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Biocatalytic Route to Chiral Precursors of β-Substituted-γ-Amino Acids

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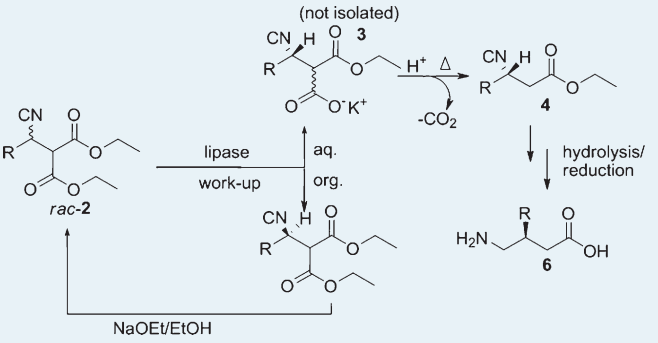
Supporting Information

ABSTRACT: In this work, we utilized commercial lipases (from *Thermomyces lanuginosa, Rhizopus delemar*, and *Mucor miehei)* as biocatalysts for the efficient synthesis of precursors of β-substituted-γ-amino acids. This biocatalytic route provides a practical and efficient synthesis of a wide range of optically active compounds by accepting a number of aliphatic and aromatic 3-substituted-3-cyano-2-(ethoxycarbonyl)propanoic acid ethyl esters (**2**) without compromising enantioselectivity or yields. The resolution step allows for the nearly quantitative recovery of the unreacted enantiomer of *R*-(**2**) as well as the newly formed 3-substituted-3-cyano-2-(ethoxycarbonyl)-propanoic acid (**3**) in high enantio and diastereoselectivity. The use of a facile thermal decarboxylation of (**3**) in aqueous solution to produce 3-substituted-3-cyanopropanoic acid ethyl esters (**4**) enable us to prepare a wide range of optically active precursors of β-Substituted-γ-Amino Acids.



KEYWORDS: Lipase resolution, ester hydrolysis, β-Substituted-γ-Amino Acids

Synthetic derivatives of γ-aminobutyric acid (GABA), the principal inhibitory neurotransmitter in the mammalian brain, have been extensively used for the treatment of several nervous system disorders including epilepsy, neuropathic pain, anxiety, and social phobia.[1] Several drugs such as Gabapentin and Pregabalin have achieved rapid success in the past two decades.[2] The use of an enzymatic kinetic resolution in the manufacturing of Pregabalin[3] was critical in providing an economical, high-yield synthesis of the final drug. The chemistry has been further developed and implemented by Pfizer, and the environmental benefits of racemizing and recycling the wrong enantiomer have been demonstrated at multiple metric ton scale.[3] This prompted research to explore the possibility of using a biocatalyst like the *Thermomyces Lanuginosa* lipase in the resolution of related compounds, namely, 3-substituted-3-cyano-2-(ethoxycarbonyl)propanoic acid ethyl esters **2** (Scheme 1). Herein, we report high enantioselectivity of three lipases toward a variety of aliphatic and aromatic substrates, producing the corresponding enantiopure 2-carbethoxy-3-substituted-3-cyanopropionate salts **3**.[4] A general decarboxylation procedure for compounds **3** is also reported, yielding the final enantiopure compounds **4**. Alkaline hydrolysis and reduction of these compounds by known methods produces the corresponding β-substituted-γ-amino acids **6** without affecting the chiral center generated in the enzymatic resolution.[3,5,6]

The substrates **2** were synthesized by a two-step method: Knoevenagel condensation of the corresponding aldehyde with diethyl malonate followed by nucleophilic cyanation of the α-β unsaturated precursor **1**. The initial Knoevenagel reaction was carried out in *n*-heptane with piperidine/acetic acid as the catalytic system with continuous removal of H_2O, and proceeded smoothly with good to high yields (Table 1). The resulting crude products **1** are used after minimal workup and without purification in the cyanation reaction. The cyanation reaction uses KCN in ethanol, and also proceeds with moderate to high yields, and the crude compound **2** thus produced can be used in the enzymatic step without purification (Scheme 2).

