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Diastereoselective hydrolysis of branched malonate diesters by Porcine Liver Esterase: Synthesis of 5-benzyl substituted Cα-methyl-β-proline and catalytic evaluation

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Abstract. Malonate diesters with highly branched side chains containing a preexisting chiral center were prepared from optically pure amino alcohols and subjected to asymmetric enzymatic hydrolysis by Porcine Liver Esterase (PLE). Recombinant PLE isoenzymes have been utilized in this work to synthesize diastereomerically enriched malonate half-esters from enantiopure malonate diesters. The diastereomeric excess of the product half-esters was further improved in the later steps of synthesis either by simple recrystallization or flash column chromatography. The diastereomerically enriched half-ester was transformed into a novel 5-substituted Cᵅ-methyl-β-proline analogue (3*R*, 5*S*)-**1c**, in high optical purity employing a stereoselective cyclization methodology. This β-proline analogue was tested for activity as a catalyst of the Mannich reaction. The β-proline analogue derived from the hydrolysis reaction by the crude PLE appeared to catalyze the Mannich reaction between an α-imino ester and an aldehyde providing decent to good diastereoselectivities. However, the enantioselectivities in the reaction was low. The second diastereomer of the 5-benzyl substituted Cᵅ-methyl-β-proline, (3*S*, 5*S*)-**1c** was prepared by enzymatic hydrolysis using PLE isoenzyme 3 and tested for its catalytic activity in the Mannich reaction. Amino acid, (3*S*, 5*S*)-**1c** catalyzed the Mannich reaction between isovaleraldehyde and an α-imino ester yielding the "*anti*" selective product with an optical purity of 99%ee.

Introduction

The demand for chiral molecules, preferentially one stereoisomer, has increased significantly due to the fact that two thirds of current prescription drugs are chiral and the majority of them being single stereoisomers.[1] These single stereoisomers can be accessed by traditional synthetic procedures that involve either asymmetric transformations or classic resolution techniques. In asymmetric chemical transformations, the stereoselectivity can be achieved by various methods that include the use of chiral auxiliaries, chemical reactions that involve chiral phase transfer catalysts or employ chiral compounds that occur in nature.[2]

Porcine Liver Esterase (PLE) as a biocatalytic tool in asymmetric synthesis

Porcine Liver Esterase (PLE) has proven to be a valuable catalyst in the field of organic chemistry. PLE has been successfully employed to carry out asymmetric transformations on a broad variety of structurally different substrates providing good to excellent stereoselective outcomes.[3] PLE is widely used in organic synthesis to catalyze the asymmetric enzymatic hydrolysis of malonate and other diesters to create optically enriched chiral synthons in a highly selective fashion.[4] These chiral synthons have been utilized in the synthesis of compounds that are of tremendous biological significance.[5] Over the past decade, our group has enjoyed success in using PLE to synthesize various optically enriched chiral synthons and transforming them into Cᵅ-methyl substituted α-, β-, γ- and δ-amino acids.[6] Our synthetic strategy involves desymmetrization of the prochiral malonate diesters with branched side chains to produce optically enriched half-ester intermediates. These malonate half-ester intermediates serve as precursors to the Cᵅ-methyl substituted amino acids. In 2012, our group published the first enantiodivergent synthesis of Cᵅ-methyl-β-proline from an optically enriched intermediate derived from PLE hydrolysis.[6c] In this study, the enantiomerically enriched chiral half-ester from PLE hydrolysis was transformed into a novel γ-lactam through a stereoselective cyclization that is then converted to Cᵅ-methyl-β-proline. This amino acid was later reported to be an efficient catalyst in the asymmetric *anti*-Mannich reactions performed using organic solvents such as methylene chloride.[7] To continue our efforts in investigating and expanding the substrate scope for the PLE-catalyzed hydrolysis reactions with malonate diester substrates, herein we present the results of PLE-catalyzed hydrolysis of chiral malonate diesters and their transformation to a novel Cᵅ-methyl-β-proline analogue that has proven to be an efficient *anti*-Mannich catalyst.

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Results and Discussion

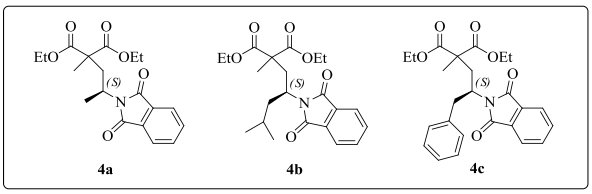
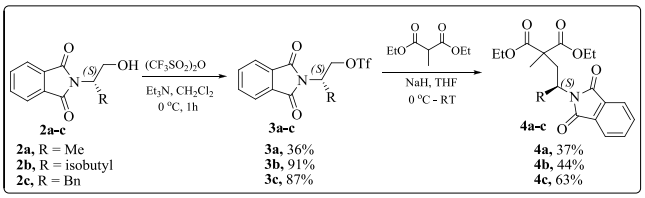


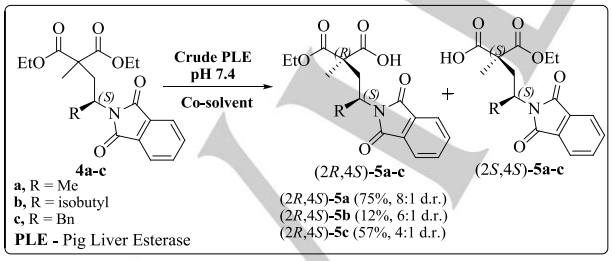
Figure 1. Substrates utilized in the PLE-catalyzed hydrolysis studies

For our PLE-catalyzed hydrolysis studies, we chose to utilize malonate diesters with a phthalimide group and a preexisting chiral center in the side chain (Figure 1). These diesters were readily prepared from optically active amino alcohols as shown in Scheme 1.[8]



