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Biocatalytic regioselective *O*-acylation of sesquiterpene lactones from chicory: a pathway to novel ester derivatives

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Abstract: We report the first biocatalytic modification of sesquiterpene lactones (STLs) found in the chicory plants, specifically lactucin (Lc), 11*B*,13-dihydrolactucin (DHLc), lactucopicrin (Lp), and 11*B*,13-dihydrolactucopicrin (DHLp). The selective *O*-acylation of their primary alcohol group was carried out by the lipase B from *Candida antarctica* (CAL-B) using various aliphatic vinyl esters as acyl donors. Perillyl alcohol, a simpler monoterpenoid, served as a model to set up the desired *O*-acetylation reaction by comparing the use of acetic acid and vinyl acetate as acyl donors. Similar conditions were then applied to DHLc, where five novel ester chains were selectively introduced onto the primary alcohol group, with conversions going from >99 % (acetate and propionate) to 69 % (octanoate). The synthesis of the corresponding *O*-acetyl esters of Lc, Lp, and DHLp was also successfully achieved with near-quantitative conversion. Molecular docking simulations were then performed to elucidate the preferred enzyme-substrate binding modes in the acylation reactions with STLs, as well as to understand their interactions with crucial amino acid residues at the active site. Our methodology enables the selective *O*-acylation of the primary alcohol group in four different STLs, offering possibilities for synthesizing novel derivatives with significant potential applications in pharmaceuticals or as biocontrol agents.

Introduction

Terpenes are the most abundant and diverse family of natural compounds, with over 64,000 structures identified to date.^[1,2] Their significance to humanity is undeniable, with plants rich in terpenes having been employed for medicinal purposes across the globe. Sesquiterpene lactones (STLs) are a group of highly diversified C₁₅ terpenoids found in plants that serve them as defense tools to cope with environmental stresses.^[3] Many of them also possess pharmacological properties. For instance, over 1,500 scientific publications between 1990 and 2010 focus on their antitumor and anti-inflammatory activities. Moreover, antimalarial, antioxidant,^[4] and antimicrobial properties have also been reported.^[4-8]

Among plants rich in STLs, Chicory (*Cichorium intybus*) is well known.^[9] This plant of the *Asteraceae* family has been historically used by Ancient Greeks, Egyptians, and Chinese as a herbal remedy to treat a variety of respiratory, liver, and digestive disorders.^[10,11] Around fifteen STLs belonging to the guaianolide sub-family have been reported, with lactucin (Lc) and its ester analog lactucopicrin (Lp) being the most well-known, as they were previously identified in wild lettuce (*Lactuca virosa*).^[12,13] Other natural analogs of Lc and Lp, such as 11*B*,13-dihydrolactucin and 11*B*,13-dihydrolactucopicrin, were also identified. Their structural characteristics have been described in detail in the literature and are reported in **Figure 1**.^[14,15,16]

Chicory-derived STLs have demonstrated promising antimicrobial properties. Both 11*B*,13-dihydrolactucin (DHLc) and lactucopicrin (Lp) inhibited the growth of *Pseudomonas aeruginosa*, DHLp was also

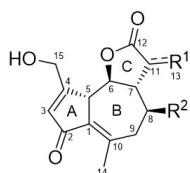
effective against *Staphylococcus aureus*, while DHLc showed promising results against different strains of *Candida*.^[17] Antiparasitic properties were reported for STLs from chicory such as lactucin and lactucopicrin, notably against *Plasmodium falciparum* strain Honduras-1.^[18,19] Moreover, lactucin has also been linked to both *in vivo* and *in vitro* anti-adipogenesis effects and anticancer activities.^[20,21]

This wide range of biological activities can be explained by the presence of a α -methylene-gamma lactone (α -MGL) and an unsaturated cyclopentenone moieties. These electrophilic moieties can interact with cysteine and other amino acid residues of certain proteins, notably *via* Michael addition reactions. While those have been identified as the main pharmacophores, there are other factors that play a role in modulating their biological activities, such as lipophilicity, the number of alkylating sites and the presence of certain ester side chains.^[22,23]

While the synthesis of new STL esters has not been explored thoroughly yet, a few examples in the literature have shown interesting results. Kitai *et al.* recently carried out the synthesis of several ester derivatives (propyl, butyl, pentyl and 2-methoxy ethynyl) of the STL sonchifolinic acid, isolated from Yacon (*Smallanthus sonchifolius*), and studied their cytotoxicity by evaluating the influence of these side chains.^[24] A similar effect was shown for the STL helenalin, where its acetate and isobutyrate ester derivatives showed a higher toxicity towards tumor cells. The difference in cytotoxicity was shown to be directly related to both the size and the lipophilicity of the side chain.^[25] Moreover, a recent work by Zhang *et al.* describes the synthesis of several semi-synthetic aryl ester derivatives of the STL

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scabertopin (isolated from *Elephantopus scaber*). Their evaluation as potential anti-cancer agents for non-small cell lung cancer also showed promising results.^[26]



STL	R ¹	R ²	clog (P)	Surface Polarity
lactucin	CH ₂	OH	-0.7	83
lactucopicrin	CH ₂	OCOCH ₂ PhOH	1.1	110
8-deoxylactucin	CH ₂	H	0.208	63.6
11β,13-dihydrolactucin	CH ₃	OH	-0.54	83
11β,13-dihydrolactucopicrin	CH ₃	OCOCH ₂ PhOH	1.27	110

Figure 1. Guaianolide skeleton and structure, clog (P) and surface polarity of the main non-conjugated STLs found in chicory root.^[1,4]

The primary allyl alcohol moiety found in chicory STLs and in many other terpenoids represents a promising starting point for the addition of side chains. Thus, we sought to develop a biocatalytic methodology which could be conveniently applied to a large variety of STLs, paving the way for the synthesis of numerous novel semi-synthetic derivatives. In this context, biological catalysts can offer distinct advantages over conventional synthesis methods, including superior selectivity and environmental friendliness.^[27,28] Lipases (EC 3.1.1.3) have already been employed as biocatalysts in the synthesis of terpenoid esters *via* esterification and transesterification reactions in organic media.^[29,30] Among commercial lipases, the lipase B from *Candida antarctica* (CAL-B) has demonstrated remarkable versatility and robustness, particularly when it comes to the synthesis of lipophilic esters.^[31] However, despite its widespread commercial availability, its application in synthesizing STL esters remains unexplored. Furthermore, the specific STLs selected for this article have not been described in any existing literature as having undergone enzymatic modification.

Given the significant cost and limited commercial availability of chicory STLs, we began our study with a simpler model compound. The monoterpenoid (*S*)-perillyl alcohol (POH), featuring an allyl-type primary alcohol moiety similar to the one present in our STL targets, was selected as the model to set up our reaction conditions. Once selected the best reaction conditions, we focused on introducing alkyl ester chains onto the primary alcohol group present in chicory STLs. This approach aimed to modulate their lipophilicity and, consequently,

their biological properties, such as interaction with plasma membranes and permeability through biological barriers. Our strategy allowed the synthesis of eight novel acyl STL derivatives. Beyond alkyl chains, we also investigated the synthesis of aryl esters using aryl vinyl esters as acyl donors. Additionally, docking simulations were conducted with the purpose of understanding the differences in reactivity (selectivity and yield) observed with the different acyl donors.

