Julia Blesl, BSc

Chemoenzymatic synthesis of α-quaternary stereogenic α-amino acids

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Gewidmet meiner Familie

Abstract

The formation or cleavage of a carbon-carbon bond is an attractive tool in organic chemistry. Aldolases are a very important group of enzymes for the formation of an asymmetric carbon-carbon bond and most of them catalyze the stereoselective aldol reaction of a ketone to an aldehyde.

Disubstituted β-hydroxy-α-amino acids with a quaternary α-carbon center are interesting building blocks for pharmaceutically relevant compounds.

This thesis presents the synthesis of a diverse set of α-quaternary β-hydroxy-α-amino acids via enzymatic aldol addition. The reaction between DL-alanine as donor and different acceptor aldehydes is catalyzed by threonine aldolase. On the one hand L-threonine aldolase from *Aeromonas jandaei* is used and on the other hand the stereocomplementary form, D-threonine aldolase from *Pseudomonas* sp., is utilized. Moreover, pyridoxal 5'-phosphate (PLP) is needed as cofactor.

A large variety of aromatic and aliphatic aldehydes are converted to the corresponding α-quaternary β-hydroxy-α-amino acids. In this aldol addition two new stereogenic centers are formed with perfect enantiospecifity at the α-carbon (>99 % ee).

Moreover, a strategy for the removal of the β-hydroxy-group is envisaged, because the stereoconfiguration is not very well defined at the β-carbon. This removal is done in two steps. In the first step the hydroxyl-group is converted to chlorine and in the second step the removal of the chlorine-group take place.

Kurzfassung

Die Bildung und Spaltung von Kohlenstoff-Kohlenstoff Bindungen ist ein vielseitig genutztes Werkzeug in der Organischen Chemie. Aldolasen sind für die Bildung von asymmetrischen Kohlenstoff-Kohlenstoff Bindungen eine wichtige Gruppe von Enzymen. Viele dieser Aldolasen katalysieren die stereoselektive Aldol Reaktion eines Ketons zu einem Aldehyd.

Disubstituierte β-Hydroxy-α-aminosäuren mit einem quaternären α-Kohlenstoff Zentrum sind interessante Bausteine für pharmazeutisch relevante Verbindungen.

In dieser Arbeit wird die Synthese von diversen β-Hydroxy-α-aminosäuren mit einem quaternären α-Kohlenstoff-Zentrum vorgestellt. Die Synthese erfolgt über eine enzymatische Aldol-Addition, ausgehend von DL-Alanin als Donor und verschiedenen Aldehyden als Akzeptor. Die Reaktion wird durch Threonine Aldolasen katalysiert. Einerseits wir L-Threonine Aldolase von *Aeromonas jandaei* verwendet und andererseits wird die stereokomplementäre Form, D-Threonine Aldolase von *Pseudomonas* sp., verwendet. Pyridoxal-5-phosphat (PLP) wird als Kofaktor benötigt.

Eine Vielzahl aromatischer als auch aliphatischer Aldehyde wird in die entsprechende α-quaternäre β-Hydroxy-α-aminosäure überführt. Bei dieser Aldol-Addition werden zwei neue stereogene Zentren gebildet und die Enantiospezifität am α-Kohlenstoff ist nahezu perfekt (>99 % ee).

Darüberhinaus wird ein Konzept zur Entfernung der β-Hydroxy-Gruppe von β-Hydroxy-α-aminosäuren vorgestellt, da die Stereokonfiguration am β-Zentrum nicht gut definiert ist. Dies erfolgt in zwei Schritten: im ersten Schritt wird die Hydroxy-Gruppe in ein Chlor überführt, im zweiten Schritt erfolgt die Entfernung des Chlors.

Table of contents

1. Introduction

The formation or cleavage of carbon-carbon bonds is a central effort in Organic Synthesis, with the aim to synthesize larger and more complex compounds starting from small and simple starting materials. For the formation of a C-C bond often tedious and time-consuming methods are used. In addition, the control of stereospecificity of organic chemical reactions remains challenging.[1]

Nature supplies diverse enzymes for the formation or breaking of C-C bonds. In the biosynthesis of many carbohydrates several C-C coupling steps are involved. For example in these reactions aldolases, transaldolases and transketolases are involved. In recent years these enzymes have received more and more attention of synthetic chemists.[1,2]

Enzymes are useful catalysts in organic chemistry. The use of enzymes has several advantages in contrast to conventional chemical reactions. One of the main benefits is the ability to perform reactions in a stereoselective manner. Additionally, they are regiospecific and therefore the necessity of protecting groups can be reduced to a minimum. Next the reactions can be carried out under mild conditions. Most enzymes work at room temperature in aqueous solution (pH around 7). Moreover enzymes are environmentally acceptable, because they are biodegradable and the use of toxic solvents and heavy metal catalysts is not necessary. Because of the high regio- and stereoselectivity and the catalytic efficiency enzymes are very useful for the synthesis of complex and highly functionalized molecules such as carbohydrates.[1,3]

One of the most powerful tools for the generation of enantiopure multifunctional molecules via the carbon-carbon bond formation is the aldol reaction. The most important group of enzymes for the formation of an asymmetric C-C bond are aldolases. They catalyze the reversible aldol reaction between a donor (nucleophile) and an acceptor (electrophile). Aldolases accept often only few compounds as donor, but a broad range of aldehydes as acceptors are possible. In most cases the enzyme controls the stereochemistry at this newly formed center. Therefore aldolases are very attractive for the synthesis of chiral complex, bioactive compounds like amino acids.[1,2,4,5]

2. Theoretical background

2.1 C-C bond-forming lyases

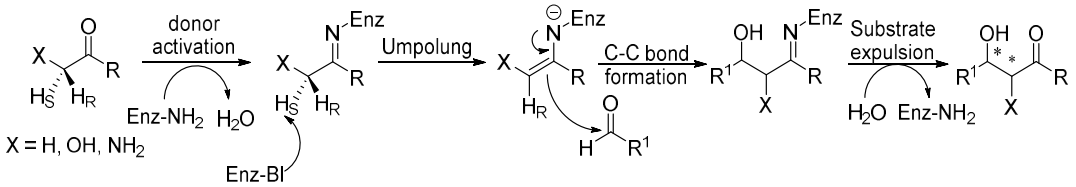
Lyases are enzymes that catalyze the formation or breaking of a bond. Such enzymes require only one substrate in one direction, but two substrates are needed for the reverse reaction.[6] In the last years more and more interesting intermediates and products were synthesized with lyase-catalyzed reactions.[1,4,7]

Lyases are classified as EC 4. They are subclassified in seven classes. The subdivision depends on the bond type which is involved. The most important and abundant subclass is carbon-carbon lyases (EC 4.1.x.x). This subclass has four subcategories: carboxy lyases (EC 4.1.1.x), aldehyde lyases (EC 4.1.2.x), oxoacid lyases (EC 4.1.3.x), and other carbon-carbon lyases (EC 4.1.99.x).[6]

For the formation of a C-C bond primary aldolases, transferases (such as transaldolases and transketolases), thiamine diphosphate-dependent enzymes, and oxynitrilases are utilized. For these enzymes, aldehydes are the most widely used group for the formation of an asymmetric C-C bond. Aldolases are actually utilized in bio-organic chemistry.[1]

2.2 Aldolases

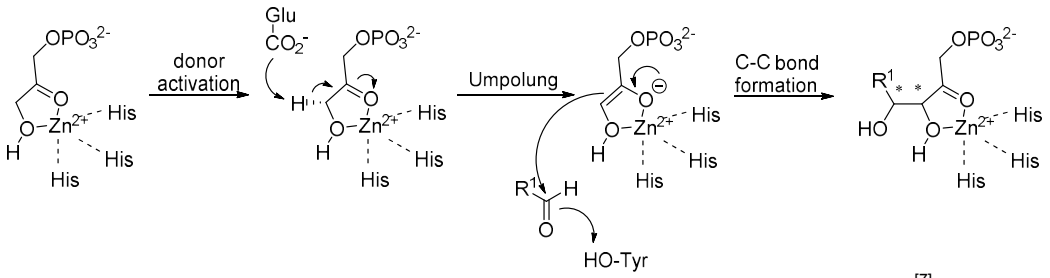
Type I aldolases are located mainly in higher plants and animals and need no metal cofactor. They catalyze the aldol reaction through formation of a Schiff base as an intermediate. In the first step the donor is covalently linked to the ε-NH_2 group of a lysine residue. Then the activated donor forms an enamine species with the acceptor aldehyde, which leads to abstraction of H_S. This enamine species attacks the carbonyl group of the acceptor. Two new stereogenic centers are formed. Depending on the enzyme the configuration is stereospecifically formed either in a *threo*- or *erythro*-configuration (Scheme 1).[1,6,7]



Scheme 1. Mechanism of type I aldolases (*: newly formed sterocenters)[7]

Type II aldolases are found in bacteria and fungi and contain a Zn^{2+} cofactor in the active site. Zn^{2+} is coordinated to three nitrogen atoms of histidin residues. The Zn^{2+} atom binds

the donor via the hydroxyl and carbonyl groups. This allows the removal of the *pro-R* (H_R) proton, forming an enolate. The enolate reacts in a nucleophilic attack with the aldehydic acceptor (Scheme 2).[1,6,7]



Scheme 2. Mechanism of type II aldolases (*: newly formed sterocenters)[7]

Aldolases are classified in four groups corresponding to the structure of the donor molecule. There are four possible donors: Dihydroxyacetone phosphate (DHAP), pyruvate (or phosphoenolpyruvate), glycine, and acetaldehyde. Group I is the best studied group and uses dihydroxyacetone or its phosphate as donor. After the reaction with an aldehyde as acceptor a ketose 1-phosphate is generated. The second group uses pyruvate or phosphoenolpyruvate as donor. The product is 3-deoxy-2-keto acid. The third group requires acetaldehyde and gives 2-deoxyaldehydes. Finally, group IV aldolases employ glycine as donor and react with an aldehyde to an α-amino-β-hydroxy acid (Figure 1).[1,6,7]

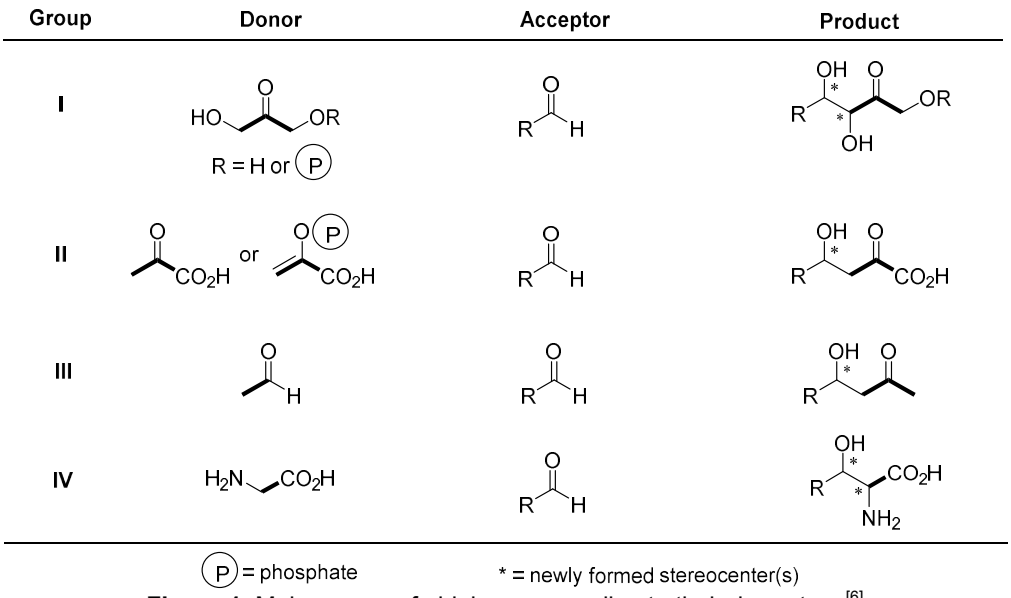


Figure 1. Main groups of aldolases according to their donor type[6]

Group II and group III aldolases produce α-methylene carbonyl compounds and hence only a single stereocenter is generated. Otherwise aldolases of the first and the fourth

group yield α-substituted carbonyl products and therefore two sterocenters at the newly formed C-C bond are generated.[6,7]

2.2.1 Dihydroxyacetone phosphate- (DHAP) and dihydroxyacetone- (DHA) dependent aldolases

Dihydroxyacetone phosphate- (DHAP) and dihydroxyacetone- (DHA) dependent aldolases are the best studied group. This group contains several enzymes and accepts a large range of substrates. For example unhindered aliphatic aldehydes, α-heteroatom-substituted aldehydes and monosaccharides are possible as acceptors.[3] Several known DHAP-dependent aldolases are of interest for Organic Synthesis and some of them are shown in Figure 2.[6,7]