Scheme 1. Synthesis of malonate diesters **4a-c**, from optically pure amino alcohols

Recently, Hoveyda et al reported a PLE-mediated diastereoselective desymmetrization of a chiral malonate diester.[9] To the best of our knowledge, there are no published reports of PLE-catalyzed hydrolysis of malonate diesters with a preexisting chiral center and a phthalimide group. Our unique approach, to utilize relatively inexpensive and optically active starting materials, helped us avoid the possibility of producing a mixture of enantiomers during the PLE-catalyzed hydrolysis reaction. Here, the malonate diester was transformed into the half-ester with the creation of a new chiral center at the quaternary carbon. Since the stereochemistry of the preexisting chiral center in the side chain of malonate diester substrate is preset to be "*S*", the PLE-catalyzed hydrolysis of this substrate produces two possible diastereomeric half-esters (2*R*, 4*S*)-**5a-c** and/or (2*S*, 4*S*)-**5a-c** as illustrated in Scheme 2.



Scheme 2. Asymmetric enzymatic hydrolysis of chiral malonate diesters catalyzed by crude PLE

Malonate diesters, **4a-c**, were subjected to asymmetric ester hydrolysis by crude PLE enzyme (crude PLE refers to the mixture of all isoenzymes). The PLE reaction was carried out at pH 7.4 in phosphate buffer using an auto burette that was set to dispense 1.0 N NaOH as the reaction progressed. The results of the PLE-catalyzed reactions for substrate and co-solvent screening are illustrated in Table 1.

Table 1. Asymmetric hydrolysis of malonate diesters, **4a-c**, by crude PLE enzyme

Substrate	EtOH (Vol %)	CH_2Cl_2 (Vol %)	% Yield	Major Product (d.r.)[a]
4a	2.5	-	75	(2*R*, 4*S*)-**5a** (8:1)
4b	2.5	-	12	(2*R*, 4*S*)-**5b** (6:1)
4c	-	0.8	10	(2*R*, 4*S*)-**5c** (3.5:1)
4c	2.5	-	30	(2*R*, 4*S*)-**5c** (4.7:1)
4c	2.5	0.6	57	(2*R*, 4*S*)-**5c** (4.1:1**)**
4c	20	-	18	(2*R*, 4*S*)-**5c** (3.1:1)

[a] d.r. determined from ^{1}H NMR of crude product

Methylene chloride and ethanol were added as co-solvents in the reaction. Ethanol is one of the common co-solvents used to enhance the selectivity in the PLE hydrolysis reactions with malonate diesters.[10] Methylene chloride was added to ensure proper stirring of the reaction with malonate diester **4c** as the diester substrate's viscous physical nature caused problems with magnetic stirring of the reaction. Crude PLE, in the absence of ethanol, produced the half-ester (2*R*, 4*S*)-**5c** with a d.r. of 3.5:1. Upon addition of 2.5% ethanol, the d.r. was improved to 4.7:1. This is in agreement with the previous results published by our group on the improvement of selectivity in PLE-catalyzed hydrolyses of malonate diesters upon addition of ethanol.[10] The diastereomeric ratios of the PLE-hydrolysed product were determined from the ^{1}H NMR analysis of the crude product.

The absolute stereochemistry of the new quaternary chiral center in the half-ester, (2*R*, 4*S*)-**5c,** was determined to be *R* from the X-ray crystal analysis of its derivative (Scheme 3). The stereochemistry of the major diastereomer, (2*R*, 4*S*)-**5b**, was determined from the key correlations (Figure 2) observed in 2D-NOESY NMR data of the lactam ester, (3*R*, 5*S*)-**7b**, that was derived from the half-ester obtained from the hydrolysis catalyzed by PLE (See experimental section for the synthesis of (3*R*, 5*S*)-**7b**). The stereochemistry at the quaternary chiral center of the half-ester **5a** created during the PLE-catalyzed hydrolysis is determined to be *R* from ^{1}H NMR data. The shifts of major diastereomeric hydrogens in the compound **5a** were compared to those in compounds (2*R*, 4*S*)-**5b** and (2*R*, 4*S*)-**5c**.

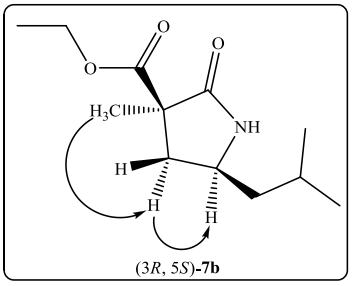


Figure 2. Key 2-D NOESY correlations of γ-lactam, (3*R*, 5*S*)-**7b**

PLE isoenzymes studies

Asymmetric hydrolysis of diesters **4a-c** catalyzed by crude PLE produced the half-esters in significant diastereomeric ratios. However, the yields of the reaction with substrates **4b** and **4c** were low compared to those using substrate **4a**. In order to optimize the enzymatic hydrolysis reaction for the yields and diastereoselectivities, we had conducted PLE isoenzyme studies with compounds **4b** and **4c** (Scheme 2). Crude PLE is a mixture of at least six different isoenzymes that have different specific activities, stereo preference, and turnover numbers compared to that of the crude isoenzyme mixture.[11] The studies were performed in phosphate buffer pH 7.4 using 2.5% ethanol and 0.8% methylene chloride as co-solvents. The yields and selectivities of the half-esters obtained from the PLE isoenzyme-catalyzed reactions are listed in Table 2.