Results and Discussion

Generic parameters

In this study, we selected the widely available lipase B from *Candida antarctica* (CAL-B) for the *O*-acylation of our target compounds. Considering the limited availability of chicory STLs and the lack of prior reports on their biocatalytic modification, we chose Novozym 435 (N435), an immobilized lipase, known for its excellent catalytic performance. The use of an immobilized lipase simplified the reaction work-up and analysis. Instead of inactivating the enzyme post-reaction—a process with potential risk of degrading the STLs—a simple filtration step was preferred. Moreover, the interfacial immobilization onto a microporous resin is known for enhancing the catalytic efficiency and robustness of N435 against denaturants such as acetaldehyde. However, for future applications, we believe that other less expensive formulations of CAL-B could be used, if a more cost-effective enzyme was required. Though it may require adjusting the amount of enzyme to compensate for any potential decrease in catalytic activity.

Our primary criterion for selecting the solvent system was its capacity to solubilize the main STLs from chicory root and the various acyl donors, while still enabling effective enzyme-catalyzed acylation reactions. For this reason, common solvents used to extract STLs, such as alcoholic solvents (methanol, ethanol) and ethyl acetate, were not considered in this case. Acetonitrile (ACN) effectively solubilized the four STLs and the acyl donors, however, based on previous experiments, we noted that methyl *tert*-butyl ether (MTBE) typically increased the overall conversion with Novozym 435. Taking this into account, we opted for a mixture of 3/1:MTBE/ACN, which proved to be the best compromise tested. In the future, MTBE could potentially be replaced by a greener alternative such as cyclopentyl methyl ether (CPME) which can be obtained from biomass.^[32]

Regarding acyl donors, we began our study with the simplest donor and then gradually increased the complexity of the acyl chain by modifying the characteristics of its substituents (length and functional groups). Consequently, we began by synthesizing the simplest ester derivative, a methyl ester. Given that the nature of the acyl donor can significantly influence the yield of the desired ester, we chose to compare two commonly used acetyl donors: vinyl acetate and acetic acid. Based on the existing literature, we used the acyl donor in excess relative to the alcohol substrate (typically 3 equivalents), this contributed to shifting the equilibrium towards the formation of the desired ester and maximizing the consumption of the valuable STLs.

Esterification of (*S*)-perillyl alcohol -model reaction-

As discussed in the introduction, perillyl alcohol (POH) was chosen as our model compound as it fitted within our criteria of accessibility/cost and, more importantly, in terms of structural similarity, sharing the allyl alcohol moiety of chicory STLs. We initiated the process by selecting the most suitable acyl donor—either acetic acid or vinyl acetate—to form the corresponding ester using immobilized CAL-B (Figure 2).

The fact that acyl donors generally lead to more favorable outcomes than corresponding carboxylic acids in lipase-catalyzed acylation reactions is well-known in the literature. This can be attributed to the favorable shift in the reaction equilibrium due to the liberation of vinyl alcohol, which subsequently isomerizes into the volatile compound acetaldehyde.^[33–35] Furthermore, acetaldehyde is unable to act as a nucleophile in the reverse transesterification reaction. Another contributing factor to the lower reactivity of carboxylic acids is their acidic character, this can lower the pH of residual water surrounding the enzyme and negatively affect its performance.^[36]

The acylation reactions and their negative controls were carried out in a small glass vial (2 mL) containing 1 mL of solvent (3/1:MTBE/ACN) in the presence of molecular sieves (5 Å). The formation of perillyl acetate was monitored by GC-FID and its structure was confirmed by NMR analysis (¹H, ¹³C and HSQC). The transesterification with 10 mg of Novozym 435, 100 mM of POH and 300 mM of vinyl acetate allowed for a remarkable >99 % conversion after 1 h of reaction at 37 °C. On the other hand, as expected, the esterification with 300 mM of acetic acid proceeded much slower, only achieving 12 % conversion in the same timeframe, and 24 % after 2 h. After 5 days, ¹H NMR analysis showed a conversion of 90 % ± 5 % which was estimated *via* the integral ratio of the hydrogens from perillyl alcohol and perillyl acetate present in the mixture.

Based on these results, we chose to proceed with vinyl esters as acyl donors instead of their corresponding acids, aiming to maximize the conversion to the desired ester. Furthermore, the complete conversion achieved with vinyl acetate can serve as a benchmark for the enzyme's ability to utilize different acyl donors. While it is known that acetaldehyde can act as an enzyme denaturant,^[37] the concentration used in this study did not significantly impact the performance of Novozym 435. Additionally, the lack of water generation when using vinyl esters as acyl donors theoretically removes the need for molecular sieves. However, these sieves may still aid in removing vinyl alcohol or acetaldehyde from the medium. Conveniently, the use of this methodology removes the need for a complicated workup procedure, as the immobilized enzyme can be easily recovered for future recycling, and a simple filtration followed by concentration (8 mbar, 35 °C, 1 h) was enough to remove any unreacted vinyl acetate and acetaldehyde from the medium. Thus, this work allowed us to set up promising reaction conditions for the acylation of terpenoids containing a primary allyl alcohol moiety, such as perillyl alcohol.

It should also be noted that while the use of lipases (particularly CAL-B) for the synthesis of various monoterpenoid methyl esters using vinyl

acetate is well known,^[29,38] to the best of our knowledge this study reports the first lipase-catalyzed synthesis of perillyl acetate. The methodology shown here could represent a viable and straightforward option for obtaining perillyl acetate in quantitative yield for future industrial applications or as an interesting potential building block.

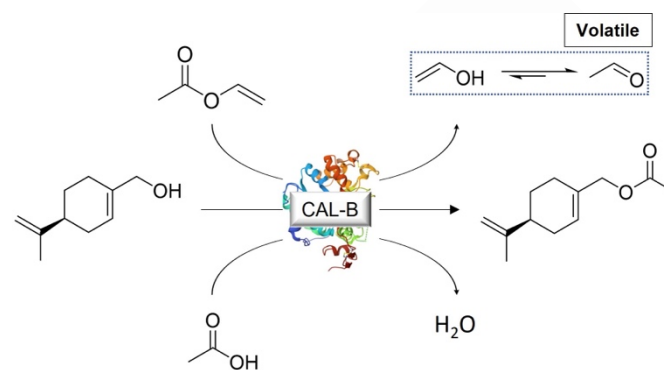


Figure 2. Lipase B from *Candida antarctica* (CAL-B) catalyzed acylation of (*S*)-perillyl alcohol with acetic acid and vinyl acetate as acyl donors. General conditions: 100 mM alcohol, 300 mM acyl donor, 10 mg Novozym 435, 3 spheres of molecular sieves 5 Å in 1 mL of 3/1:MTBE/ACN at 37 °C and 35 rpm on an orbital carousel rotating shaker.

Acylation of 11*B*,13-dihydrolactucin

Based on the promising results obtained with perillyl alcohol, we then employed the same general conditions to the *O*-acylation of the four chicory STLs of interest (Figure 3). Given that we managed to extract a relatively larger amount of DHLc from chicory root, we selected this STL as the main substrate for this section of the study.^[16]

The lipase-catalyzed acylation with vinyl acetate as the acyl donor proceeded under similar reaction conditions as before. The reaction was conducted in 1 mL of 3/1:MTBE/ACN, with 20 mg of the immobilized lipase (Novozym 435), 10 mM of DHLc at 37 °C, along with the respective negative controls. In the context of this first study, the cost of the acyl donor and the enzyme were insignificant compared to the cost of the STL. Thus, 10 equivalents of acyl donor were used in order to ensure an optimal conversion. Likewise, the amount of STL (10 mM, ~3 mg) was the minimum necessary for effective product characterization.