Conceptually, the generation of compound **3** from **2** involves the hydrolysis of one diastereotopic carboxyethyl group, a desymmetrization of the prochiral C-2 center. The desired outcome was to find an enzyme that could only perform such a reaction on the *S* enantiomer of racemic **2** (kinetic resolution), thus generating one (or two) diastereomers from a single enantiomer at the C-3 chiral center, leaving behind the *R* enantiomer of **2**. Thus, the diastereoselectivity in the desymmetrization reaction *per se* was not as important as the enantioselectivity of the kinetic resolution, as the chirality at the C-2 center will be lost while converting **3** to the desired product **4**.

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Scheme 1. Synthesis of Ethyl 2-Carbethoxy-3-cyano-3-substituted Propionate Substrates

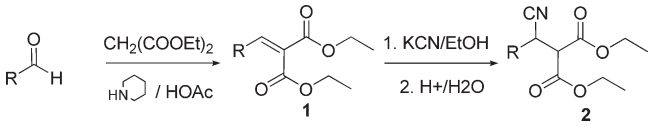


Table 1. Yields for the Knoevenagel and Addition Reactions

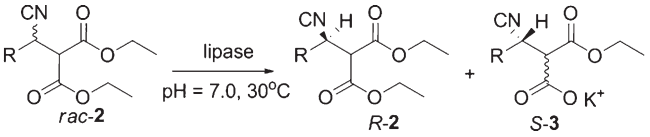
entry	R	yield **1** (%)[ref]	yield **2** (%)[ref]
a[a]	Me	84[7]	78[8]
b	Et	80[9]	83[10]
c[a]	*i*-Pr	69[11]	83[12]
d	*n*-Pr	91[7]	84[13]
e[b]	*i*-Bu	89[14]	94
f	*n*-Bu	96[7]	76
g[b]	Ph	92[7]	63
h	Bn	93[15]	65
i	$Ph(CH_2)_2$—	94[14]	64
j	*p*-anisyl	93[7]	67
k[a]	4-pyridyl	95	69

[a] Denotes **1** is commercially available. [b] **2** is commercially available.

Standards to analyze the reaction were initially prepared by base hydrolysis of one ester group in **2**, followed by acidification to produce carboxylic acids of general structure **3**. However, GC analysis of these products only displayed two products, determined (by GC-MS) to be the two enantiomers of **4**, because of decarboxylation of **3** at elevated temperatures (Scheme 3). To overcome this thermal decarboxylation, the acids **3** were derivatized prior to GC analysis to give thermally stable methyl ethyl esters **5**, thus allowing for the determination of both the enantioselectivity and the diastereoselectivity of the enzyme. The screening results (Table 2) indicate that *T. lanuginosa* Lipase (Lipolase 100 L from Novozymes) does have a broad substrate specificity for aliphatic derivatives of **2**, and limited or no selectivity for aromatic substituents examined (except substrate **2i**). Interestingly, derivatives **2b** and **2c** though accepted by lipolase, do display lower selectivity (*E* values ~100) than **2d**, **2e**, and **2f** (*E* values >200). Lipolase did not show activity with substrates **2g**, **2h**, **2j**, and **2k**, and this called for the study of additional lipases. *Rhizopus delemar* lipase (Lipase D "Amano") and *Mucor miehei* lipase (Palatase 20000 L) demonstrated high levels of activity and enantioselectivity with **2e** as previously reported,[3] and were selected on this basis. These lipases performed the efficient resolution on the aliphatic substrates **2b** and **2c** as well as the 4 aromatic substrates not resolved by Lipolase.

The *E* values[16] were obtained from the enantiomeric excess (% *ee*) of the product and of the remaining substrate at a given conversion value (Table 2). Although the diastereoselectivity of the lipases is not critical in this discussion, we note that for almost all of the compounds reported, the enzymes were highly diastereoselective as well as enantioselective. The absolute configuration of the favored diastereomer of **3** produced in the enzymatic reaction was not determined.