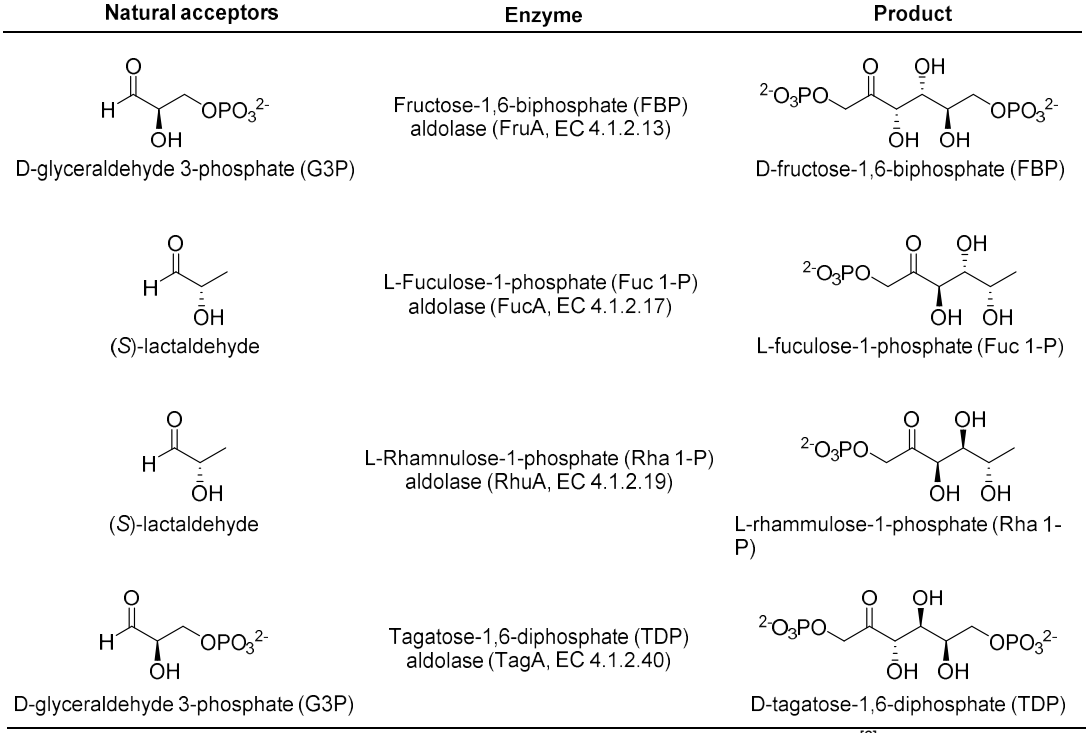


Figure 2. Dihydroxyacetone phosphate-dependent aldolases[6]

Fructose-1,6-disphosphate (FDP) aldolases are the best studied enzymes in this group. Type I and type II enzymes are known and these enzymes have been isolated from several mammalian and microbial sources. For example enzymes from rabbit muscle (RAMA), which are type I enzymes, have been used very often for synthetic purposes. Tagatose-1,6-diphosphate (TDP) aldolase is another type I aldolase in this group. These aldolases are nessesary in the galactose metabolism of cocci. Some examples of type II aldolases are for example the enzyme from *E. coli*, Fuc 1-P aldolase and Rha 1-P.[3, 7]

In the C-C bond formation with DHAP-dependent aldolases two new stereocenters are generated. The stereochemical configuration can be controlled by choosing one of four sterocomplementary DHAP aldolases, which allows the synthesis of four different stereoisomeric diol products. In each aldol reaction one single product is formed. The stereochemistry of this product is at C-3 and C-4 complementary to each other. These enzymes accept many acceptors with a high stereocontrol at C-3. In contrast, the configuration of the stereocenter at C-4 depends on the acceptor.[6,7]

2.2.2 Pyruvate- and phosphoenolpyruvate-dependent aldolases

In vivo pyruvate-dependent aldolases have catabolic functions, whereas their counterparts (= phosphoenolpyruvate-dependent aldolases) use phosphoenolpyruvate as donor. These enzymes are involved in the biosynthesis of keto acids. If the equilibrium is shifted towards the condensation product both types of enzymes can be used in Organic Synthesis. Enzymes, which employ phosphoenolpyruvate, shift the equilibrium through irreversible release of inorganic phosphate. However, these enzymes have been displaced from the original classification as lyases (EC 4.x.x.x) to the transferases group (EC 2.x.x.x). The group of pyruvate-dependent aldolases includes several enzymes, which are shown in Figure 3.[3,6]

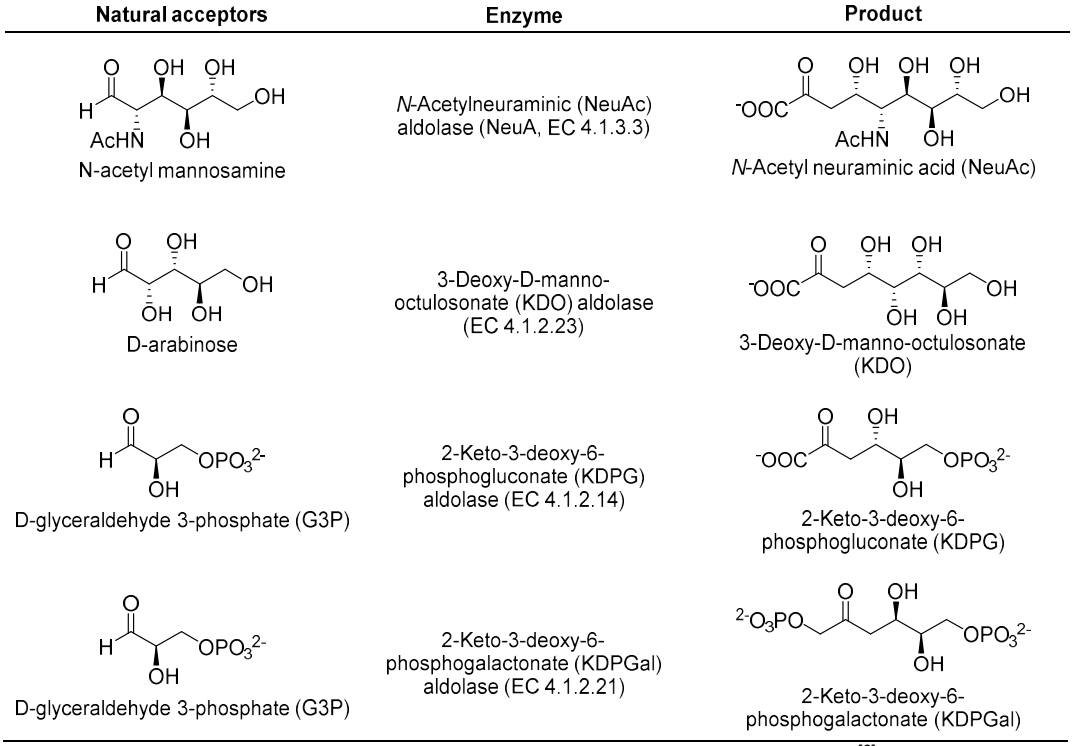


Figure 3. Pyruvate-dependent aldolases of synthetic interest[6]

2.2.3 Acetaldehyde-dependent aldolases

In the group of acetaldehyde-dependent aldolases only the enzyme 2-deoxyribose-5-phosphate aldolase (DERA, EC 4.1.2.4) is known to accept acetaldehyde as donor. DERA catalyzes the reversible aldol reaction of acetaldehyde and D-glyceraldehyde 3-phosphate (D-G3P). In this reaction 2-deoxyribose-5-phosphate is generated (Figure 4). The DERA enzyme is special because it uses an aldehyde rather than a ketone as the natural donor. But it is not unique because fructose-6-phosphate aldolase (FSA) tolerates different acceptors. For example it uses hydroxylated aldehydes with 2 or 3 carbon atoms. [1,3,7]

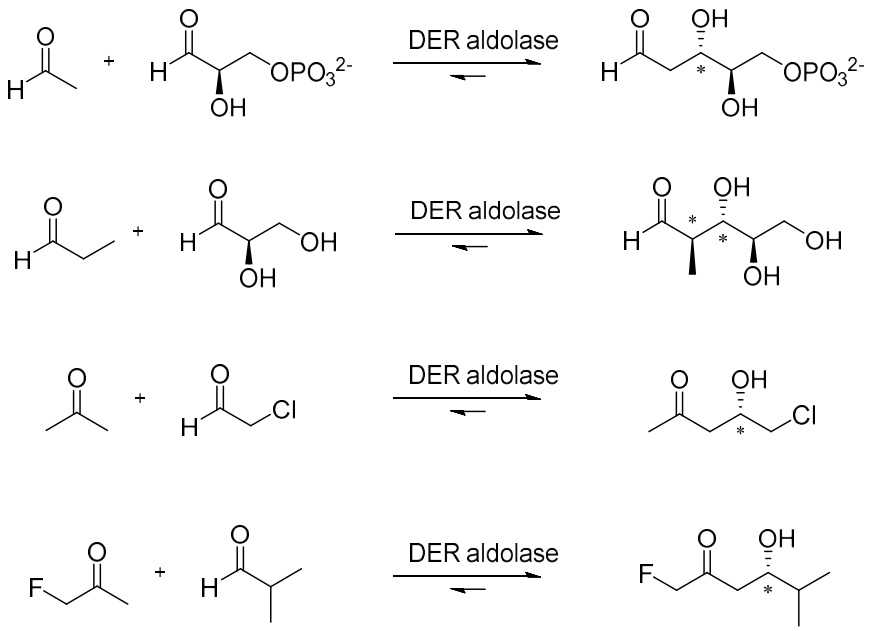
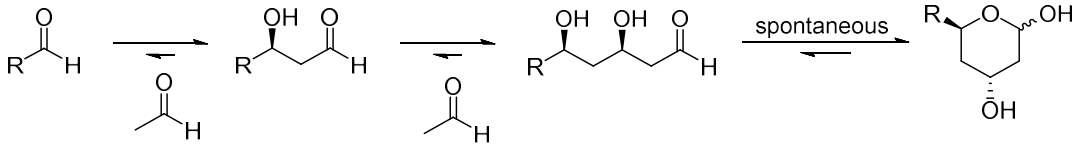


Figure 4. Aldol reactions catalyzed by 2-deoxyribose-5-phosphate aldolase (DERA)[7]

DERA is of acceptable stability and a type I aldolase. This aldolase has been isolated from animal tissues and microorganisms[8] and is commercially available.[3]

DERA is also able to accept other aldehydes not only acetaldehyde. For example also acetone, fluoroacetone and propionaldehyde are accepted as donor but are converted at a much slower rate. A broad range of acceptor substrates are tolerated. The enzyme accepts unhindered aliphatic aldehydes, α-heteroatom-substituted aldehydes and polyhydroxyaldehydes. 2-Hydroxyaldehydes for example are good acceptor molecules. In this reaction the D-isomer is preferred over the L-isomer. The enzyme defines the stereoconfiguration of the newly formed chiral center. In general the preferred configuration of the product is the *S*-configuration.[3]

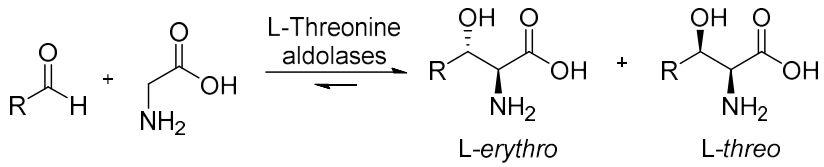
Another characteristic of this aldolase group is the fact that the substrate as well as the product is an aldehyde. The sequential aldol reaction performed in a one-pot reaction has been discovered for 2-deoxyribose-5-phosphate aldolase itself (Scheme 3).[9] Starting from an aldehyde as acceptor, first a condensation of acetaldehyde to the corresponding β-hydroxy aldehyde as intermediate takes place. Then the β-hydroxy aldehyde can react with another acetaldehyde in a second aldol reaction, forming a β,δ-dihydroxy aldehyde. The driving force of the reaction is the formation of a stable hemiacetal. This formation avoids further condensation because the hemiacetal is not tolerated as acceptor.[1,3,7]



Scheme 3. Sequential aldol reactions catalyzed by DERA[7]

2.2.4 Glycine-dependent aldolases

The glycine-dependent aldolases are the fourth group of aldolases. They catalyze the reversible aldol reaction of glycine with an aldehyde as acceptor to yield β-hydroxy-α-amino acids (Scheme 4).[10] These enzymes work with pyridoxal phosphate as cofactor and two types of glycine-dependent aldolases are known.[3]



Scheme 4. Aldol reactions catalyzed by L-threonine aldoase[7]

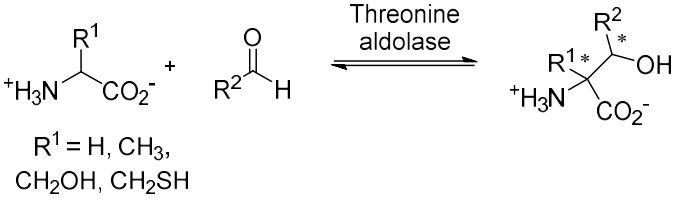
The first type is the serine hydroxymethyltransferase (SHMT, EC 2.1.2.1). It catalyzes in vivo the reversible aldol reaction to generate L-serine. The reaction starts from glycine and formaldehyde with tetrahydrofolate (THF) as a cofactor. But there are several other accepted aldehydes for which THF is not required.[1,3,7]