The reaction was stopped after 48 h and the resulting mixture was analyzed by ¹H NMR after filtration and concentration (8 mbar, 35 °C, 2 h). We observed that the signals of the two hydrogens adjacent to the primary hydroxy group of DHLc (15a and 15b), two doublet of doublets with strong roof effect at 4.23 ppm and 4.65 (Figure 4, A), shifted to higher ppm values (4.83 and 5.22 ppm) (Figure 4, C). This shift was attributed to the deshielding effect caused by the formation of an ester group, which possesses a greater electro-attractive effect than the original alcohol. This was confirmed by the negative control in the absence of the enzyme, where no shift appeared (Figure 4, B). Therefore, we followed the shifting of these two protons to monitor and validate the acylation of the primary alcohol group of DHLc for the rest of the study.

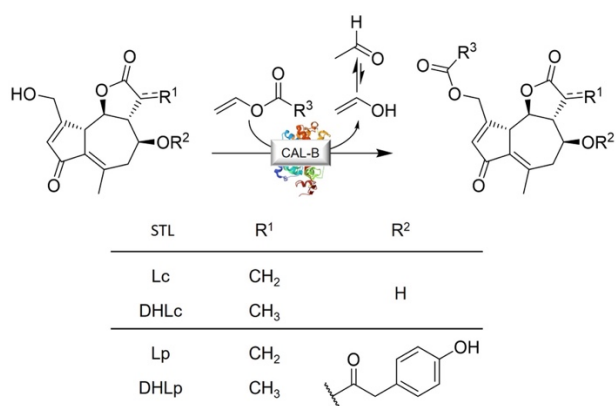


Figure 3. Representation of the lipase-catalyzed transesterification between the different sesquiterpene lactones (STLs) and vinyl esters; conducted with 10 mM of STL, 100 mM of vinyl ester, 20 mg of Novozym 435, 3 spheres of molecular sieves 5 Å in 1 mL of solvent mixture (MTBE/ACN) at 37° C and 35 rpm on an orbital carousel rotating shaker. R³ = alkyl or alkyl chloride.

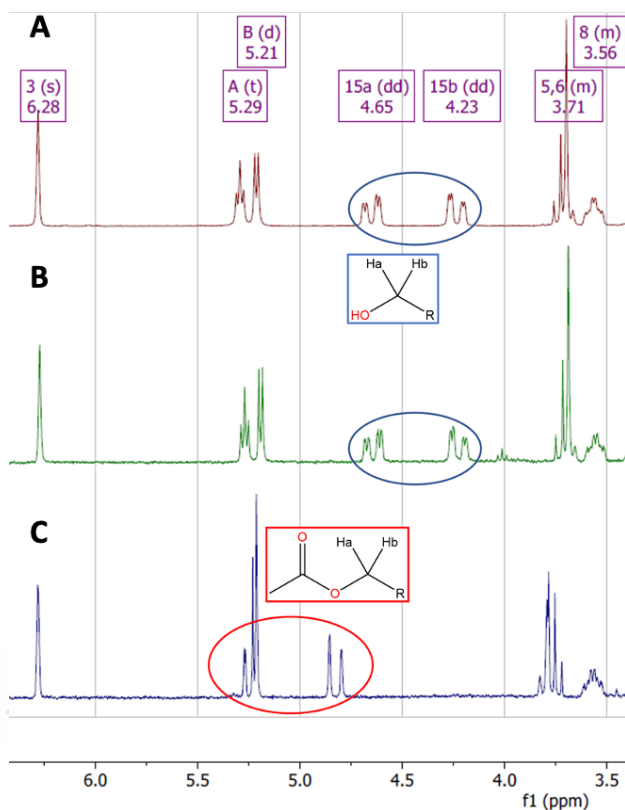


Figure 4. ¹H NMR (DMSO-D₆) comparison of the reaction mixture, the negative control and the spectra of pure DHLc. (A) Pure DHLc used for the reaction; (B) Reaction mixture of negative control without lipase after 48 h; (C) Reaction mixture after 48 h with 10 mM DHLc, 100 mM vinyl acetate and 20 mg Novozym 435.

Remarkably, no shift was observed for the hydrogen adjacent to the secondary alcohol group (hydrogen #8), or for those related to the

lactone moiety (**Figure 4, A and C**). This showed two important findings: 1) The reaction catalyzed by CAL-B is very selective for the primary alcohol; 2) The enzyme is not able to hydrolyze the lactone, allowing us to obtain only the acetyl derivative of DHLc (conversion >99 % measured by LC-MS).

Due to the cost of the starting substrate, we first maximized our chances of obtaining a detectable yield with DHLc by using a larger amount of enzyme in this first trial than in the preliminary study with perillyl alcohol (20 mg here versus 10 mg used previously). However, given the very high conversion, we repeated the operation under identical conditions, but using only 2 mg of enzyme. In this case, a conversion of 95 % ± 5 % was again obtained in only 24 h, demonstrating the very good acceptance of DHLc by the lipase.

As DHLc proved to be an excellent substrate for the enzyme, we were curious to know whether acetic acid could also be used as an effective acyl donor. Particularly in order to consider its future use on a larger scale, as vinyl esters are 1) typically more toxic than the corresponding carboxylic acid, 2) lead to the generation of acetaldehyde as side product, and 3) overall lower the carbon efficiency.^[39] The reaction was thus carried out under the same conditions as before, with 20 mg of lipase. A conversion of 74 % was achieved after 24 h and a maximum of 76 % after 48 h, demonstrating the presence of a thermodynamic equilibrium under these conditions, despite the addition of the molecular sieves. We deemed important to let the reaction run for 6 days to confirm this observation. After 6 days, the yield of DHLc acetate at the primary alcohol position reached 72 % with no other by-products, as confirmed by LC-MS and NMR analysis. This also demonstrated the high selectivity of this enzymatic reaction, irrespective of the nature of the acyl donor. While this approach was less efficient when using acetic acid as opposed to vinyl acetate, we believe that in future studies it could still be optimized for the synthesis of other DHLc esters. This might be achieved by increasing the quantity of molecular sieves or employing alternative water removal systems, such as a Dean-Stark apparatus, which would be compatible with the solvent mixture used and its boiling point.

Following the successful synthesis of the O-acetyl ester, we explored the possibility of extending the scope of the reaction to more lipophilic ester derivatives. Consequently, our methodology was extended to facilitate the esterification of DHLc with vinyl propionate, hexanoate and octanoate. Additionally, vinyl chloroacetate was also tested for two reasons: firstly, to assess the impact of a larger atom in the acyl chain; and secondly, to investigate the introduction of halogens, given their importance in the synthesis of bioactive molecules.

The same conditions were applied to the different acyl donors (20 mg Novozym 435, 10 mM DHLc and 100 mM acyl donor). As for vinyl acetate, vinyl propionate also led to a conversion of >99 % within 48 h (**Table 1**). Notably, the conversion slightly decreased with longer chain lengths, reaching 74 % and 69 % with vinyl hexanoate and vinyl octanoate, respectively. In addition, a slightly lower conversion was obtained with vinyl chloroacetate compared to vinyl propionate, suggesting that the presence of the larger chlorine atom appears to limit the ability of the enzyme to carry out the reaction. It remains plausible that near-complete conversion could be achieved with a

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longer reaction time, however, this hypothesis was not investigated in the present study. Lastly, it is worth noting that no acylation occurred on the secondary alcohol in these experiments.

