ChemPubSoc Europe

DOI: 10.1002/ejoc.201701417

EurJOC European Journal of Organic Chemistry

Full Paper

Inositols | *Very Important Paper* |

Lipase Regioselective *O*-Acetylations of a *myo*-Inositol Derivative: Efficient Desymmetrization of 1,3-Di-*O*-benzyl-*myo*-inositol

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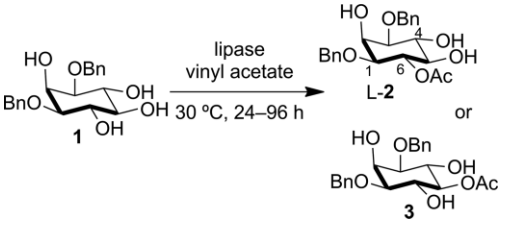
Abstract: Chiral *myo*-inositol derivatives play key roles in cell-signaling processes. Despite the relevance of these compounds, few syntheses of them rely on enantioselective catalytic reactions. Even fewer reports describe the use of desymmetrization of *myo*-inositol derivatives. In fact, most routes involve resolution by derivatization. Thus, a symmetrical partially protected *myo*-inositol derivative, 1,3-di-*O*-benzyl-*myo*-inositol (**1**), was used as a substrate in fast lipase-catalyzed desymmetrization reactions. Among the lipases tested, both Lipozyme RM-IM and Lipozyme TL-IM were effective in catalyzing the formation of the chiral acetate L-(+)-6-*O*-acetyl-1,3-di-*O*-benzyl-*myo*-inositol [L-(+)-**2**] with high conversion (98–99 %) and *ee* (>99 %). Conversely, Novozyme 435 and Lipomod 34P as biocatalysts showed different regioselectivity, leading to the formation of the symmetrical 5-*O*-acetylated product. We were able to reuse TL-IM lipase seven times without any noticeable decrease in the conversion. Acetate L-(+)-**2** is a potential precursor of biologically active *myo*-inositol derivatives and other relevant materials for cell biology studies.

Introduction

myo-Inositol derivatives, mostly chiral phosphates, are involved in a number of fundamental cell-signaling processes.[1] Thus, research directed towards the sustainable and efficient synthesis of inositol derivatives is of great interest.Despite the plethora of methods for the synthesis of *myo*-inositol derivatives that have been established since the late 1980s, a number of pitfalls remain for practical access to these compounds. Thus, the routes to these quite complex products are often lengthy and low-yielding.[2] This has led to ongoing research into improving their production.[3]

Most synthetic routes to inositols use the abundant *myo*-inositol, which has stereochemistry and functionalization of all its carbon atoms already inbuilt. As it is a symmetrical (*meso*) compound, *myo*-inositol derivatives are clearly invaluable substrates for desymmetrization techniques. In this context, enzymatic protocols,[4] especially those relying on lipases, are particularly attractive.

In this paper, we report the results of a study on lipase-catalyzed regioselective *O*-acylation reactions of 1,3-di-*O*-benzyl-*myo*-inositol (**1**), working towards its regioselective modification and desymmetrization (Scheme 1). Despite the attractive structural features and potential of this compound, it has not been explored as a substrate for lipases. The successful desymmetrization of this compound through the installation of an acyl group in an appropriate position may allow orthogonal protecting group strategies to be implemented in further steps, and so allow the synthesis of target compounds. Compound **1** bears a vicinal equatorially-oriented triol, and as such it is a distinctive and more challenging substrate for desymmetrization, if compared to the few inositol derivatives that have been tested to date (vide infra).



Scheme 1. Lipase-catalyzed mono-*O*-acetylation of 1,3-di-*O*-benzyl-*myo*-inositol (**1**).

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Supporting information and ORCID(s) from the author(s) for this article are available on the WWW under https://doi.org/10.1002/ejoc.201701417.