The second group is the threonine aldolase. These enzymes catalyze the reversible aldol reaction between glycine and acetaldehyde. In this reaction the product threonine is generated. D-Threonine aldolases as well as L-threonine aldolases are known.[1,3,7]

2.2.4.1 Threonine aldolases

Threonine aldolases (TA) catalyze the reversible cleavage of threonine into glycine and acetaldehyde. On the other hand threonine aldolases represent a powerful tool for

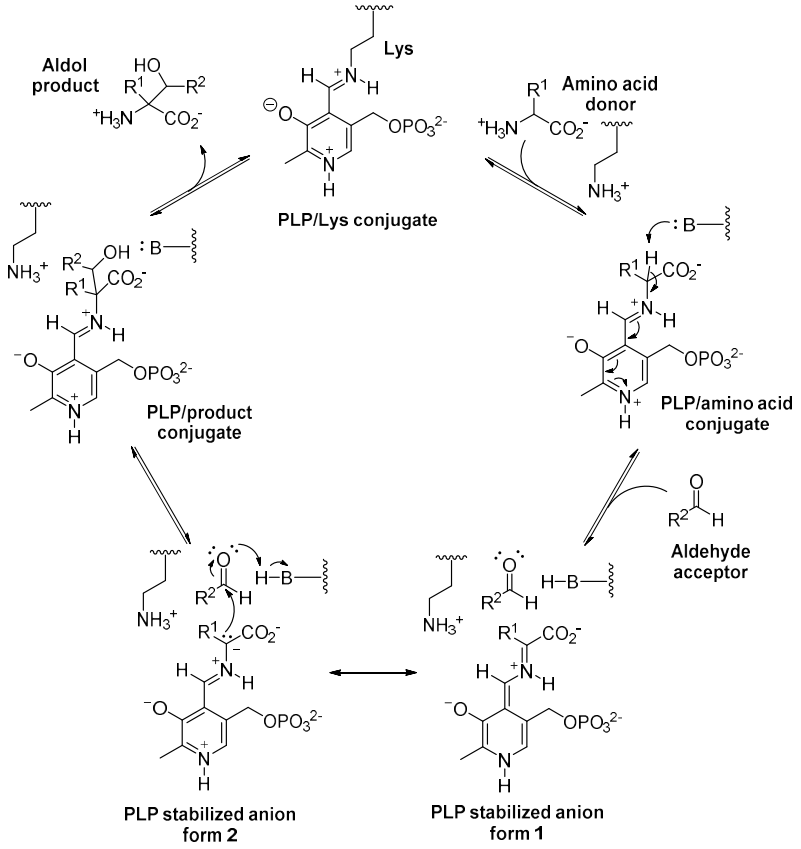
catalyzing carbon-carbon bond formation in organic chemistry. In the reverse reaction, the aldol addition, threonine derivatives can be formed from an amino acid (principally glycine) with different acceptor aldehydes. In this catalytic step two new stereogenic centers are formed (Scheme 5). Typically, threonine aldolases show very good stereoselectivity at the α-carbon, but the selectivity at the β-carbon is moderate.[11,12,13]



Scheme 5. Aldol reactions catalyzed by threonine aldoase[11]

2.2.4.1.1. Mechanism of threonine aldolases

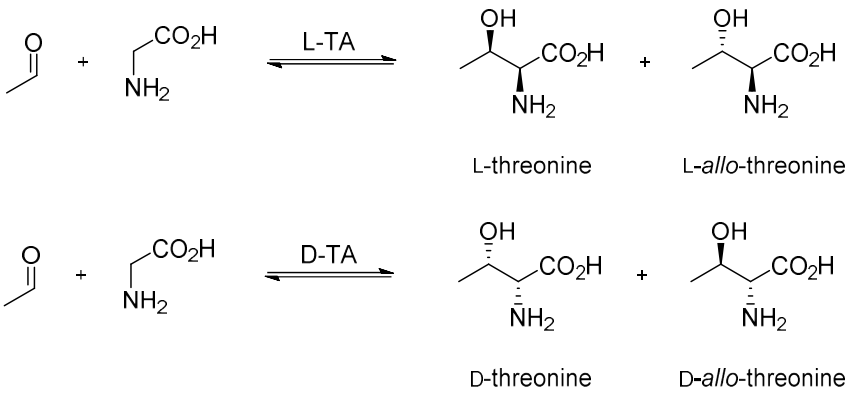
In contrast to the above mentioned aldolases threonine aldolases are pyridoxal-5-phosphate-dependent (PLP) enzymes and follow therefore a fundamentally different pathway (Scheme 6).[11]



Scheme 6. Mechanism of threonine aldolase[11]

First a Schiff base is generated between the amino group of the substrate and PLP. A cationic pyridinium ring is formed and this pyridinium ring facilitates deprotonation at the α-carbon of the imine. The accrued anion, which is highly resonance-stabilized, forms with the aldehyde acceptor a C-C bond yielding a Schiff base complex between the aldol product and the PLP cofactor. To complete the cycle the cofactor is transferred from the product back to the active site Lys side chain.[11]

Threonine aldolases are divided into two groups based on their specificity at the α-carbon: L- and D-specific threonine aldolases (Scheme 7). The L-specific threonine aldolases can be split into three subclasses: L-threonine aldolases (LTA), L-*allo*-threonine aldolases (L*allo*TA) and L-*low specificity* threonine aldolases (L*low*TA). LTA especially cleaves L-threonine (L-Thr), L*allo*TA cleaves L-*allo*-threonine (L-*allo*-Thr) and L*low*TA accepts L-Thr as well as L-*allo*-Thr as substrate. Concerning D-specific threonine aldolases only D-*low specificity* threonine aldolase (DTA) could be found in nature until now.[13]



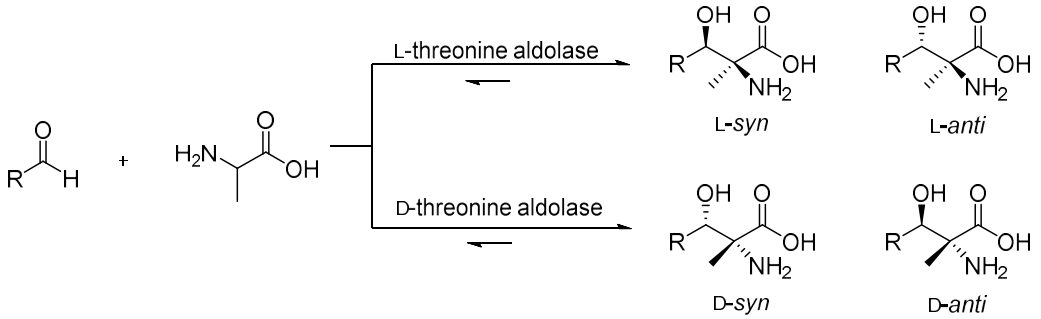
Scheme 7. Threonine aldolases catalyze addition reactions of L- and D-threonine[13]

3. Aims of this thesis

α,α-Disubstituted β-hydroxy-α-amino acids are interesting building blocks for pharmaceutically relevant compounds. Previous work from the GRIENGL group established a threonine aldolase-catalyzed approach to α,α-disubstituted β-hydroxy-α-amino acids with a quaternary α-carbon center.[14,15]

A drawback of classic organic synthesis is the use of chiral auxiliaries to establish the quaternary center. For example VISWAMBHARAN *et. al.* described an asymmetric aldol reaction by memory of chirality (MOC) to synthesize β-hydroxy α-amino acids in three steps. [16] In our synthesis β-hydroxy-α-amino acids are synthesized in only one step.

The objective of this thesis was to synthesize α-quaternary stereogenic β-hydroxy-α-amino acids. Therefore an aldol addition catalyzed by threonine aldolases was used (Scheme 8).

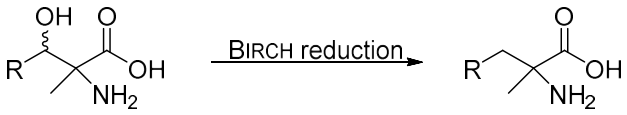


Scheme 8. Synthesis of β-hydroxy-α-amino acids

In this reaction alanine should serve as donor and through screening experiments various aldehydes should be tested to gain information about their acceptor qualities. The scope of acceptors should be as versatile as possible. Therefore aromatic aldehydes with different substituents (e. g. electron withdrawing groups versus electron donating groups) and aliphatic aldehydes should be tested. This reaction should result in the formation of two new stereocenters in one step.

For the characterization of the synthesized β-hydroxy-α-amino acids NMR and HPLC will be used for structure determination.

Another task was to remove the hydroxy function from the isolated β-hydroxy-α-amino acids since the stereochemistry at the β-carbon is not very well defined. For removing the hydroxy function BIRCH-reduction would be the preferred method (Scheme 9).

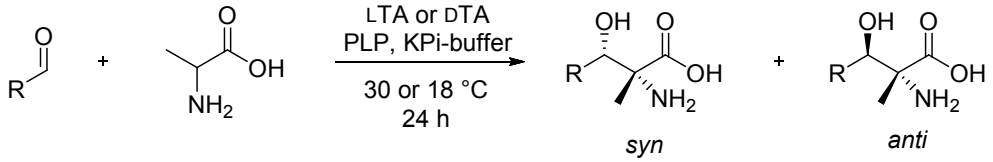


Scheme 9. Birch reduction of a β-hydroxy-α-amino acid

4. Results and discussion

4.1 Screening of different aldehyde compounds

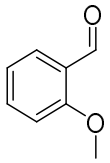
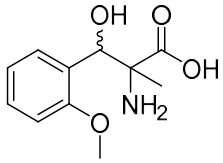
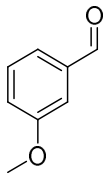
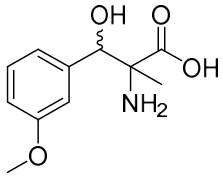
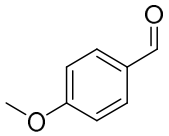
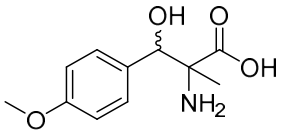
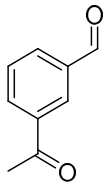
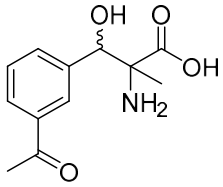
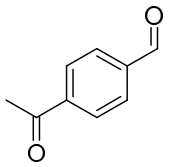
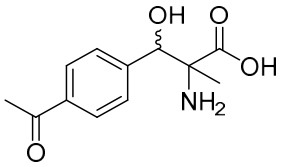
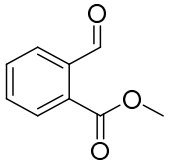
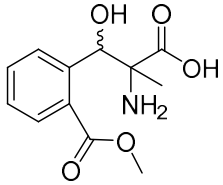
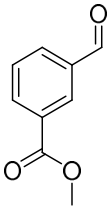
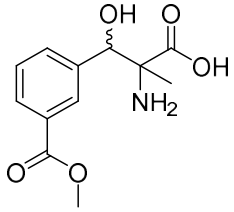
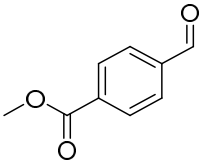
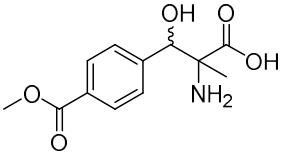
For the enzymatic aldol addition the most suitable substrates found are aromatic aldehydes bearing electron-withdrawing (EWG) groups. These EWG-groups increase the electrophilic reactivity of the aldehyde.[13,17] Therefore we screened several different aldehyde compounds as acceptors. For the screenings, the reactions were performed with alanine and glycine as donors to compare the results. In Scheme 10, the general enzymatic aldol addition with DL-alanine is shown.