Table 2. PLE isoenzyme studies of malonate diesters, **4b-c**

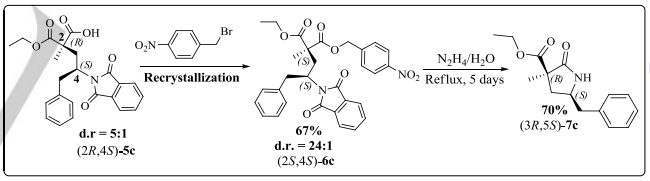
Enzyme	Substrate	Major Product	d.r.	%Yield
Crude PLE	**4b**	(2*R*, 4*S*)-**5b**	6:1	12
Crude PLE	**4c**	(2*R*, 4*S*)-**5c**	4.1:1	57
PLE 3	**4b**	(2*R*, 4*S*)-**5b**	5.7:1	24
PLE 3	**4c**	(2*S*, 4*S*)-**5c***	3.8:1	30
PLE 4	**4b**	(2*R*, 4*S*)-**5b**	1.3:1	13
PLE 4	**4c**	(2*S*, 4*S*)-**5c***	3.4:1	20
PLE 5	**4b**	-	-	-
PLE 5	**4c**	(2*R*, 4*S*)-**5c**	4.2:1	13
PLE 6	**4b**	(2*R*, 4*S*)-**5b**	11:1	25
PLE 6	**4c**	(2*R*, 4*S*)-**5c**	4.1:1	10

*Switch in diastereopreference

PLE isoenzymes 1 & 2 did not demonstrate activity with malonate diester **4c**. PLE 5 & 6 provided the diastereomer (2*R*, 4*S*)-**5c** as the major product (similar to crude PLE). Interestingly, both PLE 3 & 4 showed a switch in the diastereopreference providing the diastereomer (2*S*, 4*S*)-**5c** as the major product. These results were of interest to us as the isoenzymes 3 & 4 would readily provide access to the (2*S*, 4*S*)-**5c** diastereomer without additional chemical modifications. Even though the diastereoselectivities of hydrolysis reactions using both PLE 3 & 4 were good, the yields were low. We believe that the benzyl group, adjacent to the nitrogen, in malonate diester **4c** is probably too large to efficiently fit into the enzyme's active site. Also, these reactions proceeded rather slow and usually took 5-7 days to go to completion as determined by the delivered quantity of NaOH. The identity of the major product was determined from ^{1}H NMR chemical shifts and the reversal of diastereopreference with isoenzymes 3 & 4 was confirmed from the ^{1}H NMR data of the product half-esters. The PLE-catalyzed hydrolysis conditions for isoenzyme studies with substrate **4b** are similar to that for substrate **4c**. These reactions were also slow and normally proceeded for 4-5 days. During hydrolysis, PLE 1, 2 & 5 did not show activity towards the substrate **4b**. PLE 3 & 6 showed an improvement in the yield and diastereoselectivity of the resulting half-ester. The d.r. of the product half-ester in the hydrolysis of diester **4b** with PLE 6 was improved significantly from 6:1 to 11:1, providing an improved yield of 25%. However, the d.r. of the half-ester with PLE 4 diminished significantly (1.3:1). The stereoselective outcomes in the hydrolysis of diester **4b** with PLE isoenzymes remained the same as that with crude PLE.

*Synthesis of (3R, 5S)-5-benzyl-Cα-methyl-β-proline, (3R, 5S)-**1c***

With the product (2*R*, 4*S*)-**5c** (d.r.= 5:1) in hand we decided to investigate the stereoselective cyclization reaction[6c] that could provide access to a novel class of γ-lactams that can be converted into 5-benzyl-C$^{\alpha}$-methyl-β-proline. For this purpose the malonate half-ester (2*R*, 4*S*)-**5c** was transformed into the *p*-NO_2-benzyl ester, (2*S*, 4*S*)-**6c**, as shown in Scheme 3.



Scheme 3. Stereoselective cyclization to prepare γ-lactam (3*R*, 5*S*)-**7c**

Surprisingly the major diastereomer, (2*S*, 4*S*)-**6c**, readily crystallized out of the crude reaction mixture during the work up with diethyl ether in 67% yield. The improvement in diastereopurity was confirmed by 1H NMR analysis. Crystals of (2*S*, 4*S*)-**6c**, for X-ray analysis, were grown by the solvent diffusion technique. Single-crystal X-ray analysis data of (2*S*, 4*S*)-**6c** revealed the stereochemistry at the quaternary chiral center formed during the PLE hydrolysis to be *S* (Figure 3). Recrystallized (2*S*, 4*S*)-**6c** was treated with hydrazine hydrate to remove the phthalimide protecting group providing the primary amine, which then went on to cyclize exclusively at the *p*-nitro benzylic ester providing the lactam (3*R*, 5*S*)-**7c** in 70% yield. Interestingly, no trace of cyclization on the ethyl ester side was observed.

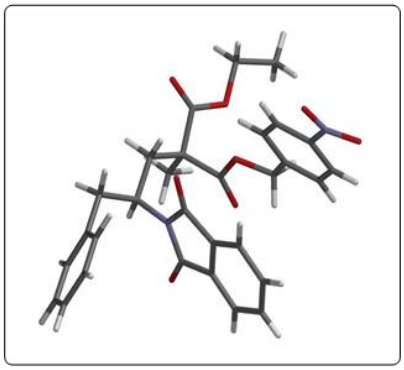
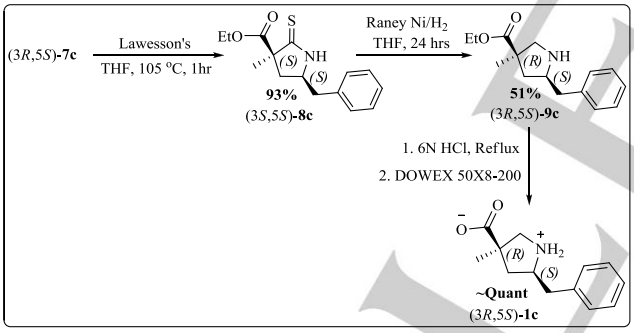


