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Asymmetric synthesis of serinol-monoesters catalyzed by amine transaminases

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ABSTRACT

The asymmetric synthesis of serinol-derivatives was investigated employing different amine transaminases as biocatalysts. Under the optimized conditions conversions up to 92% and excellent enantiomeric excesses up to 99% ee were obtained providing access to both, the (*R*)- and (*S*)-configurations of the serinol-monoester (2-amino-3-hydroxypropyl hexanoate).

1. Introduction

Serinol (2-amino-1,3-propanediol) is an amino alcohol structurally related to the amino acid serine. It is a prochiral compound and, similar to its derivatives, has several applications in medicine and the chemical industry.[1–6] For instance, monoesters of serinol containing a long or medium chain fatty acid are chiral (Scheme 1) and can exhibit a range of pharmacological properties.[7–9] Additionally, as an amino-alcohol, it can also play an important role as a building block.[1]

Due to their importance, there is a need for efficient methods to synthesize these optically active amines. Serinol-monoesters can be synthesized by either chemical or biocatalytic methods, but only with enzymes the synthesis of chiral versions is straightforward. Compared to the various chemical methods available for the synthesis of optically active amines, such as the resolution of racemates by crystallization of diastereomeric salts or asymmetric hydrogenation, the asymmetric synthesis using amine transaminases is a very attractive option. Amine transaminases can perform the synthesis in one-step using a prochiral ketone as the starting material and the use of expensive and sometimes hazardous catalysts is not required.[10,11] Also, the asymmetric synthesis is much more economical because yields of up to 100% are achievable.[11–13]

Amine transaminases are enzymes that catalyze the transfer of an amino group from an amine donor to an amine acceptor using the cofactor pyridoxal-5′-phosphate.[12] In general, the active form of a transaminase is a homodimer where the active site is positioned at the interface between the monomers and residues from each monomer participate in the architecture of the active site. The substrate binding site of amine transaminases is defined by a large and a small binding pocket. The large pocket can accommodate a rather broad range of substituents, while the small pocket does not accept ketones with large substituents next to the carbonyl functionality.[14,15]

Much effort has been directed towards engineering transaminases in such a way that the small binding pocket can accept substituents larger than a methyl group.[16,17] The classical example of this effort is the engineering of the (*R*)-selective amine transaminase-117 for the synthesis of sitagliptin.[18] The wild-type could not convert the bulky pro-sitagliptin ketone and only after extensive protein engineering (27 mutations) could high activity be achieved. The best variant enabled a 13% increase in yield and a 19% reduction in total waste compared to the transition-metal catalyzed asymmetric hydrogenation.[19]

Our group recently succeeded in engineering (*S*)-selective amine transaminases to enable the synthesis of a range of bulky amines.[14,15,20,21] Nobili et al. performed a systematic mutagenesis study of the active site residues of the amine transaminase from *Vibrio fluvialis* to expand its substrate scope towards two bulky ketones. They were able to identify two mutants (F85L/V153A and Y150F/V153A) that showed a 30-fold increased activity in the conversion of (*S*)-phenylbutylamine and (*R*)-phenylglycinol, respectively.[14] Pavlidis et al. identified a (*S*)-transaminase (3FCR) and with just four mutations (Y59W/Y87F/Y152F/T213A), they were able to synthesize important bulky amines on a preparative scale with excellent conversion, isolated yield and enantiomeric purity. They transferred these key mutations to other fold class I transaminase scaffolds that share only a modest sequence identity to the 3FCR scaffold.[20] In another work, Genz et al. used bioinformatic tools, such as 3DM, to design a mutant library that allowed the identification of a variant of the *Vibrio fluvialis* amine

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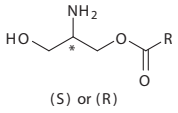


Scheme 1. Serinol-monoesters, R: –Alkyl.

transaminase (L56V/W57C/F85V/V153A) capable of catalyzing the asymmetric synthesis of 2,2-dimethyl-1-phenylpropan-1-amine, with 100% conversion and an enantiomeric excess value >99%.[15]

Due to these important examples and in order to further investigate the substrate scope of amine transaminase variants, herein we have focused on the asymmetric synthesis of serinol-monoesters using α-phenylethylamine as an amine donor (Scheme 2).

