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Influence of intramolecular hydrogen bonds in the enzyme-catalyzed regioselective acylation of quinic and shikimic acid derivatives

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Abstract—Selective mono-functionalization of 3-*epi*, 4-*epi*-, and 5-*epi* quinic and shikimic acid derivatives has been accomplished by enzymatic acylation with *Candida antarctica* lipase A (CAL-A). We propose that the selectivity of this lipase is related to both the inherent receptor selectivity and the degree of intramolecular hydrogen bonding in the ligand. Conformational analysis of the hydroxyl protons has been carried out by ^{1}H NMR spectroscopy. We have shown that exchange of the hydroxyl protons by acid catalysis provides a useful method for the detection of intramolecular hydrogen bonds. The interpretation of exchange rates and coupling constants determines the direction of the H-bonds as conditioned by the relative acceptor and donor properties of the hydroxyl groups. The selectivity of the acylation agrees fully with the effectiveness of H-bonding networks in polyol compounds and with the higher reactivity of the equatorial hydroxyl groups.

1. Introduction

The selective functionalization of one particular hydroxyl group among others of similar reactivity in polyhydroxylated molecules is extremely difficult in organic synthesis. A major problem is the lack of an efficient orthogonal protection–deprotection strategy. In the carbohydrate chemistry, Wong et al.[1] have described the synthesis of a library of oligosaccharides using a designed building-block with four selectively removable protecting groups as acceptors for glycosylation. On the other hand, regioselectivity in sugar chemistry is often solved using an approach based on complexation-induced activation of a particular OH group. For this purpose, tin[2] and boron[3] reagents have been widely used.

In some cases certain hydroxyl groups have shown an anomalous reactivity, with a secondary hydroxyl group being functionalized instead of the usually more reactive primary one.[4] However, steric interactions within themselves are not sufficient to explain this unusual reactivity. The existence of noncovalent intramolecular bonding interactions involving hydroxyls is well recognized in carbohydrate chemistry. The role of intramolecular hydrogen bonding has been used as an explanation for the low reactivity of the primary 5′-OH during the synthesis of 5′-*O*-galactosylated nucleosides. Hydrogens of such primary hydroxyls are shown to be intramolecularly hydrogen-bonded with the heteroaromatic system present in the molecule, and consequently proved to be resistant to hydrogen abstraction.[5] In another example, selective 2-*O*-benzylation of sucrose is due to the persistence of an intramolecular hydrogen bond, which makes the hydroxyl group at the 2-position the most readily deprotonated in aprotic solvents.[6] Hydrogen bonds are also confirmed to play a significant role in the acidic behavior of the various hydroxyl groups of the sucrose molecule.[7] The relative reactivities in the DMAP-catalyzed acetylation were successfully correlated with the calculated proton affinity of each OH group in carbohydrates by Yoshida et al.[8] They found that secondary OH groups were preferentially acetylated in the presence of the primary OH group at position 6. In contrast, the 6-*O*-acetylated product was the major one in the absence of DMAP. In addition, molecular recognition through hydrogen-bonding interactions is one of the challenging goals in supramolecular chemistry.[9]

Enzyme-catalyzed reactions are practical processes for the selective modifications of polyol derivatives. The regioselectivity of enzyme catalysis has been exquisitely exploited in the fields of nucleosides,[10] steroids,[11] and carbohydrates.[12] Enzymes catalyze reactions under mild conditions, diminish undesired side-reactions, and facilitate product recovery. Furthermore, enzymes are proteins, and as such they are completely biodegradable.

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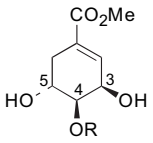

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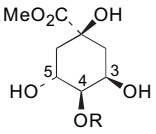
2, R= Ac



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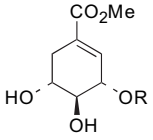
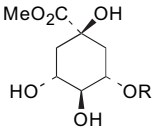
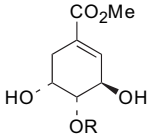
4, R= Ac



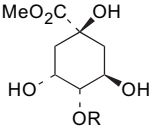
5, R= H, Methyl 3-*epi*-Shikimate
6, R= Ac



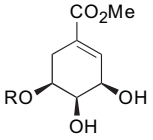
7, R= H, Methyl 3-*epi*-Quinate
8, R= Ac



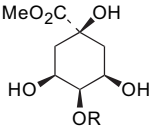
9, R= H, Methyl 4-*epi*-Shikimate
10, R= Ac



11, R= H, Methyl 4-*epi*-Quinate
12, R= Ac



13, R= H, Methyl 5-*epi*-Shikimate
14, R= Ac



15, R= H, Methyl 5-*epi*-Quinate
16, R= Ac

Figure 1. Structures of natural (**1** and **3**), 3-*epi* (**5** and **7**), 4-*epi* (**9** and **11**), and 5-*epi* (**13** and **15**) shikimic and quinic acid derivatives and their regioselective enzymatic acetylated products (**2**, **4**, **6**, **8**, **10**, **12**, **14**, and **16**) with CAL-A using vinyl acetate both as acylating agent and solvent.

In our ongoing research focused on the synthesis of quinic and shikimic acid derivatives, we carried out the regioselective enzymatic acylation of methyl shikimate (**1**, Fig. 1) and methyl quinate (**3**) with *Candida antarctica* lipase A (CAL-A).[13] This lipase allowed the selective acylation of the C-4 hydroxyl group, giving rise to a variety of ester derivatives of both acids, including the cinnamate analogues. Previous studies on the enzymatic acylation of quinic and shikimic acid derivatives have been described by Guyot et al.[14a] and Danieli et al.[14b] Although, the use of enzymes in synthesis is common, the basis of their selectivity is not well understood. The goals of this study are both to regioselectively prepare the valuable monoacyl analogues of the 3-*epi*, 4-*epi*, and 5-*epi* quinic and shikimic acid derivatives, and to identify the molecular basis of the regioselectivity of CAL-A towards these compounds as a function of the degree of intramolecular hydrogen bonding within the ligand.

2. Results and discussion

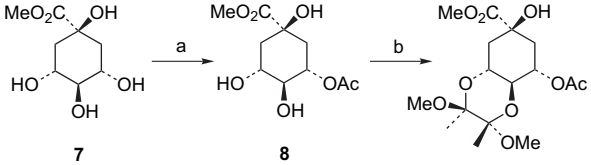
2.1. Regioselective enzymatic acylation of methyl 3-*epi*, 4-*epi*, and 5-*epi* quinic and shikimic acid derivatives

2.1.1. Enzymatic acylation of (−)-methyl 3-*epi*-shikimate (5) and *meso*-methyl 3-*epi*-quinate (7).[15] As previously reported for the natural isomer,[13b] the enzymatic transesterification of methyl 3-*epi*-shikimate (**5**) was carried out at 20 °C with vinyl acetate as acylating agent and solvent, CAL-A, and in the presence of molecular sieves 4 Å. CAL-A exhibited excellent selectivity towards the 3-position, giving rise to methyl 3-*O*-acetyl-3-*epi*-shikimate (**6**) in 88% yield after 0.5 h. In addition, methyl 3,5-di-*O*-acetyl-3-*epi*-shikimate was isolated as a minor compound (92:8 ratio **6**:diacyl derivative calculated by ^{1}H NMR). To avoid the formation of the diacyl derivative other solvents suitable for the enzymatic acylation of methyl shikimate such as *tert*-butylmethyl ether (TBME), toluene, 1,4-dioxane, chloroform or THF were used. In general, longer reaction times were achieved, although CAL-A maintained a similar degree of selectivity.

If as seems, the selectivity of CAL-A is highly dependent on the relative position of the hydroxyl groups, CAL-A would also acylate the 3-position of methyl 3-*epi*-quinate (**7**) as it did with methyl 3-*epi*-shikimate (**5**). This fact was corroborated when the latter was subjected to similar reaction conditions as those described for methyl quinate. That is, with vinyl acetate both as solvent and acylating agent, CAL-A, molecular sieves 4 Å, and at 40 °C. However, since **7** is a *meso* compound, positions 3 and 5 are prochirals. Thus, CAL-A catalyzes the acylation of methyl 3-*epi*-quinate toward the C-3 or C-5 position with 95% regioselectivity (C-3 or C-5 vs C-4) in 4.5 h. Importantly, no traces of 4-*O*-acyl or diacyl derivatives were detected by ^{1}H NMR of the crude reaction mixture.