Figure 3. Single X-ray crystal structure of (2*S*, 4*S*)-**6c**

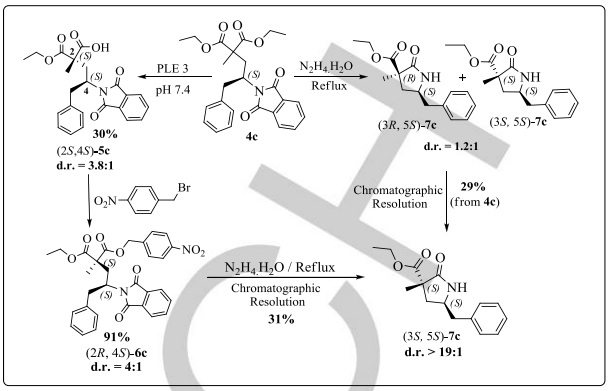
The γ-lactam (3*R*, 5*S*)-**7c** was then transformed into 5-benzyl-C$^{\alpha}$-methyl-β-proline as illustrated in Scheme 4. The reduction of the thiolactam previously reported by our group required N-benzyl protection of the thiolactam.[6c] Our attempts to install a benzyl group on the amide was not successful. So we have, instead, chosen to avoid the N-benzyl protection and deprotection steps. Upon reaction with Lawesson's reagent, lactam (3*R*, 5*S*)-**7c** was transformed into the thiolactam, (3*S*, 5*S*)-**8c**, in 93% yield.[12] Desulfurization of (3*S*, 5*S*)-**8c** was accomplished by treatment with Raney Ni under a H_2 atmosphere to provide (3*R*, 5*S*)-**9c** in 51% yield.[13] (3*R*, 5*S*)-**9c** was then subjected to ester hydrolysis under refluxing acid conditions using 6N HCl followed by cation exchange chromatography to provide amino acid, (3*R*, 5*S*)-**1c,** in quantitative yield (Scheme 4).



Scheme 4. Synthesis of 5-benzyl-C$^{\alpha}$-Methyl-β-Proline, (3*R*, 5*S*)-**1c**

Synthesis of (3S, 5S)-5-benzyl-C$^{\alpha}$-methyl-β-proline, (3S, 5S)-1c

We have developed two different routes to the intermediate γ-lactam (3*S*, 5*S*)-**7c** that was later transformed to 5-benzyl-C$^{\alpha}$-methyl-β-proline, (3*S*, 5*S*)-**1c** (Scheme 5). In the first route, which involves a non-stereoselective cyclization and separation of the diastereomers, **4c** was reacted with hydrazine hydrate to remove the phthalimide protecting group. Interestingly, upon phthalimide deprotection, compounds (3*R*, 5*S*)-**7c** and (3*S*, 5*S*)-**7c** were formed in a d.r. of 1.2:1.



Scheme 5. Synthesis of γ-lactam (3*S*, 5*S*)-**7c** from malonate diester **4c**

The crude reaction mixture from the hydrazine hydrolysis reaction was then purified via flash column chromatography to isolate the desired diastereomer of the lactam ester, (3*S*, 5*S*)-**7c** in 29% yield (d.r. > 19:1) (Scheme 5). The stereochemistry at the quaternary chiral carbon of the lactam ester (3*S*, 5*S*)-**7c** was confirmed to be *S* from single X-ray crystallographic analysis (Figure 4).

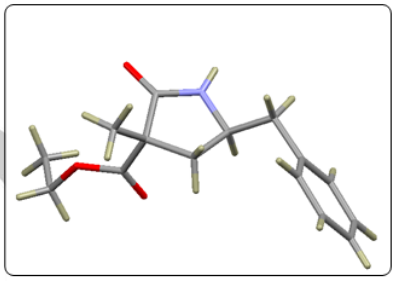
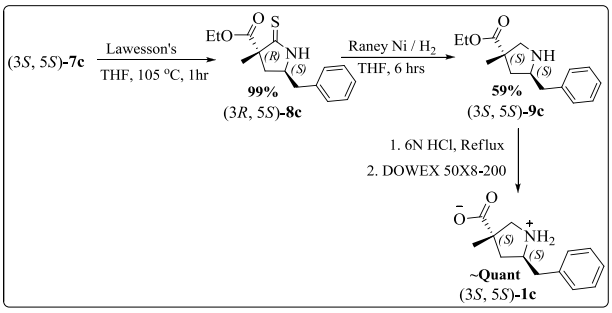