2. Results and discussion

1-Hexanoyloxy-3-hydroxyacetone **1a** was employed as model substrate. This compound was easily synthesized via esterification of 1,3-dihydroxypropan-2-one (dihydroxyacetone, DHA) with hexanoyl chloride in the presence of pyridine. A 4:1 molar ratio (DHA/hexanoyl chloride) was used in order to favor the formation of the monoester over the formation of the diester.

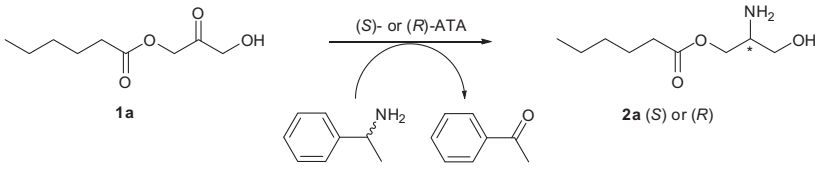
All enzymes were used as lyophilized crude lysates to minimize the cost biocatalyst production by avoiding enzyme purification.

2.1. Screening of the amine transaminases

In the first set of experiments a screening with 21 amine transaminases was employed using the acetophenone assay.[22] The amine donor α-phenylethylamine was converted into acetophenone and the initial rate of product formation was measured photometrically at 245 nm. All experiments were performed using pyruvate or pentanal as amine acceptors. Assays using the desired substrate **1a** were then used to identify active variants for the asymmetric synthesis of the serinol-monoesters.

The initial reaction conditions were chosen based on previous studies.[14,15,23] The reactions were performed in HEPES buffer (50 mM, pH 7.5), supplemented with pyridoxal-5′-phosphate (0.1 mM), at 30 °C using 2.5 mM α-phenylethylamine and 2.5 mM of amine acceptor. For transaminase reactions, the use of organic co-solvents is known to improve enzymatic performance.[24] For this reason, dimethyl sulfoxide (DMSO) was used at a final concentration of 10% v/v to increase the solubility of the substrates, but without exceeding the maximum concentration tolerated by the enzymes.

Using the acetophenone assay (Table 1), it was observed that all enzymes were active towards the amine acceptors pyruvate or pentanal, except for variant L56V/W57F/F85V/V153G/R415C (entry 18). This variant only accepted pentanal due to a mutation of the flipping arginine (R415).[15,23] The screening for activity towards the target ketone **1a** showed that most of the variants



Scheme 2. Asymmetric synthesis of serinol-monoesters.

Table 1
Screening of amine transaminases using the acetophenone assay

Entry	Variant	Activity[a] (U mL^{-1})		
		Pyruvate	Pentanal	**1a**
1	*Vibrio fluvialis* wild-type	1.87 ± 0.09	0.91 ± 0.01	N.A.
2	*Vibrio fluvialis* F85L	0.11 ± 0.01	0.11 ± 0.01	N.A.
3	*Vibrio fluvialis* Y150F	0.50 ± 0.01	0.26 ± 0.08	N.A.
4	*Vibrio fluvialis* Y150M	0.34 ± 0.01	0.03 ± 0.00	N.A.
5	*Vibrio fluvialis* V153A	0.89 ± 0.01	0.56 ± 0.01	N.A.
6	*Vibrio fluvialis* F85L/Y150F	0.27 ± 0.03	0.31 ± 0.00	0.02 ± 0.00
7	*Vibrio fluvialis* F85L/Y150M	0.38 ± 0.00	0.08 ± 0.00	N.A.
8	*Vibrio fluvialis* F85L/V153A	0.63 ± 0.02	0.40 ± 0.02	0.08 ± 0.00
9	*Vibrio fluvialis* F19C	0.09 ± 0.01	0.04 ± 0.00	0.02 ± 0.00
10	*Vibrio fluvialis* F19V	0.08 ± 0.01	0.05 ± 0.00	0.02 ± 0.00
11	*Vibrio fluvialis* F19Y	0.39 ± 0.02	0.18 ± 0.01	0.08 ± 0.00
12	*Vibrio fluvialis* Y150M/V153A	0.19 ± 0.01	0.01 ± 0.00	N.A.
13	*Vibrio fluvialis* F85L/Y150M/V153A	0.02 ± 0.00	0.00 ± 0.01	N.A.
14	*Vibrio fluvialis* L56V/W57F/F85V	0.09 ± 0.00	0.05 ± 0.00	N.A.
15	*Vibrio fluvialis* L56V/W57C	0.34 ± 0.00	0.12 ± 0.00	N.A.
16	*Vibrio fluvialis* L56V/W57C/F85V	0.80 ± 0.08	0.23 ± 0.01	N.A.
17	*Vibrio fluvialis* L56V/W57C/F85V/V153A	0.20 ± 0.01	0.04 ± 0.00	N.A
18	*Vibrio fluvialis* L56V/W57F/F85V/V153G/R415C	N.A.	0.54 ± 0.01	N.A.
19	*Vibrio fluvialis* L56V/W57C/V153A	0.98 ± 0.01	0.17 ± 0.01	N.A.
20	Aspfum wild-type	1.68 ± 0.10	0.80 ± 0.01	0.06 ± 0.01
21	Amine transaminase 117	3.43 ± 0.02	0.81 ± 0.01	0.03 ± 0.00