The enantiopurity of compound **8** was determined by derivatization to the corresponding *trans*-acetal **17** (Scheme 1). In addition, (±)-**17** was synthesized by protection of *trans*-1,2-diol in *meso*-methyl 3-*epi*-quinate and subsequent treatment with acetic anhydride as described in Scheme 2.

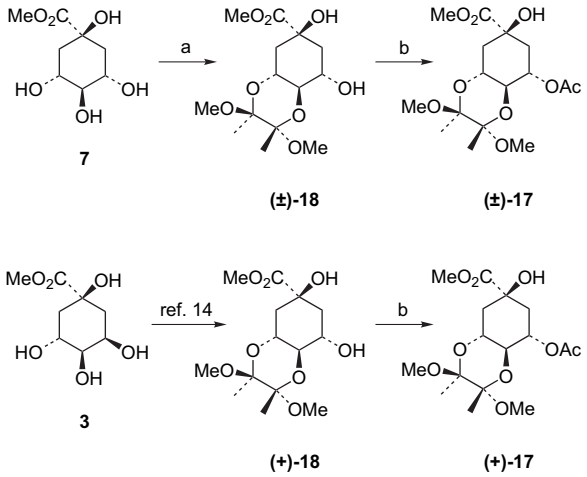
Chiral HPLC analysis of (±)-**17** and derivative of **17** obtained from the enzymatic reaction revealed an enantiomeric excess of 60% (see page S2 in Supplementary data). The assignment of the relative configuration of **17** was determined by comparison of the sign of the optical rotation of our synthesized derivative of **17** and that of the enantiomerically pure derivative prepared according to Scheme 2.[15] This was corroborated by chiral HPLC analysis of both compounds, which clearly establishes the configuration (1*R*,3*S*,4*S*,5*R*) for our compound. This means that acetylation of methyl 3-*epi*-quinate takes place with moderate selectivity towards 3-position. We did not try to find the optimal conditions for asymmetrization of **7** since it was not the aim of this study.



7 **8** **17**

(a) **CAL-A**, CH_2=CHOAc, sieves 4 Å, 40 °C, 4.5 h; (b) butane-2,3-dione, $CH(OMe)_3$, (±)-CSA, MeOH, 65 °C, 2 h

Scheme 1.



(a) butane-2,3-dione, $CH(OMe)_3$, (±)-CSA, MeOH, 65 °C, 2 h;
(b) Ac_2O, DMAP, Py, CH_2Cl_2, 0 °C, 4 h

Scheme 2.

2.1.2. Enzymatic acylation of (−)-methyl 4-*epi*-shikimate (9) and (−)-methyl 4-*epi*-quinate (11).[16] We extended the aforementioned methodology to 4-*epi* derivatives. First, we studied the acylation reaction of methyl 4-*epi*-shikimate (**9**) with vinyl acetate at 20 °C in the presence of molecular sieves, and catalyzed by CAL-A. The transesterification takes place in 1.5 h with total selectivity towards the C-4 hydroxyl group, methyl 4-*O*-acetyl-4-*epi*-shikimate (**10**) being isolated exclusively in 80% yield after flash chromatography.

Similarly, methyl 4-*epi*-quinate (**11**) was subjected to reaction with CAL-A under identical conditions as **7**. However, the presence of corresponding 1,5-lactone **19** (see below, Fig. 8D) was observed and a mixture of acylated derivatives was obtained. Formation of the lactone has been previously observed in the attempted recrystallization of **11**. To avoid this by-product, the reaction was carried out in the absence of molecular sieves since these remove the MeOH and shift the equilibrium towards the lactone. Also, the temperature was decreased from 40 to 20 °C. In these conditions, CAL-A furnished exclusively the 4-*O*-acetyl derivative **12** after 24 h.

2.1.3. Enzymatic acylation of (−)-methyl 5-*epi*-shikimate (13) and *meso*-methyl 5-*epi*-quinate (15).[17] When the reaction was done with methyl 5-*epi*-shikimate (**13**), CAL-A showed total selectivity towards the C-5 hydroxyl group, a 100% conversion being achieved after 0.3 h at 20 °C.

In contrast to other *epi*-isomers, ^{1}H NMR of the 5-*epi*-shikimate derivative revealed different half-chair conformations in MeOH-d_4 and $CDCl_3$. Change from MeOH-d_4 to $CDCl_3$ resulted in an upfield shift of H-4 and a downfield shift of H-5 (see pages S47 and S55 in Supplementary data). This is in line with exchanges in the axial and equatorial positions. Moreover, the coupling constants of H-5 are very significant, indicating an axial position in MeOH-d_4 ($^3J_{HH}$ 9.6 Hz) and an equatorial position in $CDCl_3$ ($^3J_{HH}$ 5.0 Hz). Thus, in MeOH-d_4 solution, triol **13** prefers a conformation with OH-3 and OH-5 in equatorial orientation and OH-4 in axial one (A, Fig. 2). On the other hand, OH-3 and OH-5 are in axial orientation and OH-4 is in equatorial one, which are the proposed conformation in $CDCl_3$ (B, Fig. 2). In that conformation, strong 1,3-diaxial intramolecular hydrogen bonds are present and as a result this should be the favored conformation in nonhydrogen bonding solvents.

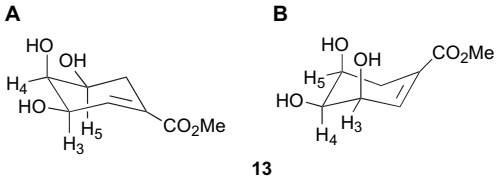


Figure 2. Chair conformations of methyl 5-*epi*-shikimate in MeOH-d_4 (A) and in $CDCl_3$ (B).

A comparison of the acylation of **13** in hydrogen (acetone, vinyl acetate) and nonhydrogen (CH_2Cl_2, $CHCl_3$) bonding solvents has been performed. No changes in the regioselectivity were observed with any of the solvents tested.

Finally, selective acylation at the C-4 position of methyl 5-*epi*-quinate (**15**) was successfully accomplished using CAL-A at 40 °C for 4.5 h. As with the 5-*epi*-shikimate derivative, methyl 5-*epi*-quinate showed different conformation in MeOH-d_4 and $CDCl_3$ solvents. Protons H-6a and H-2a exhibited in MeOH-d_4 a $^3J_{HH} \approx 12.1$ Hz corresponding to an axial–axial vicinal coupling to H-3 and H-5, in addition to a large geminal coupling ($^2J_{HH} \approx 12.1$ Hz). Therefore, OH-3 and OH-5 are in equatorial orientation and OH-4 in axial one (A, Fig. 3). On the other hand, in $CDCl_3$ the value of this vicinal coupling constant is ≈3.8 Hz, which is consistent with an axial position for OH-3 and OH-5, and an equatorial orientation for OH-4 (B, Fig. 3).

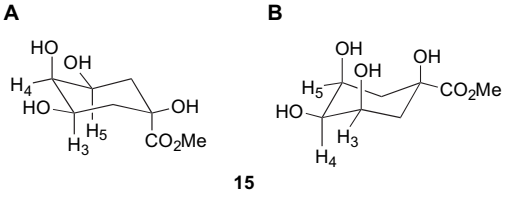


Figure 3. Chair conformations of methyl 5-*epi*-quinate in MeOH-d_4 (A) and in $CDCl_3$ (B).

Although different solvents were tested (acetone, vinyl acetate, CH_2Cl_2, and $CHCl_3$) CAL-A maintains the excellent regioselectivity; methyl 4-*O*-acetyl-5-*epi*-quinate (**16**) in all cases being isolated.

2.2. Conformational analysis of intramolecular hydrogen bonds and their influence in the enzymatic catalysis

In order to explain the regioselectivity exhibited by CAL-A in quinic and shikimic acid derivatives, we next study the role of intramolecular hydrogen bonds between vicinal alcohols within each ligand.