Figure 4. Single X-ray crystal structure of γ-lactam (3S, 5S)-**7c**

Compound (3*S*, 5*S*)-**7c** was also prepared from the malonate diester **4c** via the stereoselective cyclization methodology using the half-ester derived from PLE isoenzyme 3 hydrolysis (Scheme 5). We have utilized PLE isoenzyme 3 to cause the asymmetric hydrolysis of diester **4c** to provide the desired diastereomer (2*S*, 4*S*)-**5c** in 30% yield and a d.r. of 4:1. The half-ester (2*S*, 4*S*)-**5c** was treated with *p*-nitro benzyl bromide to provide the ester (2*R*, 4*S*)-**6c** in 91% yield and a d.r. of 4:1. Our attempts to improve the diastereopurity at this point were not successful. The 3.8:1 diastereomeric mixture of (2*R*, 4*S*)-**6c** was reacted with hydrazine hydrate under refluxing conditions. The crude reaction mixture was analyzed by ^{1}H NMR and the data suggested that the cyclization had occurred exclusively towards the *p*-nitro benzyl ester. The reaction mixture was then purified via flash column chromatography to resolve the diastereomers and provide the desired γ-lactam (3*S*, 5*S*)-**7c** in 31% yield. The γ-lactam, (3*S*, 5*S*)-**7c**, was then transformed into 5-benzyl-C$^{\alpha}$-methyl-β-proline, (3*S*, 5*S*)-**1c**, in three steps (Scheme 6). Treatment of compound (3*S*, 5*S*)-**7c** with Lawesson's reagent produced the thiolactam (3*R*, 5*S*)-**8c** in 99% yield. In the next step, desulfurization of (3*R*, 5*S*)-**8c** was carried out using Raney Ni under a H_2 atmosphere to provide the amino ester (3*S*, 5*S*)-**9c** in 59% yield. In the final step, hydrolysis of the ethyl ester (3*S*, 5*S*)-**9c** in refluxing 6N HCl followed by

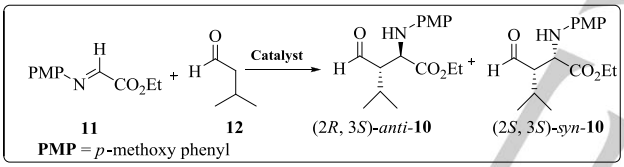
cation exchange chromatography furnished the target diastereomer of 5-benzyl-Cᵅ-methyl-β-proline, (3*S*, 5*S*)-**1c**, in quantitative yield (Scheme 6).



Scheme 6. Synthesis of Cᵅ-Methyl-β-Proline analogue from γ-lactam (3*S*, 5*S*)-**7c**

Mannich Reaction catalyzed by (3*R*, 5*S*)-**1c** & (3*S*, 5*S*)-**1c**

With both diastereomers of 5-benzyl-Cᵅ-methyl-β-proline in hand, we proceeded to investigate the activity of these amino acids in the Mannich reaction between a preformed *N*-PMP protected α-imino ester (**11**) and isovaleraldehyde (**12**) (Scheme 7). We have performed this reaction with L-proline, which provides "*syn*" selective products[14] and used it as a reference with which to compare our results.



Scheme 7. Mannich reaction for catalytic evaluation of 5-benzyl-Cᵅ-Methyl-β-proline

The Mannich reaction, catalyzed by (3*R*, 5*S*)-**1c** in various solvent systems, yielded *anti* products in moderate to excellent diastereoselectivities (Table 3). However, the enantioselectivities in the reaction were poor, suggesting a possible steric hindrance in the transition state with amino acid (3*R*, 5*S*)-**1c** as catalyst. We have performed this reaction in three different solvent systems and observed some solvent dependent enantioselectivity when the reaction was performed in methylene chloride. Interestingly, we observed a switch in the enantioselectivity of the *anti* diastereomer when the reaction was performed in methylene chloride and in a 1:1 mixture of 2-propanol/methylene chloride solvent systems (Table 3). According to the enamine mechanism, the enamine formed from the amino acid and the aldehyde component reacts with the preformed imine (Figure 5). The carboxylic acid of the amino acid should provide a hydrogen bond to the imine and control the selective attack of the enamine on the imine component. However, in the transition state the benzyl group and the carboxylic acid on the pyrrolidine ring of amino acid (3*R*, 5*S*)-**1c** are in the same side causing steric congestion between the benzyl group of amino acid and imine as illustrated in Figure 5.[15] As a result of this steric congestion, we hypothesize that the hydrogen bonding interaction between the carboxylic acid of the amino acid (3*R*, 5*S*)-**1c** and the α-imino ester is diminished, and thus results in lower enantioselectivities of the Mannich reaction.

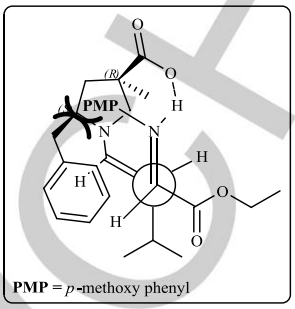


Figure 5. Possible steric hindrance in the transition state for the Mannich reaction by (3*R*, 5*S*)-**1c**

On the other hand, the second diastereomer of the designed amino acid (3*S*, 5*S*)-**1c** proved to be a very efficient catalyst, for the Mannich reaction between a preformed α-imino ester (**11**) and isovaleraldehyde (**12**), yielding *anti* selective Mannich products in a diastereomeric ratio of 23:1 (*anti: syn*) with enantiomeric excess of 99% ee. Given the hydrophobic quaternary methyl group and a benzyl group on the pyrrolidine core of the amino acid, (3*S*, 5*S*)-**1c**, we chose to carry out the Mannich reaction in methylene chloride at room temperature. The catalyst, however, in its zwitterionic form was initially insoluble in methylene chloride. The reaction mixture was heterogeneous in the beginning and became more homogenous as the reaction progressed. We were able to see improved enantioselectivities in the Mannich reaction with the designed amino acid (3*S*, 5*S*)-**1c** as catalyst. Based on the enamine mechanism of proline catalyzed Mannich reaction we propose a transition state (Figure 6) for the reaction, between preformed α-imino ester and isovaleraldehyde, which accounts for the observed enantioselectivities in the reaction. In the transition state, the benzyl group and the carboxyl group on the pyrrolidine core of amino acid, (3*S*, 5*S*)-**1c**, are on opposite sides as shown in the Figure 6. This eliminates the possibility of steric hindrance in the transition state and thus favors the strong hydrogen bond interaction between the carboxylic acid and imine.[15]