Lipases are among the most widely used enzymes as biocatalysts due to their very broad specificity, which enables their use in reactions involving different compounds.[5] Thus, they are able to catalyze quite diverse reactions, ranging from reactions related to their physiological function (hydrolysis of oils and fats) to other quite different reactions (esterifications, transesterifications, acidolysis, amidations, etc.).[6] They are the enzymes with the widest promiscuity described to date.[7] Lipases are extensively used as catalysts for enantioselective reactions,[8] just as they are in this paper, but in this paper a regioselectivity issue arises as well. However, the use of lipases in regio- and enantioselective reactions with *myo*-inositol derivatives as substrates is rather limited.[9] And indeed, examples of lipase-catalyzed desymmetrizations are even scarcer.[10]

In this study, we screened commercially available immobilized lipases such as Lipozyme TL-IM (immobilized lipase from *Thermomyces lanuginosus*),[11] Lipozyme RM-IM (immobilized lipase from *Rhizomucor miehei*),[12] and the most widely used lipase in the literature, Novozyme 435 (immobilized lipase B from *Candida antarctica*).[13] The acylation reactions were carried out in a kinetically controlled process, using vinyl acetate as an activated acyl donor; this prevents any competition between the target nucleophile and any released nucleophile (as would occur when, for example, a standard ester was used, which would release an alcohol).[14] In these reactions, the maximum yields may be transient; and they may depend on the kinetic properties of the biocatalysts, the enzyme source, and even the immobilization protocol.[15]Vinyl acetate may also have some negative side-effects on the enzyme performance, e.g., through chemical modification of the enzyme.

Results and Discussion

Screening of Lipases for the Desymmetrization of *myo*-Inositol Diether 1

Different lipases were evaluated with diether **1** as the substrate and vinyl acetate acting as both acyl donor and solvent (Scheme 1 and Table 1). PS-D, A-Amano, PS C- Amano, and F-AP 15 were not able to use *myo*-inositol **1** as a substrate under these conditions. However, Lipozyme TL-IM, Lipozyme RM-IM, Novozyme 435, and Lipomod 34P catalyzed transesterification reactions (Scheme 1) leading to different products. Novozyme 435 and Lipomod 34P catalyzed the formation of *meso* compound 1,3-di-*O*-benzyl-5-*O*-acetyl-*myo*-inositol (**3**) with high regioselectivity. Conversely, Lipozyme TL-IM and Lipozyme RM-IM produced L-(+)-1,3-di-*O*-benzyl-6-*O*-acetyl-*myo*-inositol [L-(+)-**2**].

Table 1. Results of screening of lipases against *myo*-inositol derivative **1**.

Entry[a]	Lipase	Time [h][b]	Product	*ee* [%]
1	PS-D	96	–	–
2	A-Amano	96	–	–
3	Novozym 435	24	**3**	–
4	RM-IM	72	**2**	>99
5	TL-IM	48	**2**	99
6	Lipomod 34P	24	**3**	–
7	F-AP 15	96	–	–

The formation of both monoesters was completely regioselective in all cases.

For the determination of the *ee* of chiral product **2**, a mixture of monoacetates [including (±)-**2**] was chemically synthesized and separated by HPLC. HPLC analysis on a chiral column confirmed that both Lipozyme TL-IM and Lipozyme RM-IM were highly enantioselective (Table 1). It is noteworthy that the choice of lipase can determine whether a chiral or a symmetrical product is obtained from the same substrate, an uncommon situation.

Effect of the Acylating Agent on the Desymmetrization of *myo*-Inositol Diether 1 Using Different Immobilized Commercially Available Lipases

The effect of the acyl donor on the performance of the monoacetylation reactions of 1,3-di-*O*-benzyl-*myo*-inositol (**1**) using TL-IM, RM-IM, and Novozyme 435 was investigated. It is known from the literature that the nature of the acyl donor has a strong influence on enzyme activity, and that acylations catalyzed by lipases involve the formation of an acyl–enzyme intermediate, therefore the nature of the acyl donor needs to be explored. Acetic anhydride (data not shown), vinyl acetate (VA), and ethyl acetate (EtOAc) were compared as acyl donors in this reaction using three lipases (TL-IM, RM-IM, and Novozyme 435; Table 2). As our goal was to develop practical and economical routes to *myo*-inositol derivatives, we only tested these immobilized enzymes, which could be recycled. The use of acetic anhydride did not result in acylation of substrate **1** with any of the enzymes. Only vinyl acetate and ethyl acetate were recognized by the enzymes as acyl donors in the desymmetrization of **1**. The results are presented in Table 2.