Table 1. Conversion % after 48 h at 37° C, 35 rpm in 1 mL MTBE/ACN:3/1 with 10 mM of DHLc, 100 mM of vinyl ester or acetic acid, 20 mg of Novozym 435 and 3 molecular sieves 5 Å.

	Vinyl acetate (1)	Vinyl propionate (2)	Vinyl hexanoate (3)	Vinyl octanoate (4)	Vinyl chloroacetate (5)	Acetic acid (6)
DHLc	>99 %	>99 %	74 %	69 %	92 %	76 %

To continue to broaden the substrate scope of the reaction, we also tested a panel of vinyl esters containing aromatic substituents (Figure 5). All of these compounds were synthesized in-house as, to the best of our knowledge, only compound (8) was commercially available. These compounds were tested in excess (10 equivalents) relative to DHLc, following the same general conditions as before.

Despite the use of identical conditions, none of these new aromatic acyl donors proved effective for the acylation of DHLc, as no esters were detected. However, some of them underwent hydrolysis into their corresponding carboxylic acids, which was likely attributable to residual water in the reaction medium. This was particularly prominent for vinyl-2-(4-methoxyphenyl) acetate (9) which experienced complete hydrolysis, and to a lesser extent for vinyl-4-formylbenzoate (11) and vinyl-4-nitrobenzoate (12), exhibiting over 50 % hydrolysis.

On a related note, the lipase A from *Candida antarctica* and a lipase from *Pseudomonas cepacia* were also tested with compounds 9 and 12, but both failed to catalyze the desired acylation reaction. These results are not entirely surprising, as several lipases have already been tested on other occasions with aromatic acyl donors and have typically demonstrated poor activity. Such is the case for CAL-B, for which another article mentions its inability to catalyze transesterification reactions with aryl vinyl esters as acyl donors.^[40] Consequently, future research may necessitate the identification of a specific enzyme capable of accepting aryl vinyl esters as acyl donors.

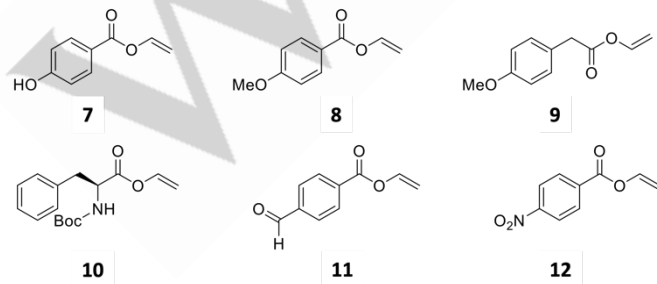


Figure 5. Vinyl esters with aromatic side chains tested for the CAL-B catalyzed transesterification with DHLc.

Acylation of other STLs from chicory root

Having demonstrated that our methodology enabled the regioselective enzymatic acylation of the primary allyl alcohol group of DHLc with very high conversion rates, we moved to lactucin (Lc). Lc shares the same overall structural characteristics as DHLc, except for the presence of an α -methylene- γ lactone moiety (α -MGL), which may have been problematic due to its electrophilicity. For this experiment, vinyl acetate was chosen as the preferred acyl donor and the same reaction conditions were used.

The ¹H NMR analysis of the reaction mixture after 48 h showed a very promising 95 % \pm 5 % (>99 % from LC-MS) conversion with perfect selectivity towards the primary alcohol. Remarkably, the α -MGL moiety also remained unchanged as shown in the ¹H NMR spectra (Figure 6).

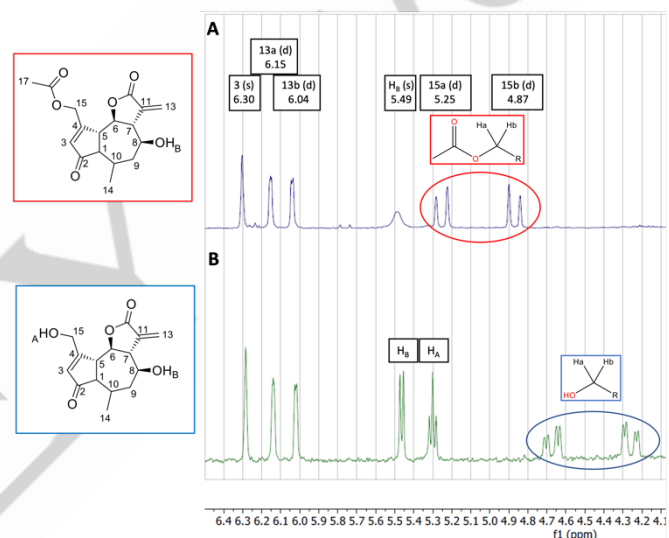


Figure 6. ¹H NMR (DMSO-*d*₆) comparison of the reaction mixture for lactucin and the negative control. (A) Reaction mixture after 48 h with 10 mM of Lc, 100 mM of vinyl acetate and 20 mg of Novozym 435; (B) Reaction mixture of negative control without lipase after 48 h.

Building upon these promising results and aiming to extend this concept to encompass the majority of STLs found in chicory root, we replicated the previous experiment with lactucopicrin (Lp) and 11 β ,13-dihydroxylactucopicrin (DHLp). These two molecules correspond to the respective ester analogues of Lc and DHLc, formed at their secondary alcohol sites with a 4-hydroxyphenylacetic acid moiety. The high selectivity exhibited by CAL-B was of significant interest to us in this instance, as it raised the prospect of selective esterification of the primary alcohol, without the risk of degrading the pre-existing ester of these two substrates. Additionally, the feasibility of the reaction with these two STLs was of interest, considering that the presence of the secondary ester makes them bulkier than the previous substrates. Remarkably, we managed to achieve the same excellent results as before, with 95 % \pm 5 % conversion and complete selectivity towards the primary alcohol group.

Study of enzyme-substrate binding modes in STL acylation reactions

Our experiments showed that CAL-B exclusively targeted the primary alcohol group of STLs (e.g. DHLc) in acylation reactions using various alkyl vinyl esters as donors. To better understand this specificity, we conducted molecular docking simulations to explore the preferred binding modes between different acyl-enzyme complexes and the four STLs discussed in this study.

In the transesterification with vinyl acetate, flexible docking calculations indicated that steric effects alone were inadequate to account for the selectivity towards the primary alcohol. Indeed, two main orientations of DHLc were observed: with either the primary or the secondary alcohol group pointing towards the catalytic residues. A detailed analysis of the distances showed 30 % of the poses with either the primary or the secondary alcohol group close to the acyl-enzyme carbonyl function. Among these poses, both hydroxy groups came at a distance below 4 Å that is necessary for the nucleophilic attack and the subsequent establishment of the ester bond (Figure 7A). A similar result was obtained with the vinyl propionate chain. Thus, in the case of short acyl chains, these results suggest that the selectivity observed is due to the intrinsic reactivity of both hydroxy groups, rather than steric hindrance or the bad orientation of the substrates. On the other hand, when dealing with longer alkyl chains, such as those present in vinyl hexanoate and octanoate, steric hindrance became more significant. This led to less buried poses and increased distances between DHLc and the catalytic residues. Only 3 % and 1 % of the poses came close to the catalytic residues in the presence of the hexanoyl chain and the octanoyl chain, respectively. Moreover, only the primary alcohol of DHLc could reach the acyl-enzyme carbonyl function. These results suggest that as the acyl donor chain length increases, the binding modes of the enzyme-substrate complex become less favorable and the steric effect plays a more significant role in determining selectivity. This also correlates with a reduced reactivity compared to vinyl esters with a shorter side chain, resulting in a lower conversion.

