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Article

Co-catalyst enabled biotransformation of polyunsaturated fatty acids for bio-based monomers

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Abstract. Enzymatic lipid epoxidation offers a promising approach to obtain renewable intermediates for biomaterials, but regiochemical control of these reactions has remained elusive. Here we report the discovery and application of artificial co-catalysts to direct the regioselectivity of catalytic epoxidation with the lipase CpLIP2. The methyl esters of alkyl diacids show the unique ability to direct regioselective epoxidation of polyunsaturated fatty acids, major components of plant and algae-based oils. We apply this transformation to the conversion of linoleic acid into sebacic acid, a dicarboxylic acid precursor valuable for bio-based polyester polyurethanes, through a six-step pathway involving a regioselective Meinwald rearrangement. To highlight the route's significance, sebacic acid was used to prepare a 100% bio-based thermoplastic polyurethane (TPU), illustrating the relevance of this pathway to industrial applications. This regioselective chemoenzymatic oxidation and rearrangement process can be used to access multiple dicarboxylic acids that have remained previously unexplored as bio-based monomers.

Introduction

Chemoenzymatic transformations have gained recent importance as a tool to develop and produce renewable polymeric materials, a current need in today's global plastic crisis.[1] While many enzymes have been explored, lipases have found particular utility in the preparation of polyester polyols *via* polycondensation of dicarboxylic acids or esters with diols and in tandem with chemical routes. These bioprocesses can increase efficiency, alleviate costs associated with the production of bio-based monomers and associated polymers, and employ greener chemistry into manufacturing methods.[2–5] The ability to leverage enzymes in these chemical reactions paves the way for environmentally-friendly products, improving access to both known monomers as well as new or underexplored precursors.[6–8]

We recently reported a chemoenzymatic lipase transformation that catalyzes olefin oxidation to an epoxide, a highly functional synthetic intermediate.[9] We observed that the lipase CpLIP2 from the yeast *Candida parapsilosis* exhibits a preference for hydrogen peroxide (H_2O_2) over water as a nucleophile, enabling access to perhydrolysis in aqueous medium at low H_2O_2 concentration (< 5%).[10,11] When in the presence of an ester co-catalyst, H_2O_2, and an olefin substrate, CpLIP2 rapidly converts the ester into a peracid, which in turn serves to epoxidize the olefin. This two-step *in situ* process allows for peroxidation using mild conditions and the development of reactions with precise regulation of peracid identity and levels. We showed that this reaction can be conducted in a single vessel in water with a significant range of substrates, including unsaturated fatty acids, terpenes, and sterols.[9] These studies suggested that the choice of co-catalyst and lipase mutant could be tailored for each substrate to significantly impact selectivity and turnover, critical for process fine tuning. As there is an ongoing need for renewable monomers from sustainable sources, we therefore saw an opportunity to adapt the chemoenzymatic epoxidation for use on bio-based oils while utilizing chemical conversions that reduce environmental risks to give rise to polyurethane monomeric units.

Bio-based oils as feedstock for polymer precursors have great promise for realistic applicability in industrial practices since the oils are renewable, readily obtainable, and high in unsaturated fatty acids.[12–15] Linoleic acid and linolenic acid are the most common polyunsaturated fatty acids (PUFAs) found in plant and algae biomass,[16] and these lipids can serve as basic building blocks that can be transformed into other useful compounds, including dicarboxylic acids (Figure 1).

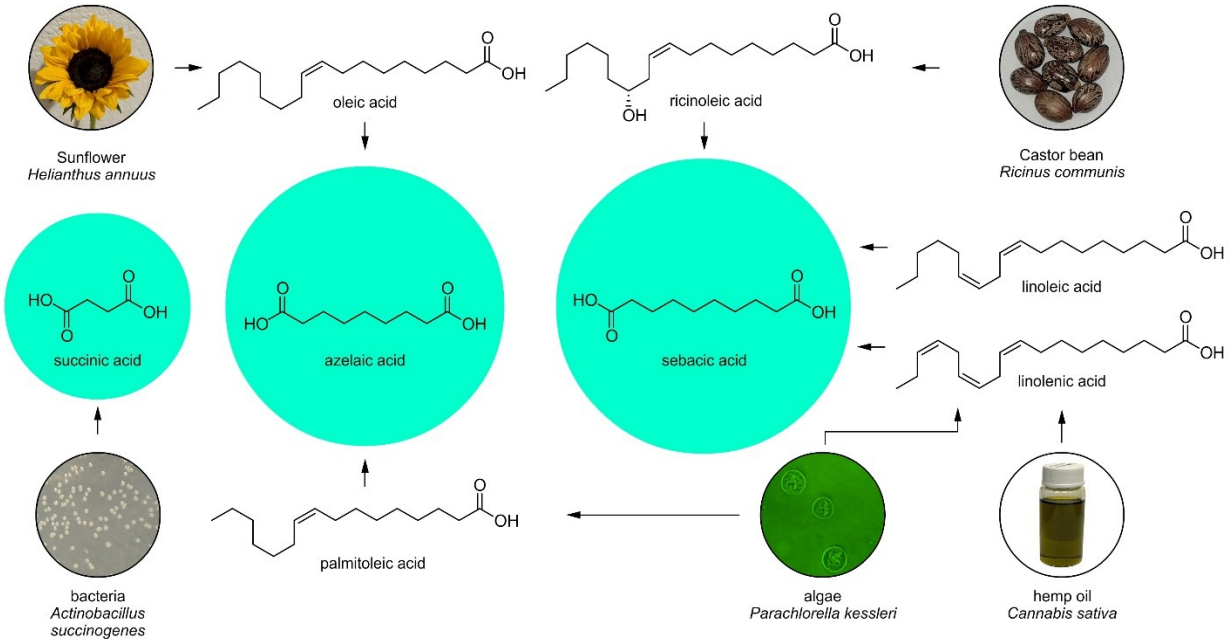

Figure 1. Sources of common dicarboxylic acid feedstocks. Dicarboxylic acids produced from bio-based sources, such as succinic, azelaic, and sebacic acid, can be generated from mono- and polyunsaturated fatty acids found in high concentrations in bacteria, sunflower oil, algae oil, hemp oil, or castor beans.