IR and ^{1}H NMR spectroscopy are common methods to determine intra- and intermolecular H-bonding. IR allows direct

observation of the free and hydrogen-bonded hydroxyl stretch resonances (ν_{OH}), which are subsequently used to determine the extent of intramolecular hydrogen bonding.[18] Since our molecules possess several OH groups this technique is not adequate due to the superposition of the OH bands. NMR spectra potentially provide more useful information in the form of coupling constants, chemical shifts, temperature coefficients, and NOEs, although in order to obtain this data the suppression of intermolecular exchange of hydroxyl groups is necessary. In these conditions, vicinal coupling constants (^3J) for hydroxyl protons calculated by ^{1}H NMR can provide important structural information in terms of hydrogen-bonding. Since the magnitude of ^3J depends on the dihedral angle, according to the Karplus equation[19] derived for hydroxyl protons,[20] the values of $^3J_{CH,OH}$ can predict the orientation of the hydroxyl groups.

For the detection of intramolecular hydrogen bonds sample dilution is a powerful method.[21] The limiting chemical shifts and the concentrations required for fast exchange are characteristically different for protons that are intramolecularly hydrogen-bonded. Intramolecular H-bonds can also be detected by a weak dependence of the chemical shift of OH groups upon the temperature.[22] Due to the low solubility of our substrates in chloroform or methylene chloride (commonly used deuterated solvents), which are nonhydrogen-bonding, a concentration dependent hydroxyl proton exchange is not adequate. Similarly, since a conformational equilibrium is present in the target molecules, a dependence upon the temperature of the OH signals in ^{1}H NMR is not suitable. These facts oblige us to study the exchange process by acid catalysis. As a consequence, rigorous removal of acid and water from sample, solvent, and NMR tube should be controlled. To the best of our knowledge, it is the first time that hydrogen bond interactions are determined by exchange of the hydroxyl protons with acid in an organic solvent solution.

2.2.1. Study of hydrogen bonds network in methyl shikimate (1). After careful preparation of the NMR samples (see Section 4) the rate of exchange of the hydroxyl protons with the solvent is slow enough, because of which the coupling pattern is detectable. Figure 4 shows the ^{1}H NMR spectra of methyl shikimate (**1**) in $CDCl_3$ solution before (spectrum A) and after addition of subsequent portions of 0.05 mM trifluoroacetic acid in $CDCl_3$ solution (spectra B and C). Ester

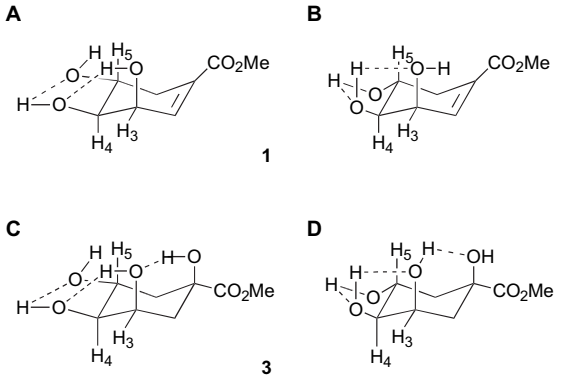


Figure 5. Clockwise and reverse clockwise orientations of methyl shikimate (A and B) and methyl quinate (C and D) hydrogen-bonding networks.

1 exists preferentially in the chair conformation indicated in Figure 5 (A and B). To assign the spectra and determine the structures, ^{1}H–^{1}H homonuclear correlation experiments have been performed. The analysis of the spectrum of **1** provides the coupling constants of the hydroxyl groups: $^3J_{H\text{-}3,OH\text{-}3}$=4.7 Hz, $^3J_{H\text{-}4,OH\text{-}4}$=5.5 Hz, and $^3J_{H\text{-}5,OH\text{-}5}$= 3.5 Hz. Comparison of spectra A–C in Figure 4 suggests that the signal corresponding to OH-5 is altered first, since it appears as a singlet instead of a doublet. Spectrum C shows that the next hydroxyl to loose its coupling is OH-3, whereas OH-4 still maintains certain residual coupling. These results of hydroxyl exchange indicated an acidity order of OH-5>OH-3>OH-4.

There are two possible orientations for the hydrogen bonds in the cyclohexene ring of **1** (A and B, Fig. 5). The hydroxyl OH-5 forms a weak H-bond with the adjacent hydroxyl OH-4 both are in equatorial position. Diequatorial *trans*-1,2-diols are expected to form weaker intramolecular bonds. The strong hydrogen bond is formed by OH-3 and OH-4, which are in cis orientation. Equatorial–axial vicinal diols in six-membered rings have stronger intramolecular hydrogen bonds than equatorial–equatorial diols. On the other hand, OH-3 is more acidic than OH-4 and this suggests that the axial OH-3 and not the equatorial OH-4 acts preferentially as H-acceptor.[22b] Thus, the directional hydrogen-bonding network is that indicated in Figure 5B. In addition, an equatorial OH group in a six-membered ring system can be

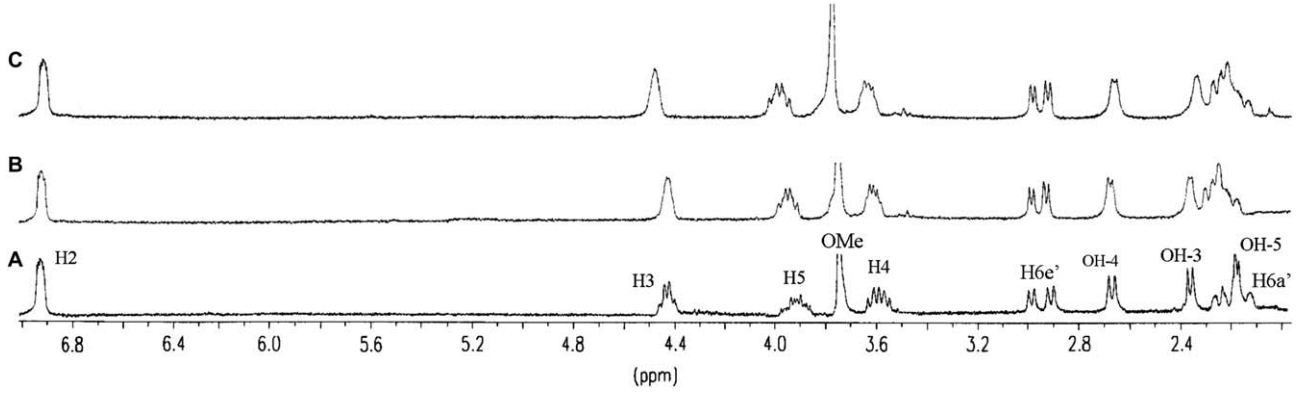


Figure 4. ^{1}H NMR spectra (300 MHz) of **1** in $CDCl_3$ solution (2 mM): (A) in absence of acid; (B) first change observed after acid addition; and (C) second change observed after acid addition.

functionalized preferentially in the presence of secondary axial partners.

According to the points mentioned above, when OH-4 results acylated the positive charge in the transition state is delocalized through a strong intramolecular hydrogen bond. In contrast, OH-5 forms a less-efficient hydrogen bond to delocalize the charge. If these parameters have an influence in the transition state of the enzymatic acylation, the major regioisomer formed should be the 4-*O*-acetylated derivative. In fact, acylation of **1** catalyzed by CAL-A takes place with excellent selectivity at OH-4.[13b]

2.2.2. Study of hydrogen bonds network in methyl quinate (3). The ^{1}H NMR spectra of methyl quinate (**3**) in $CDCl_3$ or MeOH-d_4 solution shows that the preferred chair conformation is close to the one observed for methyl shikimate (C and D, Fig. 5). Compound **3** is characterized by two large $^3J_{H\text{-}3,OH\text{-}3}$=8.6 Hz and $^3J_{H\text{-}4,OH\text{-}4}$=9.1 Hz, and a smaller $^3J_{H\text{-}5,OH\text{-}5}$=2.5 Hz (A, Fig. 6). Comparison of the ^{1}H NMR spectra A–C (Fig. 6) reveal that the order of acidity of the hydroxyl groups is OH-5>OH-4>OH-3.