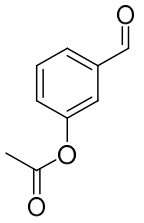
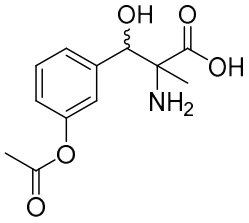
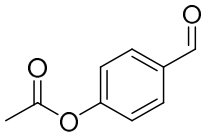
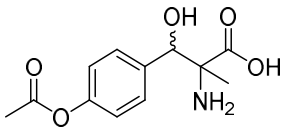
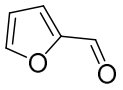
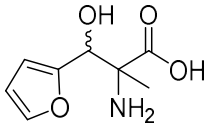
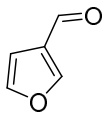
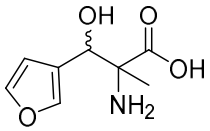
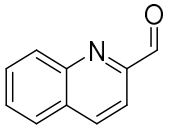
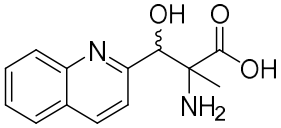
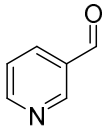
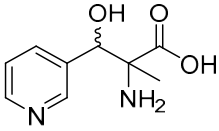
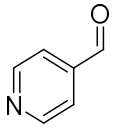
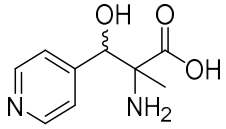
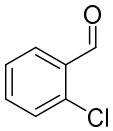
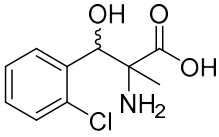
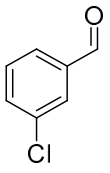
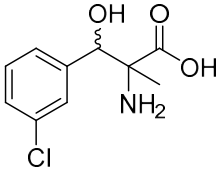
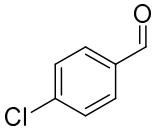
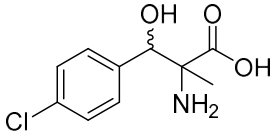


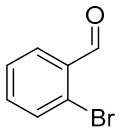
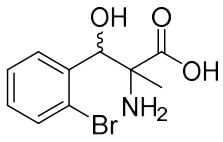
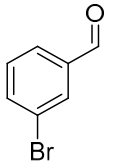
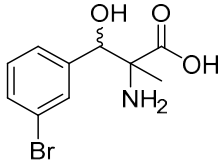
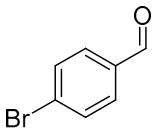
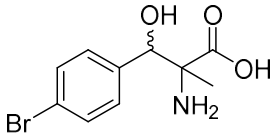
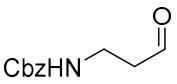
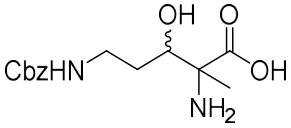
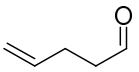
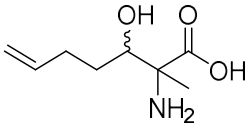
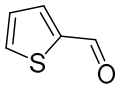
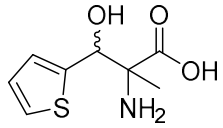
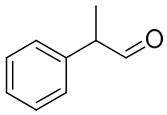
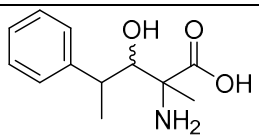
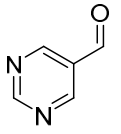
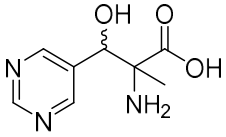
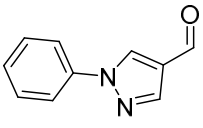
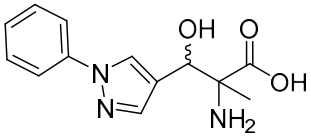
Scheme 10. Enzymatic aldol reaction with **DL**-alanine

The screening reactions were carried out at 1 mL scale in the presence of aqueous potassium phosphate buffer (pH = 8.0) and pyridoxal 5'-phosphate (PLP) was used as cofactor. Due to the limited solubility of the aldehydes in water, a cosolvent was used to partly overcome this limitation. Moreover, better conversions were obtained when solid aldehydes were first dissolved in a suitable cosolvent. DMSO proved to be as a good cosolvent, because of the high solubility of all aldehydes in it. Furthermore, a large excess of donor (alanine or glycine) was used. This is required to shift the reaction equilibrium towards the aldol products. Therefore, a 20-fold excess of donor (1000 mM of donor, 50 mM of acceptor) were used in all cases. As enzymes, LTA from *Aeromonas jandaei* (310 U/mL) or DTA from *Pseudomonas* species (190 U/mL) were used. The reaction mixtures were shaken at 850 rpm in an Eppendorf thermomixer for 24 h at 30 °C for LTA and 18 °C for DTA. After 24 h, 50 µL of the reaction mixture were diluted with 450 µL 0.3 M HCl/MeOH (1:1). Afterwards, the mixture was centrifuged for 3 min at 13,000 rpm to remove particulate matter. All reactions were monitored by analytical HPLC to evaluate the conversion and stereoselectivity. In Table 1, the results of all tested combinations of aldehydes with DL-alanine are shown.

Table 1. Results of threonine aldolase-catalyzed reactions of selected aldehydes with DL-alanine

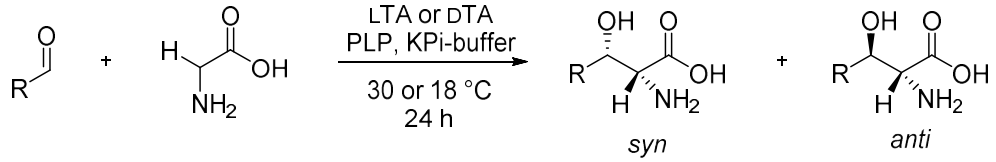
	aldehyde	product	LTA		DTA	
			conv.*	d.e.*	conv.*	d.e.*
1			< 1 %	-	-	-
2			3 %	-	-	-
3			< 1 %	-	-	-
4			74 %	14 %	8 %	70 %
5			74 %	9 %	2 %	28 %
6			15 %	-	< 1 %	-
7			53 %	16 %	2 %	-
8			44 %	6 %	< 1 %	-

9			1 %	-	< 1 %	-
10			n. f.	-	n. f.	-
11			n. f.	-	n. f.	-
12			n. f.	-	n. f.	-
13			60 %	4 %	-	-
14			n. f.	-	n. f.	-
15			n. f.	-	n. f.	-
16			34 %	47 %	4 %	-
17			28 %	17 %	4 %	84 %
18			24 %	15 %	< 1 %	-

19			4 %	20 %	< 1 %	-
20			5 %	21 %	< 1 %	-
21			15 %	15 %	< 1 %	-
22			17 %	6 %	66 %	31 %
23			n. f.	-	n. f.	-
24			n. f.	-	n. f.	-
25			24 %	-	11 %	-
26			n. f.	-	n. f.	-
27			3 %	63 %	10 %	8 %

* Monitored by reversed-phase HPLC, n. f. = product not found

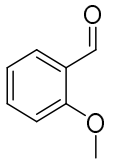
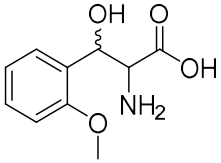
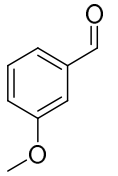
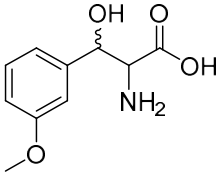
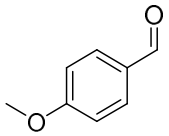
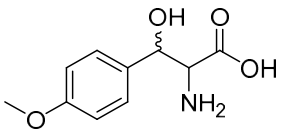
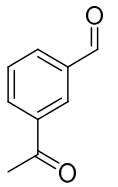
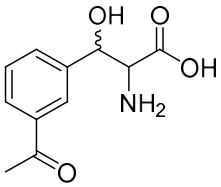
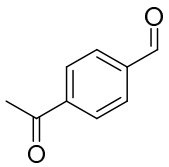
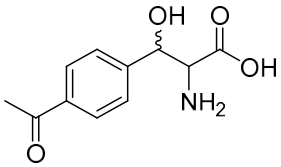
For the aldol addition with glycine, the same protocol as for alanine was used. In Scheme 11, the enzymatic aldol addition reaction with glycine is shown.

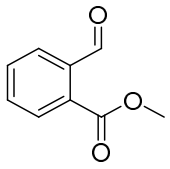
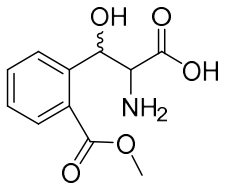
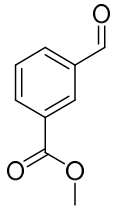
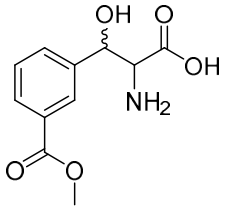
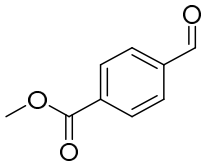
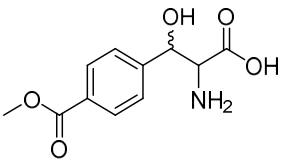
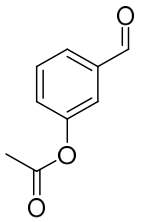
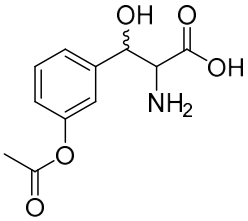
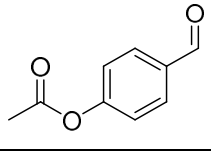
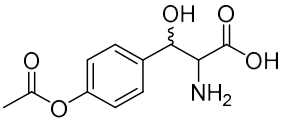
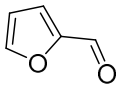
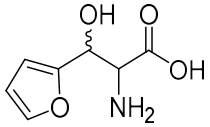
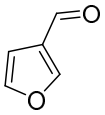
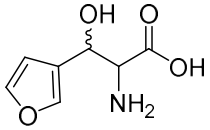
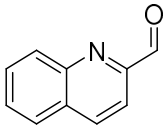
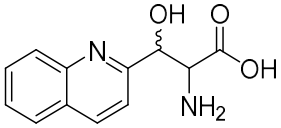
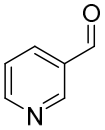
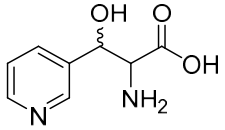


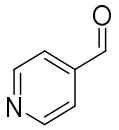
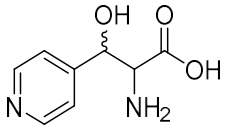
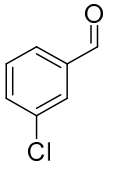
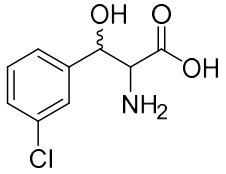
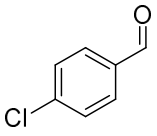
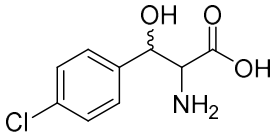
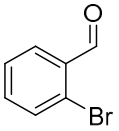
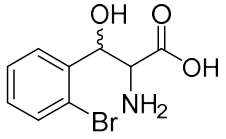
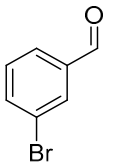
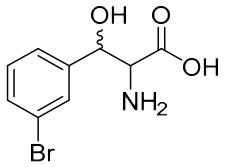
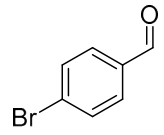
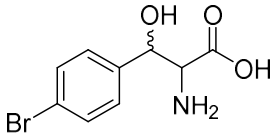
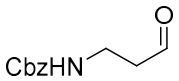
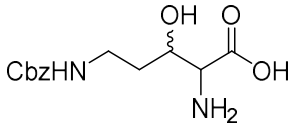
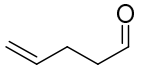
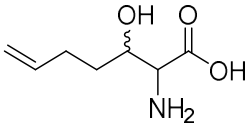
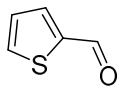
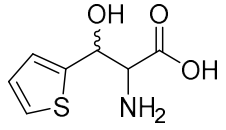
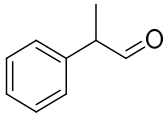
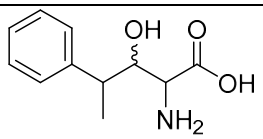
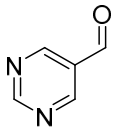
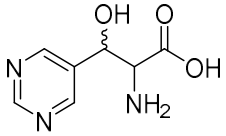
Scheme 11. Enzymatic aldol reaction with glycine

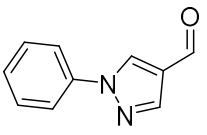
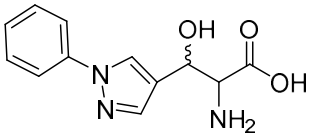
Table 2 shows the tested aldehydes with the conversions and d.e.s obtained in the aldol reaction with glycine and the two stereocomplementary enzymes.

Table 2. Results of threonine aldolase-catalyzed reactions of selected aldehydes with glycine

	aldehyde	product	LTA		DTA	
			conv.*	d.e.*	conv.*	d.e.*
1			11 %	-	-	-
2			11 %	-	-	-
3			< 1 %	-	-	-
4			90 %	-	92 %	-
5			85 %	-	92 %	-

6			94 %	43 %	82 %	27 %
7			69 %	32 %	79 %	26 %
8			87 %	26 %	92 %	24 %
9			< 1 %	-	22 %	33 %
10			n. f.	-	n. f.	-
11			n. f.	-	n. f.	-
12			n. f.	-	n. f.	-
13			96 %	26 %	-	-
14			n. f.	-	n. f.	-

15			n. f.	-	n. f.	-
16			64 %	24 %	69 %	21 %
17			64 %	17 %	71 %	23 %
18			5 %	25 %	6 %	25 %
19			7 %	24 %	6 %	21 %
20			54 %	16 %	50 %	20 %
21			40 %	-	63 %	-
22			n. f.	-	n. f.	-
23			n. f.	-	n. f.	-
24			75 %	-	78 %	-
25			n. f.	-	n. f.	-

26			18 %	37 %	33 %	34 %

* Monitored by reversed-phase HPLC, n. f. = product not found

First benzaldehydes with a methoxy-group were used for the screening experiments. In the reaction with glycine and 2-methoxybenzaldehyde or 3-methoxybenzaldehyde a conversion of 11 % was reached (Table 2, Entries 1 – 3). In the reaction with alanine only conversions up to 3 % were obtained (Table 1, Enteris 1 – 3).