We have previously shown that ozonolysis offers a useful method to create dicarboxylic acids from palmitoleic acid, a waste stream fatty acid from algae biomass.[17] This approach is limited by its lack of regioselectivity, in addition to bio-based unsaturated fatty acids predominantly featuring a Δ9 unsaturation. To address this, we have developed a regioselective oxidation method to capture PUFA metabolites to prepare sebacic acid (decanedioic acid), an industrially relevant aliphatic diacid utilized as a precursor of multiple commercial products, including nylon-6,10, lubricants, plasticizers, cosmetics and polyurethanes.[18,19] Currently, 40,000 tons/year of sebacic acid are synthesized from castor oil through a high-energy (250 °C) process with caustic soda that involves the *in situ* hydrolysis of glyceryl ricinoleate into ricinoleic acid, followed by isomerization and hydrolysis into sebacic acid and octan-2-ol.[20,21] The cultivation of castor at this scale presents similar environmental concerns as plant-based oils used for biofuels

and commodity chemicals, including use of fresh water and diverting land that could be used for food crops, thereby promoting deforestation, fertilizer and pesticide pollution, and toxic manufacturing waste generation.[22] We have therefore focused on more sustainable feedstocks, including oils from microalgae biomass and waste hemp oil. Microalgae offer an ideal sustainable feedstock, as they are able to be grown on non-arable land with saline or waste water and do not require intensified farming practices, while hemp oil is a byproduct of hemp fiber production and presents an opportunity to transform a waste product into a valuable commodity.[23]

Here, we report the development of a six-step method, encompassing a lipase-catalyzed chemoenzymatic epoxidation and rearrangement relay, to produce sebacic acid from both commercial linoleic acid and a hemp oil-derived mix of linoleic acid and linolenic acid. Through screening a range of co-catalyst substrates, we found that the CpLIP2 lipase can infer regioselectivity of the epoxidation of PUFAs based on chain length of the co-catalytic ester. This unprecedented biotransformation provides access to previously inaccessible oxidation products.

Results

Tuning chemoenzymatic epoxidation to enable regioselectivity control of PUFAs

We began by understanding the synergistic relationship between the lipase and ester co-catalyst on unsaturated fatty acid conversion based on our prior tuning of CpLIP2 mutation with the ester co-catalyst to maximize the epoxidation yield.[9] First, lipase mutants known to exhibit higher transesterification over hydrolysis activity were surveyed (Figure S1a). A 3:2 mixture of methyl palmitoleate to the co-catalyst methyl palmitate, extracted from *Nannochloropsis salina*, was chosen as our model to observe the olefin to epoxide conversion.[24] As opposed to the CpLIP2 Y179F mutant previously employed on a broader range of substrates, WT CpLIP2 demonstrated the highest turnover rate for the unsaturated fatty acid methyl ester (FAME). Notably, increasing

the WT CpLIP2 concentration two-fold significantly improved the epoxidation yield from 57% to 85%, exemplifying the robust reactivity of the enzyme. The type of co-catalyst for the peracid precursor was next investigated using the established procedure with WT CpLIP2, screening aliphatic mono- and dimethyl esters and lactones (Figure S1b). Monitoring by NMR, these reactions were examined for conversion of the methyl palmitoleate olefin. Methyl palmitate and the lactones led to conversion rates of 81-91%, while methyl hexanoate and dimethyl dodecanedioate gave rise to 98% and 100% epoxide formation, respectively. As a result, we hypothesized the employment of a dimethyl ester would enhance the efficiency of the reaction, presumably due to one ester interacting with the lipase and the other available for *in situ* peracid formation (Figure S2).

To evaluate the utility of the dimethyl esters, additional unsaturated substrates commonly found in bio-based oils, specifically linoleic acid and linolenic acid, were tested. PUFAs offer an ideal starting substrate for monomer synthesis, as they are abundant in renewable feedstocks and few synthetic applications for them have been established. Rather than the shorter methyl hexanoate originally used as the co-catalyst for the chemoenzymatic epoxidation, the effect of dimethyl ester chain length on the epoxidation of these substrates was investigated. Upon analyzing the results, we were surprised to discover selectivity in epoxidation depending on co-catalyst identity. We achieved reproducibly selective degrees of epoxidation of linoleic acid **1**, along with preferential regioselectivity toward the Δ9 olefin, when evaluating co-catalysts ranging from 3 to 18 carbon chain length (Figure 2). Shorter chain lengths (C3-C4) led to mostly unreacted starting material, while medium chain length dimethyl esters (C5-C11) delivered the Δ9-mono epoxide **2a** in the highest yields. Interestingly, dimethyl dodecanedioate gave rise to the highest

turnover to di-epoxide **2b**, while increasing to the longest chain length dimethyl octadecanedioate (C18) resulted in the mono-epoxide as the major product.

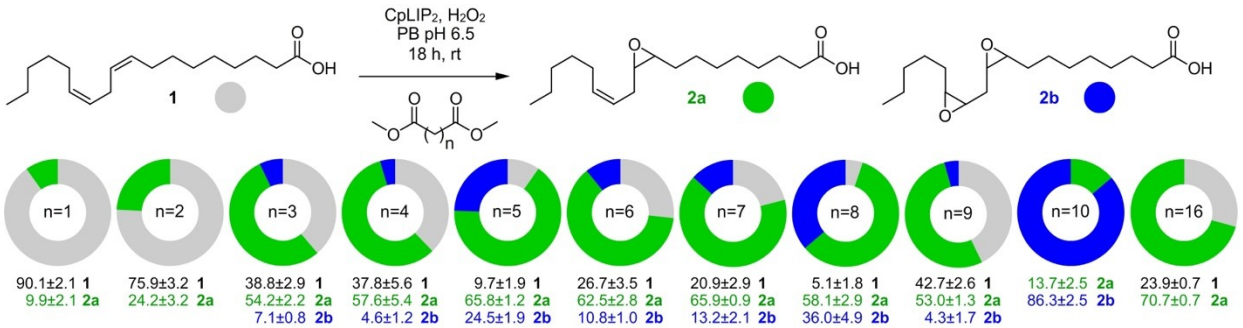

Figure 2. Chain-length selectivity in enzymatic epoxidation. The regioselectivity of the chemoenzymatic epoxidation was tested on linoleic acid **1** by varying the chain length of the co-catalytic dimethyl ester and analyzing reaction products after 18 h. Chain lengths of 3-12 and 18 carbons from carbonyl-to-carbonyl were screened; grey represents starting material, green represents the mono-epoxide product **2a**, and blue represents the di-epoxide **2b**.