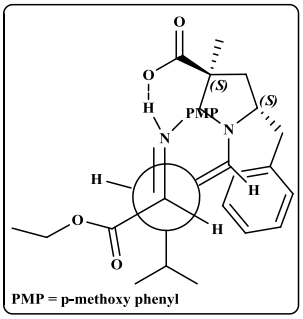


Figure 6. Predicted transition state for the Mannich reaction catalyzed by (3*S*, 5*S*)-**1c**

Table 3. Table Caption Mannich reaction catalyzed by L-proline, and designed C^{α}-methyl-β-proline

Catalyst	Loading	Solvent	Time, Temp	% Yield	d.r.	%ee (Major)
L-Proline	20 mol%	DMSO	4h, RT	96	24:1 (*syn*)	97% (2*S*, 3*S*)
(3*R*, 5*S*)-**1c**	5 mol%	DMSO	6h, RT	79	4:1 (*anti*)	9% (2*R*, 3*S*)
(3*R*, 5*S*)-**1c**	5 mol%	*i*-PrOH:CH_2Cl_2 (1:1)	2h, RT	82	19:1 (*anti*)	11% (2*S*, 3*R*)
(3*R*, 5*S*)-**1c**	5 mol%	CH_2Cl_2	5h, RT	82	19:1 (*anti*)	32% (2*S*, 3*R*)
(3*S*, 5*S*)-**1c**	5 mol%	CH_2Cl_2	2.5h, RT	92	23:1 (*anti*)	99% (2*R*, 3*S*)
(3*S*, 5*S*)-**1c**	1 mol%	CH_2Cl_2	2.5h, RT	85	23:1 (*anti*)	98% (2*R*, 3*S*)

Conclusions

Our unique strategy to employ optically pure starting materials, enzyme Porcine Liver Esterase (PLE) and stereoselective cyclization provides access to a novel class of pyrrolidine-3-carboxylic acids that could have significant biological applications in the field of chemistry. In this work, we were able to show that PLE can be used as a valuable biocatalyst in the asymmetric hydrolysis of chiral malonate diesters providing decent diastereoselectivities. PLE isoenzyme studies showed that PLE 3 & 4, when used in hydrolysis of substrate **4c**, switch the diastereoselectivity. We were also able to demonstrate that the half-esters (2*R,* 4*S*)-**5c** and (2*S,* 4*S*)-**5c**, derived from the PLE-catalyzed hydrolysis can be used as valuable synthons that provide access to a novel class of C^{α}-methyl-β-proline analogues. Asymmetric hydrolysis of substrate **4c** by crude PLE provided the half-ester (2*R*, 4*S*)-**5c** in a d.r. of 4.6:1. This half-ester was then transformed to 5-benzyl-C^{α}-methyl-β-proline analogue, (3*R*, 5*S*)-**1c**, via a diastereoselective cyclization strategy. Substrate **4c**, when subjected to hydrolysis with PLE isoenzyme 3, provided half-ester (2*S*, 4*S*)-**5c** in a d.r. of 3.8:1 that was then transformed into 5-benzyl-C^{α}-methyl-β-proline analogue, (3*S*, 5*S*)-**1c**, via a diastereoselective cyclization. Both diastereomers of 5-benzyl-C^{α}-methyl-β-proline analogue **1c** were tested for catalytic activity in the Mannich reaction between α-imino ester and isovaleraldehyde. Designed amino acid (3*S*, 5*S*)-**1c** proved to be an efficient catalyst providing "*anti*" selective products in a d.r. of 23:1 and %ee of up to 99%.

Experimental Section

General Experimental Information

All anhydrous solvents were obtained by passage through a column of activated silica. Flash column chromatography was performed using SiliaFlash® P60 40-63µm (230-400 mesh) silica gel. NMR experiments were performed on a Bruker 400 MHz NMR instrument and chemical shifts were reported with reference to either TMS (for $CDCl_3$) or residual solvent proton peak (for CD_3OD and D_2O). Infra-Red analysis was performed on a Thermo Nicolet nexus 470 FT-IR instrument. Melting point analysis was performed using Thomas-Hoover "Uni-Melt" Melting Point Apparatus and are uncorrected. Triflic anhydride was freshly distilled on phosphorous pentoxide under Nitrogen atmosphere before use according to the reported procedure.[16] High Resolution mass spectra were obtained using positive electrospray ionization on a Bruker 12 Tesla APEX -Qe FTICR-MS with an Apollo II ion source.

Procedure for PLE Isoenzyme reactions

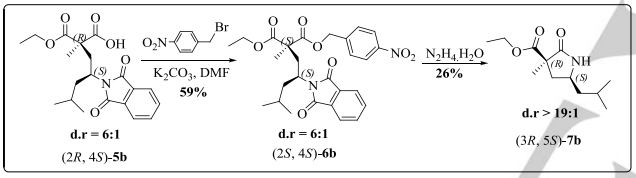
The reaction set up procedure for PLE isoenzyme reactions is similar to that of the preparation of half-ester **5a**. All the reactions were performed in 60 mL of phosphate buffer pH 7.4 and PLE isoenzyme (250 units/mmol of the diester) was added as a suspension in 3.0 M Ammonium Phosphate. For the PLE isoenzyme studies of diester **4b**, diester **4b (**0.150g, 0.37 mmol) of was dissolved in appropriate co-solvents as listed and added to the reaction buffer. For PLE isoenzyme reactions of diester substrate **4c**, diester **4c** (0.150g, 0.34 mmol) was dissolved in appropriate co-solvents and added to the reaction buffer. All the reactions were carried out at room temperature and the pH of the reaction was maintained at 7.4 with an auto burette set up that is set to add 1.0N NaOH solution as the reaction proceeded. After the reaction was completed, to the reaction was added 1.0N NaOH to bring the pH to 9.0. 40 mL of ethyl acetate was added to the above reaction buffer and a gentle extraction was performed to remove any unreacted starting material. The aqueous buffer solution was filtered through celite bed to remove the enzyme. The filtered buffer solution was taken and the pH of the solution was adjusted to 2.0 by adding 50% sulfuric acid slowly in a drop wise manner while stirring. Then the aqueous buffer solution was extracted carefully with dichloromethane (8 x 40 mL) to prevent the formation of emulsion. All the organic portions were combined, dried over MgSO4, filtered and the solvent was removed under vacuum to provide crude half-esters. ^{1}H NMR was obtained on the crude half-ester products and the diastereomeric ratios were determined. The ^{1}H NMR data of the product half-esters looked essentially pure in most cases. The yields of the product half-esters was determined from the amount of half-esters isolated after purification. Purification was performed using a flash column chromatography, 40% ethyl acetate/60% hexanes to 100% ethyl acetate (product eluted at 100% ethyl acetate). Our attempts to improve diastereomeric ratio on flash column were not successful as the diastereomers seemed to be inseparable.