N.A.: not active or below the detection limit.

[a] Acetophenone assay conditions: HEPES buffer (50 mM, pH 7.5) and 0.1 mM pyridoxal-5′-phosphate, and 10 μL of enzyme solution (10 mg mL^{-1}), DMSO 10%, 2.5 mM racemic α-phenylethylamine and 2.5 mM amine acceptor, 200 μL final reaction volume and 30 °C. The production of acetophenone was followed at 245 nm. The activities were calculated as U mL^{-1} of enzyme solution. One unit is defined as the formation of 1 μmol product per minute. Values and standard deviations given are based on three measurements. For these activity tests α-phenylethylamine was used with pyruvate (or pentanal) as acceptors. For the asymmetric synthesis of serinol-monoesters, **1a** served as amine acceptor and α-phenylethylamine as amine donor.

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Table 2
Asymmetric synthesis of serinol-monoester using **1a** as amine acceptor and α-phenylethylamine as amine-donor

Entry	Variant	Conversion (%)[a]	ee[a] (%)
1	*Vibrio fluvialis* F85L/Y150F	34	93 (*S*)
2	*Vibrio fluvialis* F85L/V153A	47	92 (*S*)
3	*Vibrio fluvialis* F19C	29	95 (*S*)
4	*Vibrio fluvialis* F19V	30	96 (*S*)
5	*Vibrio fluvialis* F19Y	36	81 (*S*)
6	AspFum wild-type	41	>99 (*R*)
7	Amine transaminase 117	27	>99 (*R*)

Asymmetric synthesis conditions: 5 mM racemic α-phenylethylamine and 5 mM **1a** in 50 mM HEPES buffer (pH 7.5) containing 1 mM pyridoxal-5′-phosphate, 10% DMSO at 30 °C, 100 μL enzyme solution (10 mg/mL of lyophilized enzyme). Reaction time was 72 h.
[a] Conversion and % ee-values were determined by chiral HPLC.

had little to no activity towards this compound. However, it was possible to identify seven hits: *Vibrio fluvialis* F85L/Y150F, *Vibrio fluvialis* F85L/V153A, *Vibrio fluvialis* F19C, *Vibrio fluvialis* F19V, *Vibrio fluvialis* F19Y, amine transaminase 117 and *Aspergillus fumigatus* wild-type.

These hits were used in the asymmetric synthesis and the results are shown in Table 2. After 72 h, the variants *Vibrio fluvialis* F85L/V153 and *Aspergillus fumigatus* wild-type showed the best conversion and highest enantiomeric excess and so these enzymes were chosen for further studies.

Having selected the most adequate biocatalysts, we optimized the asymmetric synthesis process. The transamination catalyzed by amine transaminases suffers from equilibrium issues, i.e., the equilibrium does not favor the formation of the desired products and hence we used a 20-fold excess of α-phenylethylamine to overcome this problem. This strategy was chosen because it is easy to conduct and the only limitations are co-substrate solubility and possible enzymatic inhibition.[12] In fact the procedure allowed us to reach up to 83% conversion within 72 h for both enzymes (Table 3).