Lipases have previously been reported to demonstrate varying chain length selectivity, with alcoholysis, hydrolysis, and esterification activities for CpLIP2 peaking with an acyl ester chain length of 12 carbons, with lower and higher chain lengths showing a decrease in activity.[25–27] Therefore, we reasoned that the enzyme's ability to preferentially react with certain chain lengths impacts the amount of peracids produced *in situ*. Since the epoxidation of linolenic acid could lead to different product combinations, it proved challenging to measure but allowed us to observe a comparable selectivity trend with medium chain length dimethyl esters yielding mono-epoxide in the highest yield and longer esters (C10-C12) leading to higher quantities of di- and tri-epoxides (Figure S3). The ability to program epoxidation regioselectivity to preferentially yield the Δ9-mono-epoxide based on the activity of the lipase with the co-catalyst allows PUFAs to serve as key building blocks in the synthesis of renewable products.

To probe the reaction parameters further, we examined the impact of both the enzyme and co-catalyst on the epoxidation of linoleic acid by focusing on the dimethyl azelate result (n=7) and

varying the amounts of the two components. With the additional available site for the co-catalyst to undergo perhydrolysis compared to methyl hexanoate, the amount added to the chemoenzymatic transformation was initially halved from five equivalents while keeping the enzyme concentration (0.4 mg/mL, $<10^{-4}$ equivalents) fixed (Figure S4). This led to a 20% decrease in mono-epoxide, suggesting that only one of the two terminal esters may be serving as a peracid at a time. Increasing the CpLIP2 concentration to 0.8 mg/mL under this reduced co-catalyst condition generated a similar profile in mono-epoxidation formation to the preliminary dimethyl azelate reaction, shown in Figure S4, both at 6- and 18-hour timepoints. This exemplifies the key role the enzyme plays in catalyzing peracid formation *in situ* and how efficient the reaction can be. Due to the reaction occurring at the interface of the water and organic layers, increasing either the ester equivalents or enzyme concentration would allow for greater reactivity and therefore enhance the reaction. To further validate the influence of the enzyme, we conducted an additional study in which the amount of co-catalyst was reduced from five equivalents to one equivalent while maintaining the lipase at 0.4 mg/mL and increasing to 0.8 mg/mL. Surprisingly, with 0.4 mg/mL CpLIP2, over 60% linoleic acid was left unreacted, reducing the mono-epoxide formation by half compared to the original result. However, the 0.8 mg/mL trial yielded a nearly identical distribution to the original reaction, demonstrating the significant function of the lipase. These conditions may therefore be used interchangeably with the general chemoenzymatic reaction protocol to sustainably limit the amount of co-catalyst or utilize lesser quantities of enzyme at larger scales.

Design of hybrid synthetic pathway from linoleic acid to sebacic acid

Using the tunability of the lipase and co-catalyst to create the desired level of epoxidation, we next explored its ability to be incorporated into a chemoenzymatic hybrid oxidative synthesis of a dicarboxylic acid from linoleic acid.[28] In view of the lipase capable of inferring regioselectivity,

we elected to use dimethyl azelate as the co-catalyst due to its ability to be sustainably produced from renewable algae oil and generate the Δ9 mono-epoxide **2a** as the major product.[29] The mono-epoxide also features desirable traits over the di-epoxide, given that the Δ12 olefin aids in providing an additional functional handle. From this intermediate, two main strategies arose to implement downstream chemical reactions toward a monomeric unit, functionalization at either C9 or C10. Considering our interest in using algae and hemp oil as sustainable feedstocks in the production of dicarboxylic acids, we hypothesized that the regioselective chemoenzymatic epoxidation could provide access to monomers not easily attainable from PUFAs present in these sources. We therefore focused our attention on accessing sebacic acid to complement our previously established method generating algae-based azelaic acid.[17]

With our sights set on functionalizing the Δ9 mono-epoxide to selectively position the oxygen at C10, an epoxide rearrangement was explored. The Meinwald-House rearrangement (Figure 3a), which converts epoxides to ketones *via* a 1,2-hydride shift or to aldehydes by a 1,2-alkyl shift, presented a viable approach toward attaining the targeted rearranged product, and previous studies indicate the reaction may be optimized for carbonyl selectivity.[30,31] A thorough screen of Lewis acids was therefore evaluated to identify conditions that would lead to selective conversion, including $BF_3 \bullet Et_2O$, $ZnCl_2$, ZnI_2, $AlCl_3$, $MgCl_2 \bullet 6\ H_2O$, CuCl, $CuCl_2$, YCl_3, $MgBr_2$, and p-TsOH.[32,33] Initial attempts to perform this transformation were hindered, however, by the terminal carboxylic acid on the epoxy-linoleic acid **2a** complexing the Lewis acids and preventing access to the ketone. To address this, we opted to esterify the acid to a methyl ester. As expected, esterification of linoleic acid prior to the epoxidation led to hydrolysis of the FAME by the enzyme, while epoxidation of the PUFA followed by esterification with methyl iodide proved successful.

The synthesized methyl linoleate epoxide **3** was subsequently subjected to the same screen of Lewis acids, and it was observed that most rearrangements yielded either the C9 ketone or a diol as the major product, in addition to some showing aldehyde formation. Nevertheless, the rearrangement was successfully executed with ZnI_2 providing selectivity toward the desired C10 ketone **4** (3:1 C10:C9 ketone) (Figure 3a). While initial experiments were carried out with ZnI_2 in benzene, the reaction was further optimized to identify solvents that increase the safety of the reaction. Of the solvents tested, dichloromethane and toluene produced results comparable to benzene, and toluene was selected as the ideal solvent due to its more favorable environmental and health rating .[34]