Next aromatic aldehydes with a ketone function or an ester function were tested. The reactions with glycine worked quiet well. Conversions of 69 – 94 % were reached in the reactions catalyzed by L-threonine aldolase (Table 2, Entries 4 – 8). The aldol addition with alanine as donor worked also fine. In these reactions catalyzed by LTA conversions of 74 % with 3-acetylbenzaldehyde and 4-acetylbenzaldehyde were achieved (Table 1, Entries 4 – 5). The reactions with methylformylbenzoate as acceptor worked not as good as the aldol addition with acetylbenzaldehyde. Conversions of 15 – 53 % were obtained (Table 1, Entries 6 – 8). The best conversion (53 %) was reached with 3-methylformylbenzoate (Table 1, Entry 7). Only 15 % conversion to the desired product was achieved in the reaction with 2-methylformylbenzoate (Table 1, Entry 6). However, another unknown product was formed in this reaction.

Other good acceptors for the aldol addition are halogenated aromatic aldehydes. Therefore chlorobenzaldehydes and bromobenzaldehdes served as acceptors. In reactions with glycine as well as in reactions with alanine chlorobenzaldehydes are better acceptors for the aldol addition (Table 1, Entries 16 – 21; Table 2, Entries 16 - 20). Only low conversions were achieved in the reactions with bromobenzaldehydes (Table 1, Entries 19 – 21; Table 2, Entries 18 - 20). One exception is the reaction with glycine and 4-bromobenzaldeyde. In this case a conversion of 54 % was obtained (Table 2, Entry 20).

Moreover, good conversions were achieved with benzyl (3-oxopropyl)carbamate as acceptor and glycine as well as alanine as donor. Almost a quantitative conversion was obtained, but only 17 % conversion to the desired product in the reaction with alanine catalyzed by LTA (Table 1, Entry 21). In the reaction catalyzed by DTA better conversion (66 %) was achieved (Table 1, Entry 21). However, in these reactions side reactions like dimerization took place.

In conclusion, the reactions with glycine worked typically much better than those with alanine as donor. One of the reasons for this is the higher steric demand of the methyl-group in alanine which acts to some extent as a barrier for the aldol addition. Another

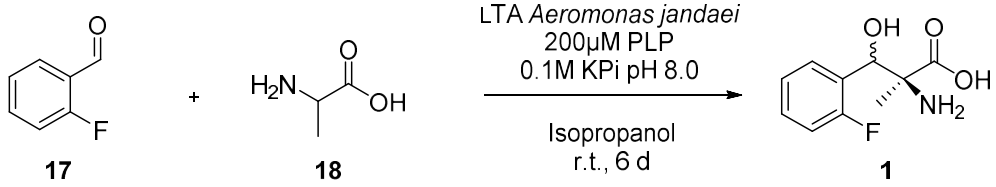
minor reason is the higher effective concentration of glycine in the system to promote the reactions, as only the D-configured part of DL-alanine can be used by the enzymes.

4.2 Preparative scale reactions with halogenated aromatic aldehydes

Good substrates for the aldol condensation are aromatic aldehydes with electron-withdrawing (EWG) groups, as mentioned before. Therefore halogenated aromatic aldehydes were used as starting materials.

4.2.1 Synthesis of (2S)-2-amino-3-(2-fluorophenyl)-3-hydroxy-2-methyl-propanoic acid (1)

(2*S*)-2-Amino-3-(2-fluorophenyl)-3-hydroxy-2-methylpropanoic acid (**1**) was synthesized in an aldol addition of 2-fluorobenzaldehyde (**17**) with DL-alanine (**18**), which was used as a donor in excess. The reaction was catalyzed by L-threonine aldolase from *Aeromonas jandaei* (Scheme 12).



Scheme 12. Synthesis of (2*S*)-2-amino-3-(2-fluorophenyl)-3-hydroxy-2-methylpropanoic acid (**1**)

First an excess of alanine (**18**) had to be dissolved completely in aqueous 100 mM KPi-buffer (pH = 8.0). The large excess (6-fold) of donor was necessary to shift the reaction equilibrium towards the aldol products. Then the cofactor pyridoxal 5'-phosphate (PLP) and the cosolvent isopropanol were added and the reaction solution stirred for 15 min. In this case 10 vol% of cosolvent was added. It is not beneficial to use too much cosolvent, because adverse effects on the enzymes can be expected. However, up to 20 vol% of isopropanol or DMSO was found to be acceptable for both enzymes used.

To possibly improve the performance of the reaction, only parts of the aldehyde and enzyme were added at the beginning. The aldehyde **17** was added in portions. Therefore, one portion (350 µL) of the aldehyde **17** was added immediately. After 3 h an additional amount of 350 µL 2-fluorobenzaldehyde (**17**) was added. The third portion of aldehyde **17** was added after 2 h. 2.5 mL LTA (310 U/mL) were also added immediately. After 24 h fresh enzyme LTA (1.5 mL) was added to compensate for the loss of activity. The

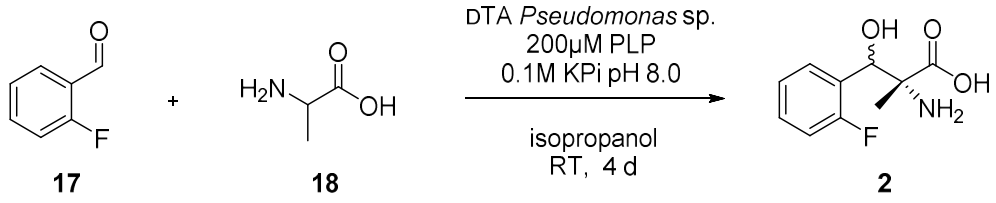
aldehyde and the enzyme were added in portions, because the presence of too much aldehyde can harm the enzyme as well. Moreover the aldehyde had to be added not too fast. As soon as the enzyme was added stirring had to be turned back, because too much stirring is not benefical for the enzyme too.

After 6 d the reaction reached the equilibrium. Therefore, the reaction was stopped by adding 250 mL MeOH to the reaction mixture. The formed precipitate did not contain any product and was filtered off. After concentrating the filtrate under reduced pressure, the product was purified via normal-phase flash chromatography. To avoid losing any product, all chromatography fractions were carefully monitored via TLC and the product **1** was isolated in 20 % yield.

One explanation for the gap between the lower isolated yield and the higher estimated conversion is a consequence of the methods used. As the conversions are determined by HPLC methods, different absorption coefficients of the analytes at the analysis wavelength (210 nm) lead to over- or underestimation of concentrations in the samples.

4.2.2 Synthesis of (2*R*)-2-amino-3-(2-fluorophenyl)-3-hydroxy-2-methyl-propanoic acid (2)

To obtain the stereoisomer of compound **1**, the aldol condensation for the synthesis of (2*R*)-2-amino-3-(2-fluorophenyl)-3-hydroxy-2-methylpropanoic acid (**2**) was done with 2-fluorobenzaldehyde (**17**) as acceptor and alanine (**18**) as donor in the presence of a different enzyme. In this case, the stereocomplementary D-threonine aldolase from *Pseudomonas* sp. was used (Scheme 13).



Scheme 13. Synthesis of (2*R*)-2-amino-3-(2-fluorophenyl)-3-hydroxy-2-methylpropanoic acid (2)

The protocol for this reaction is similar to the one for the synthesis of (2*S*)-2-amino-3-(2-fluorophenyl)-3-hydroxy-2-methylpropanoic acid (**1**). First DL-alanine (**18**) had to be dissolved completely in the buffer. Also in that case an excess of DL-alanine (10-fold) was necessary. Then the cofactor pyridoxal-5-phosphate (PLP) was added and the reaction solution stirred for 15 min. In the case of DTA from *Pseudomonas* sp., the reaction had to be carried out at 17 - 20 °C to enable a better performance. Next, 10 vol% of isopropanol

were added as cosolvent (3 mL). Moreover $MnCl_2$ was added, as this additive had been shown to be beneficial for the performance of the reaction.

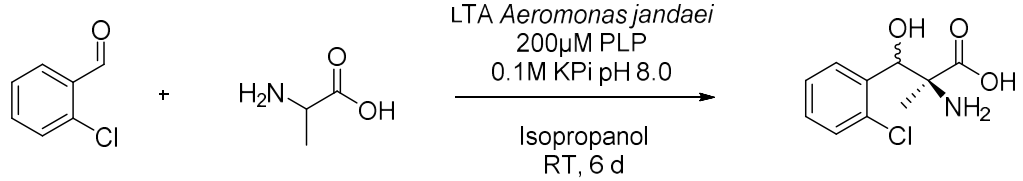
The aldehyde **17** was added in 3 portions to keep the possible inhibition because of too much aldehyde low. 80 µL aldehyde **17** were added immediately, the second portion (80 µL) after 4 h and the last 156 µL were added after 24 h. 0.5 ml DTA (190 U/mL) were added immediately and after 24 h 0.5 mL fresh enzyme were added to the reaction mixture to compensate for the loss of activity.

After 4 d a maximum of conversion was reached and the reaction was stopped by heating the reaction mixture to 80 °C for 2 h. The resulting precipitate was filtrated and washed with water + 0.01 % formic acid (3 x 15 mL) to ensure no loss of the product. After removing the solvent under reduced pressure the product was purified via reversed-phase flash chromatography using C18-modified silica gel and H_2O/MeOH mixtures as eluent. The advantage of C18-modified silica gel is that mainly water is used as solvent and the column can be regenerated after use with acetonitrile and methanol. The column can be used several times without significant loss of performance.

The desired product **2** was isolated in 27 % yield. Compared to the synthesis of (2*S*)-2-amino-3-(2-fluorophenyl)-3-hydroxy-2-methylpropanoic acid (**1**), the yield obtained with the D-threonine aldolase is higher. Also the diastereomeric ratio (d.r.) is much better. Whereas for the reaction with LTA a d.r. of 3.5 (*syn*) was obtained, the one with DTA topped this by far by reaching a d.r. of >100 (*syn*). This result supports the fact that DTAs are frequently more diastereoselective than their LTA counterparts.

4.2.3 Synthesis of (2*S*)-2-amino-3-(2-chlorophenyl)-3-hydroxy-2-methyl-propanoic acid (3)

2-Chlorobenzaldehyde (**19**) was selected for the synthesis of (2*S*)-2-amino-3-(2-chlorophenyl)-3-hydroxy-2-methyl-propanoic acid (**3**) as this aldehyde showed the best conversions in the screening experiments with 2-, 3- or 4-substituted chloro- and bromobenzaldehydes as acceptors (Scheme 14).



19 **18** **3**

Scheme 14. Synthesis of (2*S*)-2-amino-3-(2-chlorophenyl)-3-hydroxy-2-methyl-propanoic acid (**3**)

The reaction was carried out in an aqueous buffer at pH 8.00, which contained a 10-fold excess of alanine (**18**) and the cofactor PLP. The reaction solution was stirred for 30 min until the whole amount of alanine was dissolved.

2-Chlorobenzaldehyde (**19**) was dissolved in isopropanol and added via a syringe pump in a time range of 20 h. With this modified procedure, we wanted to guarantee that the enzyme works best. 5 mL of the enzyme (310 U/mL) were added directly and another 5 mL fresh enzyme were added after 18 h to compensate for a loss of activity.

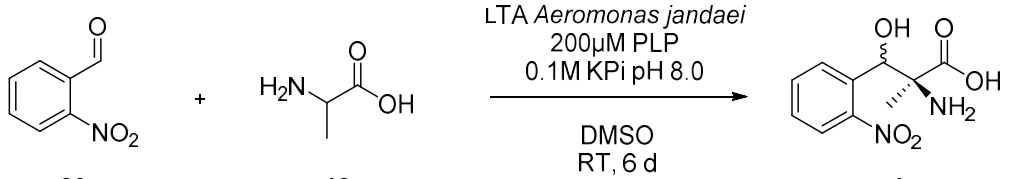
After 2 d the conversion to the desired product **3** was too low and so the reaction ran longer. After 5 d the reaction was stopped by adding 1 mL formic acid and the reaction mixture was heated to 80 °C for 1 h. The resulting precipitate did not contain any product **3** and was filtered off. To remove most of the unconverted aldehyde the filtrate was extracted with ethyl acetate (1 x 100 mL). The aqueous phase was then concentrated under reduced pressure and the product **3** was purified via reversed-phase flash chromatography. The product **3** was isolated in 16 % yield.