Table 2. Effect of acylating agents on the conversion of lipase-catalyzed *O*-acylation reactions of *myo*-inositol **1** (conversion after 24 and 96 h).[a]

Entry	Lipase	Acyl donor[b]	Product	Conversion [%]	
				24 h	96 h
1	Novozym 435	VA	**3**	93	97
2	Novozym 435	EtOAc	**3**	64	80
3	TL-IM	VA	**2**[c]	92	93
4	TL-IM	EtOAc	**2**[c]	8	9
5	RM-IM	VA	**2**[c]	79	90
6	RM-IM	EtOAc	**2**[c]	6	34
7	control	VA	–	–	–
8	control	EtOAc	–	–	–

[a] Conditions (Scheme 1): lipase (200 U; esterification activity), acyl donor (2 mL), no other solvent. [b] VA = vinyl acetate. [c] $E > 100$ in all cases.

The use of EtOAc led to a conversion of 80 % after 96 h when Novozyme 435 was used as the biocatalyst. Lower conversions were obtained with Lipozyme TL-IM (9 %) and Lipozyme RM-IM (34 %).

Vinyl acetate gave much better results than EtOAc when it was used as solvent/acylating agent, and higher conversion (≥90 %) was obtained for all three enzymes. The higher conversions with vinyl acetate were expected, as this acyl donor forms a carbonyl by-product rather than an alcohol, so competition between the released alcohol (ethanol) and the target substrate (**1**) is avoided.[16]

Effects of Cosolvents on the Desymmetrization of *myo*-Inositol Diether 1 Using RM-IM and TL-IM Lipases

We also investigated the effect of solvents, using vinyl acetate as an activated acyl donor. It is well known that the solvent affects enzyme activity, selectivity, and enantioselectivity.[16,17] In choosing a solvent, the solubility and the stability of the substrate and the activity and stability of the immobilized commercially sourced enzymes must be considered.[18] The effect of different solvents [EtOAc, hexane, CH_2Cl_2, and MTBE (methyl *tert*-butyl ether)] was studied, used in a 1:1 ratio together with vinyl acetate as an activated acyl donor, with RM-IM and TL-IM as biocatalysts.

In all reactions of inositol derivative **1**, a single enantiomer of the product (L-**2**; Scheme 1) was detected. This shows that the cosolvents do not affect the enantioselectivity of the enzyme. In the reactions with vinyl acetate catalyzed by Lipozyme TL-IM (Table 3), the highest conversion to acetate L-**2** occurred with EtOAc (99 % in 96 h) and MTBE (98 % in 96 h) as cosolvents (Table 3, entries 3 and 5, respectively). CH_2Cl_2 also gave a high conversion (Table 3, entry 4). For the same reaction, but catalyzed by Lipozyme RM-IM, EtOAc was found to be the best solvent (>99 % in 96 h).

Table 3. Effect of cosolvent on the conversion[a] and rate (V_i) of the desymmetrization of **1** catalyzed by TL-IM with VA.[b]

Entry	Cosolvent	Conversion [%]	V_i [μmol min^{-1} g^{-1}]	Error
1	none	93	493.1	26.6
2	hexane	93	230.4	15.9
3	EtOAc	99	835.9	28.9
4	CH_2Cl_2	98	416.3	78.3
5	MTBE	98	1444.2	15.3

[a] >99 % *ee* in all cases; Reaction time of 24 h. [b] Conditions for the desymmetrization of **1**: lipase (200 U), acyl donor (1 mL), substrate (5 mg mL^{-1}), in the respective solvent (1 mL).

In the desymmetrizations of **1** by TL-IM with vinyl acetate, the use of cosolvents improved the conversions (Table 3, entry 1) except in the case of hexane. No change in conversion was observed, possibly due to solubility reasons. The use of hexane as cosolvent has been shown to improve the transformation of other *myo*-inositol derivatives by lipases.[9h,10d] In fact, it is surprising that the conversion remained high in hexanes, as the polar substrate **1** was not soluble in that reaction mixture.