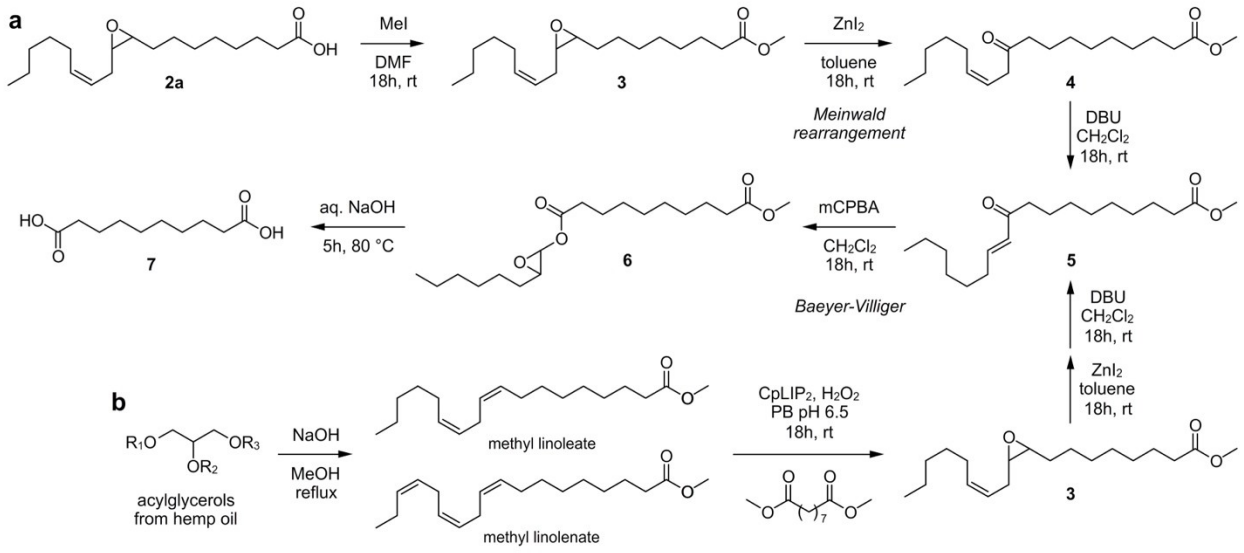

Figure 3. Transformation of linoleic acid into sebacic acid. (a) A five-step route from linoleic acid Δ9 mono-epoxide **2a**, obtained by the regioselective chemoenzymatic epoxidation of linoleic acid **1** (Figure 2) was developed, employing a Meinwald rearrangement to provide access to sebacic acid **7** in a 5.9% overall yield. (b) The route was then applied to a mix of methyl linoleate and methyl linolenate from acylglycerols in hemp oil to produce sebacic acid **7**.

With the carbonyl installed at the C10 position, we then strove to cleave the C10-C11 bond through a Baeyer-Villiger oxidation using mCPBA, a common peracid for this reaction. Unsurprisingly, during these experiments the secondary alkene was also epoxidized. Initial

experiments, however, did not yield selectivity for the oxidation, and insertion of the oxygen occurred equally between C10-C11 and C10-C9 carbons. In order to infer specificity of the oxygen insertion between the C10-C11 carbons, we reasoned the β,γ-unsaturated ketone needed to be isomerized to its respective α,β-unsaturated ketone **5**. Several acidic and basic conditions were explored to perform this isomerization, including NaOH, HCl, and organic bases such as DABCO and DBU. Only DBU delivered the desired transformation. With the isomerized carbonyl in hand, we then observed selectivity for the Baeyer-Villiger oxidation with insertion between C10 and C11, and the product **6** was consistent with similar literature spectra.[35] Finally, sebacic acid **7** was successfully isolated through hydrolysis of the oxidized product with KOH, resulting in a 5.9% overall yield from linoleic acid over six steps.

Translation of hybrid pathway to hemp oil biomass

To demonstrate the effectiveness of this method, it was then tested and adapted to a linoleic-linolenic acid mixture isolated from hemp oil to yield sebacic acid with comparable purity to commercial grade sebacic acid. While we have developed a method to successfully extract these PUFAs from *Parachlorella kessleri* microalgae, the oil yield optimization is not fully refined (see Supporting Information). Therefore, hemp oil, which is readily accessible, was used to evaluate the application of the pathway. Based on our earlier study that linolenic acid exhibited similar epoxidation trends to linoleic acid in the chemoenzymatic reaction, we chose to use the blend of the acids in our process to optimize the amount of sebacic acid that could be generated from the oil. Hemp oil, high in linoleic and linolenic acid triglycerides, is generated as a byproduct of other hemp products and then cold pressed to purify out the fatty acids. After methyl transesterification of the oil to remove the glycerol, the linoleic and linolenic methyl esters were then used as the

starting material in the synthesis of sebacic acid (Figure 3b). The esterification step post-epoxidation was therefore bypassed compared to the established methodology. During our experiments, it was found that the Baeyer-Villiger reaction required an additional equivalent of mCPBA in order to obtain the same level of conversion due to the additional olefin in the linolenic acid. Incorporation of these minor modifications with the hemp oil at small scale led to the production of sebacic acid at an overall yield of 2.0%, exemplifying the route's versatility in using different starting sources in the presence of additional PUFAs. The lower yield in sebacic acid from hemp oil compared to commercial sources may be attributed to impurities from the hemp oil biomass carried through the route, hydrolysis of the methyl esters by the lipase, as well as the loss of linolenic acid intermediates during the purification process. However, since a mixture of unsaturated fatty acids are typically seen in algae and plant oils and can be difficult to purify from one another, this method proves advantageous in that isolation of the desired PUFA starting substrate is not required and can be applied to crude oils.

Utilizing sebacic acid to generate a new bio-based TPU

To highlight the value of sebacic acid for renewable polymers, a 100% bio-based thermoplastic polyurethane (TPU) was formulated using commercial sebacic acid as the main monomer (Figure 4a). As our developed sebacic acid has similar purity to commercial sebacic acid, this test is pertinent to demonstrate its industrial application. A polyester polyol was first created via a polycondensation reaction between sebacic acid and a common bio-based diol, 1,3-propanediol (PDO).[36] In parallel, additional sebacic acid was transformed into octamethylene diisocyanate (ODI) using our previously established flow methodology.[29,37] To create the TPU, the polyol was then mixed with ODI and PDO, allowed to harden in a silicone tray, and confirmed by IR and NMR analyses (Figure S5a and Figure S6). After curing, the semi-rigid TPU (SbPDO-ODI)