Hence, it appears that, for acyl donors possessing a small alkyl side chain, steric factors cannot explain the complete selectivity towards the primary alcohol group. This implies, as discussed, that such effect may be partially mediated by other factors, for instance, the superior nucleophilicity of primary hydroxy groups which is related to more favorable electronic effects. In addition, our findings suggest that the flexibility of the acyl chain is crucial in forming favourable enzyme-substrate complexes. This helps explain why aryl vinyl esters, with their rigid aromatic ring, failed to react with DHLc. This was the case even for substrates **11** and **12**, possessing an electron-deficient aromatic cycle which theoretically increases the electrophilic character of the carbonyl carbon. Docking simulations between DHLc and methoxybenzoyl- or methoxyphenoyl-CAL-B targets confirmed this, as no pose came within a 5 Å radius from the acyl-enzyme carbonyl function.

For all the acyl acceptors (DHLc, DHLp, Lc, Lp), the main interactions within enzyme/substrates complexes were hydrophobic in nature. More specifically, the residues Ile189, Ile285 and Val154 located on

both sides of the cavity entrance interact with cycle B and the methyl groups of the STLs (Figure 1 and Figure 7B).

Also, the residues constituting the hydrophobic wall of the cavity interact with the acyl donor chain (Figure 7B). Aliphatic alkyl chains, particularly those with high flexibility, are preferred in the acylation of DHLc and similar STLs due to their ability to adhere to the hydrophobic wall, optimizing the available space in the catalytic cavity. This efficient use of space favors the formation of key transition states in the acylation process. Indeed, a substrate like DHLc already takes up a significant amount of space in the enzyme's cavity, even without an acyl donor. Thus, Figure 7D shows that a flexible alkyl chain, such as octanoyl, makes better use of the cavity space compared to a rigid chain.

In addition to the primary hydrophobic interactions, we also observed various hydrogen bond interactions involving the STLs. The hydroxy groups of the STLs formed hydrogen bonds with both Thr40 from the oxyanion hole and the less buried residue Gln157. Notably, when one hydroxy group interacts with Thr40, the other tends to interact with Gln157, and vice versa. Furthermore, hydrogen bonds were observed between Ala282 and the α,β -unsaturated ketone in cycle A (cyclopentenone), as illustrated in Figure 7C.

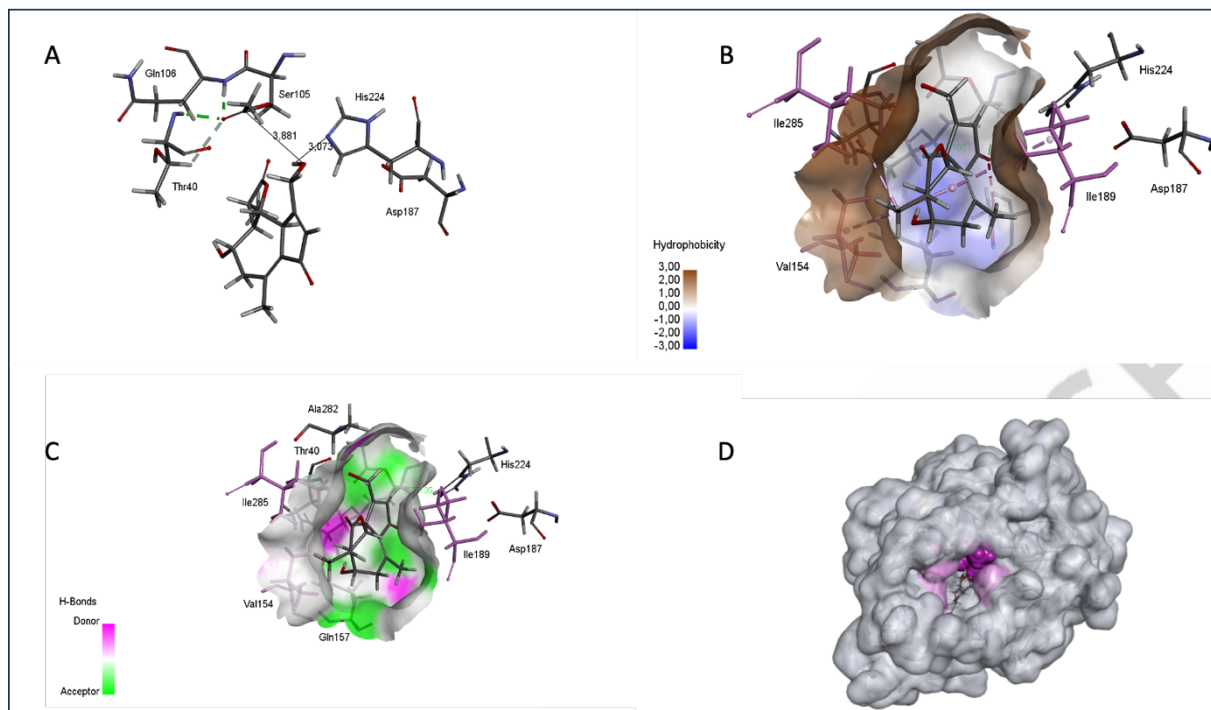


Figure 7. Main binding modes and interactions between DHLc and CAL-B acyl enzyme. A) proximity of DHLc primary hydroxy group to catalytic residues in acetylation reaction. H bonds stabilizing the acyl enzyme within the oxyanion hole are shown in dot lines. B) Hydrophobic interactions between DHLc and the residues Ile189, Ile285, Val154 (coloured in purple). Hydrophobic and hydrophilic regions are coloured in red and blue, respectively. C) H bond interactions between DHLc and the residues Gln157, Ala282. Regions with H bond donor residues and H bond acceptor residues are coloured in pink and green, respectively. D) Binding mode between DHLc and CAL-B in the presence of long acyl chain. Octanoyl chain is shown in CPK representation and coloured in dark purple; the Connolly accessible surface of CAL-B is coloured in grey except the region made by the hydrophobic residues Ile189 and Ile285 that is coloured in light purple.

Conclusions

In conclusion, our work showed the remarkable selectivity and efficiency of the immobilized lipase B from *Candida antarctica* (Novozym 435) in the *O*-acylation of the primary alcohol group of STLs, performed with various alkyl chains (acetate, propionate, hexanoate, octanoate and chloroacetate). The initial development of this method was facilitated by using the *O*-acylation of perillyl alcohol with vinyl acetate and acetic acid as a model reaction, allowing for the first reported lipase-catalyzed synthesis of perillyl acetate, achieving >99 % conversion in just 1 h at 37 °C. The corresponding ester derivatives of DHLc were then obtained with excellent conversions going from >99 % (acetate and propionate) to 69 % (octanoate). As for Lc, Lp and DHLp, their corresponding acetate derivatives were obtained with >99 % conversion. Thus, we report a versatile and very selective method for the biocatalytic synthesis of semi-synthetic ester derivatives of STLs found in chicory root.

In addition, the study of the enzyme-substrate binding modes in the biocatalytic acylation of STLs brought us a more comprehensive understanding of their reactivity and the nature of the interactions with important amino acid residues in the active site of CAL-B. Our findings indicate that lipophilic acyl chains with sufficient flexibility are

more effectively incorporated into STL targets, especially compared to aryl chains which are unreactive.