Finally, in order to demonstrate the applicability of the biocatalytic concept developed, the reaction was performed on a preparative scale and this resulted in similar conversions. The products (*S*)-**2a** and (*R*)-**2a** were isolated in good yield (Table 4).

3. Conclusions

In conclusion, several variants of amine transaminases catalyzed the asymmetric reductive amination of ketone **1a**. This method opens up a new route to access optically active serinol-monoesters as demonstrated for (*S*)-2-amino-3-hydroxypropyl hexanoate and (*R*)-2-amino-3-hydroxypropyl hexanoate. Among the various enzymes tested, *Aspergillus fumigatus* wild-type afforded the corresponding (*R*)-amine in a conversion of 93% with excellent enantiomeric excess (>99% ee). The (*S*)-enantiomer was obtained with 92% conversion and 92% ee when the *Vibrio fluvialis* F85L/V153A variant was used. In this way, attractive building blocks have been obtained while overcoming the limitations of a classical chemical synthesis. Furthermore, the reactions were performed on a preparative scale affording the desired amines in enantiomerically pure form and good isolated yields.

Table 4
Preparative scale asymmetric synthesis

Transaminase	Product	Conversion[a] (%)	Yield[b] (%)
Vibrio fluvialis F85L/V153A	(*S*)-**2a**	92	54
Aspfum wild-type	(*R*)-**2a**	93	37

The reactions were conducted using 100 mM of the amine donor and 5 mM **1a** in 50 mM HEPES buffer (pH 7.5) containing 1 mM pyridoxal-5′-phosphate, 10% DMSO at 30 °C using 100 mg lyophilized enzymes for 3 days.
[a] Conversion was determined by measurements of acetophenone by HPLC.
[b] Isolated product.

4. Experimental

4.1. General

All chemicals were purchased from commercial suppliers and used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance II 300 MHz. The infrared analysis was performed on a IR Affinity-1 Fourier Transform Infrared Spectrometer from Shimadzu. Biocatalysis reactions were performed on an Eppendorf® Comfort Thermomixer shaker and preparative scale reactions were performed in an orbital incubator shaker (Infors HT Unitron).

4.2. Synthesis of 1-hexanoyloxy-3-hydroxyacetone 1a

Substrate **1a** was synthesized according to the following procedure.[25] In a 500 mL three-neck flask fitted with a thermometer, stirring bar and a 100 mL addition funnel, were added 56 mmol dihydroxyacetone and 27.75 mL dry pyridine. Next 14 mmol hexanoyl chloride was dissolved in 27.75 mL chloroform (freshly distilled over P_2O_5), added dropwise over a period of 2.5 h while maintaining a temperature of −6 to −2 °C. After complete addition, the reaction mixture was allowed to warm to 25 °C and then allowed to stir for 1 h at this temperature. Finally, 0.5 mL of water was used to stop the reaction. To a 500 mL three-neck flask fitted with a thermometer, stirring bar and a 100 mL addition funnel was added 25 mL of 3 M HCl and 50 g ice. This mixture was cooled to −10 °C. Then 50 mL 6 M HCl and the pyridine-chloroform reaction mixture were added slowly, at the same rate, over a period of 2 h while maintaining a temperature of −5 to −2 °C. The chloroform phase was separated and the aqueous phase was washed with 2 × 10 mL chloroform. The chloroform fractions were

Table 3
Effect of the concentration of amine donor in the enzymatic transamination of ketone **1a**

Entry	Transaminase	α-Phenylethylamine (mM)	Conversion[a] (%)		
			24 h	48 h	72 h
1	*Vibrio fluvialis* F85L/V153A	5	12	29	45
2		50	20	33	53
3		100	45	50	83
4	AspFum wild-type	5	9	23	50
5		50	33	49	66
6		100	52	62	84

The reactions were conducted at different concentrations of the amine donor using 5 mM **1a** in 50 mM HEPES buffer (pH 7.5) containing 1 mM pyridoxal-5′-phosphate, 10% DMSO at 30 °C with 100 μL of enzyme solution (10 mg/mL of lyophilized enzyme).
[a] Conversion was determined by measurements of formed acetophenone by HPLC.