underwent various mechanical and thermal analyses to determine its material properties. To understand how SbPDO-ODI would behave in industrial manufacturing such as injection molding, we analyzed thermal transitions and stability via differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). DSC showed a glass transition temperature (T_g) of -49.3 °C which highly dictates the physical and mechanical properties of the material (Figure S5b). Below the T_g, the material exhibits glassy and brittle characteristics due to the lack of polymer chain mobility. Above the T_g, the material exhibits its desired elastomeric or rubbery properties resulting in stretchy yet durable materials. This transition temperature is similar to other materials in this class such as polyethylene (PE) and polypropylene with T_g's of -100 °C and -25 °C, respectively, finding applications in bags, coatings, and the packaging industry.[38] The melting temperature was determined to be 116 °C with a degree of crystallinity of 29 %, resulting in an opaque color as shown in Figure 4b. The thermal decomposition temperature (T_d) was determined to be 276 °C once 5 wt% of the material was lost during the increased heat treatment during TGA (Figure S5c). The thermal properties of SbPDO-ODI are comparable to that of PE which melts between 125-132 °C, has degrees of crystallinity between 30-90%, and has processing temperatures of ~200 °C, which is typical for PE and TPU.[39] Thus, we believe this material could be molded within current commercial processing systems such as injection molding without thermal decomposition.

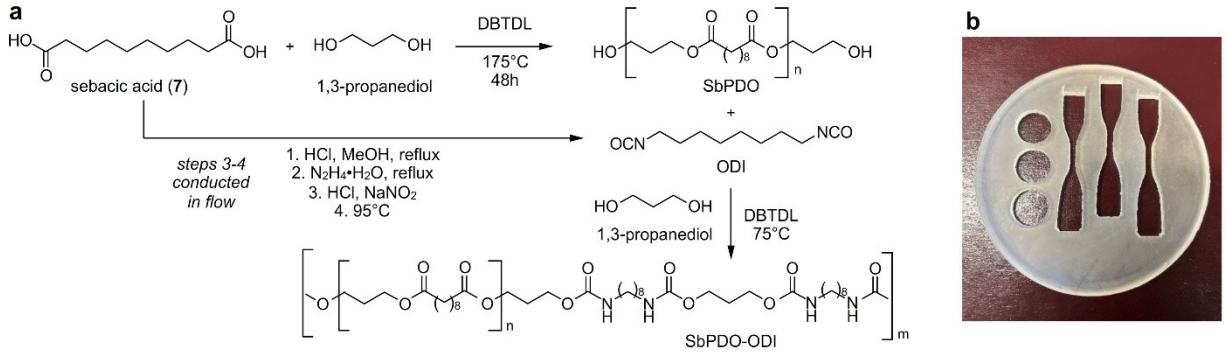

Figure 4. Preparation of a TPU plastic from sebacic acid. (a) Commercial sebacic acid was used as the dicarboxylic acid portion of the polyol (SbPDO) and was also incorporated as the diisocyanate (ODI) using a method previously published by our team in the synthesis of SbPDO-ODI. (b) Image of the final TPU, SbPDO-ODI, after curing and die cuts for property testing.

After determining the thermal processability of the material, commercial applications could be elucidated based on mechanical strength and robustness of SbPDO-ODI. First, tensile strength and elongation were determined on a universal testing machine (UTM) to measure the amount of force required to break the material and how long the material stretched before mechanical failure. SbPDO-ODI exhibited a tensile strength of 39 MPa and an elongation of 626% past its original length (Figure S5d). The hardness of the material was determined to be 97 Shore A, which is comparable to commercial products such as shoe heels or grocery cartwheels. From these results, we again observed comparable metrics to PE which exhibit a tensile strength of 21-45 MPa, elongations between 200-800%, and hardness between 80-95 Shore A.[40,41]

Discussion

The unique regioselective chemoenzymatic epoxidation reported here provides access to bio-based monomers not easily accessible from PUFAs. Through the co-catalytic ester screen with CpLIP2, we uncovered an unprecedented trend that shows site-selective oxidation based on co-catalyst chain length. The preference for mono-epoxide (Δ9) formation as the major product generally increased as the chain length of the co-catalysts increased from C5 to C9, then began to decrease for longer chains, with the exception of C18 that showed high conversion to the mono-epoxide. In contrast, the di-epoxide (Δ9, Δ12) showed the highest yield with dimethyl dodecanedioate without a noticeable trend related to the co-catalyst chain lengths. We hypothesize that the co-catalyst peracids serve as directing groups toward the PUFAs in which the ester hydrogen bonds with the acid's terminal carboxylic acid, allowing reactivity of the peracid with

certain alkenes depending on length, leading to the regioselectivity we observe. This hypothesis could account for the differences in mono- and di-epoxide ratios for shorter versus longer co-catalysts. As a result, it is feasible that PUFAs with alternative degrees of unsaturation may exhibit preference for shorter or longer co-catalytic esters depending on the location of the first unsaturation, granting access to a larger suite of dicarboxylic acids that are challenging to obtain.

The substrate specificity of the lipase toward the ester co-catalysts is also a key factor in the epoxidation reaction. Peracid formation occurs within the active site of CpLIP2, though it is currently unknown whether the peracid epoxidation is achieved inside or outside of the pocket. Previous studies with CpLIP2 show it exhibits peak activity in transesterification and alcoholysis reactions with long-chain acyl ester substrates containing a chain length of 12 carbons, decreasing in activity as this chain length is shortened or lengthened.[25,27] This coincides with our findings where the C12 dimethyl ester gave rise to the highest formation of di-epoxide **2b** from linoleic acid, as well as di- and tri-epoxides from linolenic acid, amongst the different chain lengths tested. These results demonstrate that increased selectivity and catalysis of the C12 ester by the lipase leads to greater peracid production, driving higher levels of epoxidation. As the chain length of the dimethyl ester co-catalysts we screened increase up to 12 carbons, we observe a general increase in epoxidation formation, aligning with the previous studies and supporting the role the lipase substrate specificity has on the extent of epoxidation. Based on our lipase screens testing alternative equivalents of the dimethyl esters, our hypothesis is that only one terminal ester may be peroxidized by the lipase at a time, mimicking the long-chain acyl esters as substrates. We would therefore expect that as the chain lengths of the dimethyl esters extend past 12 carbons, the extent of epoxidation will follow the same trend and decrease as observed with CpLIP2 and long-chain