Existing literature suggests that enhancing the lipophilicity of STLs by introducing alkyl side chains may increase their reactivity. These side chains could play a role in modulating the biological activities associated with their pharmacophores, namely the α -MGL and unsaturated cyclopentenone moieties. Consequently, we hypothesize that the semi-synthetic ester derivatives discussed in this article might be able to cross biological barriers more readily, potentially leading to enhanced antimicrobial properties. Future research should focus on conducting biological tests against specific microbial targets, as this could provide valuable insights and potentially lead to the development of new antimicrobial agents.

Materials and Methods

Chemicals and Enzymes

(*S*)-Perillyl alcohol (96 %) and glacial acetic acid (99.7 %) were purchased from Sigma-Aldrich. Vinyl propionate (98 %, stabilized with MEHQ), vinyl hexanoate (99 %, stabilized with MEHQ), vinyl octanoate (99 %, stabilized with MEHQ) and vinyl chloroacetate (99 %, stabilized

with MEHQ) were purchased from TCI Chemicals. Vinyl acetate (99 %, stabilized with hydroquinone) was purchased from Alfa Aesar. Solvents methyl-tert-butyl ether (MTBE) (99 %) and acetonitrile (99.9 %, LCMS grade) were obtained from Alfa Aesar and Sigma-Aldrich respectively. Reference standards lactucin (# 3809), 11 β ,13-dihydroxylactucin (# 3810), lactucopicrin (# 3813) and 11 β ,13-dihydroxylactucopicrin (# 3811) were purchased from ExtraSynthese at 95 % purity. 11 β ,13-dihydroxylactucin used in this study was extracted in-house from lyophilized chicory root and purified *via* flash-column chromatography. The complete extraction protocol is described by Ruggieri *et al.*^[16]

Novozym 435 (Immobilized lipase B from *Candida antarctica*), with a specific activity of 10,000 propyl laurate units per gram, was obtained from Novozymes.

Lipase-catalyzed acylation general procedure

A mixture of 3/1:MTBE and ACN was prepared and dried under 3 Å molecular sieves (previously activated at 350 °C for 48 h) for 24 h prior to the reactions. The acylation reactions were conducted with 1 mL of the solvent mixture in 2 mL HPLC vials with screw-on cap (8 mm) and unpierced septa in the presence of 3 spheres of 5 Å molecular sieves (zeolite with 3–5 mm diameter previously activated), the vials were placed in an orbital carousel rotating shaker (Thermo Scientific Tube Revolver) at 35 rpm in an oven at 37 °C.

Acetylation of (S)-perillyl alcohol

100 mM of perillyl alcohol and 300 mM of acetic acid or vinyl acetate were dissolved in the solvent mixture in the presence of 3 Å molecular sieves. 1 mL of the resulting solution was introduced into a 2 mL vial containing 10 mg of Novozym 435 and 3 spheres of 5 Å molecular sieves. Controls were performed in the same conditions in the absence of the enzyme. 25 μ L samples were taken at different time intervals and dissolved in 225 μ L of acetonitrile (LCMS grade). They were then filtered on 0.2 μ m PTFE filter and introduced into GC vials for analysis. Perillyl acetate was obtained as viscous and slightly yellow oil at >99 % yield. ¹H NMR (300 MHz, DMSO-*d*₆) δ = 5.72 (s, 1H, 2), 4.71 (s, 2H, 9), 4.40 (s, 2H, 7), 2.16 – 2.06 (m, 2H, 3b-4), 2.06 – 1.98 (m, 2H, 5), 2.00 (s, 3H, 13), 1.97 – 1.86 (m, 1H, 3a), 1.83 – 1.73 (m, 1H, 6a), 1.70 (s, 3H, 10), 1.49 – 1.30 (m, 1H, 6b). ¹³C NMR, DEPT135 (75 MHz, DMSO-*d*₆) δ = 125 (C2), 109.28 (C9), 67.68 (C7), 40.49 (C13), 30.06 (C3), 27.10 (C6), 26.06 (C5), 20.97 (C10), 20.89 (C4).

Acetylation of STLs from chicory root

10 mM of Lc (2.76 mg), DHLc (2.78 mg), Lp (4.10 mg) or DHLP (4.12 mg) and 100 mM of vinyl acetate (8.60 mg, 9.22 μ L) were dissolved in the solvent mixture in the presence of 3 Å molecular sieves. 1 mL of the resulting solution was introduced into a 2 mL vial containing 20 mg of Novozym 435 and 3 particles of 5 Å molecular sieves. The vials were placed in an orbital carousel rotating shaker (Thermo Scientific Tube Revolver) at 35 rpm in an oven at 37 °C for 48 h. The reaction mixtures were then filtered on 0.2 μ m PTFE filter into 2 mL Eppendorf tubes and concentrated under 8 mbar at 35 °C in a Thermo Scientific SpeedVac system for 2 h until dry.

Lactucin acetate was obtained at >99 % yield. ¹H NMR (300 MHz, DMSO-*d*₆) δ = 6.30 (s, 1H, 3), 6.15 (dd, 1H, 13a, *J* = 3.05 Hz, 1.48 Hz), 6.04 (dd, 1H, 13b, *J* = 3.25 Hz, 1.48 Hz), 5.49 (d, 1H, OHb, *J* = 5.56 Hz), 5.25 (d, 1H, 15a, *J* = 17.53 Hz), 4.87 (d, 1H, 15b, *J* = 17.50 Hz), 3.99 – 3.67 (m, 3H, 5-6-8), 3.20 – 3.04 (m, 1H, 7), 2.84 – 2.68 (m, 1H, 9a), 2.38 – 2.31 (m, 3H, 14), 2.32 – 2.23 (m, 1H, 9b), 2.13 (s, 3H, 17). Main fragments = 317.1 [M-H]⁻(ESI-) and 319.1 [M+H]⁺(ESI+)

11 β ,13-dihydroxylactucin acetate was obtained at >99 % yield. ¹H NMR (300 MHz, DMSO-*d*₆) 6.28 (s, 1H, 3), 5.30 – 5.18 (m, 2H, 15a-OHb), 4.83 (d, 1H, 15b *J* = 17.83 Hz), 3.76 (m, 2H, 5-6), 3.57 (m, 1H, 8), 2.68 (m, 2H, 9a-11), 2.35 (s, 3H, 14), 2.32 – 2.13 (m, 2H, 7-9b), 2.12 (s, 3H, 17), 1.26 (d, 3H, 13, *J* = 6.96 Hz). ¹³CAPT NMR (75 MHz, ns= 20480, DMSO-*d*₆) δ = 194.49 (C2), 178.10 (C12), 170.44 (C16), 168.07 (C4), 148.64 (C10), 132.72 (C3), 131.84 (C1), 80.67 (C6), 68.29 (C8), 63.35 (C15), 60.40 (C7), 48.83 (C9), 48.65 (C5), 40.96 (C11), 21.65 (C17), 20.98 (C14), 15.67 (C13). Main fragment = 321.12 [M+H]⁺

Lactucopicrin acetate was obtained at >99 % yield. ¹H NMR (300 MHz, DMSO-*d*₆) δ = 7.10 (d, 2H, 4B-8B, *J* = 8.44 Hz), 6.71 (d, 2H, 5B-7B, *J* = 8.50 Hz), 6.33 (s, 1H, 3), 5.91 (d, 1H, 13a, *J* = 3.19 Hz), 5.39 (d, 1H, 13b, *J* = 2.96 Hz), 5.23 (d, 1H, 15a, *J* = 17.72 Hz), 4.88-4.85 (m, 2H, 8-15b), 4.02 – 3.97 (m, 2H, 5-6), 3.66 (s, 2H, 2B), 3.56 (m, 1H, 7), 2.86 (m, 1H, 9a), 2.33 (m, 3H, 14), 2.27 (m, 1H, 9b), 2.12 (s, 3H, 17). Main fragments = 453.1 [M+H]⁺(ESI+) and 451.1 [M-H]⁻(ESI-)