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combined and dried over anhydrous sodium sulfate. The chloroform solution was filtered and the solvent was removed by evaporation under reduced pressure. The residual oil was distilled under vacuum (fraction boiling at 114–116 °C) and the purified compound was characterized by NMR and IR. **1a** (oily liquid, 50% yield), ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) = CH_3 0.86 (3H); CH_2 1.27 (4H); CH_2 1.52 (2H); CH_2 2.18 (2H); CH_2 4.54 (2H); CH_2 4.85 (2H). ^{13}C NMR (DMSO-d_6; 300 MHz) δ (ppm) = CH_3 13.79; CH_2 21.14; CH_2 24.14; CH_2 30.74; CH_2 33.60; CH_2 65.62; CH_2 65.62; C=O 174.45. FT-IR (neat): 3400, 2958, 2901, 2662, 1710, 1465, 1380, 1246, 1111, 619 cm^{-1}.

4.3. Synthesis of the racemic mixture of 2-amino-3-hydroxypropyl hexanoate 2a

A racemic mixture of the serinol-monoester **2a** was prepared by chemical reductive amination. Ammonium acetate (4.50 mmol, 0.350 g) and sodium cyanoborohydride (1.50 mmol, 0.094 g) were added to a solution of dihydroxyacetone-monoester **1a** (0.38 mmol, 0.071 g) in anhydrous methanol (1.1 mL). The reaction mixture was then stirred for 24 h at room temperature. The solvent was evaporated and the remaining residue was suspended in 0.5 M NaOH (6 mL) and extracted with ethyl acetate (3 × 2 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The crude amine was purified by column chromatography (CH_2Cl_2/MeOH 90:10). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) = CH_3 0.90 (3H); CH_2 1.29 (2H); 1.31 (2H); CH_2 1.63 (2H); NH_2 2.0 (2H); CH_2 2.32 (2H); CH 3.15 (H); OH 3.63 (H); CH_2 3.74 (2H); CH_2 4.22 (2H).

4.4. Preparation of enzymes

The amine transaminases used herein include the variants of the *Vibrio fluvialis* enzyme described by Nobili et al. [14] and Genz et al.,[15] *Vibrio fluvialis* wild-type and the (*R*)-amine transaminases: *Aspergillus fumigatus* wild-type (AspFum)[11] and amine transaminase-117[18] from Codexis (Table 5).

The enzymes were expressed as previously described.[14,15] The crude lysates obtained were frozen overnight and lyophilized for 24 h. The solid obtained from each variant was maintained in the fridge until use.

4.5. Acetophenone assay

For each lyophilized enzyme a 10 mg/mL solution in HEPES buffer (50 mM, pH 7.5) was prepared and 10 μL of this solution were used to determine the activity. The activity assay was performed in 96 well plates at the following conditions: HEPES buffer (50 mM, pH 7.5) and 0.1 mM pyridoxal-5′-phosphate, DMSO 10%, 2.5 mM of α-phenylethylamine and 2.5 mM amine acceptor (pyruvate, pentanal or **1 a**), 200 μL final reaction volume and 30 °C. The production of acetophenone was followed at 245 nm, as described by Schätzle et al.[22] The activities were calculated as U/mL enzyme solution. One unit is defined as the formation of 1 μmol product per minute. Values and standard deviations given are based on three measurements.

Table 5
Variants of *Vibrio fluvialis* amine transaminases used herein

Entry	Mutations	Entry	Mutations
1	F85L[a]	10	F19Y[a]
2	Y150F[a]	11	Y150M/V153A[a]
3	Y150M[a]	12	F85L/Y150M/V153A[a]
4	V153A[a]	13	L56V/W57F/F85V[a]
5	F85L/Y150F[a]	14	L56V/W57C[b]
6	F85L/Y150M[a]	15	L56V/W57C/F85V[b]
7	F85L/V153A[a]	16	L56V/W57C/F85V/V153A[b]
8	F19C[a]	17	L56V/W57F/F85V/V153G/R415C[b]
9	F19V[a]	18	L56V/W57C/V153A[b]

[a] Variant described by Nobili et al. [14].
[b] Variant described by Genz et al. [15].