Scheme 2. Enzymatic Kinetic Resolution of 2



Once the resolution is complete, the desired product is present as the potassium salt **3**, which is easy separated from the unreacted enantiomer of **2** by solvent extraction. The final step to form **4** made used of a facile thermal decarboxylation of **3** (performed in the same aqueous phase obtained after resolution). This method was previously demonstrated by the conversion of **3e** to **4e**,[3] and it proved to be a general method for all the substrates screened herein. The 2 h reflux enabled the thermal decarboxylation of **3** to afford **4**, which is obtained as a water-insoluble oil/solid of high purity (Scheme 4). As an added benefit, the water-soluble enzyme and other buffer components that may interfere with the efficiency of subsequent steps are removed without the need for additional purification.

This method provides a practical and efficient synthesis of a wide range of optically active compounds. Several lipases were identified with broad substrate scope including aliphatic and aromatic compounds, high enantioselectivities and yields, allowing the nearly quantitative recovery of both enantiomers. The absence of purification steps and demonstrated scalability of the reactions also allows this method to be used on an industrial scale to give products in high yields. This has resulted in significant environmental savings in terms of both material and energy usage when applied to the synthesis of Pregabalin (**6e**).[22]

EXPERIMENTAL METHODS

General Considerations. All ^1H NMR spectra were obtained at 400 MHz; ^{13}C NMR data was obtained at 100 MHz. For the chiral products we report single enantiomer GC traces on a Chiraldex G-TA column (30 m), or HPLC traces on Chiralpak HPLC columns. For enzymes commercially available as solids, 10% (w/v) solutions were prepared in 0.1 M potassium phosphate buffer of pH 7.0. The following products have previously been reported, and can be found in the references: **1a**−**k**, **2a**−**e**, **4a**−**b**, **4g**, **4i**−**j**. The remaining compounds are all properly characterized by ^1H and ^{13}C NMR. The compounds **4** that have been reported previously are reported as their racemic mixtures, thus GC or HPLC traces of the single enantiomers are also reported herein. Absolute (R/S) configurations were not determined for **4g**, **4j**, or **4k**.

General Procedure for 3-Substituted-2-(ethoxycarbonyl)-propanoic Acid Ethyl Esters (1). A solution was prepared of diethyl malonate (1.0 equiv) and piperidine[23] (0.1 equiv) in *n*-heptane (30 mL). To this was added aldehyde (10.0 g) and glacial acetic acid (0.1 equiv). The solution was refluxed under Dean−Stark conditions for 16−23 h, until water collection ceased. The reaction was then diluted with 30 mL of H_2O and 30 mL of methyl *t*-butyl ether, and the layers separated. The organic phase was then washed sequentially with 10% aqueous $NaHCO_3$, brine, and H_2O (30 mL each), then dried over Na_2SO_4, and the solvent removed by rotary evaporation (80−96% yield). The crude product was used without purification in the next step.

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Scheme 3. Generation of GC Standards for Selectivity Analyses

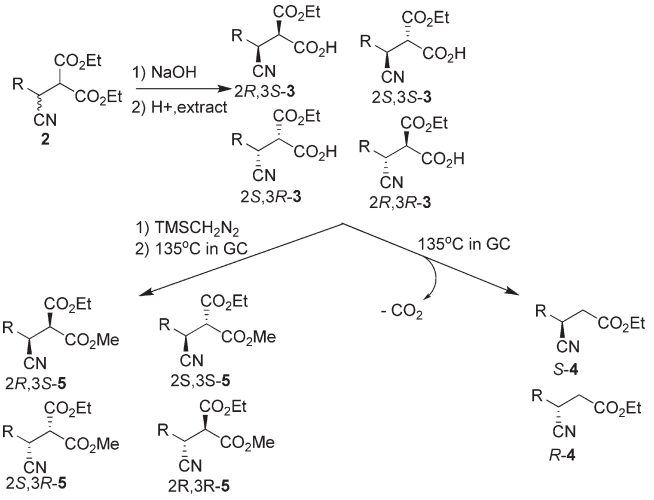
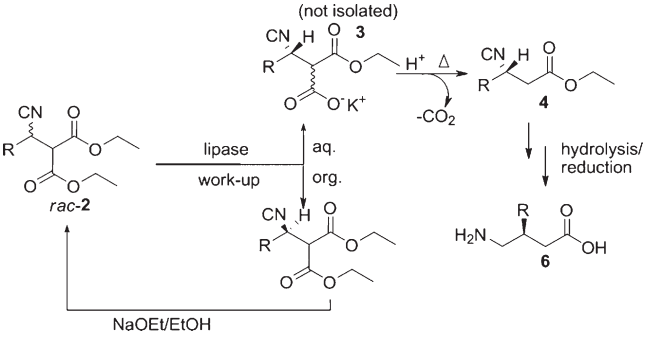