Since we have an additional hydroxyl proton in the molecule (OH-1) for which ^{1}H NMR spectrum does not provide coupling information (it is a tertiary alcohol), acidity does not indicate the direction of the hydrogen bonds. For that, vicinal coupling constants were employed. As previously mentioned, the value of $^3J_{CH,OH}$ depends on the dihedral angle between the hydroxyl proton and the hydrogen of the vicinal carbon. $^3J_{CH,CH}$ values of 11–15 Hz are characteristic of axial–axial vicinal couplings, while the presence of electronegative atoms give rise to ^3J values of 8–10 Hz. According to the Karplus equation, the large $^3J_{H\text{-}4,OH\text{-}4}$=9.1 Hz evidences a predominant conformation in which the O–H bond is almost *anti* to the C–H bond (dihedral angle close to 150–180°).[23] The same interpretation can be applied to OH-3, suggesting that the direction of the hydrogen bonds is that which is indicated in Figure 5D. OH-3 and OH-1 form a strong 1,3-diaxial hydrogen bond. Also, the hydrogen-bonding between OH-4 and OH-3 is effective. Thus, the OH-4 should exhibit much higher reactivity than the other hydroxyl groups in the enzyme-catalyzed acetylation reaction. The positive charge in the transition state is stabilized through two consecutive efficient hydrogen bonds. The OH-5 group does not effectively participate in the hydrogen-bonding network (trans-diequatorial hydrogen bond between OH-5 and OH-4), and acetylation at the OH-3 reduces the extent of charge delocalization (through only one effective hydrogen bond). Interestingly, in the presence of CAL-A, the regioselectivity of the acetylation of **3** agrees fully with cooperative H-bond effects and by a higher reactivity of the equatorial groups, methyl 4-*O*-acetylquinate (**4**) being obtained exclusively.[13a]

2.2.3. Study of hydrogen bonds network in methyl 3-*epi*-shikimate (5). The ^{1}H NMR spectra of methyl 3-*epi*-shikimate (**5**) in MeOH-d_4, acetone-d_6, or $CDCl_3$ solutions show the chair conformation indicated in Figure 7A, in which the large coupling constants of H-4 (dd, $^3J_{HH}$=9.8, 7.8 Hz) and H-5 (ddd, $^3J_{HH}$=9.7, 9.7, 6.2 Hz) suggest an axial disposition (see pages S44 and S51 in Supplementary data).

The exchange of hydroxyl protons of alcohols after addition of acid to NMR sample implies an acidity order of OH-4≈OH-5>OH-3 (see pages S62 and S63 in Supplementary data). The two hydrogen bonds are between trans-diequatorial OH groups, and, evidently, none of them are involved in a strong intramolecular H-bond. The fact that OH-3 is the lesser acidic hydroxyl, and thus a H-bond donor, indicates the direction of the H-bonds (Fig. 7A). The enzymatic acylation of **5** led almost exclusively to 3-acetyl derivative **6**. This is in keeping with the more efficient hydrogen bond

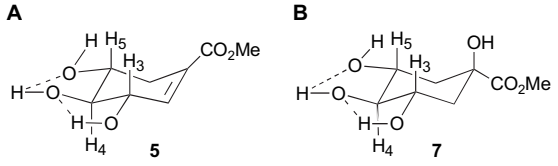


Figure 7. Hydrogen-bonding orientations of methyl 3-*epi*-shikimate (A) and methyl 3-*epi*-quinate (B).

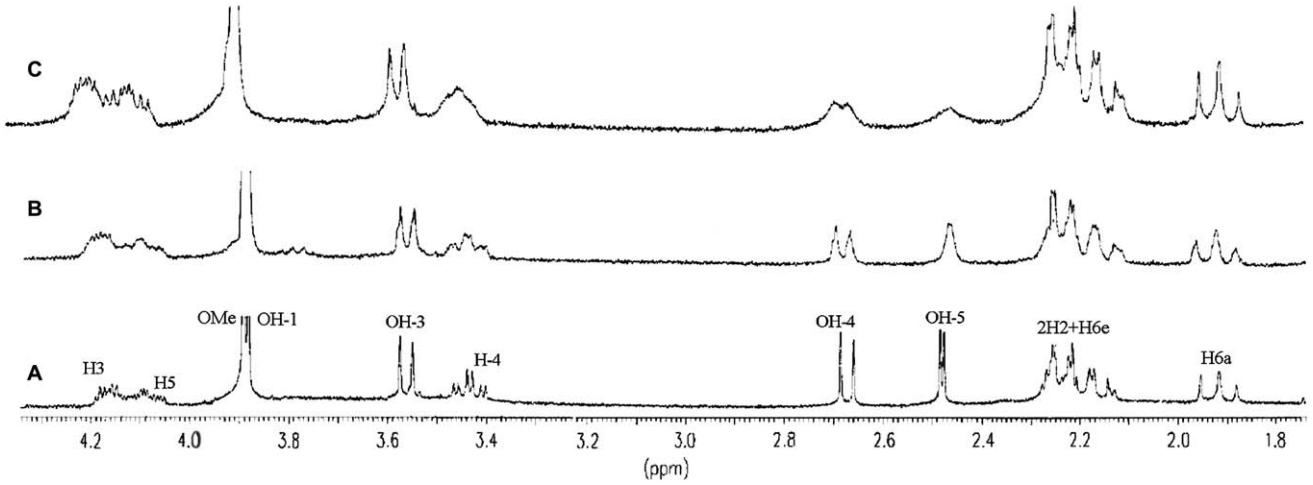


Figure 6. ^{1}H NMR spectra (300 MHz) of **3** in $CDCl_3$ solution (2 mM): (A) in absence of acid; (B) first change observed after acid addition; (C) second change observed after acid addition.

between OH-3 and OH-4 due to the *pseudo*equatorial position of OH-3.

2.2.4. Study of hydrogen bonds network in methyl 3-*epi*-quinate (7). As with the shikimate derivative, analysis of the coupling constants of *meso*-methyl 3-*epi*-quinate (**7**) evidences the preferred chair conformation, shown in Figure 7B. Large coupling constant values are observed for axial H-2 and H-6 (dd, $^{2}J_{HH}$=12.5 Hz, $^{3}J_{HH}$=12.5 Hz), and thus H-3 and H-5 are in axial orientation.

The three secondary OH groups of **7** are involved in weak intramolecular H-bonds. Hydroxyl exchange is similar, although the H-bond formed by OH-3 (or OH-5) seems to be more stable (see pages S64 and S65 in Supplementary data). In addition, the two possible orientations of the hydroxyl network are equivalent (Fig. 7B), since the molecule possesses a plane of symmetry.

Acetylation of **7** catalyzed by CAL-A led to exclusive acylation at OH-3 or OH-5. The larger value of the coupling constant for OH-3 (or OH-5) ($^{3}J_{H\text{-}3,OH\text{-}3}$=3.7 Hz and $^{3}J_{H\text{-}4,OH\text{-}4}$=2.9 Hz) indicates a more adequate dihedral angle for effective hydrogen-bonding.

2.2.5. Study of hydrogen bonds network in methyl 4-*epi*-shikimate (9). The analysis of (−)-methyl 4-*epi*-shikimate (**9**) by ^{1}H NMR in several deuterated solvents ($CDCl_3$, MeOH-d_4, and acetone-d_6) determines the chair conformation indicated in Figure 8A. The coupling constants of H-5 with the two H-6 protons show values ≤5 Hz, which indicate an equatorial position for H-5. This was corroborated by $^{3}J_{HH}$=∼7.8 Hz between H-3 and H-4, which is in agreement with an axial–*pseudo*axial disposition for both protons.

The study of hydroxyl exchange indicates an acidity order of OH-5>OH-3≈OH-4 (see pages S66 and S67 in Supplementary data). The analysis of the acidity order determines the direction of the hydrogen bonds. Since OH-5 is the most acidic group, it is an acceptor, and as a consequence the direction of the hydrogen bonds network is as shown in Figure 8A. Thus, the enzymatic acylation of **9** should proceed regioselectively towards the 4-position: OH-4 is equatorial, and the positive charge in the transition state is stabilized through the most efficient hydrogen bond. In practice, CAL-A catalyzes the acylation at OH-4 with total selectivity, the 4-*O*-acetyl derivative **10** being isolated with excellent yield.

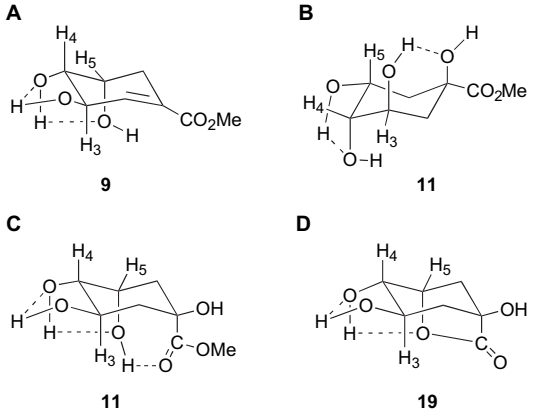


Figure 8. Hydrogen bond networks of methyl 4-*epi*-shikimate (A), methyl 4-*epi*-quinate (B and C), and methyl 4-*epi*-quinate lactone derivative **19** (D).