11 β ,13-dihydroxylactucopicrin acetate was obtained at >99 % yield. ¹H NMR (300 MHz, DMSO-*d*₆) δ = 7.06 (d, 2H, 4B-8B *J* = 8.31 Hz), 6.71 (d, 2H, 5B-7B, *J* = 8.50 Hz), 6.31 (s, 1H, 3), 5.23 (d, 1H, 15a, *J* = 17.40 Hz), 4.85-4.77 (m, 2H, 15b-8), 3.90 (m, 2H, 5-6), 3.58 (s, 2H, 2B), 2.82 (m, 1H, 9a), 2.64 (m, 1H, 11), 2.33 (s, 3H, 14), 2.27 (m, 2H, 7-9b), 2.12 (s, 3H, 17), 1.09 (d, 3H, 13, *J* = 6.61 Hz). Main fragments = 455.3 [M+H]⁺(ESI+) and 453.2 [M-H]⁻(ESI-)

Synthesis of ester derivatives of DHLc

10 mM of DHLc (2.76 mg) and 100 mM of the corresponding vinyl esters were dissolved in the solvent mixture in the presence of 3 Å molecular sieves. 1 mL of the resulting solution was introduced into a 2 mL vial containing 20 mg of Novozym 435 and 3 particles of 5 Å molecular sieves. The vials were placed in an orbital carousel rotating shaker (Thermo Scientific Tube Revolver) at 35 rpm in an oven at 37 °C for 48 h. The reaction mixtures were then filtered on 0.2 μ m PTFE filter into 2 mL Eppendorf tubes and concentrated under 8 mbar at 35 °C in a Thermo Scientific SpeedVac system for 2 h.

DHLc-propionate was obtained at >99 % yield. ¹H NMR (300 MHz, DMSO-*d*₆) δ = 6.26 (s, 1H, 3), 5.31 – 5.17 (m, 2H, 15a-OHb), 4.85 (d, 1H, 15b, *J* = 18.22 Hz), 3.87 – 3.68 (m, 2H, 5-6), 3.56 (m, 1H, 8), 2.78 – 2.59 (m, 2H, 9a-11), 2.4 (t, 2H, 17, *J* = 7.40 Hz), 2.35 (s, 3H, 14), 2.31 – 2.09 (m, 2H, 7-9b), 1.26 (d, 3H, 13, *J* = 6.95 Hz), 1.06 (t, 3H, 18, *J* = 7.52 Hz). Main fragment = 335.2 [M+H]⁺

DHLc-chloroacetate was obtained at 92 % (\pm 5 %) yield. ¹H NMR (300 MHz, DMSO-*d*₆) δ = 6.35 (s, 1H, 3), 5.35 (d, 1H, 15a, *J* = 17.50 Hz), 4.97 (d, 1H, 15b, *J* = 17.50), 4.54 (s, 2H, 17), 3.88 – 3.70 (m, 2H, 5-6), 3.57 (m, 1H, 8), 2.70 (m, 2H, 9a-11), 2.35 (s, 3H, 14), 2.31 – 2.10 (m, 2H, 9b-7), 1.26 (d, 3H, 13, *J* = 6.96 Hz). Main fragment = 355.1 [M+H]⁺

DHLC-hexanoate was obtained at 74 % (\pm 5 %) yield. ^1H NMR (300 MHz, DMSO- D_6) δ = 6.25 (s, 1H, 3), 5.31 – 5.16 (m, 2H, 15a-OHb), 4.85 (d, 1H, 15b, J = 18.07 Hz), 3.76 (m, 2H, 5-6), 3.56 (m, 1H, 8), 2.79 – 2.56 (m, 2H, 9a-11), 2.40 (t, 2H, 17, J = 7.36 Hz), 2.34 (s, 3H, 14), 2.31 – 2.09 (m, 2H, 9b-7), 1.64 – 1.41 (m, 2H, 18), 1.35 – 1.15 (m, 7H, 13-19-20), 0.86 (t, 3H, 21, J = 6.91 Hz). Main fragment = 377.3 [M+H] $^+$

DHLC-octanoate was obtained at 69 % (\pm 5 %) yield. ^1H NMR (300 MHz, DMSO- D_6) δ = 6.25 (s, 1H, 3), 5.30 – 5.15 (m, 2H, 15a-OHb), 4.85 (d, 1H, 15b, J = 17.52 Hz), 3.85 – 3.66 (m, 2H, 5-6), 3.56 (m, 1H, 8), 2.77 – 2.59 (m, 2H, 9a-11), 2.40 (t, 2H, 17, J = 7.30 Hz), 2.34 (s, 3H, 14), 2.31 – 2.23 (m, 2H, 9b-7), 1.61 – 1.40 (m, 2H, 18), 1.33 – 1.15 (m, 9H, 13-19-20-21-22), 0.86 (t, 3H, 23, J = 7.00 Hz). Main fragment = 405.3 [M+H] $^+$

General Procedure for the Synthesis of Vinyl Esters 8 and 9

The synthesis proceeded in accordance to the literature.^[41] Potassium hydroxide (0.5 eq.), palladium (II) acetate (0.4 eq.) and 1 eq. of carboxylic acid were weighed in a 25 mL round-bottom flask and dissolved in vinyl acetate (0.1 M). The reaction mixture was stirred overnight at 40 °C. The resulting mixture was cooled to room temperature, filtered over a celite pad and washed with dichloromethane. The solvents were then removed under vacuum and the crude product was purified by flash column chromatography.

8 was obtained as a white powder after flash column chromatography on a 15 g-cartridge (cyclohexane/ethyl acetate:9/1) (182 mg, 78 % yield). ^1H NMR (300 MHz, CDCl_3), δ = 8.06 (m, 2H), 7.50 (dd, 1H, J = 6.3 Hz, 14.0 Hz), 6.94 (m, 2H), 5.03 (dd, 1H, J = 1.6 Hz, 14.0 Hz), 4.66 (dd, 1H, J = 1.6 Hz, 6.3 Hz), 3.87 (s, 3H). ^{13}C NMR (75 MHz, CDCl_3), δ = 164.24, 163.71, 141.84, 132.46 (2C), 121.54, 114.15 (2C), 98.02, 55.82.

9 was obtained as a colorless oil after flash column chromatography on a 15 g-cartridge (cyclohexane/ethyl acetate:9/1) (55 mg, 24 % yield). ^1H NMR (300 MHz, CDCl_3), δ = 7.28 (dd, 1H, J = 6.3 Hz, 13.9 Hz), 7.20 (m, 2H), 6.87 (m, 2H), 4.90 (dd, 1H, J = 1.6 Hz, 13.9 Hz), 4.58 (dd, 1H, J = 1.6 Hz, 6.3 Hz), 3.78 (s, 3H), 3.62 (s, 2H). ^{13}C NMR (75 MHz, CDCl_3), δ = 169.31, 159.25, 141.68, 130.70 (2C), 125.51, 114.47 (2C), 98.29, 55.62, 40.38.