acyl esters. This is supported by our C18 dimethyl ester co-catalyst result that favored mono-epoxidation over di-epoxidation. In addition, while the even-numbered dimethyl esters exhibit an increased trend of epoxidation levels as they are lengthened to C12, the odd-numbered chains, not previously explored, appear to show distinct substrate selectivity by the lipase with peak epoxidation observed with the C7 dimethyl ester, decreasing as it is shortened to C3 and lengthened to C11. This difference in specificity by the lipase could be attributed to orientation of the ester carbonyls within its pocket affecting their catalysis, the amount of peracids produced *in situ*, and overall epoxidation levels. Therefore, the chain length preference of the enzyme for medium chain dimethyl esters impacts the peracid levels and tuning the reaction parameters allows us to obtain the desired level of epoxidation. While we focused on the mono-epoxide in this study, the di- and tri-epoxides of linoleic and linolenic acid, respectively, could also be used for other applications. This bio-catalytic transformation therefore serves as a robust oxidative tool with low enzymatic loading, high turnover, and mild conditions in aqueous medium, serving as an ideal green reaction in monomer synthesis.

The chemoenzymatic epoxidation, particularly its regioselectivity, was critical for the development of a new route toward sebacic acid. Its preference for the Δ9 epoxidation of linoleic acid created access to the C10 diacid through the substrate's unique dual functionality with the alkene. As expected, rearrangement of the epoxide to yield a carbonyl at the desired position required the secondary alkene but also proved challenging due to the mixture of side products that formed. Implemented through the Meinwald rearrangement, it was thought that the reaction would show selectivity toward C10 over C9 due to the presence of the β,γ-unsaturation at Δ12 with a variety of acids. However, the only Lewis acid to selectively yield the C10 product was ZnI_2, which is attributed to Zn coordinating with both the alkene and the oxygen of the epoxide, enabling the

preferential hydride shift to C9 and thereby yielding the β,γ-ketone **4**. The sequence of these two steps remarkably provided the framework for a hybrid synthetic route toward sebacic acid, with both reactions exemplifying selectivity control under mild conditions. In addition, while six steps are involved in this methodology, only two chromatographic purifications were required. While this method effectively introduces a novel route toward sebacic acid and utilizes a sustainable starting material, its scalability for industrial use is currently limited by a low overall yield. Additional studies are currently underway to further optimize these reactions and increase its scalability so that substantial amounts of alternatively sourced sebacic acid can be produced for high-value products.

Along with finding greener reactions for dicarboxylic acid production, utilizing lipid waste streams of large industrial processes offers a unique opportunity to transform what is otherwise considered useless byproducts into value-added products. Since we previously used ozonolysis with algae waste oils to isolate azelaic acid, this method presents a mild, complementary approach to access alternative dicarboxylic acids for polymer synthesis.[14] Polyester polyurethane plastics have attracted significant interest for their tunable properties and potential for biodegradability. Previous studies using similar diacids and aliphatic diols have shown high biodegradability, reinforcing the strong likelihood that our material will degrade under environmentally relevant conditions.[19] Our sebacic acid-based TPU SbPDO-ODI exhibits thermal and mechanical properties comparable to widely used industrial plastics such as polyethylene and polypropylene, demonstrating the versatility and suitability of sebacic acid in the polymer industry. These observed characteristics allow us to conclude that our material is suitable for manufacturing a broad range of products requiring high durability and strength, such as coatings, shopping cart

wheels, safety helmets, etc. In polyurethane synthesis, even small chain length changes in the monomers can greatly diversify the properties that can be achieved.[37] For example, a polyol that contained 50% succinic and 50% sebacic acid was found to be significantly more viscous and nearly doubled the tensile strength of a similar molecular weight polyol consisting of 50% succinic and 50% azelaic acid.[19] Therefore, creating a diverse portfolio of sustainable and bio-based monomers, like with the process described here, is crucial in order to increase the scope and properties that can be achieved by the resulting green polyurethanes.

Conclusion

In this study, we developed regioselective epoxidation methods through a highly sustainable chemoenzymatic reaction and exemplified how its product could be used in the synthesis of an industrially relevant monomer. Tuning this reaction to prepare sebacic acid demonstrated a highly relevant application for the biotransformation of polyunsaturated fatty acids. The epoxidation and rearrangement sequence was then successfully applied to a hemp oil waste stream to demonstrate the utility of the process toward creating sustainable bio-based monomers for polyurethane synthesis. The utility of a lipase in aqueous medium to access regioselective oxidation products that otherwise require harsh conditions serves as a stepping stone to convert this process into a greener alternative. Several of the steps toward sebacic acid, such as the synthesis of the co-catalytic dimethyl esters, transesterification of triglycerides in the hemp oil, esterification of the PUFAs after epoxidation, Baeyer-Villiger oxidation, and final hydrolysis, have been shown to be achievable through similar enzymes, and these lipases could also be immobilized and recycled for greater efficiency.[42–44] Further studies that explore increasing the use of enzymatic processes in the described pathway and increasing the yields of the individual steps are currently underway.

Experimental Section

General experimental methods

Chemical reagents were purchased from Acros, Fluka, Sigma-Aldrich, VWR, or TCI. Deuterated NMR solvents were purchased from Cambridge Isotope Laboratories. All reactions were performed in glass round bottom flasks or 20 mL scintillation vials and were stirred with a Teflon coated stir bar using an IKAMAG RCT-basic mechanical stirrer (IKA GmbH). Analytical Thin Layer Chromatography (TLC) was performed on Silica Gel 60 F254 precoated glass plates (EM Sciences). Visualization was achieved with UV light and/or an appropriate stain (potassium permanganate, bromocresol green, dinitrophenylhydrazine, ninhydrin and ceric ammonium molybdate). Flash chromatography was carried out with Geduran Silica Gel 60 (40-63 mesh) from EM Biosciences. Yields and characterization data correspond to isolated, chromatographically and spectroscopically homogeneous materials. ^{1}H NMR spectra were recorded on a JEOL ECA500, JEOL ECA400, JEOL ECZ400, or Varian VX500 spectrometer equipped with an Xsens Cold probe. Chemical shifts for ^{1}H NMR and ^{13}C NMR analyses were referenced to the reported values of Gottlieb[45] using the signal from the residual solvent for ^{1}H spectra, or to the ^{13}C signal from the deuterated solvent. All ^{13}C NMR spectra were recorded with complete proton decoupling. FID files were processed using MestreNova 14.3.1 (MestreLab Research). Electrospray (ESI) mass spectrometric analyses were performed using a ThermoFinnigan LCQ Deca spectrometer and high-resolution analyses were conducted using a ThermoFinnigan MAT900XL mass spectrometer with electron impact (EI) ionization. A Thermo Scientific LTQ Orbitrap XL mass spectrometer was used for high-resolution electrospray ionization mass spectrometry analysis (HR-ESI-MS). Spectral data and procedures are provided for all new compounds and copies of select spectra have been provided.