4.6. Biocatalysis (analytical scale)

The biocatalysis reactions had a final volume of 1.0 mL: 100 μL enzyme solution, 5 mM **1a** and 5 mM α-phenylethylamine were dissolved in HEPES buffer (50 mM, pH 7.5) including 1 mM pyridoxal-5′-phosphate and 10% DMSO. The reaction was performed at 30 °C and 600 rpm. For the analysis of the reaction, a sample of the reaction mixture was taken (200 μL) and treated with a saturated solution of Na_2CO_3 (300 μL). The extraction of substrates and products was performed with ethyl acetate (3 × 200 μL) after mixing (30 s). Separation of the two phases was obtained via centrifugation (2 min, 13,000 rpm). The organic phase was transferred into a clean Eppendorf tube and dried over $MgSO_4$. The solution was mixed again and centrifuged for the removal of the $MgSO_4$.

4.7. Analytical methods

4.7.1. TLC

The reactions were monitored by thin-layer chromatography. The mobile phase used was dichloromethane/methanol (9:1). The spots were identified by UV light (254 nm). Amines were stained using ninhydrin and for staining the ketones, 2,4-dinitrophenylhydrazine was used. The retention factors (R_f) found were: α-phenylethylamine (R_f = 0.29), **2a** (R_f = 0.51); **1a** (R_f = 0.67) and acetophenone (R_f = 0.88).

4.7.2. HPLC

Determination of the conversion and enantiomeric excess was performed by HPLC analysis.[26] 400 μL of an extracted sample (in ethyl acetate) was placed in an Eppendorf tube. The ethyl acetate was evaporated using a continuous flow of nitrogen. Then the sample was diluted in 1 mL 2-propanol (HPLC grade), 10 mg 9-anthraldehyde and 60 mg $MgSO_4$ were added. This mixture was stirred at 600 rpm at room temperature for 12 h. After this time, the supernatant was separated by centrifugation (2 min, 13000 rpm) and the sample was directly injected into the HPLC. The separation of the chiral amines as 9-anthraldimine derivatives using a coated covalently bonded polysaccharide-derived chiral column was achieved. Chromatography was performed at room temperature using the LaChrom Elite HPLC system, with automatic injector, UV detector at 254 nm and a Lux Cellulose-1 column (250 mm L × 4.6 mm I.D., 5 μm). The volume injected was 10 μL. The mobile phase and the flow were optimized. The best conditions for the separation were 10% 2-propanol in hexane (v/v) and a flow rate of 0.3 mL/min. Under these conditions the retention times (t_R) were: acetophenone: 17.2 min; **1a**: 19.5 min; (*S*)-**2a**: 20.5 min; (*R*)-**2a**: 22.5 min; (*S*)-α-phenylethylamine: 25.9 min and (*R*)-α-phenylethylamine: 28.2 min, see also Figure 1.

4.8. Preparative scale

The volume of the reactions was 100 mL and 5.3 mM of 1-hexanoyloxy-3-hydroxyacetone **1a**, were used. The reaction mixtures contained HEPES buffer (50 mM, pH 7.5), pyridoxal-5′-phosphate (1 mM), DMSO (10% v/v) and 100 mM α-phenylethylamine. The reactions were performed in a shaker at 30 °C, 180 rpm and were monitored by TLC. After 72 h, the reactions were acidified with HCl to pH 2.0 to precipitate protein, which was removed by centrifugation. Acetophenone and **1a** were extracted with ethyl acetate. The pH was then increased to 10 by the addition of