Furthermore, the use of EtOAc as a cosolvent in the TL-IM-catalyzed desymmetrization of **1** increased the initial rate by almost two times (Table 3, entry 3) compared to the rate with neat vinyl acetate; with MTBE (entry 5), the initial rate increased by almost three times. A decrease of the reaction rate was observed for the reactions in hexanes and in CH_2Cl_2.

For reactions catalyzed by RM-IM, the best performance after 24 h (conversion yields and enzyme activity) was obtained using MTBE as solvent. As in the reactions by TL-IM lipase, the use of EtOAc gave the highest yields, but only after a reaction time of 48 h; the activity was around 55 % of that with MTBE. Hexane and CH_2Cl_2 did not perform well.

EtOAc was selected for further studies because of the improved yields with TL-IM. Moreover, MTBE has a higher toxicity than EtOAc.[19] The kinetics of the desymmetrization reactions catalyzed by TL-IM and RM-IM in EtOAc are shown in Figure 1. The reaction was significantly faster when TL-IM was used as the biocatalyst, as expected.

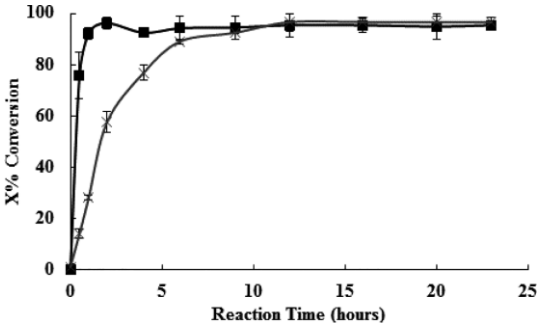


Figure 1. Progression of the kinetic resolution reactions of **1** in EtOAc with TL-IM (■) and RM-IM (X). Conditions: substrate (5 mg mL^{-1}), lipase (200 U), 30 °C, in EtOAc.

The use of the enzyme Lipozyme TL-IM led to conversions of 96 % within only 2 h. On the other hand, when Lipozyme RM-IM (data not shown) was used, the maximum conversion achieved after 6 h was 89 %. The reaction catalyzed by RM-IM only reached a plateau of 96 % conversion after a reaction time of 12 h. Thus, the TL-IM conversion rate between 0 and 2 h was 836 μmol min^{-1} (g of immobilized enzyme)$^{-1}$. The conversion rate in the reaction catalyzed by the RM-IM enzyme between 0 and 6 h was 277 μmol min^{-1} (g of immobilized enzyme)$^{-1}$, as shown in Table 3. This result was expected, since the *T. lanuginosus* enzyme had higher conversion rates than the *R. miehei* lipase in all solvents.

Biocatalyst Reuse

Figure 2 shows that the best catalyst found in this work, Lipozyme TL-IM, could be used for at least seven cycles (each cycle lasting 1 h) without any decrease in conversion when EtOAc was used as cosolvent. In addition, the selectivity was maintained over all these cycles. On the other hand, it was not possible to study the reusability of Lypozyme TL-IM in the presence of MTBE as cosolvent, since the support loses its physical characteristics under these conditions. This phenomenon has already been reported for transesterification reactions of oleic acid in a medium containing ethanol.[20]

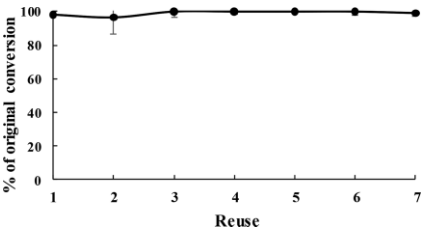
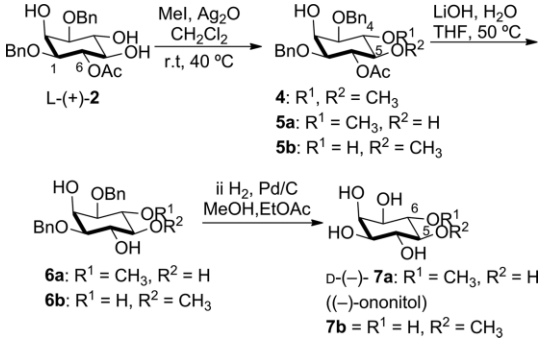


Figure 2. Operational stability in the kinetic resolution of **1** by TL-IM (filled circles). Conditions: substrate (5 mg mL^{-1}), enzyme (200 U), 30 °C, in vinyl acetate/EtOAc.