Procedure for Mannich reactions

The preformed imine, **11**, **(**0.207g, 1.0 mmol) was weighed and added to a 5 mL sealed tube containing 2 mL anhydrous methylene chloride. Then appropriate amount of catalyst was weighed and added to the reaction vessel followed by 0.173 g (2.0 mmol) of isovaleraldehyde (**12**) as a solution in 2.0 mL of anhydrous methylene chloride. Reaction tube was tightly sealed and left to stir at room temperature. Reaction was monitored by TLC for the disappearance of imine. Once all the starting material had disappeared, reaction mixture was washed with saturated NaCl (1 x 5 mL). The organic portion was dried over $MgSO_4$, filtered and the solvent was removed under vacuum to afford crude product. Crude product was analyzed by ^{1}H NMR to determine diastereomeric ratio of the Mannich product. The crude product was then purified immediately by flash column chromatography (20% ethyl acetate/80% hexanes) to provide pure product. Flash column purification did not result in any improvement of the diastereomeric ratio. The % yield of the reaction was determined from the pure product isolated in the reaction. The pure product was immediately analyzed by the HPLC using chiral stationary phase to determine the percent enantiomeric excess. The data of the products was compared to the reported to the literature and determined to be consistent.[14-15, 17]

Synthesis of γ-lactam, (3*R*, 5*S*)-**7b**

Half-ester **5b** (d.r. = 6:1) from PLE hydrolysis was reacted with 4-nitro benzyl bromide to afford the ester **6b** in 59% yield (Scheme 8).



Scheme 8. Synthesis of lactam (3*R*, 5*S*)-**7b**, via stereoselective cyclization

The 4-nitro benzyl ester (2*S*, 4*S*)-**6b** was then treated with hydrazine hydrate to cause the phthalimide deprotection. This resulted in stereoselective cyclization of the amine onto the carbonyl with 4-nitro benzyl group to provide γ-lactam (3*R*, 5*S*)-**7b** (Scheme 8). There was no trace of cyclization of the amine towards the ethyl ester observed from the NMR data of the crude product. The crude material was then purified via flash column chromatography to separate the diastereomers and provide diastereopure γ-lactam (3*R*, 5*S*)-**7b** in 26% yield. The resulting lactam (3*R*, 5*S*)-**7b** was analyzed by 2D-NOESY experiments to determine the stereochemistry at the quaternary chiral center.

(S)-1-ethyl 3-(4-nitrobenzyl) 2-((S)-2-(1, 3-dioxoisoindolin-2-yl)-4-methylpentyl)-2-methylmalonate, (2*S*, 4*S*)-**6b**

(2*R*, 4*S*)-**5b** (0.160g, 0.426 mmol; d.r = 6:1) was dissolved in 4 mL anhydrous DMF in a 100 mL, three neck flask at room temperature and under N_2 atmosphere. K_2CO_3 (0.059g, 0.426 mmol) was added to the above flask and the contents were stirred for 10 minutes. Then, 4-nitrobenzyl bromide (0.092g, 0.426 mmol) was added in a dropwise manner as a solution in 2 mL anhydrous DMF to the above reaction flask. Then the reaction mixture was allowed to stir overnight at room temperature and under N_2 atmosphere. After stirring overnight, 6 mL water was added to the reaction flask and the organic contents were extracted with diethyl ether (4 x 6 mL). All organic portions were combined and washed with water (7 x 6 mL) followed by brine (3 x 6 mL). The organic portion was dried over $MgSO_4$ and the solvent was removed under vacuum to provide crude product. Crude compound was purified by flash column chromatography (10% ethyl acetate / 90% hexanes) to provide pure (2*S*, 4*S*)-**6b** **(**0.129 g, 0.253 mmol) in 59% Yield. Our attempts to resolve diastereomers, to improve diastereomeric ratio, via flash column purification were not successful at this point. (R_f = 0.38, 30% ethyl acetate / 70% hexanes). IR (cm^{-1}): 2957, 1729, 1705. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 – 8.11 (m, 2H), 7.87 – 7.66 (m, 4H), 7.39 (dd, *J* = 9.0, 2.1 Hz, 2H), 4.83 (dd, *J* = 74.9, 13.5 Hz, 2H), 4.58 – 4.42 (m, 1H), 4.07 (q, *J* = 7.1 Hz, 2H), 3.10 – 2.97 (m, 1H), 2.28 – 2.09 (m, 2H), 1.53 (d, *J* = 6.0 Hz, 3H), 1.50 – 1.34 (m, 2H), 1.12 (t, *J* = 7.1 Hz, 3H), 0.89 (dt, *J* = 12.8, 6.4 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.7, 171.3, 168.4, 167.3, 147.7, 142.5, 134.0, 128.2, 123.7, 65.4, 61.7, 52.9, 45.8, 42.5, 37.7, 25.0, 23.1, 21.8, 19.7, 13.9. HRMS ($C_{27}H_{30}N_2O_8$ Na^+) calcd = 533.189437 m/z, obsd = 533.189437 m/z.