General Procedure for the Synthesis of Vinyl Esters 7, 10, 11 and 12

The synthesis proceeded in accordance to the literature.^[42] In a 25 mL round-bottom flask copper(II) triflate (1 eq.) and 1,3-diethylurea (1 eq.) were added. Then, anhydrous THF (0.1 M) was added and the mixture was stirred to give a clear solution. Eventually, triethylamine (1 eq.) was added to give a dark solution, followed by carboxylic acid (1 eq.) addition. At last, trivinylboroxine pyridine complex (0.66 eq.) was added and the reaction was stirred overnight at 50 °C under a balloon filled with air. The solvent was evaporated under vacuum and the desired product was obtained after flash column chromatography. 7 was obtained as a white powder after flash column chromatography on a 15 g-cartridge (cyclohexane/ethyl acetate:9/1) (43 mg, 18 % yield). ^1H NMR (300 MHz, CDCl_3), δ = 8.02 (m, 2H), 7.48 (dd, 1H, J = 6.3 Hz, 14.0 Hz), 6.88 (m, 2H), 5.57 (bs, 1H), 5.03 (dd, 1H, J = 1.7 Hz,

14.0 Hz), 4.67 (dd, 1H, J = 1.7 Hz, 6.3 Hz). ^{13}C NMR (75 MHz, CDCl_3), δ = 163.8, 160.7, 141.8, 132.8 (2C), 121.8, 115.76 (2C), 98.24.

10 was obtained as a yellow oil after flash column chromatography on a 15 g-cartridge (cyclohexane/ethyl acetate:9/1) (184 mg, 84 % yield). ^1H NMR (300 MHz, CDCl_3), δ = 7.12 – 7.30 (m, 6H), 4.93 (dd, 2H, J = 1.8 Hz, 14.0 Hz), 4.65 (dd, 2H, J = 1.8 Hz, 6.2 Hz), 3.13 (m, 2H), 1.42 (s, 9H). ^{13}C NMR (75 MHz, CDCl_3), δ = 169.54, 155.39, 141.22, 135.93, 129.65(2C), 129.0 (2C), 127.52, 99.30, 80.49, 54.60, 38.37, 28.61 (3C).

11 was obtained as a white powder after flash column chromatography on a 15 g-cartridge (cyclohexane/ethyl acetate:9/1) (140 mg, 60 % yield). ^1H NMR (300 MHz, CDCl_3), δ = 10.12 (s, 1H), 8.27 (m, 2H), 7.98 (m, 2H), 7.50 (dd, 1H, J = 6.3 Hz, 13.9 Hz), 5.12 (dd, 1H, J = 1.8 Hz, 13.9 Hz), 4.77 (dd, 1H, J = 1.8 Hz, 6.3 Hz). ^{13}C NMR (75 MHz, CDCl_3), δ = 191.80, 162.0, 141.54, 139.87, 137.20, 130.89 (2C), 129.90 (2C), 99.38.

12 was obtained as a white powder after flash column chromatography on a 15 g-cartridge (cyclohexane/ethyl acetate:9/1) (130 mg, 56 % yield). ^1H NMR (300 MHz, CDCl_3), δ = 8.30 (m, 4H), 7.49 (dd, 1H, J = 6.2 Hz, 13.8 Hz), 5.15 (dd, 1H, J = 1.9 Hz, 13.8 Hz), 4.80 (dd, 1H, J = 1.9 Hz, 6.2 Hz). ^{13}C NMR (75 MHz, CDCl_3), δ = 162.14, 151.22, 141.44, 134.68, 131.44 (2C), 124.02 (2C), 99.85.

NMR spectroscopy

All reaction mixtures were filtered on 0.2 μm PTFE filter into 2 mL Eppendorf tubes and concentrated under 8 mbar at 35 °C in a Thermo Scientific SpeedVac system for 1-2 h until dry. The concentrate was then dissolved in 650 μL of DMSO- D_6 (99.9 % from Dutscher) and placed into the NMR glass tube. Analysis was carried out in a 300 MHz Bruker NMR spectrometer. The spectra were analyzed on MestreNova software (version 14.2.3).

Gas Chromatography

Samples were analyzed on a GC-FID from Shimadzu equipped with a Phenomenex ZB-5MS (30 m x 0.25 mm x 0.25 μm) column. The following temperature programming was used: starting at 50 °C for 2 min, then 20 °C/min until 310 °C and hold for 5 min. The injector and the FID were both set at a temperature of 320 °C. A split ratio of 10 with a splitless sampling time of 1 min was used. Total flow was 21.6 mL/min with a linear velocity of 47.2 cm/s and a purge flow of 3 mL/min.

Liquid Chromatography

Chromatograms and mass spectra from the acylation reactions of lactucin, lactucopicrin and dihydrolactucopicrin with vinyl acetate, as well as the acylation of DHLC with vinyl chloroacetate, propionate, hexanoate and octanoate were obtained on the following system : LC-MS Waters ACQUITY UPLC I-Class system equipped with a UPLC I BIN SOL MGR solvent manager, a UPLC I SMP MGR-FTN sample manager, an ACQUITY UPLC I-Class eK PDA Detector photodiode array detector (210–400 nm) and an ACQUITY QDa (Performance) as mass detector (full scan ESI+/- in the range 30–1250). Acquity BEH C18 column (1.7 μm particle size, dimensions 50 mm x 2.1 mm) was used for UPLC

analysis. The injection volume was 0.5 μ L. For a 5 min analysis, the elution was done at pH 3.8 from 100 % H₂O/0.1 % ammonium formate to 2 % H₂O/98 % CH₃CN/0.1 % ammonium formate over 3.5 min. A flow rate at 600 μ L/min was used. For a 30 min analysis, the elution was performed at pH 3.8 from 100 % H₂O/0.1 % ammonium formate to 100 % CH₃CN/0.1 % ammonium formate over 25 min. A flow rate of 600 μ L/min was used.

The acetylation of DHLc was followed by UPLC-QTOF using a Phenomenex Luna Omega Polar C18 column (50 x 2.1 mm x 1.6 μ m) using water and CH₃CN containing 0.1 % of trifluoroacetic acid and an injection volume of 0.5 μ L via the following gradient: starting at 40 % CH₃CN during 2 min, gradually increasing to 100 % CH₃CN from 2 to 5 min, 100 % CH₃CN was maintained for 3 more minutes. The percentage of CH₃CN was then decreased back to 40 % over 2 more minutes, for a total run time of 10 min.

Molecular docking simulations

The targets for docking simulations were prepared as previously described by Dettori *et al.* (2018).^[43] Briefly, the CAL-B crystal structure (PDB entry: 1LBS) that contains ethylhexylphosphonate (HEE) inhibitor covalently bound to the catalytic serine was chosen as starting structure. The inhibitor was removed and acyl-enzyme systems were built by binding the different acyl donors to the catalytic serine. The building strategy consists of following the placement of the inhibitor hexyl chain that was assumed to indicate the localization of the acyl-enzyme acyl moiety. Then, a structure relaxation procedure was performed with constraints and restraints that were progressively removed in order to preserve the organization of protein atoms. Docking simulations were run using the Flexible Docking module of the software Discovery Studio 4.5. the flexible zone was defined by the residues 40, 105, 106, 134, 140, 141, 144, 149, 154, 157, 187, 189, 224, 278, 281, 282, 285 and 286.

Conflicts of interest

There are no conflicts to declare.

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Keywords: •Lipase •CAL-B •Sesquiterpene Lactone, •Biocatalytic Acylation •Chicory Root

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