Lipase production

WT CpLIP2 and mutants of CpLIP2 (Y179F, S369A, Y179F/S369A) were prepared as previously described[9] through recombinant expression in *Komagataella pastoris* X-33 using the pPICZαB expression vector. Briefly, the corresponding expression vectors containing CpLIP2 or its mutant genes were transformed into *K. pastoris* X-33 followed by cultivation in synthetic medium to initiate enzyme production at larger scales. The culture supernatant containing the recombinant enzymes was concentrated, diafiltered with ultrapure water, and lyophilized prior to storage at -20 °C. Prior to reactions, the lyophilized proteins were resuspended in water (2.5 mg ml^{-1}) and aliquots of 160 µL (0.4 mg) were stored at -20 °C.

General chemoenzymatic screens with CpLIP2

The screens of the chemoenzymatic epoxidation with unsaturated fatty acid methyl esters or acids were carried out according to our previous publication[9]. Briefly, the unsaturated substrate (0.1 mmol) and a co-catalyst (0.5 mmol) were suspended on phosphate buffer at pH 6.5 (200 µL), 30% H_2O_2 (153 µL), 2.5 mg/mL CpLIP2 (160 µL), and water up to a total volume of 1 mL. The reaction was allowed to stir overnight for 18 h at rt, followed by extraction with EtOAc (3 × 2 mL). The combined organic layers were dried over Na_2SO_4 and then concentrated by rotary evaporation. Conversion of the olefin(s) to epoxide(s) was measured by their respective ^{1}H NMR peaks.

Synthesis of sebacic acid from commercial linoleic acid. The following section provides an overview of the synthetic methods used to prepare sebacic acid from linoleic acid.

(*Z*)-8-(3-(oct-2-en-1-yl)oxiran-2-yl)octanoic acid (2a). Linoleic acid (1.09 mL, 3.51 mmol, 1.0 eq) and dimethyl azelate[29] (3.76 mL, 17.5 mmol, 5.0 eq) were suspended on 500 mM sodium phosphate buffer at pH 6.5 (7.00 mL), 30% H_2O_2 (5.36 mL, 1.5 M) and water (12.2 mL). WT

CpLIP2 (5.60 mL of 2.5 mg/mL stock solution) was then added, and the reaction was allowed to stir at rt for 18 h. The mixture was then extracted with EtOAc (3 × 10 mL), and the combined organic layer was dried over Na_2SO_4 and concentrated by rotary evaporation. The crude oil mixture containing acid **2a** was used in the next reaction without further purification.

Methyl (*Z*)-8-(3-(oct-2-en-1-yl)oxiran-2-yl)octanoate (3). The crude epoxide mixture containing acid **2a** (~1.00 g, 3.37 mmol, 1.0 eq) and cesium carbonate (6.59 g, 20.2 mmol, 6.0 eq) were dissolved in DMF (8.42 mL). The reaction was cooled to 0 °C and iodomethane (1.48 mL, 23.6 mmol, 7.0 eq) was added dropwise. The reaction was then warmed to rt and stirred overnight. After 18h, water was added and then extracted with EtOAc (3 × 20 mL). The organic layers were combined, washed with water to remove residual DMF, dried over Na_2SO_4, and concentrated by rotary evaporation. The crude oil was purified by flash chromatography, eluting with a gradient of 98/2% hexanes/diethyl ether to 86/14% hexanes/diethyl ether by 2% increments to yield epoxide **3** as a yellow oil (298 mg, 27.4% over two steps), which was confirmed with literature NMR spectra.[46]

Epoxide **3**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.46 (m, 2H), 3.66 (s, 3H), 2.92 (t, J = 4.8 Hz, 2H), 2.38 (m, 1H), 2.30 (t, J = 7.7 Hz, 2H), 2.18 (m, 1H), 2.04 (q, J = 7.1 Hz, 2H), 1.62 (m, 2H), 1.53 (m, 4H), 1.33 (m, 12H), 0.90 (t, J = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.5, 132.7, 124.0, 57.4, 56.7, 51.6, 34.2, 31.9, 29.6, 29.3, 29.2, 29.2, 27.9, 27.5, 26.4, 26.3, 25.1, 22.7, 14.2; HRMS (ESI) *m/z* $[M+H]^+$ calculated for $C_{19}H_{35}O_3$: 311.2586; found 311.2578.

Methyl (*Z*)-10-oxooctadec-12-enoate (4). Zinc iodide (306 mg, 0.960 mmol, 1.0 eq) was added to a solution of epoxide **3** (298 mg, 0.960 mmol, 1.0 eq) in toluene (4.8 mL). After stirring the reaction overnight at rt, water (5 mL) was added to the vial and the layers were separated. The aqueous layer

was extracted with EtOAc (3 × 5 mL). The combined organic phase was dried over Na_2SO_4 and concentrated by rotary evaporation, affording ketone **4** as a crude orange oil, which was used in the next step without further purification.

Methyl (*E*)-10-oxooctadec-11-enoate (5). DBU (141 µL, 0.934 mmol, 1.0 eq) was added to a solution of ketone **4** (290 mg, 0.934 mmol, 1.0 eq) in dichloromethane (4.67 mL). The reaction was then stirred overnight at rt. After 18 h, the solution was washed with 1M HCl (2 × 5 mL) and brine (1 × 5 mL), dried over Na_2SO_4, and concentrated by rotary evaporation. The isomerized product **5** was obtained as a yellow oil and was carried forward in the next reaction without additional purification.