4.3 Preparative scale reaction with nitrobenzaldehydes

Nitro-substituted benzaldehydes bearing an electron-withdrawing group turned out as attractive acceptors. Preparative scale aldol condensations were therefore done with 2-nitro-, 3-nitro- and 4-nitrobenzaldehyde.

4.3.1 Synthesis of (2*S*)-2-amino-3-hydroxy-2-methyl-3-(2-nitrophenyl) propanoic acid (4)

The synthesis of (2*S*)-2-amino-3-hydroxy-2-methyl-3-(2-nitrophenyl) propanoic acid (**4**) was done with 2-nitrobenzaldehyde (**20**) as acceptor and DL-alanine (**18**) as donor in the presence of LTA (Scheme 15).



20 **18** **4**

Scheme 15. Synthesis of (2*S*)-2-amino-3-hydroxy-2-methyl-3-(2-nitrophenyl) propanoic acid (**4**)

As in the reactions before, DL-alanine (6-fold excess) had to be dissolved completely in aqueous KPi-buffer (pH = 8.00) while stirring for 15 min. Pyridoxal 5'-phosphate (PLP) as cofactor was added to reach a concentration of 200 µM.

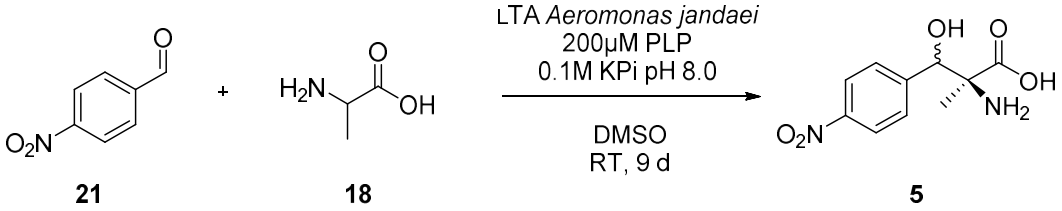
Because of the insolubility of 2-nitrobenzaldehyde (**20**) in water or low solubility in isopropanol, 15 vol% DMSO was used as cosolvent. It was necessary to dissolve the aldehyde, because better conversions were obtained when solid aldehydes were first dissolved in a particular cosolvent. Therefore the aldehyde (1.81 g) was first dissolved in 3 mL DMSO. The mixture was heated up for complete dissolution. 2.5 mL of the solution of aldehyde **20** and DMSO and 4 mL DMSO were added immediately. Then the enzymatic transformation was started via addition of 3 mL LTA (310 U/mL). After 20 h the remaining amount of aldehyde (0.5 mL) as well as 1 mL DMSO and 2.5 mL LTA were added.

After 6 d the reaction reached equilibrium and the reaction was stopped by lowering the pH upon the addition of 7 mL 37 % hydrochloric acid. The precipitate was filtered and washed with 0.1 M HCl (50 mL). The filtrate was concentrated under reduced pressure and the product **4** was purified via flash chromatography.

The isolation of the reaction product turned out to be quite difficult. The major problem in this case was the used DMSO. First a reversed-phase flash chromatography with C18-modified silica gel was tried. Therefore 100 % water was used, but only parts of the DMSO could be removed. As a consequence a second reversed-phase flash chromatography had to be performed. Fortunately, DMSO was then completely removed, but alanine residues were detected in the NMR spectrum. To remove the alanine, a silica gel filtration was envisaged. The crude material was treated with CH_2Cl_2/MeOH 80:20 + 5 % NH_3 and the filtration through silica gel were done with the same eluent. A final yield of 14 % a pure product **4** was obtained.

4.3.2 Synthesis of (2*S*)-2-amino-3-hydroxy-2-methyl-3-(4-nitrophenyl) propanoic acid (5)

The synthesis of (2*S*)-2-amino-3-hydroxy-2-methyl-3-(4-nitrophenyl)propanoic acid (**5**) was done via aldol addition of 4-nitrobenzaldehyde (**21**) as acceptor and DL-alanine (**18**) as donor using the L-threonine aldolase from *Aeromonas jandaei* (Scheme 16).



Scheme 16. Synthesis of (2*S*)-2-amino-3-hydroxy-2-methyl-3-(4-nitrophenyl)propanoic acid (**5**)

The reaction was done in a similar way like the synthesis of (2*S*)-2-amino-3-hydroxy-2-methyl-3-(2-nitrophenyl)propanoic acid (**4**) using a 6-fold excess of DL-alanine. DL-Alanine and the cofactor pyridoxal-5-phosphate (PLP) were added to the aqueous potassium phosphate buffer and stirred for 15 min to obtain a homogeneous solution.

4-Nitrobenzaldehyde (**21**) could only be dissolved well in DMSO. 3 mL DMSO were needed to dissolve the whole amount of aldehyde (1.81 g). The solution of 4-nitrobenzaldehyde (**21**) was added in two portions over 20 h to keep the possible inhibition low. First 2.5 mL of the solution of aldehyde **21** in DMSO and 4 mL DMSO were added. After 20 h the remaining 0.5 mL aldehyde **21** were added to the reaction mixture. The enzyme was added in two portions. 3 mL of enzyme (310 U/mL) were added immediately and further 2.5 mL LTA after 20 h.

The reaction was stopped after 9 d when no further increase of conversion became evident. Hence the reaction was quenched by the addition of 7 mL 37 % hydrochloric acid. The formed precipitate was filtered off and washed with 1M HCl (15 mL). After concentrating the filtrate under reduced pressure 5 mL NH_3 were added to neutralize the acid and the solution was again concentrated by rotary evaporation. The crude yellow solid was pre-purified via silica gel filtration to remove most of the alanine. For that CH_2Cl_2/MeOH 80:20 + 5 % NH_3 (200 mL) and then CH_2Cl_2/MeOH 65:35 + 5 % NH_3 (200 mL) were used as solvent. After removing the solvent under reduced pressure a flash chromatography was tried, but the product could not be purified. The reason for this unsuccessful attempt must be largely attributed to the presence of DMSO which causes adverse effects on the chromatography. So we had to continue with the separation and selected next a reversed phase flash chromatography on C18-modified silica gel. However, main parts of DMSO could not be separated from the product. Finally, a flash

chromatography with CH_2Cl_2/MeOH 85:15 + 2.5 % NH_3 (fraction 1 - 49) and CH_2Cl_2/MeOH 75:25 + 5 % NH_3 (fraction 50 - 65) as eluent was done and the desired product **5** was isolated in 7 % yield.

Next time DMSO as cosolvent for the reactions should be avoided, because DMSO cannot easily be removed before doing a purification. To avoid further problems during the work-up process, the use of DMSO as cosolvent was limited to the scale-up reactions with nitrobenzaldehydes as acceptors.

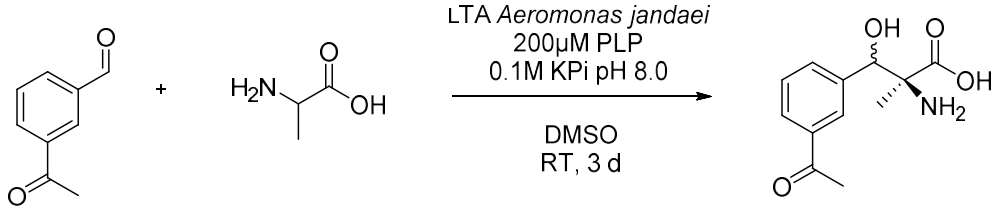
But if the nitrobenzaldehydes should completely dissolve in cosolvent, which is beneficial for the enzymatic aldol reaction, only DMSO or DMF can be used to dissolve the aldehydes. DMSO is no problem if a ion exchanger is used for the work-up process. But in our case lots of optimization have to be done to separate the amino acids with this method.

4.4 Preparative scale reactions with aromatic aldehydes bearing a ketone or an ester function

Aromatic aldehydes with a ketone function or an ester turned out to be an attractive group for the enzymatic aldol addition. As examples, the aldol addition was done with 3-acetylbenzaldehyde (**22**), 4-acetylbenzaldehyde (**23**) and methyl-3-formylbenzoate (**24**) as acceptor.

4.4.1 Synthesis of (2*S*)-3-(3-acetylphenyl)-2-amino-3-hydroxy-2-methyl-propanoic acid (6)

The synthesis of (2*S*)-3-(3-acetylphenyl)-2-amino-3-hydroxy-2-methylpropanoic acid (**6**) was done in an aldol addition with 3-acetylbenzaldehyde (**22**) as acceptor and alanine (**18**) as donor catalyzed by LTA (Scheme 17).



22 **18** **6**

Scheme 17. Synthesis of (2*S*)-3-(3-acetylphenyl)-2-amino-3-hydroxy-2-methylpropanoic acid (**6**)

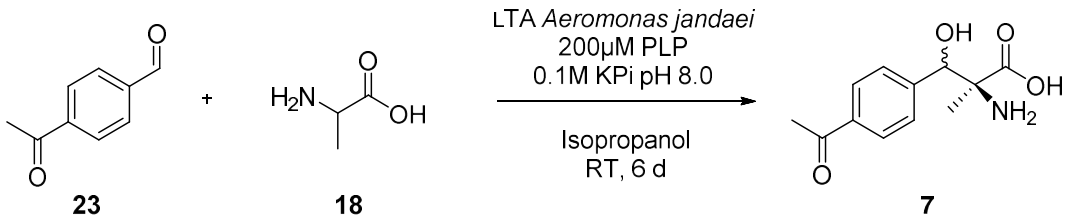
The reaction was done in the same way as for the syntheses before. Because of the separation problems during the work-up process with DMSO in the syntheses of (2*S*)-2-amino-3-hydroxy-2-methyl-3-(2-nitrophenyl) propanoic acid (**4**) and (2*S*)-2-amino-3-hydroxy-2-methyl-3-(4-nitrophenyl)propanoic acid (**5**), isopropanol was used as cosolvent. The aldehyde (**22**) was dissolved in isopropanol, but only a small amount of the material was dissolved. So the aldehyde/isopropanol mixture had to be heated up to obtain a homogeneous solution of the whole amount of 3-acetylbenzaldehyde (**22**).

In this reaction the solution of 3-acetylbenzaldehyde **22** in isopropanol was added in one portion at the beginning. The enzyme was added in two portions. The first 1 mL of enzyme (310 U/mL) was added directly and a second portion fresh enzyme was added after 20 h to compensate for the loss of activity.

After 3 d equilibrium was reached and the reaction was stopped by the addition of 2.5 mL formic acid. The reaction mixture was concentrated under reduced pressure until most of the cosolvent was removed. It must be noted that the presence of isopropanol in the mixture would have also effects on the chromatographic separation, although not as severely as DMSO. Fortunately, isopropanol is much more volatile and can be quickly removed after the reaction. The product was purified via reversed-phase flash chromatography. Because the NMR-spectra showed contamination of the product with alanine, a silica gel filtration had to be done. The silica gel filtration was done with CH_2Cl_2/MeOH 80:20 + 5 % NH_3. Thus alanine could easily be removed with this method. A possible reason that the removal of alanine by the first flash chromatography partly failed is that too much of the cosolvent was still present. The desired product **6** was isolated in 21 % yield.

4.4.2 Synthesis of (2*S*)-3-(4-acetylphenyl)-2-amino-3-hydroxy-2-methyl-propanoic acid (7)

The next synthesis was done with another acetyl-substituted benzaldehyde. Therefore 4-acetylbenzaldehyde (**23**) was used as acceptor and alanine (**18**) as donor for the synthesis of (2*S*)-3-(4-acetylphenyl)-2-amino-3-hydroxy-2-methyl-propanoic acid (**7**). The reaction was done via an aldol addition and catalyzed by the enzyme LTA (Scheme 18).



23 **18** **7**

Scheme 18. Synthesis of (2*S*)-3-(3-acetylphenyl)-2-amino-3-hydroxy-2-methylpropanoic acid (**7**)

The reaction was also done in KPi-buffer (pH = 8.00) with pyridoxal 5'-phosphate (PLP) as cofactor. It was necessary to dissolve alanine (6-fold excess) completely in the buffer. Then the cofactor (PLP) was added and the reaction solution had to be stirred for 15 min.

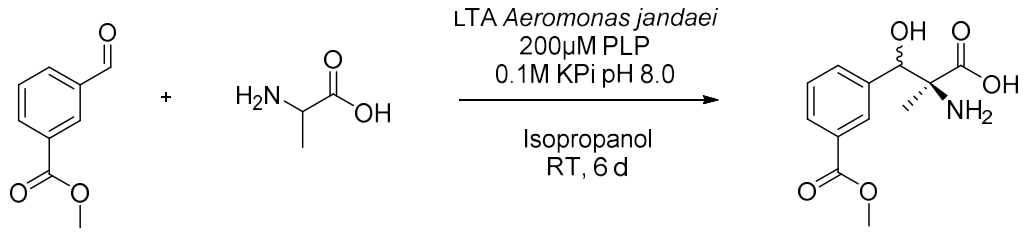
As DMSO had to be avoided in this case too isopropanol was used as cosolvent. The solubility of 4-acetylbenzaldehyde (**23**) was not very good in isopropanol. Hence, the solution of aldehyde **23** and isopropanol had to be heated up to dissolve the whole amount.