Establishment of the Desymmetrization Product Configuration

The establishment of the configuration of product **2** as L was carried out through its conversion into a known derivative. We initially chose to convert acetate **2** into the known derivative 5,6-di-*O*-methyl-*myo*-inositol.[21] However, the Ag_2O-mediated *O*-methylation[22] of L-(+)-**2** did not lead to the desired diether **4**, but to a mixture of monoethers **5a** and **5b**. This was still a useful result for our purposes. This inseparable mixture (flash chromatography, TLC) was subjected to hydrolysis with LiOH to give a mixture of compounds **6a** and **6b**. Analytical samples of pure **6a** and **6b** were obtained by HPLC, and were separately analyzed by HRMS, which confirmed their presence. The mixture was then subjected to hydrogenolysis, leading once again to an inseparable (TLC, flash chromatography) mixture of the chiral monoether D-6-*O*-methyl-*myo*-inositol [D-(–)-**7a**] with its symmetrical regioisomer **7b** (Scheme 2, also see the Experimental Section $[\alpha]_D^{20}$ (mixture) =–0.52). The ^{1}H NMR spectroscopic data of this material was consistent with the presence of the enantiomer of (+)-ononitol,[23] thus establishing the absolute configuration of the desymmetrization product L-**2**.



Scheme 2. Assignment of the configuration of chiral product (+)-**2** by conversion into D-(–)-**7a**.

Compound L-(+)-**2** is a potential precursor of biologically active phosphates, such as Ins(1,4)P_2 and Ins(1,3,4)P_3.[24] One might also envisage the conversion of L-(+)-**2** into other relevant inositol derivatives, e.g., D-2,4,5-*myo*-inositol trisphosphate and D-1,3,4,5-*myo*-inositol tetrakisphosphate.[25] The currently available practical syntheses of these compounds rely on optical resolutions.[26]

Conclusions

Two commercially available lipases, Lipozyme TL-IM and Lipozyme RM-IM, were found to catalyze, in selected solvents, the desymmetrization of 1,3-di-*O*-benzyl-*myo*-inositol (**1**), leading to high conversions and *ee*. When TL-IM lipase was used in EtOAc as a cosolvent (but not in TBME), it maintained its activity and selectivity through seven desymmetrization cycles.

Our investigation also revealed that the commercially available lipases Novozym 435 and Lipomod 34P selectively acetylate *myo*-inositol derivative **1** to produce *meso* compound **3**. In vinyl acetate, the first of these lipases gave a high conversion (93 %). Triol **3** may be a useful intermediate for the synthesis of inositol derivatives.

The efficient protocol described here for the enantioselective synthesis of L-(+)-**2**, through an unprecedented desymmetrization process, will streamline practical, short syntheses of biologically active *myo*-inositol derivatives (including phosphoinositides),[27] as discussed above. As already noted, the desymmetrization of *myo*-inositol derivatives has hardly been exploited, despite the synthetic potential of this concept.

Experimental Section

General Remarks: Unless otherwise noted, all chemicals were used as obtained from commercial sources, and all reactions were carried out under an argon atmosphere. 1,3-Di-*O*-benzyl-*myo*-inositol (**1**) may be synthesized by a literature procedure,[28] or, more practically, by an nonoptimized procedure based on the stannylene-catalyzed direct multiple *O*-alkylation of polyols[29] (see Supporting Information). Analytical thin-layer chromatography (TLC) was carried out on precoated silica gel F-254 plates. Column chromatography was carried out on silica gel (200–300 mesh). Nuclear magnetic resonance spectra were recorded with NMR spectrometers at the following frequencies: ^{1}H, 400 MHz; ^{13}C{^{1}H}, 101 MHz. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane, or using the residual solvent peak as an internal reference. Multiplicities are reported as follows: singlet (s), doublet (d), doublet of doublets (dd), triplet (t), quartet (q), and multiplet (m). Infrared (IR) spectra were recorded as KBr pellets, and wavenumbers are given in cm^{-1}. High-resolution mass spectra (HRMS) were recorded with a TOF MS instrument using the ESI technique.