2.2.6. Study of hydrogen bonds network in methyl 4-*epi*-quinate (11). The hydroxyl exchange study of (−)-methyl 4-*epi*-quinate (**11**) was more difficult than in previous cases because of the low solubility of this derivative in $CDCl_3$, and even in CD_2Cl_2. The ^{1}H NMR of **11** in $CDCl_3$ shows the coupling constants of two hydroxyls ($^{3}J_{CH,OH}$=8.7 Hz and $^{3}J_{CH,OH}$=3.1 Hz), which were assigned by COSY spectrum as OH-3 and OH-4, respectively, (page S69 in Supplementary data). Taking into account that proton spectra in $CDCl_3$, MeOH-d_4, and acetone-d_6 evidence the chair conformation indicated in Figure 8B, the small value of the coupling constant for OH-4 indicates that the axial OH-4, and not the equatorial OH-5, acts preferentially as H-acceptor. In addition, the large value of $^{3}J_{H\text{-}3,OH\text{-}3}$ suggests a dihedral angle of 180°, showing the donor character of OH-3, and compatible with a strong 1,3-diaxial H-bond (see Fig. 8B). In accordance with this, the acylation should take place at OH-3, although this hydroxyl is in axial configuration. However, CAL-A exhibited total selectivity towards OH-4. This is in keeping with the chair conformation similar to that previously shown for methyl 4-*epi*-shikimate (Fig. 8C). In this case, an efficient hydrogen bond between OH-5 and the oxygen of the ester group increases the hydrogen-bonding network. In addition, OH-4 is in equatorial orientation. Similarly, enzymatic acetylation of the corresponding lactone **19** (Fig. 8D), which is restricted in this chair conformation, also afforded exclusively the 4-*O*-acetyl derivative **20** (Fig. 9).

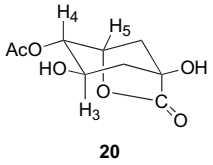


Figure 9.

Furthermore, we previously observed that the selectivity of CAL-A was independent of the solvent employed in the acylation of 5-*epi*-derivatives, despite both compounds having different conformations in different solvents. These facts suggest that the enzyme active site fits the most effective hydrogen-bonding network conformation, since the later better stabilizes the transition state.

2.2.7. Study of hydrogen bonds network in methyl 5-*epi*-shikimate (13). The ^{1}H NMR spectrum of **13** in $CDCl_3$ exhibits well-resolved OH signals ($^{3}J_{H\text{-}3,OH\text{-}3}$=9.9 Hz, $^{3}J_{H\text{-}4,OH\text{-}4}$=6.3 Hz and $^{3}J_{H\text{-}5,OH\text{-}5}$=5.5 Hz). Hydroxyl exchange after acid addition provided an acidity order of OH-5>OH-4>OH-3 (see pages S70 and S71 in Supplementary data). Thus, OH-5 should be the acceptor of OH-3, the two hydroxyl groups being involved in the molecule's strongest hydrogen bond, which is almost 1,3-diaxial. This observation is consistent with the large coupling constant value of OH-3, suggesting a dihedral H–C–O–H angle close to

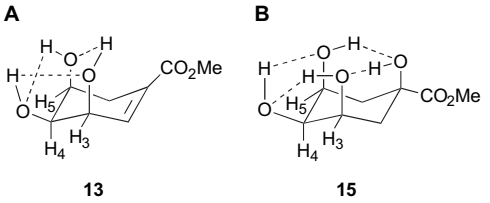


Figure 10. Hydrogen bonds network of methyl 5-*epi*-shikimate (A) and methyl 5-*epi*-quinate (B).

180°. The coupling constants of OH-4 and OH-5 also corroborated the direction of the hydrogen bonds indicated in Figure 10A.

Thus, if acylation takes place at the equatorial OH-4 group, the transition state is stabilized through an effective hydrogen bond network. However, we isolated exclusively the 5-*O*-acyl derivative. This result would be explained due to acyl migration from position 4 to 5 (acyl migrations were previously observed for methyl *O*-acylshikimate derivatives in certain conditions[13]). To confirm this fact, we tried to prepare the corresponding 4-*O*-acyl analogue by independent synthesis. On doing so, 3,5-di-*O*-TBDMS protection of methyl 5-*epi*-shikimate and subsequent acetylation of the OH-4 group afforded the 4-acetyl-3,5-di-*O*-TBDMS derivative. All attempts to obtain methyl 4-*O*-acetyl-5-*epi*-shikimate, after silyl deprotection, gave rise to a mixture of 3-, 4-, and 5-*O*-acetyl derivatives, in which the 5-*O*-acyl derivative was the major compound.

2.2.8. Study of hydrogen bonds network in methyl 5-*epi*-quinate (15). Finally, we also studied the hydrogen bonds in methyl 5-*epi*-quinate (**15**). The exchange of the hydroxyl protons of alcohols by acid shows the hydrogen bonds to be of similar strength. As previously mentioned, in $CDCl_3$ the chair conformation is indicated in Figure 10B. The $J_{CH,OH}$ values for hydroxyl groups ($^3J_{H\text{-}3,H\text{-}5,OH\text{-}3,OH\text{-}5}$= 7.6 Hz and $^3J_{H\text{-}4,OH\text{-}4}$=8.2 Hz) imply that the O–H bond is almost *anti* to the C–H bond and confirm that OH-1, OH-3, and OH-5 are involved in 1,3-diaxial hydrogen bonding. As methyl 3-*epi*-quinate, this is a *meso* form and the two possible orientations of the hydroxyl network are equivalent. Since equatorial hydroxyls are more reactive than axials, the acylation reaction should take place towards that position. In fact, the enzymatic process proceeds selectively at the OH-4 group.

In view of these results, we propose that the cooperative effect of hydrogen bonds is responsible for the enzyme–substrate interactions, and leads to the selectivity detected. Unusually strong hydrogen bonds, known as low barrier hydrogen bonds (LBHB), have emerged as a new rationale for the exceptional catalytic abilities of some enzymes.[24] It has been suggested that these LBHB are significant contributors to the intermediate stabilization and catalytic power of the enzyme.

The catalytic mechanism of lipases is based on a 'catalytic triad' composed of a nucleophilic serine residue activated by a hydrogen bond in relay with histidine and aspartate or glutamate. The interaction between the enzyme and the acylating agent yields the acyl–enzyme intermediate. A nucleophile attacks the latter intermediate, assisted by the catalytic histidine, yielding a second tetrahedral intermediate (TI-2). This intermediate collapses to expel the acylated product and gives the free enzyme.

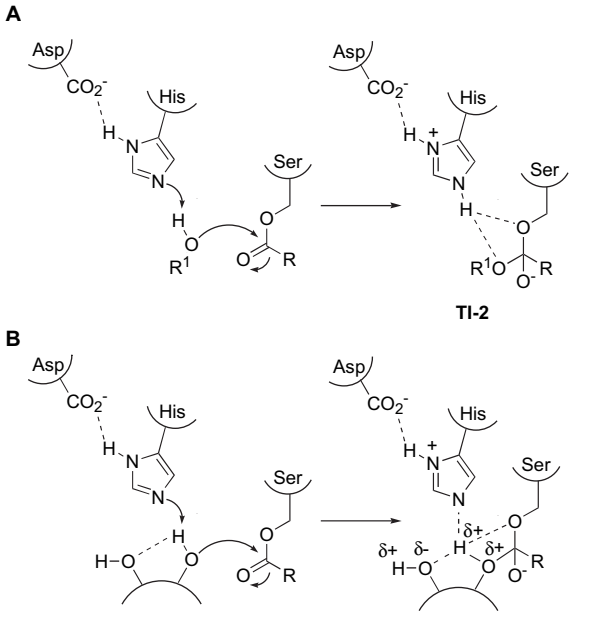


Figure 11. (A) Tetrahedral intermediate (TI-2) in the serine mechanism catalysis. (B) General representation of TI-2 in which positive charge is delocalized through hydrogen-bonding networks in quinic and shikimic acid derivatives.