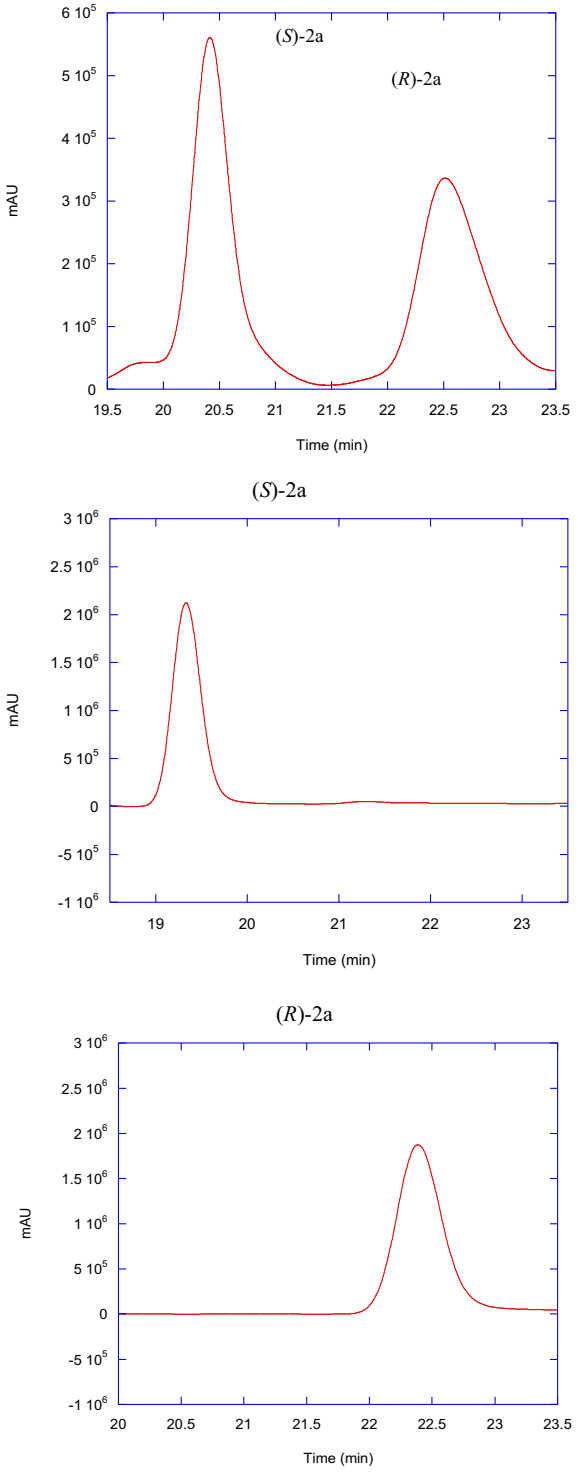

Figure 1. HPLC analysis of the product **2a** from the asymmetric synthesis: (a) racemic mixture of **2a**; (b) product (*S*)-**2a** obtained from the reaction catalyzed by the (*S*)-selective amine transaminase and (c) product (*R*)-**2a** obtained from the reaction catalyzed by the (*R*)-selective amine transaminase.

Na_2CO_3. The product and α-phenylethylamine were extracted with ethyl acetate. The organic phase was transferred to a clean flask and dried over $MgSO_4$. The solution was centrifuged for the removal of the $MgSO_4$ and the solvent was evaporated by a rotary evaporator. The purification of the product **2a** was performed by preparative TLC. The desired band (containing the product) was scraped from the TLC plate and extracted from the silica with ethyl acetate and after filtration, the solvent was removed by evaporation. The purified products obtained were characterized by NMR.

(*S*)-2-Amino-3-hydroxypropyl hexanoate **2a**: ^{1}H NMR (CD_3OD, 300 MHz) δ (ppm) = CH_3 0.90 (3H); CH_2 1.29 (2H); CH_2 1.31 (2H); CH_2 1.63 (2H), NH_2 2.0 (2H); CH_2 2.32 (2H), CH 3.30 (1H), CH_2 3.67 (2H); CH_2 4.15 (2H). ^{13}C NMR (CD_3OD; 300 MHz) δ (ppm) = CH_3 14.90; CH_2 21.04; CH_2 30.74; CH_2 33.9; CH 52.4 CH_2 67.10; C═O 173.45.

(*R*)-2-Amino-3-hydroxypropyl hexanoate **2a**: ^{1}H NMR (CD_3OD, 300 MHz) δ (ppm) = CH_3 0.90 (3H); CH_2 1.29 (2H); CH_2 1.31 (2H); CH_2 1.63 (2H), NH_2 2.0 (2H); CH_2 2.32 (2H), CH 3.30 (1H), CH_2 3.67 (2H); CH_2 4.15 (2H). ^{13}C NMR (CD_3OD; 300 MHz) δ (ppm) = CH_3 14.90; CH_2 21.04; CH_2 30.74; CH_2 33.9; CH 52.4 CH_2 67.10; C═O 173.45.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetasy.2017.08.012.

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