A solution of 4-acetylbenzaldehyde (**23**) and isopropanol were added all at once and the enzyme in two portions. 1 mL enzyme (310 U/mL) was added directly and another 1 mL fresh enzyme was added after 20 h to compensate for the loss of activity.

The reaction was stopped after 4 d. 2.5 mL formic acid were added to the reaction mixture. After removing the solvent under reduced pressure, the product was purified via reversed-phase flash chromatography. In this case the cosolvent was almost completely removed before separation and thus the product **7** was purified with a single flash chromatography step. The product **7** was isolated in 22 % yield.

4.4.3 Synthesis of (2*S*)-2-amino-3-hydroxy-3-(3-(methoxycarbonyl)-phenyl)-2-methylpropanoic acid (8)

Having successfully used acetylated benzaldehyde derivatives, we continued with an aromatic aldehyde bearing an ester functionality as EWG. Thus, we used the commercially available methyl-3-formylbenzoate as acceptor for the synthesis of (2*S*)-2-amino-3-hydroxy-3-(3-(methoxycarbonyl)phenyl)-2-methylpropanoic acid (**8**), which is shown in Scheme 19.



24 **18** **8**

Scheme 19. Synthesis of (2*S*)-2-amino-3-hydroxy-3-(3-(methoxycarbonyl)phenyl)-2-methylpropanoic acid (**8**)

The reaction was done in an aqueous buffer at pH = 8.00. First the excess of alanine (6-fold) had to be dissolved in the buffer. Then the cofactor pyridoxal-5-phosphate was added and the solution had to be stirred for 15 min.

The aldehyde was dissolved in isopropanol. The solubility of methyl-3-formylbenzoate (**24**) was not very good in this cosolvent at RT. Hence the suspension of aldehyde **24** in isopropanol had to be heated up to dissolve the whole amount of it. The complete aldehyde/isopropanol solution was added at once. 1 mL of the enzyme LTA (310 U/mL) was added directly and another 1 mL fresh enzyme was added after 20 h.

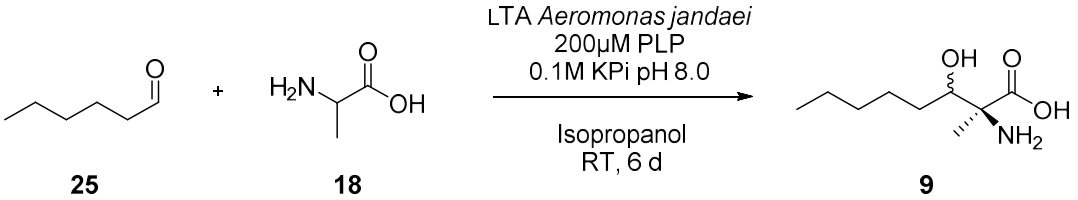
After 6 d a maximum of conversion was achieved and the reaction was stopped by adding 2.5 mL formic acid to the reaction mixture. After removing most of the solvent under reduced pressure the product was purified via reversed-phase flash chromatography. In this case we removed the cosolvent almost completely and thus we could purify the product **8** with a single flash chromatography and a yield of 28 % was achieved.

4.5 Preparative scale reactions with aliphatic aldehydes

To show the large diversity of threonine aldolase-accepted aldehydes, scale up reactions with aliphatic aldehydes were done.

4.5.1 Synthesis of (2*S*)-2-amino-3-hydroxy-2-methyloctanoic acid (9)

Driven by the results of the screening experiments, hexanal (**25**) was selected as acceptor, alanine as donor and LTA as catalyst for the aldol condensation to generate (2*S*)-2-amino-3-hydroxy-2-methyloctanoic acid (**9**). This reaction is outlined in Scheme 20.



Scheme 20: Synthesis of (2*S*)-2-amino-3-hydroxy-2-methyloctanoic acid (**9**)

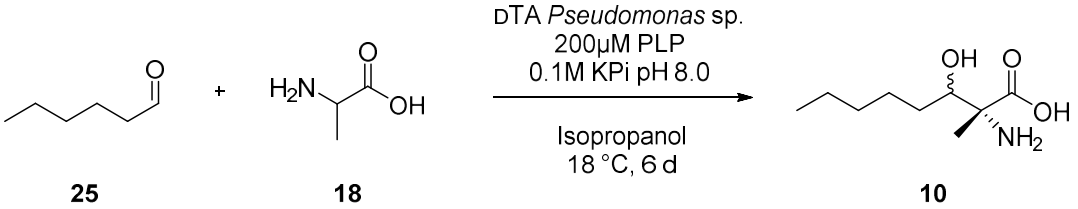
The 10-fold excess of alanine had to be completely dissolved in the aqueous buffer (pH 8.00) which was achieved by stirring for 10 min. Then the cofactor and 3 mL cosolvent (isopropanol) were added.

The liquid aldehyde **25** can be added without the need to dissolve it in the cosolvent. To keep the possible inhibiting concentration low the addition of hexanal (**25**) was done within 6 h. 1 mL of the enzyme (310 U/mL) was added directly and another 1 mL fresh enzyme was added after 24 h to compensate for the loss of activity.

After 4 d the reaction was stopped by heating the reaction mixture to 90 °C for 1 h to inactivate the enzyme. After removing most of the solvent under reduced pressure the product was purified via reversed-phase flash chromatography. In this case we removed the solvent almost completely and thus we could purify the product in a single flash chromatography and a yield of 10 % was achieved. For the detection of the product **9** LC-MS-based analysis method had to used for the detection of the UV-transparent compound.

4.5.2 Synthesis of (2*R*)-2-amino-3-hydroxy-2-methyloctanoic acid (10)

The same reaction like before but with D-threonine aldolase as enzyme was carried out to form the corresponding stereoisomer (2*R*)-2-amino-3-hydroxy-2-methyloctanoic acid (**10**) (Scheme 21).



25 **18** **10**

Scheme 21. Synthesis of (2*R*)-2-amino-3-hydroxy-2-methyloctanoic acid (**10**)

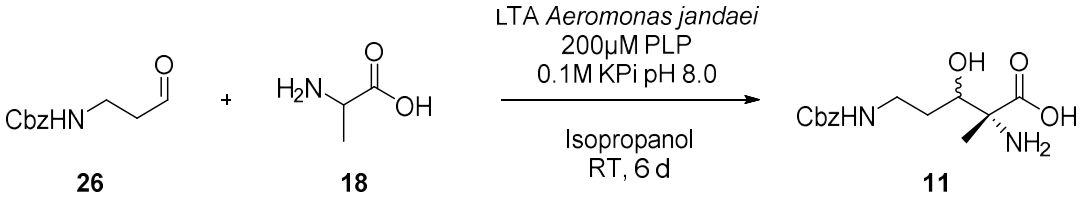
Alanine (**18**) was used in an excess (6-fold) and the whole amount had to be dissolved completely in an aqueous buffer (pH 8.00). Then the cofactor PLP was added and the reaction solution was stirred for 10 min. Moreover, 3 mL isopropanol were added immediately.

The liquid aldehyde **25** could be directly added. The addition of hexanal (**25**) was done within 24 h to keep the possible inhibiting concentration low. 0.5 mL DTA (190 U/mL) were added directly and another 0.5 mL were added after 24 h to compensate for the loss of activity.

After 3 d the reaction was stopped by heating the reaction mixture to 90 °C for 1 h. After removing the solvent under reduced pressure the product was purified via a single reversed-phase flash chromatography. The pure product **10** was isolated in a yield of 27 %. In that case the diasteromeric ratio (d.r. = 2.7 (*syn*)) is not as high as in the case of the synthesis of (2*R*)-2-amino-3-(2-fluorophenyl)-3-hydroxy-2-methylpropanoic acid (**2**).

4.5.3 Synthesis of (2*R*)-2-amino-5-(((benzyloxy)carbonyl)amino)-3-hydroxy-2-methylpentanoic acid (11)

To introduce more diversity, carboxybenzyl-protected 3-amino-propionaldehyde **26** was used for the aldol addition. For the reaction benzyl (3-oxopropyl)carbamate (**26**) was used as acceptor and DTA to catalyze the reaction (Scheme 26).



26 **18** **11**

Scheme 22. Synthesis of (2*R*)-2-amino-5-(((benzyloxy)carbonyl)amino)-3-hydroxy-2-methylpentanoic acid (**11**)

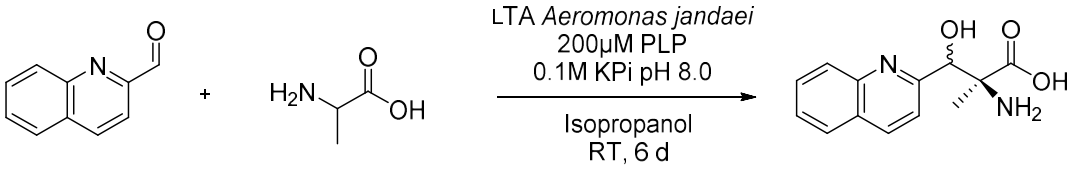
An excess (6-fold) alanine (**18**) was dissolved completely in an aqueous buffer (pH 8.00). Then the cofactor PLP was added and the reaction solution was stirred for 10 min. Moreover 30 µL $MnCl_2$ were added immediately. The solution was cooled to 15 – 18 °C for an optimal reaction performance.

The solid aldehyde **26** was dissolved in isopropanol. The suspension of aldehyde **26** and isopropanol mixture had to be heated up to dissolve the whole amount. The solution of aldehyde **26** in isopropanol was added in 6 portions over 10 h to keep the possible inhibiting concentration low. 0.5 mL of the enzyme LTA (310 U/mL) were added directly and another 0.5 mL were added after 24 h to compensate for the loss of activity.

After 2 d a maximum of conversion was reached and the reaction was stopped by the addition of 85 mL MeOH to the reaction mixture. The formed precipitate of alanine and other compounds was filtered off and the filtrate concentrated under reduced pressure. The solvent was removed almost completely to avoid problems during the work-up process and the product was purified in a single flash chromatography. The pure product **11** was achieved in 32% yield.

4.6 Synthesis of (2*S*)-2-amino-3-hydroxy-2-methyl-3-(quinolin-2-yl) propanoic acid (12)

Quinoline-2-carbaldehyde (**27**) showed very good conversion (60 %) in the screening experiments with alanine (**18**) and LTA. Therefore an aldol addition at larger scale with this aldehyde **27** as acceptor and alanine (**18**) as donor was done to generate (2*S*)-2-amino-3-hydroxy-2-methyl-3-(quinolin-2-yl)propanoic acid (**12**) (Scheme 23).



Scheme 23. Synthesis of (2*S*)-2-amino-3-hydroxy-2-methyl-3-(quinolin-2-yl)propanoic acid (**12**)

The reaction was basically done as described in the reactions before. In this case the aldehyde **27** should have been dissolved in 2 mL ethanol as isopropanol proved to be inappropriate and DMSO had to be avoided in general. However, the solubility of quinoline-2-carbaldehyde (**27**) in ethanol was very poor and also heating up the aldehyde/ethanol suspension, the aldehyde **27** could not be dissolved completely in ethanol. Therefore the aldehyde/ethanol suspension was added in 5 portions over 9 h. 1 mL of the enzyme LTA (310 U/mL)was added directly and another 1 mL was added after 21 h to compensate for the loss of activity.

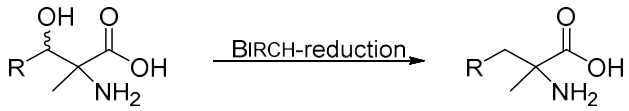
The conversion was monitored by rp-HPLC. After 3 d only low conversion was reached and therefore the reaction ran longer. After 7 d the reaction was stopped by heating up the brown reaction mixture to 80 °C for 2 h. Because of impurities in the aldehyde starting material, it seemed beneficial to remove parts of the unpolar ones via extraction of the reaction mixture with ethyl acetate. The aqueous phase was then monitored via rp-HPLC and some impurities could be removed by this extraction step. The aqueous phase was concentrated under reduced pressure. Product **12** was purified via reversed-phase flash chromatography, but only a yield of 4% was achieved. The diastereomers could be separated by using the standard protocol. Therefore a reversed-phase flash chromatography was used, starting with H_2O/MeOH 98:2 + 0.01 % HCOOH to remove alanine. Then the eluent was changed to H_2O/MeOH 90:10 + 0.01 % HCOOH.

One reason for the low yield is maybe the poor solubility of aldehyde **27** in the reaction mixture which was obviously not much improved by the addition of a cosolvent like ethanol. Quinoline-2-carbaldehyde (**27**) could not be dissolved completely in ethanol and so a suspension of quinoline-2-carbaldehyde (**27**) in ethanol had to be added to the

reaction mixture. In that case maybe an inhibition of the enzyme took place. On the other hand, the screening reaction was done with DMSO as cosolvent which completely dissolved quinoline-2-carbaldehyde (**27**). In fact, we got a much better conversion in the screening-type experiments and this would have clearly supported the use of DMSO also for the scale up.