We propose that transition state hydrogen-bonding plays an important role in enzyme selectivity, and can be understood as one result of the effectiveness of hydrogen-bonding networks (the original hydrogen bond network from the enzyme conjugates with intramolecular hydrogen bonding in the ligand). It is worth noting that a web of hydrogen-bonding interactions links the substrate binding sites to the catalytic triad. Two key hydrogen bonds in the TI-2 are that from His to the oxygen of Ser, and that with the OR^1 group (A, Fig. 11). In the case of quinic and shikimic acid derivatives, the positive charge in the transition state can also be delocalized through a hydrogen-bonding network present in the polyhydroxylated molecule (B, Fig. 11). The acylation reaction takes place preferentially at the hydroxyl group that participates in the more effective hydrogen-bonding network.

Acylation of quinic and shikimic acid derivatives was also carried out in the presence of DMAP as catalyst and in 1 mM concentration in $CHCl_3$, suitable conditions for efficient intramolecular hydrogen bonding.[8] In some cases, the regioselectivity of the acetylation agrees with the H-bonding scheme shown and a rise in the reactivity of certain OHs was observed. However, the reaction is not as selective as the process catalyzed by CAL-A and a mixture of acylated products was obtained.

3. Summary

Selective acylated derivatives of 3-*epi*, 4-*epi*, and 5-*epi*-isomers of quinic and shikimic acids have been efficiently synthesized in a one-step enzymatic transesterification reaction

with vinyl acetate as acyl donor and *C. antarctica* lipase A as catalyst. These analogues are useful as chiral building blocks and as optically active synthetic precursors for natural products. A study of the exchange rate of hydroxyl protons by ^{1}H NMR spectroscopy has been performed to determine the strength of the intramolecular H-bonds in these substrates. In addition, vicinal coupling constants $J_{CH,OH}$ provide structural information in terms of dihedral H–O–C–H angles and evidence the directional hydrogen-bonding network. We have established that selectivity of *C. antarctica* lipase A is related to both the inherent receptor selectivity and the degree of intramolecular hydrogen bonding in the ligand. We found a satisfactory correlation between reactivity of hydroxyl groups and effectiveness of the corresponding hydrogen-bonding network.

4. Experimental

4.1. General spectroscopy and experimental data

C. antarctica lipase A (CAL-A, chirazyme L-5, c-f, lyophilized, 25 U/g using 1-phenylacetate) was obtained from Roche. TLC chromatograms were visualized by heating after spraying with a 5% aqueous sulfuric acid solution containing cerium sulfate (1%) and molybdophosphoric acid (2.5%). Column chromatography was performed over silica 60 Å (230–400 mesh) except for **10**, **12**, **14**, and **20** in which silica 60 Å (32–63 μm) pH 7 was used. Molecular sieves 4 Å were dried at 180 °C in a vacuum over 2 h. Methyl 3-*epi*-, 4-*epi*-, and 5-*epi*-shikimate and quinate derivatives were dried in a high vacuum (10^{-5} mbar) before use. ^{1}H and ^{13}C NMR signals assignment is based on selective homonuclear and heteronuclear decoupling experiments.

Chiral HPLC analysis were performed using a chiralcel OD-H column, a flow of 0.5 mL/min, 5% EtOH/hexane as eluent, and at 35 °C. Sample concentration was 0.5 mg/mL. For (±)-**17**, t_R 12.7 and 13.9 min.

4.1.1. Sample preparation for ^{1}H NMR hydroxyl exchange studies. $CDCl_3$ was filtered over anhydrous K_2CO_3, which serves to deacidify and partially dry the solvent. $CDCl_3$ was stored under N_2 over K_2CO_3 and powdered 4 Å molecular sieves (predried under vacuum at 180 °C during 2 h) to completely remove the water. Syringes and NMR tubes were dried on a vacuum line at room temperature. The samples were prepared by dissolving a portion of the quinic and shikimic acid derivative in $CDCl_3$ to make a 2 mM solution. For the hydroxyl exchange study successive portions of 0.05 mM trifluoroacetic acid solution in $CDCl_3$ were added to the NMR tube and corresponding ^{1}H NMR spectra (300.13 MHz) were recorded.

4.2. Enzymatic acylation of methyl 3-*epi*-, 4-*epi*-, and 5-*epi*-shikimate and quinate derivatives. General procedure for the synthesis of 6, 8, 10, 12, 14, and 16

In a standard procedure, vinyl acetate (0.75 mL) was added to an Erlenmeyer flask that contained **5**, **7**, **9**, **11**, **13**, or **15** (0.069 mmol), CAL-A (13 mg), and 4 Å molecular sieves (13 mg) under nitrogen. The suspension was shaken at 250 rpm at 20 °C (except for **7** and **15** at 40 °C) for 0.5 h for **5**, 4.5 h for **7**, 1.5 h for **9**, 24 h for **11**, 0.3 h for **13**, and 4.5 h for **15**. The mixture was filtered, and the organic solvent was evaporated. Then, the reaction crude was subjected to flash chromatography (gradient eluent 50–80% Et_2O/CH_2Cl_2 for **6**; gradient eluent EtOAc–3% MeOH/EtOAc for **8**; 40% acetone/CH_2Cl_2 for **10**; 45% acetone/CH_2Cl_2 for **12**; 20% acetone/CH_2Cl_2 for **14**; and gradient eluent EtOAc–5% MeOH/EtOAc for **16**) to give **6** (white solid, 88% yield), **8** (colorless oil, 84% yield), **10** (colorless oil, 80% yield), **12** (colorless oil, 82% yield), **14** (colorless oil, 88% yield), and **16** (colorless oil, 85% yield).

4.2.1. Methyl 3-*O*-acetyl-3-*epi*-shikimate (6). R_f (Et_2O): 0.13; mp: 106–107 °C; IR (NaCl): υ 3406, 2952, 2921, 1719, 1654, 1438, 1374, 1240, and 1072 cm^{-1}; ^{1}H RMN (300 MHz, $CDCl_3$): δ 2.17 (s, 3H, OAc), 2.30 (dddd, 1H, $H_{6a'}$, $|^2J_{HH}|$ 17.8, $^3J_{HH}$ 9.8, $|^4J_{HH}|$ 3.7, $^5J_{HH}$ 3.1 Hz), 2.95 (ddd, 1H, $H_{6e'}$, $|^2J_{HH}|$ 17.7, $^3J_{HH}$ 5.7, $|^4J_{HH}|$ 1.1 Hz), 3.71 (dd, 1H, H_4, $^3J_{HH}$ 10.0, 8.0 Hz), 3.78 (s, 3H, OMe), 3.87 (ddd, 1H, H_5, $^3J_{HH}$ ~10.0, ~10.0, 5.9 Hz), 5.43 (m, 1H, H_3), and 6.62 (m, 1H, H_2) ppm; ^{13}C RMN (75.5 MHz, $CDCl_3$): δ 21.0 (C_{10}), 31.7 (C_6), 52.2 (OMe), 69.5 (C_5), 74.7, 74.9 (C_3+C_4), 130.4 (C_1), 134.4 (C_2), 166.0 (C_7), and 171.5 (C_9) ppm; $[\alpha]_D^{20}$ +38 (*c* 1.07, $CHCl_3$); MS (ESI$^+$, *m/z*): 253 [(M+Na$^+$), 100%] and 269 [(M+K$^+$), 6%]; Anal. Calcd (%) for $C_{10}H_{14}O_6$: C, 52.16; H, 6.13. Found: C, 52.2; H, 6.1.