Table 2. E Values and Conversions of the CNDE Substrates

R	*ee* **4** (%)[ref]	*ee* **2** (%)	*de* **3** (%)[a]	E (Enzyme)	conv (%)[b]
a	95.1[17]	69.4	20.7	102 (Lipolase 100 L)	42
b	93.0[18]	>99	84.8	>200 (Lipase D Amano)	52
c	91.4	>99	>99	179 (Palatase 20 000 L)	52
d	98.4	81.4	>99	>200 (Lipolase 100 L)	45
e	>99[5]	95.0	>99	>200 (Lipolase 100 L)	47
f	>99	75.0	>99	>200 (Lipolase 100 L)	43
g	94.3[19]	>99	>99	>200 (Palatase 20 000 L)	51
h	>99[20]	95.9	>99	>200 (Lipase D Amano)	49
i	94.4	>99	>99	>200 (Lipolase 100 L)	51
j	92.5[21]	>99	>99	>200 (Palatase 20 000 L)	52
k	90.6	>99	>99	158 (Lipase D Amano)	52

[a] The % *de* of **3** is as determined by the % *de* of derivatives **5** observed by GC analysis for compounds **a**−**f**. [b] Conversion data reflects measurements taken at 22 h. for **a**−**f**, and 16.5 h for **g**−**k**.

Scheme 4. Isolation of the Enzymatic Resolution Products



Generalized Procedure for 3-Substituted-3-cyano-2-(ethoxycarbonyl)propanoic Acid Ethyl Esters (2). The crude propenoic acid ester **1** is dissolved in ethanol (~40% w/v); to this solution is added solid KCN (0.96 equiv). The resulting slurry is stirred at reflux for 16−20 h. After reflux, the mixture is diluted with 1 volume of MTBE and treated with glacial acetic acid (1.0 equiv) in water (1 volume). The layers are separated, the organic phase washed with brine (2 × 1 volume portions), and the solvent removed by rotary evaporation under vacuum. This product can be used crude in the enzymatic resolution.

Generalized Procedure for Screening 3-Substituted-3-cyanopropanoic Acid Ethyl Esters (4). Ten microliters of **2** were suspended in 1.0 mL of potassium phosphate buffer at pH 7.0 (0.10 M). To this was added 1 μL of enzyme solution, and the mixture stirred and maintained at 30.0 °C for 16−23 h. The mixture was then extracted with 3 mL of MTBE to remove the undesired enantiomer. The aqueous layer containing the potassium salt of **3** was refluxed for 1−2 h, causing an insoluble product to settle over the aqueous layer. The resulting solution was extracted with 1 mL of MTBE and washed with water (1 mL), leaving the desired enantiopure ethyl 3-substituted-3-cyanopropionate **4**. The procedure as given is satisfactory for small substrate loads. At higher substrate concentrations, a decrease in activity is observed, because of enzyme inhibition by the carboxylate salts **3**. In such cases, the addition of 10% molar equivalent of $Ca(OAc)_2$ or $Zn(OAc)_2$ serves to restore full enzymatic activity. Further discussion of this phenomenon may be found in ref 3. Additionally, the use of buffer solution becomes impractical; pH control by means of an autotitrator (with 4N NaOH) is preferred.

■ ASSOCIATED CONTENT

Supporting Information. The spectral characterization data for products reported. This material is available free of charge via the Internet at http://pubs.acs.org.

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(4) Throughout this communication, both the 3-substituted-3-cyano-2-(ethoxycarbonyl)-propanoate salts and their corresponding free acids will be referred to as **3**. The text will explicitly state which compound is being discussed in all cases.

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