The lipases Novozym 435 (Lipase B from *Candida antarctica* immobilized on a macroporous acrylic resin), Lipozyme TL-IM (lipase from *Thermomyces lanuginosus* immobilized on acrylic resin), and Lipozyme RM-IM (lipase from *Rhizomucor miehei* immobilized on ion-exchange resin) were supplied by Novo Nordisk. The lipases PS-D (lipase from *Pseudomonas* species immobilized on diatomaceous earth), PS-C Amano (lipase from *Pseudomonas cepacia* immobilized on ceramic acrylic), A-Amano (lipase from *Aspergillus niger*), and F-AP 15 (lipase from *Rhizopus javonicus*) were supplied by Amano. Lipomod 34P (lipase from *Candida rugosa*) was supplied by Biocatalysts. The esterification activities were measured before and after each reaction.

Preparation of (±)-2: 1,3-Di-*O*-benzyl-*myo*-inositol (**1**; 0.05 g, 0.1387 mmol) and DMAP (0.0017 g, 0.1387 mmol) were put into a round-bottomed flask (10 mL) containing a teflon magnetic stirrer bar, and the flask was closed with a rubber stopper. Then, DMF (1.0 mL, 0.0129 mmol) and Et_3N (0.029 mL, 0.2081 mmol) were added under Ar. The resulting stirred mixture was cooled to 0 °C with an ice-water bath. Then, Ac_2O (0.020 mL, 0.2081 mmol) was added, and the mixture was kept at the same temperature for 90 min. After this time, sat. aq. $NaHCO_3$ (5 mL) was added. After 15 min, the cooling bath was removed, and the mixture was allowed to warm to room temp. The reaction mixture was transferred to a test tube with EtOAc (5 mL). The phases were separated, and the aqueous phase was extracted further with EtOAc (2 × 5 mL). The combined organic phases were dried with Na_2SO_4, and filtered. The volatiles were evaporated under vacuum to give a mixture of monoacetates, containing (±)-**2**. This mixture was separated by HPLC on a reverse-phase column.

Screening of Lipases for the Mono-*O*-acetylation of 1,3-Di-*O*-benzyl-*myo*-inositol (1): Compound **1** (5 mg) was mixed with vinyl acetate (2.0 mL) in a capped vial (10 mL, with a Teflon liner). The lipase (50 mg) was then added. The resulting mixture was kept at

30 °C for 24–96 h. The biocatalyst was then removed by filtration, and the volatiles were evaporated to give the monoacylated product **2** or **3**.

Preparative Desymmetrization of 1,3-Di-*O*-benzyl-*myo*-inositol with TL-IM Lipase (Under Improved Conditions): 1,3-Di-*O*-benzyl-*myo*-inositol (0.150 g, 0.3728 mmol) was added to round-bottomed flask (100 mL) containing a magnetic stirrer bar, and then vinyl acetate (15 mL) and ethyl acetate (15 mL) were added. TL-IM lipase (1 g) was added, the flask was stoppered, and the resulting mixture was stirred at 30 °C for 24 h. After this time, the mixture was filtered through a 2 cm pad of silica gel, which was washed with EtOAc (30 mL). The volatiles were evaporated from the resulting solution under vacuum. The residue was purified by flash chromatography (EtOAc/hexanes, 50:50, 70:30, 90:10, and neat EtOAc) to give pure chiral acetate L-(+)-**2** (0.132 g, 87 %).

Acetate L-(+)-2: $[\alpha]_D^{20}$ = +1.090 (*c* = 1.035, $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 1738 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.34–7.26 (m, 10 H), 5.31 (t, *J* = 9.8 Hz, 1 H), 4.72–4.47 (m, 4 H), 4.20 (t, *J* = 2.7 Hz, 1 H), 3.99 (t, *J* = 9.5 Hz, 1 H), 3.36 (t, *J* = 9.6 Hz, 1 H), 3.31 (dd, *J* = 9.8, 2.7 Hz, 1 H), 3.18 (dd, *J* = 9.6, 2.7 Hz, 1 H), 2.04 (s, 3 H) ppm. ^{13}C NMR (125.00 MHz, $CDCl_3$): δ = 170.4, 138.4, 138.3, 128.6, 128.5, 128.1, 128.0, 127.0, 127.6, 80.8, 79.5, 75.3, 75.1, 74.8, 73.4, 71.3, 69.9, 21.0 ppm. HRMS (ESI): calcd. for $C_{22}H_{27}O_7$ $[M + H]^+$ 403.1757; found 403.1751; calcd. for $C_{22}H_{26}NaO_7$ $[M + Na]^+$ 425.1576; found 425.1571.