4.2.2. Methyl 3-*O*-acetyl-3-*epi*-quinate (8). R_f (EtOAc): 0.18; IR (KBr): υ 3396, 2956, 2927, 1732, 1435, 1371, 1259, 1130, 1067, and 1036 cm^{-1}; ^{1}H RMN (300 MHz, acetone-d_6): δ 1.76 (ddd, 2H, H_{6a}+H_{2a}, $|^2J_{HH}|$ 12.9, $^3J_{HH}$ 11.7, $|^2J_{HH}|$ 5.7 Hz), 1.98 (s, 3H, OAc), 2.01–2.1 (m, 2H, H_{6e}+H_{2e}, under solvent), 2.9 (br s, 1H, OH), 3.40 (dd, 1H, H_4, $^3J_{HH}$ ~9.2, ~9.2 Hz), 3.71 (s, 3H, OMe), 3.83 (ddd, 1H, H_5, $^3J_{HH}$ 11.6, 9.0, 4.6 Hz), and 5.02 (ddd, 1H, H_3, $^3J_{HH}$ 11.6, 9.6, 4.8 Hz) ppm; ^{13}C RMN (75.5 MHz, acetone-d_6): δ 21.7 (C_{10}), 39.4, 42.0 (C_2+C_6), 53.3 (OMe), 70.7 (C_5), 73.7 (C_3), 74.8 (C_1), 78.8 (C_4), 171.5 (C_9), and 176.2 (C_7) ppm; $[\alpha]_D^{20}$ (compound from the enzymatic reaction) +19 (*c* 1.37, MeOH); $[\alpha]_D^{20}$ (compound enantiomerically pure) +31 (*c* 1.31, MeOH); MS (ESI$^+$, *m/z*): 271 [(M+Na$^+$), 100%], 287 [(M+K$^+$), 7%], and 287 [(M+H$^+$), 1%]; Anal. Calcd (%) for $C_{10}H_{16}O_7$: C, 48.39; H, 6.5. Found: C, 48.4; H, 6.6.

4.2.3. Methyl 4-*O*-acetyl-4-*epi*-shikimate (10). R_f (CH_2Cl_2): 0.13; IR (NaCl): υ 3420, 2950, 1710, 1655, 1430, 1358, 1250, and 1182 cm^{-1}; ^{1}H RMN (300 MHz, $CDCl_3$): δ 2.16 (s, 3H, OAc), 2.58 (dd, 1H, $H_{6a'}$, $|^2J_{HH}|$ 18.5, $^3J_{HH}$ 4.5 Hz), 2.68 (dddd, 1H, $H_{6e'}$, $|^2J_{HH}|$ 18.6, $^3J_{HH}$ 4.4, $|^4J_{HH}|$ ~2.2, $^5J_{HH}$ ~2.2 Hz), 3.78 (s, 3H, OMe), 4.28 (ddd, 1H, H_5, $^3J_{HH}$ 4.5, 4.3, 2.4 Hz), 4.59 (m, 1H, H_3), 4.91 (dd, 1H, H_4, $^3J_{HH}$ 6.9, 2.1 Hz), and 6.84 (m, 1H, H_2) ppm; ^{13}C RMN (75.5 MHz, $CDCl_3$): δ 21.1 (C_{10}), 31.1 (C_6), 52.1 (OMe), 66.4 (C_5), 67.3 (C_3), 76.8 (C_4), 128.6 (C_1), 136.9 (C_2), 166.7 (C_9), and 171.4 (C_7) ppm; $[\alpha]_D^{20}$ −56 (*c* 0.86, $CHCl_3$); MS (ESI$^+$, *m/z*): 253 [(M+Na$^+$), 100%] and 231 [(M+H$^+$), 3%]; Anal. Calcd (%) for $C_{10}H_{14}O_6$: C, 52.17; H, 6.13. Found: C, 52.2; H, 6.1.

4.2.4. Methyl 4-*O*-acetyl-4-*epi*-quinate (12). R_f (EtOAc): 0.2; IR (KBr): υ 3402, 2946, 1733, 1447, 1434, 1237, 1128, and 1091 cm^{-1}; ^{1}H RMN (300 MHz, MeOH-d_4): δ 1.70 (dd, 1H, H_{2e}, $|^2J_{HH}|$ 13.8, $^3J_{HH}$ 6.6 Hz), 1.87 (dd, 1H, H_{6e}, $|^2J_{HH}|$ 13.3, $^3J_{HH}$ 3.4 Hz), 2.11 (s, 3H, H_{10}), 2.28–2.36

(m, 2H, H_{2a}+H_{6a}), 3.75 (s, 3H, OMe), 4.11 (1H, m, H_3), 4.23 (ddd, 1H, H_5, $^3J_{HH}$ ~6.4, ~6.4, 4.1 Hz), and 4.89 (dd, 1H, H_4, $^3J_{HH}$ 6.4, 2.9 Hz) ppm; ^{13}C RMN (75.5 MHz, MeOH-d_4): δ 19.6 (C_{10}), 36.7 (C_2), 38.6 (C_6), 51.38 (OMe), 63.8 (C_5), 66.7 (C_3), 74.1 (C_1), 75.7 (C_4), 171.1 (C_9), and 174.0 (C_7) ppm; $[\alpha]_D^{20}$ −19 (*c* 0.48, H_2O); MS (ESI$^+$, *m/z*): 271 [(M+Na)$^+$, 100%] and 249 [(M+H)$^+$, 10%]; Anal. Calcd (%) for $C_{10}H_{16}O_7$: C, 48.39; H, 6.5. Found: C, 48.2; H, 6.5.

4.2.5. Methyl 5-*O*-acetyl-5-*epi*-shikimate (14). R_f (Et_2O): 0.18; IR (NaCl): υ 3422, 2955 1718, 1654, 1438, 1372, 1259, and 1036 cm^{-1}; ^{1}H RMN (300 MHz, $CDCl_3$): δ 2.13 (s, 3H, OAc), 2.52–2.78 (m, 2H, $H_{6a'}$+$H_{6e'}$), 3.78 (s, 3H, OMe), 4.12 (m, 1H, H_4), 4.42 (m, 1H, H_3), 5.07 (ddd, 1H, H_5, $^3J_{HH}$ 8.2, 6.0, 2.0 Hz), and 6.80 (m, 1H, H_2) ppm; ^{13}C RMN (75.5 MHz, $CDCl_3$): δ 21.1 (C_{10}), 26.0 (C_6), 52.1 (OMe), 67.5 (C_3), 68.5 (C_4), 70.9 (C_5), 128.6 (C_1), 137.5 (C_2), 166.3 (C_7), and 170.3 (C_9) ppm; $[\alpha]_D^{20}$ −60 (*c* 0.5, MeOH); MS (ESI$^+$, *m/z*): 253 [(M+Na)$^+$, 100%] and 231 [(M+H)$^+$, 5%]; Anal. Calcd (%) for $C_{10}H_{14}O_6$: C, 52.17; H, 6.13. Found: C, 52.2; H, 6.1.

4.2.6. Methyl 4-*O*-acetyl-5-*epi*-quinate (16). R_f (10% MeOH/EtOAc): 0.30; IR (KBr): υ 3387, 2957, 1727, 1651, 1455, 1435, 1258, 1128, and 1045 cm^{-1}; ^{1}H RMN (300 MHz, acetone-d_6): δ 1.81 (dd, 2H, H_{6a}+H_{2a}, $|^2J_{HH}|$ ~12.0, $^3J_{HH}$ ~12.0 Hz), 2.09 (s, 3H, OAc), 2.18 (dd, 2H, H_{6e}+H_{2e}, $|^2J_{HH}|$ 11.9, $^3J_{HH}$ 4.6 Hz), 3.71 (s, 3H, OMe), 3.80 (m, 2H, H_3+H_5), 3.99 (d, 2H, OH_3+OH_5, $^3J_{HOH}$ 6.6 Hz), 4.82 (s, 1H, OH_1), and 5.27 (dd, 1H, H_4, $^3J_{HH}$ ~2.4, ~2.4 Hz) ppm; ^{13}C RMN (75.5 MHz, acetone-d_6): δ 21.2 (C_{10}), 39.4 (C_2+C_6), 52.5 (OMe), 66.91 (C_3+C_5), 73.58 (C_1), 75.0 (C_4), 171.0 (C_9), and 175.3 (C_7) ppm; MS (ESI$^+$, *m/z*): 271 [(M+Na)$^+$, 100%]; Anal. Calcd (%) for $C_{10}H_{16}O_7$: C, 48.39; H, 6.5. Found: C, 48.4; H, 6.4.

4.3. Methyl (1*S*,3*R*,4*R*,5*S*,2′*S*,3′*S*)-3-acetoxy-4,5-[2,3-dimethoxybutan-2,3-di(yloxy)]-1-hydroxycyclohexan-1-carboxylate (17)

A solution of **8** (20 mg, 0.081 mmol) in MeOH (1 mL) was treated with trimethyl orthoformate (43 μL, 0.39 mmol), 2,3-butanedione (14 μL, 0.165 mmol), and camphorsulfonic acid (0.93 mg, 0.004 mmol). The mixture was refluxed under nitrogen for 2 h. The resulting solution was treated with powdered $NaHCO_3$ (spatula tip) and filtered. The filtrate was concentrated and subjected to flash chromatography (gradient eluent 50–80% Et_2O/CH_2Cl_2) to afford **17** as a colorless oil (89% yield).