1-(3-hexyloxiran-2-yl) 10-methyl decanedioate (6). Isomerized product **5** (233 mg, 0.750 mmol, 1.0 eq) was added to a solution of 3-chloroperbenzoic acid (mCPBA) (407 mg, 1.65 mmol, 2.2 eq) suspended in dichloromethane (3.3 mL). The reaction was stirred overnight for 18 h at rt.[5] Once the reaction was complete, the solution was filtered, and the solids were washed with cold dichloromethane. The filtrate was then concentrated by rotary evaporation and the crude solid was purified by flash chromatography (hexanes to 85/15% hexanes/EtOAc), yielding ester **6** (85.0 mg, 25.9 % over 3 steps) as an oil.

Ester **6**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.33 (s, 1H), 3.66 (s, 3H), 3.10 (m, 1H), 2.32 (m, 4H), 1.61 (m, 4H), 1.43 (m, 2H), 1.29 (m, 16H), 0.88 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 174.5, 173.8, 76.7 (minor isomer), 76.6, 57.7 (minor isomer), 57.7, 51.7, 34.2, 34.2, 31.8 (minor isomer), 31.5, 29.9 (minor isomer), 29.7, 29.4, 29.3, 29.2, 29.2, 29.2 (minor isomer), 29.0 (minor isomer), 28.8, 25.4, 25.0, 24.6, 22.7 (minor isomer), 22.6, 14.2 (minor isomer), 14.2; HRMS (ESI) *m/z* $[M+H]^+$ calculated for $C_{19}H_{35}O_5$: 343.2484; found 343.2481.

Sebacic acid, or decanedioic acid (7). Ester **6** (85.0 mg, 0.248 mmol) was dissolved in a 3M solution of potassium hydroxide (KOH) (500 μL), heated to reflux (80 °C), and stirred for 5 h. The solution was cooled to rt and then 1M HCl was added until the solution reached a pH of 3, followed by extraction with EtOAc (3 × 5 mL). The combined organic phase was dried over Na_2SO_4 and concentrated to afford a crude white solid. Water was then added to the white solid, and the mixture was heated to 90 °C until all the sebacic acid was solubilized. The solution was then extracted with hexanes while still hot to remove impurities. The aqueous phase was then concentrated by rotary evaporation to afford sebacic acid **7** as a white solid (42 mg, 83.7%, 5.94% overall from linoleic acid), which was confirmed with literature NMR spectra.[47]

Acid **7**: ^{1}H NMR (400 MHz, CD_3OD): δ 2.27 (m, 4H), 1.59 (m, 4H), 1.34 (s, 8H); ^{13}C NMR (101 MHz, CD_3 OD): δ 177.9, 35.1, 30.3, 30.2, 26.1; HRMS (ESI) *m/z* $[M-H]^-$ calculated for $C_{10}H_{17}O_4$: 201.1127; found 201.1133.

Isolation of polyunsaturated fatty acids from hemp oil

Linoleic and linoleic acids were isolated from hemp oil (Manitoba Harvest) via an adaptation of previously published methods.[48] 50 g (1.0 eq) of hemp oil was mixed with 0.3 M methanolic NaOH (44 mL). The reaction was stirred and refluxed at 65 °C for 2 h. Hexanes were then added and the mixture separated into two layers; the organic phase containing the FAMEs was washed three times with water and then dried by rotary evaporation. GCMS analysis showed methyl linoleate and methyl linolenate representing 94% of the isolated FAME mixture, with methyl linoleate representing 58% of the oil.

Synthesis of sebacic acid based polyester polyol (SbPDO)

An approximately equimolar ratio of sebacic acid (commercial) and 1,3-propanediol (1,3-PDO) was used to prepare the corresponding polyester polyol (SbPDO) (Figure 4a). Sebacic acid (120.2 g, 0.594 mol, 1.0 eq) was heated until melted, followed by addition of 1,3-PDO (51.2 g, 0.671 mol, 1.13 eq) under nitrogen atmosphere in a three-necked round bottom flask fitted with a Dean-Stark apparatus. The polymerization was initiated around 150 - 160 °C and the temperature was increased up to 175 °C over a period of 2 h. The rapid release of water as a by-product was observed in the initial 4-5 h of the reaction. A few drops of the catalyst dibutyltin dilaurate (DBTDL) were added to the reaction when around 80% of the theoretical amount of by-product water was collected. The completion of reaction was determined via acid number and hydroxyl number titrations of polyol which were found to be 0.2 and 68.8, respectively. The molecular weight was calculated from hydroxyl number to be 1630.8 g/mol.

Preparation of TPU SbPDO-ODI

The polyester polyol (SbPDO) and 1,3-PDO were dried at 80 °C under vacuum for ~48 h prior to use. SbPDO, 1,3-PDO, and octamethylene diisocyanate (ODI) were taken in a ratio of 1:1:2.2 to maintain the isocyanate index of 1.1 for the corresponding TPU. In a plastic cup, polyester polyol (31 g, 0.019 mol, 1.0 eq), 1,3-PDO (1.44 g, 0.019 mol, 1.0 eq) and catalyst DBTDL (15 µL) were mixed in a speed mixer for about one minute. To this mixture, octamethylene diisocyanate (ODI)[37] (8.2 g, 0.042 mol, 2.2 eq), which was heated to 75 °C, was added followed by mixing the contents for another one minute. The contents were further poured into a Teflon mold and cured at 75 °C for 48 h to give SbPDO-ODI (Figure 4a). The TPU was then prepared for mechanical testing by extracting dog-bone and tab die cuts (Figure 4b).

ASSOCIATED CONTENT

Supporting Information. Experimental data including additional synthetic methods, supplementary figures, and spectral data. This material is available free of charge.

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Author Contributions

M. D. B., J. J. L., M. S., R. N. R., and C. H. conceived the project. M. S. prepared the proteins. M. S., J. J. L. and R. N. R. developed the enzymatic epoxidation conditions. R. N. R. and A. D. conducted the enzymatic screening. R. N. R., C. H., A. D., and N. H. conducted the chemical synthetic steps and purified each compound. R. N. R. and C. H. collected and processed the synthesis data. A. J. conducted the synthesis of the polyester polyol and TPU. M. H. conducted the synthesis of ODI. A. B. conducted the thermal and physical property testing of the TPU. K. M. J. W. extracted and analyzed *P. kessleri* lipids and collected algae microscopy images. M. D. B. guided the project. All authors analyzed the data. All authors contributed to the writing and editing.

Notes

M.D.B. is a founder and holds an equity position in Algenesis materials, a biotechnology company developing commercial products from renewable sources.

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