Acetate 3: IR (KBr): $\tilde{\nu}$ = 1728 cm^{-1}. ^1H NMR (400.00 MHz, $CDCl_3$): δ = 7.42–7.28 (m, 10 H), 4.85 (t, *J* = 9.7 Hz, 1 H), 4.69 (dd, *J* = 35.3, 11.7 Hz, 4 H), 4.24 (s, 1 H), 4.03 (t, *J* = 9.6 Hz, 2 H), 3.29 (dd, *J* = 9.5, 2.4 Hz, 2 H), 2.15 (s, 3 H) ppm. ^{13}C NMR (400 MHz, CD_3OD): δ = 171.4, 138.2, 127.9, 127.8, 127.3, 72.2, 76.1, 71.9, 70.2, 66.3, 19.9 ppm. HRMS (ESI): calcd. for $C_{22}H_{26}NaO_7$ $[M + Na]^+$ 425.1576; found 425.1565.

Establishment of the Configuration of L-(+)-2: Compound L-(+)-**2** (0.090 g, 0.2237 mmol), Ag_2O (0.052 g, 0.2237), CH_2Cl_2 (1.0 mL), and CH_3I (4 drops, excess) were sequentially added to a capped vial (8 mL, with a Teflon liner) containing a magnetic stirrer bar. The resulting mixture was stirred at r.t. for 1 h, and then it was heated to 40 °C for 7 h. As unreacted starting material remained, additional Ag_2O (0.02 g) was added, and after 3 h under the same conditions, additional MeI (5 drops, excess) was added. After a further 3 h, the reaction mixture was cooled and subjected to flash chromatography (EtOAc/hexanes, 30:70, 50:50, and 60:40) to give a mixture of regioisomeric monoethers **5a** and **5b** (0.066 g).

The mixture of **5a** and **5b** (0.058 g, 0.134 mmol), THF (2.0 mL), distilled H_2O (1.0 mL), and LiOH (0.032, 1.340 mmol) were added to a capped vial (8 mL, with a Teflon liner) containing a magnetic stirrer bar. The resulting mixture was heated to 50 °C for 2 h. After this time, the mixture was cooled to room temp. The mixture was transferred to a tube with EtOAc (15 mL), and washed with distilled H_2O (5 mL). The organic phase was dried with Na_2SO_4, and filtered, and the volatiles were evaporated. The residue was purified by flash chromatography (EtOAc/hexanes, 50:50, 80:20, and 90:10, and neat EtOAc) to give a mixture of triols **6a** and **6b** (0.027 g). Analytical amounts of these compounds were separated by HPLC, and analyzed by HRMS. HRMS: calcd. for $C_{21}H_{26}NaO_6$ $[M + Na]^+$ 397.1729; found 397.1657.

The mixture of **6a** and **6b** (0.020 g, 0.0515 mmol) was dissolved in EtOAc/MeOH (1:1; 6 mL) in a pressure bottle, and Pd/C (10 %; 0.02 g) was added. The mixture was pressurized with H_2 (45 psi) after the needed air purges, and was shaken for 20 h. Then, after pressure relief, the reaction mixture was filtered. The filter residue containing the catalyst was washed with a H_2O/MeOH mixture (10:90). The filtrate was then filtered again through a 0.45 µm PVDF (polyvinylidene difluoride) filter. Finally, the resulting solution was concentrated. The residue was triturated with a EtOAc/hexanes mixture (80:20) to give a mixture of reference compound D-(–)-**7a** and **7b** (0.01 g, 95 %) as a white solid. $[\alpha]_D^{20}$ (mixture) =–0.52 (*c* = 1.15, H_2O); lit.[22] +6.4 for the pure enantiomer. ^1H NMR [400 MHz, D_2O; data for this mixture related to D-(–)-**7a**]: 4.07 (t, *J* = 2.8 Hz, 1 H), 3.72 (t, *J* = 9.7 Hz, 1 H), 3.61 (s, 3 H), 3.57 (dd, *J* = 10.0, 2.9 Hz, 1 H), 3.51 (dd, *J* = 10.0, 2.8 Hz, 1 H). HRMS (ESI): calcd. for $C_7H_{14}NaO_6$ $[M + Na]^+$ 217.0790; found 217.0683.