(3R, 5S)-ethyl 5-isobutyl-3-methyl-2-oxopyrrolidine-3-carboxylate, (3*R*, 5*S*)-**7b**

(2*S*, 4*S*)-**6b** (0.120g, 0.242 mmol; d.r = 6:1) was dissolved in a solvent mixture of 4 mL methanol and 2 mL methylene chloride in a 25 mL single neck round bottom flask. 0.1 mL of hydrazine hydrate (35% in water) was added to the reaction flask and reaction mixture was heated to reflux the solvent. Reaction was monitored by TLC. After stirring for 24 hrs an additional 0.060 mL of hydrazine hydrate was added to the reaction to maintain the basic pH. Reaction was continued to stir for another 17 hrs (total reaction time of 41 hrs) and then cooled to room temperature. The white precipitate was washed with methylene chloride (5 x 6 mL) and the contents were filtered via a micro column to remove any white precipitate. The solvent was then removed under vacuum to provide crude material. The crude product was taken up in methylene chloride and the organic portion was washed with water (1 x 20 mL) followed by brine (1 x 20 mL). Organic portion was then dried over magnesium sulfate, filtered and the solvent was removed under vacuum. The resulted crude product was carefully purified via gradient flash column chromatography (45% ethyl acetate/55% hexanes to 80% ethyl acetate/ 20% hexanes). Purification via flash column provided diastereopure (3*R*, 5*S*)-**7b** **(**14 mg, 0.062 mmol) in 26% yield. The improvement in diastereopurity was confirmed from the ^{1}H NMR data analysis. 2D-NOESY experiment was performed on this diastereopure lactam and the stereochemistry at the quaternary chiral carbon was determined to be *R*. R_f = 0.11(80% ethyl acetate / 20% hexanes). $[\alpha]_D^{24}$ = +6.1 (*c* = 0.3, CH_2Cl_2).IR (cm^{-1}): 3218, 2957, 2872, 1737, 1701.^{1}H NMR (400 MHz, $CDCl_3$) δ 6.26 (s, 1H), 4.28 – 4.14 (m, 2H), 3.69 (p, *J* = 7.0 Hz 1H), 2.33 – 2.24 (m, 1H), 2.13 (dd, *J* = 12.8, 7.0 Hz, 1H), 1.64 (tt, *J* = 13.1, 6.6 Hz, 1H), 1.58 – 1.48 (m, 1H), 1.47 – 1.44 (m, 3H), 1.39 (ddd, *J* = 11.8, 7.3, 6.5 Hz, 1H), 1.32 – 1.24 (m, 3H), 0.93 (dt, *J* = 6.7, 3.3 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.4, 172.6, 61.6, 51.5, 49.6, 45.6, 40.3, 25.4, 22.8, 22.5, 20.3, 14.1. HRMS ($C_{12}H_{21}NO_3H^+$) calcd =228.159420 m/z, obsd = 228.159218 m/z.

Supporting Information

NMR spectra and HPLC data are provided in the supporting information. CCDC 1538677 and CCDC 1538678 contain the supplementary crystallographic data for this paper. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures/

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Keywords: Biocatalysis • *anti*-Mannich Catalyst • Organocatalysis • Porcine Liver Esterase • C$^{\alpha}$-Methyl-β-Proline analogue

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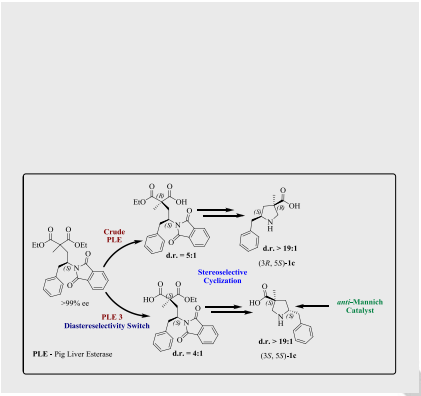
FULL PAPER

Recombinant Porcine Liver Esterase (PLE) has been used in this work to hydrolyse optically pure chiral malonate diesters and provide diastereoenriched half-esters. Crude PLE (mixture of six different isoenzymes, PLE1-6) hydrolysed the malonate diester **4c** to provide the half-ester in a *d.r.* of *5:1*. PLE isoenzyme 3 hydrolysed the diester **4c** to yield the half-ester in a *d.r.* of *4:1*. These half-esters were transformed to novel 5-benzyl Cα-methyl-β-proline analogues (3*R*, 5*S*)-**1c** and (3*S*, 5*S*)-**1c** via a stereo selective cyclization methodology. The synthesized amino acid (3*S*, 5*S*)-**1c** is proved to be an excellent catalyst in the Mannich reaction between an aldehyde and an α-imino ester providing *anti* selective products in a *d.r.* of *23:1* and *ee* of *99%*.



Key Topic*

Enzyme catalysis, Organocatalysis

Authors:

*Hari Kiran Kotapati, Jamarii D. Robinson, Daniel R. Lawrence, Kimberly R. Fortner, Caleb W. Stanford, Douglas R. Powell, Rainer Wardenga, Uwe T. Bornscheuer, Douglas S. Masterson**

Page No. – Page No.

Title:

Diastereoselective hydrolysis of branched malonate diesters by Porcine Liver Esterase: Synthesis of 5-benzyl substituted Cα-methyl-β-proline and catalytic evaluation