4.3.1. Synthesis of (±)-17. Acetic anhydride (0.3 mL, 0.312 mmol) was added to a mixture of (±)-**18** (50 mg, 0.156 mmol), pyridine (63 μL, 0.78 mmol), and DMAP (1 mg, 1.56 mmol) in CH_2Cl_2 (2 mL) at 0 °C. After 4 h at 0 °C, the mixture was partitioned between CH_2Cl_2 and H_2O. The aqueous phase was reextracted with CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4. Filtration and concentration afforded a crude, which was purified by flash chromatography (gradient eluent 50–80% Et_2O/CH_2Cl_2) to give (±)-**17** (80% yield).

R_f (20% Et_2O/CH_2Cl_2): 0.29; IR (NaCl): υ 3448, 2954, 1739, 1438, 1370, 1240, and 1132 cm^{-1}; ^{1}H RMN (300 MHz, $CDCl_3$): δ 1.30, 1.31 (2s, 6H, 2Me), 1.83 (dd, 1H, H_{2a}, $|^2J_{HH}|$ 12.9, $^3J_{HH}$ 11.2 Hz), 1.88–1.99 (m, 2H, H_{6a}+H_{6e}), 2.08 (s, 3H, OAc), 2.18 (m, 1H, H_{2e}), 3.26, 3.29 (2s, 6H, OMe), 3.70 (dd, 1H, H_4, $^3J_{HH}$ ~9.9, ~9.9 Hz), 3.80 (s, 3H, OMe), 4.08 (ddd, 1H, H_5, $^3J_{HH}$ 11.7, 9.9, 5.1 Hz), and 5.17 (ddd, 1H, H_3, $^3J_{HH}$ 11.2, 10.1, 5.1 Hz) ppm; ^{13}C RMN (75.5 MHz, $CDCl_3$): δ 17.6, 17.63 (C_{13}+C_{14}), 21.0 (C_{18}), 37.3, 38.5 (C_2+C_6), 53.1 (OMe), 47.5, 47.8 (C_{15}+C_{16}), 65.3 (CH), 69.2 (CH), 72.9 (CH), 73.2 (C_1), 99.5, 99.54 (C_9+C_{10}), 170.1 (C_{17}), and 175.1 (C_7) ppm; MS (ESI$^+$, *m/z*): 385.1 [(M+Na$^+$), 100%] and 401.1 [(M+K$^+$), 33%]; Anal. Calcd (%) for $C_{16}H_{26}O_9$: C, 53.03; H, 7.23. Found: C, 52.9; H, 7.2.

4.4. Methyl (1*S*,3*R*,4*R*,5*S*,2′*S*,3′*S*)-1,3-dihydroxy-4,5-[2,3-dimethoxybutan-2,3-di(yloxy)]cyclohexan-1-carboxylate [(±)-18]

The same procedure as previously described for **17** (from **8**) afford (±)-**18** starting from **7**. The crude was purified by flash chromatography (70% EtOAc/hexane). White solid. Yield: 91%. Spectroscopical data in agreement with the enantiomerically pure compound previously described.[15,25] R_f (20% MeOH/EtOAc): 0.6; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.3, 1.34 (2s, 6H, Me), 1.92 (dd, 1H, H_{6a}, $|^2J_{HH}|$ 13.4, $^3J_{HH}$ 10.1 Hz), 2.08 (m, 3H, H_{6e}, H_{2a}, H_{2e}), 3.26 (2s, 6H, OMe), 3.59 (dd, 1H, H_4, $^3J_{HH}$ 8.6, 3.2 Hz), 3.77 (s, 3H, OMe), 4.18 (m, 1H, H_3), and 4.28 (ddd, 1H, H_5, $^3J_{HH}$ 10, 8.6, 4.5 Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 17.5, 17.7 (C_{11}+C_{12}), 37.3 (CH_2), 38.5 (CH_2), 47.8, 52.8 (C_{13}+C_{14}), 62.3 (CH), 69.0 (CH), 72.6 (CH), 75.7 (C_1), 99.6, 100.2 (C_9+C_{10}), and 174.1 (C═O) ppm.

4.5. (1*S*,3*R*,4*S*,5*R*)-1,3,4-Trihydroxycyclohexan-1,5-carbolactone (19)

This compound was obtained in the attempted recrystallization of **11**. R_f (EtOAc): 0.35; mp: 148–150 °C; IR (KBr): υ 3396, 3311, 2997, 2963, 1765, 1445, 1362, 1256, 1122, and 1062 cm^{-1}; ^{1}H NMR (300 MHz, MeOH-d_4): δ 1.96 (dd, 1H, H_{2a}, $|^2J_{HH}|$ 11.9, $^3J_{HH}$ 10.6 Hz), 2.27 (d, 1H, H_{6a}, $^3J_{HH}$ 11.5 Hz), 2.42–2.29 (m, 1H, H_{2e}), 2.63 (ddd, 1H, H_{6e}, $|^2J_{HH}|$ 11.7, $^3J_{HH}$ 6.7, $|^4J_{HH}|$ 3.5 Hz), 3.68–3.86 (m, 2H, H_3+H_4), and 4.79 (d, 1H, H_5, $^3J_{HH}$ 6.6 Hz) ppm; ^{13}C NMR (75.5 MHz, MeOD-d_4): δ 41.9 (C_2), 42.3 (C_6), 71.0 (C_3), 74.1 (C_1), 76.1 (C_4), 80.5 (C_5), and 179.5 (C_7) ppm; MS (ESI$^+$, *m/z*): 371 [(2M+Na)$^+$, 100%] and 197 [(M+Na)$^+$, 70%]; $[\alpha]_D^{20}$ −94 (*c* 0.51, MeOH); Anal. Calcd (%) for $C_7H_{10}O_5$: C, 48.28; H, 5.79. Found: C, 48.2; H, 5.8.

4.6. (1*S*,3*R*,4*S*,5*R*)-4-Acetoxy-1,3-dihydroxycyclohexane-1,5-carbolactone (20)

Using the general procedure for enzymatic acylation previously described yield **20** (colorless oil, 70% yield) from **19**. The reaction mixture was stirred at 40 °C for 4.5 h. The crude material was purified by flash chromatography (40% acetone/CH_2Cl_2). R_f (EtOAc): 0.31; IR (NaCl): υ 3358, 2995, 2971, 1771, 1440, 1453, 1360, 1258, 1122, and 1067 cm^{-1}; ^{1}H NMR (300 MHz, MeOH-d_4): δ 2.03 (dd, 1H, H_{2a}, $|^2J_{HH}|$ 12.3, $^3J_{HH}$ 11.2 Hz), 2.31 (s, 3H, OAc), 2.38 (d, 1H, H_{6a}, $|^2J_{HH}|$ 11.7 Hz), 2.39 (m, 1H, H_{2e}), 2.67 (ddd, H_{6e}, $|^2J_{HH}|$ 11.7, $^3J_{HH}$ 6.7, $|^4J_{HH}|$ 3.5 Hz), 3.98

(ddd, 1H, H_3, $^3J_{HH}$ 11.0, 8.3, 7.3 Hz), 4.89 (dd, 1H, H_5, $^3J_{HH}$ 6.7, 1.1 Hz), and 4.96 (dd, 1H, H_4, $^3J_{HH}$ 8.3, 1.1 Hz) ppm; ^{13}C NMR (75.5 MHz, MeOH-d_4): δ 20.9 (C_9), 41.6 (C_2), 42.0 (C_6), 68.2 (C_3), 73.8 (C_1), 77.3 (C_5), 78.1 (C_4), 172.3 (C_8), and 178.9 (C_7) ppm; $[\alpha]_D^{20}$ −116 (*c* 0.48, MeOH); MS (ESI$^+$, *m*/*z*): 239 [(M+Na)$^+$, 100%]; Anal. Calcd (%) for $C_9H_{12}O_6$: C, 50.0; H, 5.59. Found: C, 50.1; H, 6.0.

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