Esterification Activity: The enzyme activity was determined as the initial rate in esterification reactions between oleic acid and ethanol at a molar ratio of 1:1, temperature of 30 °C, and enzyme concentration of 1.8 wt.-% in relation to the substrates. One lipase activity unit (U) was defined as the amount of enzyme necessary to consume 1 µmol of oleic acid per minute under the experimental conditions established previously. All enzymatic activity determinations were replicated at least three times.

Conditions for the Enzymatic Assays: The enzymatic reactions were carried out with magnetic stirring in closed thermostatted flasks (water bath). Substrate **1** (5 mg), the selected enzyme (0.25 % w/v), the selected acyl donor, and the selected solvent (1:1 ratio; 2 mL) were mixed. After 2 h, the reactions were stopped by removal of the catalyst by filtration. The assays were run in triplicate. The volatiles were evaporated from the samples containing product **2** or **3**. The resulting material was subjected to HPLC analysis to determine the conversion and the *ee*.

Determination of Conversion to 2 and 3 by HPLC Analysis: Conversion analysis carried out by HPLC on an Agilent C18 column (40 °C in an Agilent 1260 Infinity oven), eluting with an acetonitrile/H_2O (40:60) mixture (0.5 mL min^{-1}) by using an Agilent 1260 infinity quaternary pump. An Agilent 1260 Infinity UV/Vis detector with a variable-wavelength detector was used, with the detection set at 215 nm, and the Agilent LC solution software was used for chromatogram integration. The samples to be analyzed were filtered through a 0.22 µm PTFE filter. The retention times of the substrate **1** and the products **2** and **3** were 4.7, 8.2, and 7.3 min, respectively.

Determination of Enantiomeric Excesses (*ee*): Unreacted substrate **1** and mono-*O*-acetylated product **2** were separated by HPLC on a Shimadzu C18 column (40 °C in a CTO-20A oven), eluting with an acetonitrile/H_2O (40:60) mixture (0.5 mL min^{-1}) with a Shimadzu LC-20AT pump. A Shimadzu SPD-M20A UV/Vis detector with variable wavelength was used, 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 0.22 µm PTFE filter. Then, the solvents of the resulting solutions were evaporated before direct analysis. Chromatographic determination of the *ee* of **2** was carried out using the Shimadzu/Agilent equipment mentioned above, on a Chiralcel OD-H column (5 µm; 4.6 mm × 250 mm), eluting with a hexane/isopropanol mixture (1:1; 0.5 mL min^{-1}). The retention times of these enantiomorphs were 17 and 25 min, respectively. The enantiomeric ratio (*E*) was calculated using the equation of Chen et al.[30]

Enzyme Reusability: In the Lipozyme TL-IM reuse assays, run as described above, after each 1 h batch run (2 mL), the reaction mixture was centrifuged. Then, the liquid phase (for chromatographic analysis) was decanted, and the solid catalyst was used in the next run under the optimum conditions. This procedure was repeated seven times. In each run, a sample of 100 µL was taken to determine the enzyme activity. No decline in activity was observed. When washing of the catalyst (with ethyl acetate) was carried out after each run, no significant loss of activity was observed.

Acknowledgments

We acknowledge CNPq, CAPES, and Faperj for fellowships and funding, Central Analítica-IPPN for analytical data, and CTEx and Samir Cavalcante for polarimetric analyses.

Keywords: Inositols · Cyclitols · Enzyme catalysis · Acylation · Desymmetrization · Asymmetric synthesis

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Received: October 10, 2017