1,3,6-tri-*O*-benzyl-*myo*-inositol, DL-3[15a]. ^{1}H NMR (300 MHz, $CDCl_3$), δ 2.47 (s, 1H), 2.61 (s, 2H), 3.23 (dd, 1H, *J* = 2.67 9.52), 3.38–3.43 (m, 2H), 3.82 (t, 1H, *J* = 9.52), 3.96 (t, 1H, *J* = 9.28), 4.24 (s, 1H), 4.63–4.98 (m, 6H), 7.25–7.35 (m, 15H); ^{13}C NMR (75.00 Hz, $CDCl_3$), δ 66.9, 71.8, 72.2, 72.4, 74.1, 75.5, 79.0, 79.8, 80.4, 127.8, 127.87, 127.9, 128.1, 128.5, 128.58, 137.6, 137.7, 138.6.

4.4. Resolution of DL-3

The enzymatic reactions were realized in closed thermostatized flasks containing DL-**3** (5 mg), acylating agent (1.0 mL) and lipase (50 mg) in the solvent (1.5 mL) under stirring at 30 °C. Aliquots were taken after 0.5, 6, 24, 48, 72, 96, and 112 h, dried in a Speed Vac Concentrator (Savant SPD 1010-Thermo scientific) and re-solubilized in a 60:40 acetronitrile:H_2O mixture (1 mL) and analyzed by HPLC.

4.5. HPLC analysis of conversion of DL-3 to L-(−)-6

Conversion analyses were carried out via HPLC on a Kromasil C18 column (40 °C in a CTO-20A oven), eluted with a acetonitrile–H_2O (60:40) mixture (0.5 mL/min) by a Shimadzu LC-20AT pump. A Shimadzu SPD-M20A variable-wavelength UV/vis detector was employed, with the detection set at 215 nm, and the Shimadzu LC solution software was used for chromatogram integration. The samples to be analyzed were filtered through a 0.45 μm PTFE filter. The retention times of the substrate D-(+)-**3** and L-(−)-**6** were 8 and 13 min, respectively. The analysis of the synthetic mixture (prepared by acylation of DL-**3** with Ac_2O) containing DL-**6** also showed peaks at 22 and 39 min, corresponding to diacetylated DL-**3** and triacetylated DL-**3**.

4.6. Determination of the enantiomeric excesses (ee) and enantiomeric ratio (*E*)

Unreacted triol D-(+)-**3** and acetate L-(−)-**6** were separated by HPLC (vide supra). Chromatographic determinations of the enantiomeric excesses (*ee*) of D-(+)-**3** (ee_s) were undertaken on the same equipment mentioned above carrying a Chiralcel OD-H column (5 μm; 4.6 × 250 mm), eluted with a 7:3 hexane–2-propanol mixture (0.6 mL/min). The retention times of the enantiomers D-(+)-**3** and L-(−)-**3** were 24.5 min and 28.5 min, respectively. As for the ee of L-(−)-**6** (ee_p), the sample was subjected to methanolysis ($MeOH/K_2CO_3$) prior to the HPLC analyses.[9c]

The enantiomeric ratio (*E*) was calculated by using the equation of Chen et al.[22]

4.7. Quantitative resolution of DL-3: synthesis of L-5-acetyl-1,3,6-tri-*O*-benzyl-myo-inositol L-(−)-6 and D-1,3,6-tri-*O*-benzyl-myo-inositol D-(+)-3

Following the protocol of Section 4.4, DL-**3** (95 mg), CaLB (750 mg), vinyl acetate (27 mL) and TBME (18 mL) were mixed at 30 °C for 120 h. The obtained residue was purified by flash chromatography (column eluted with 5:95, 20:80, 30:70, 70:30, 80:20, 100:0 EtOAc/hexane mixtures) to give L-(−)-**6** (37 mg, 39%) and D-(+)-**3** (45 mg, 43%):

4.7.1. L-5-*O*-Acetyl-1,3,6-tri-*O*-*myo*-inositol L-(−)-6
$[\alpha]_D^{25} = -24.2$ (*c* 1.36, $CHCl_3$); ^{1}H NMR (400 Mhz, $CDCl_3$), δ 7.28–7.41 (m, 15H), 4.96 (t, *J* = 9.8 Hz, 1H), 4.87–4.61 (m, 6H), 4.21 (t, *J* = 2.7 Hz, 1H), 4.03 (t, *J* = 9.7 Hz, 1H), 3.94 (t, *J* = 9.6 Hz, 1H), 3.45 (dd, *J* = 9.5, 2.7 Hz, 1H); 3.27 (dd, *J* = 9.6, 2.6 Hz, 1H); 1.99 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 170.8, 138.5, 137.7, 137.4, 128.6, 128.5, 128.3, 128.2, 127.99, 127.96, 127.91, 127.7, 127.6, 79.8, 79.3, 78.8, 75.5, 74.5, 72.9, 72.4, 70.5, 66.9, 21.0. IR (film): 3417; 3062; 3030; 2922; 2872; 1732; 1494; 1454; 1359 cm^{-1}. EM-ESI: *m*/*z* = 493.2220 $[M+H]^+$; 515.2035 $[M+Na]^+$.

4.7.2. D-1,3,6-Tri-*O*-benzyl-*myo*-inositol D-(+)-3
$[\alpha]_D^{25} = +17.8$ (*c* 0.45, $CHCl_3$) Lit.[13a] $[\alpha]_D = +20$ (*c* 1.00 $CHCl_3$) Lit.[13b] $[\alpha]_D = +16.2$ (*c* 1.00 $CHCl_3$).

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