4.7 β-Dehydroxylation via BIRCH-reduction

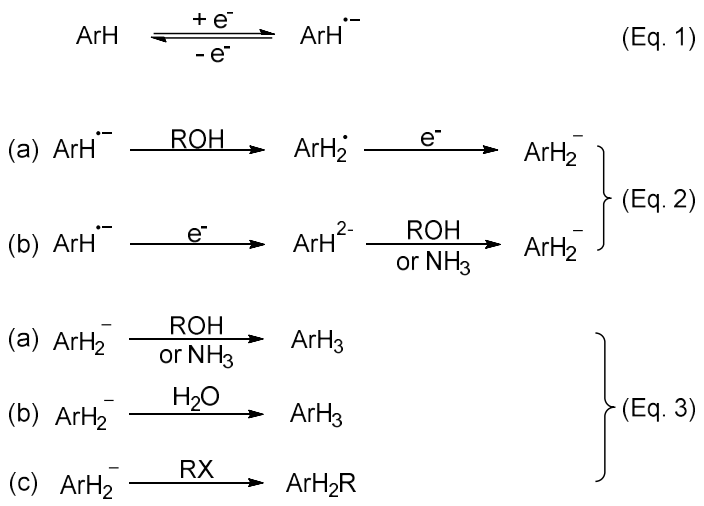
The first attempt for removing the β-hydroxyl group was conducted by a BIRCH-reduction (Scheme 24).



Scheme 24. BIRCH-reduction of a β-hydroxy-α-amino acid

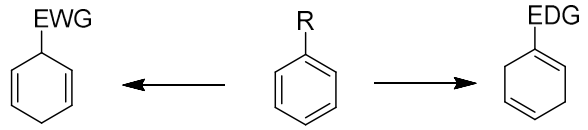
The BIRCH reduction is one of the basic reactions in organic chemistry. The reaction is named after Arthur BIRCH, who reported about the reaction in 1944. [18] In fact WOOSTER and GODFREY had first discovered the reaction in 1937.[19] The BIRCH-reduction is a partial reduction of aromatic compounds using group I metals, commonly Na or Li, in liquid ammonia and in presence of weak acids which act as proton source, for example alcohols (mostly *tert*-butyl alcohol). These weak acids are necessary for the reduction of benzene and unactivated derivatives of benzene. Moreover cosolvents like ether or THF are often used to improve the solubility of the substrate.[20,21]

The mechanism is shown in Scheme 25. First an electron attacks an aromatic system ArH. This forms a radical anion, which is in equilibrium with the reactant (Eq. 1). Then this radical can be protonated by a proton donor, for example an alcohol, to generate the radical $ArH_2^{\bullet}$. ArH_2^{-} is formed after addition of another electron (Eq. 2a).
ArH_2^{-} can also be formed without a proton donor in the system. In this case an electron attacks the radical anion $ArH^{\bullet-}$ and generates a dianion. This dianion can be protonated by an alcohol as well as ammonia to give ArH_2^{-} (Eq. 2b). Next ArH_2^{-} can on the one hand be protonated to generate ArH_3 (Eq. 3a and 3b) and on the other hand ArH_2^{-} can be alkylated to produce ArH_2R (Eq. 3c).[20]



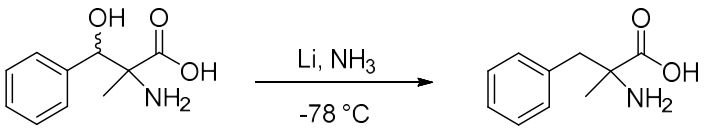
Scheme 25. Mechanism of the BIRCH-reduction[20]

The substituents attached to the aromatic ring influence the regiochemical outcome of the Birch-reduction. The regiochemical outcome of the BIRCH-reactions can be predicted via the "BIRCH rule". Electron-donating substituents, for example alkoxy or alkyl groups, direct the reduction so that the EDG ends up on the electron poor double bond. Electron-withdrawing substituents, like carboxyl or amide groups, avoids the electron poor double bond (Scheme 26).[20]



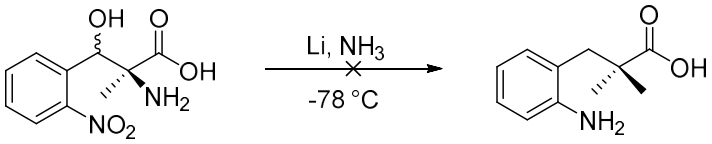
Scheme 26. Influence of substituents on the regiochemistry in the BIRCH-reduction[20]

The BIRCH-reduction of 2-amino-3-hydroxy-2-methyl-3-phenylpropanoic acid, which was done by Melanie TROBE, is outlined in Scheme 27. This reaction worked very well and gave an isolated yield of the β-dehydroxylated product in 91 %.



Scheme 27. BIRCH-reduction of (2*S*)-2-amino-3-hydroxy-2-methyl-3-phenylpropanoic acid

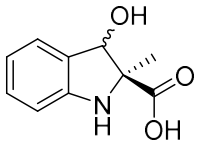
First the BIRCH-reduction was done with other β-hydroxy-α-amino acids. Therefore β-hydroxy-α-amino acids with aromatic moieties were used. The next BIRCH-reduction was done with (2*S*)-2-amino-3-hydroxy-2-methyl-3-(2-nitrophenyl) propanoic acid (**4**) (Scheme 28).



4

Scheme 28. BIRCH-reduction of (2S)-2-amino-3-hydroxy-2-methyl-3-(2-nitrophenyl)propanoic acid (**4**)

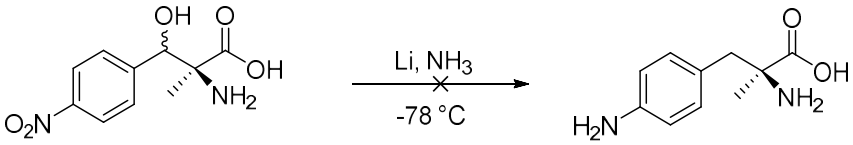
The desired product could not be observed, but another interesting product **13**, shown in Scheme 29, was isolated. Although the isolated yield (8 %) is very low and moreover the hydroxy group could not be removed.



13

Scheme 29. Isolated product from the BIRCH-reduction of (2*S*)-2-amino-3-hydroxy-2-methyl-3-(2-nitrophenyl) propanoic acid (**13**)

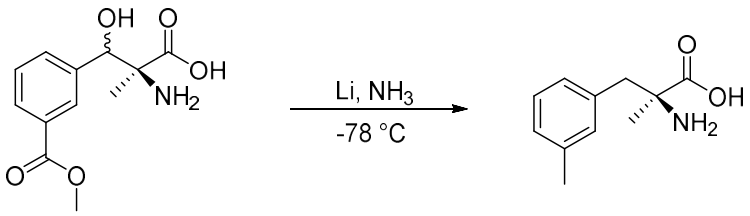
Next (2*S*)-2-amino-3-hydroxy-2-methyl-3-(4-nitrophenyl)propanoic acid (**5**) was treated with Li in liquid ammonia in a BIRCH-reduction to possibly generate 2-amino-3-(4-aminophenyl)-2-methylpropanoic acid (Scheme 30). With this compound several by-products were formed, but the desired one was not observed.



5

Scheme 30. Attempted Birch-reduction of (2S)-2-amino-3-hydroxy-2-methyl-3-(4-nitrophenyl)propanoic acid (**5**)

Then the BIRCH-reduction was applied to (2*S*)-2-amino-3-hydroxy-3-(3-(methoxycarbonyl)phenyl)-2-methylpropanoic acid (**8**) (Scheme 31).



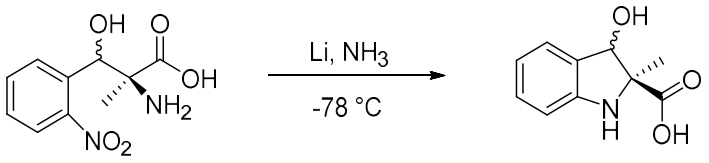
8 **14**

Scheme 31. Attempted BIRCH-reduction of (2S)-2-amino-3-hydroxy-3-(3-(methoxycarbonyl)phenyl)-2-methylpropanoic acid (**8**)

In this reaction several products were formed but only product **14** could be isolated via preparative HPLC in a yield of 11 %.

4.8 Attempted synthesis of (2*S*)-2-methylindoline-2-carboxylic acid

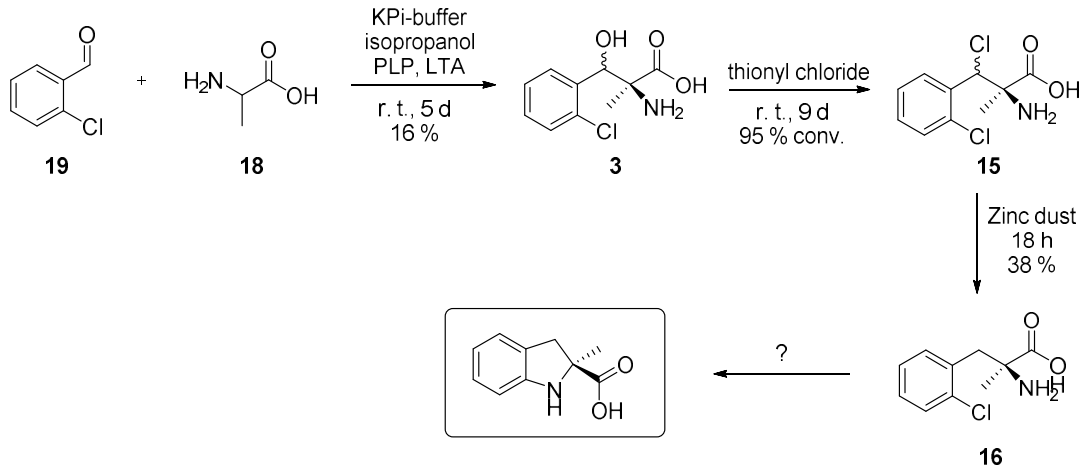
(2*S*)-2-Methylindoline-2-carboxylic acid (**13**) was synthesized via BIRCH-reduction in low yield (8 %) but the hydroxyl-group was not removed by this method (Scheme 36). So we sought another strategy to generate this interesting product in higher yields and without the β-OH-group.



4 **13**

Scheme 32. Synthesis of (2*S*)-3-hydroxy-2-methylindoline-2-carboxylic acid via BIRCH-reduction (**13**)

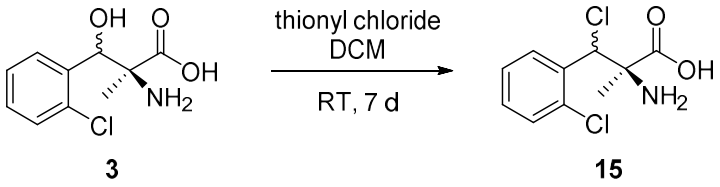
Hence a synthesis of (*S*)-2-methylindoline-2-carboxylic acid was envisaged. This synthesis starts from the commercially available and cheap chemicals 2-chlorobenzaldehyde (**19**) and alanine (**18**) (Scheme 33).



Scheme 33. Attempted synthesis of (*S*)-2-methylindoline-2-carboxylic acid

The first step was an aldol addition of 2-chlorobenzaldehyde (**19**) and alanine (**18**) to yield (2*S*)-2-amino-3-(2-chlorophenyl)-3-hydroxy-2-methyl-propanoic acid (**3**). This reaction was catalyzed by the enzyme LTA and is described in section 4.2.3.

In the next step, the β-hydroxy group should be removed by conversion of the OH-group into the corresponding chlorine-group. Therefore (2*S*)-2-amino-3-(2-chlorophenyl)-3-hydroxy-2-methyl-propanoic acid (**3**) was treated with thionyl chloride (Scheme 34).



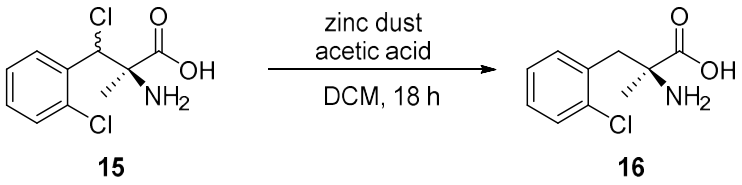
Scheme 34. Synthesis of (2*R*)-2-amino-3-chloro-3-(2-chlorophenyl)-2-methylpropanoic acid (**15**)

First (2*S*)-2-amino-3-(2-chlorophenyl)-3-hydroxy-2-methyl-propanoic acid (**3**) was suspended in dichloromethane. Then 750 µL thionyl chloride were added and the reaction mixture was stirred at RT. After 2 d only very low conversion was achieved, therefore dichloromethane was removed and the reaction was stirred for another 5 d.

A problem was the very long reaction time required to reach completion. After 7 d, a conversion of 95 % was obtained. The reaction was stopped at this point because of time reasons. Furthermore in this reaction some unknown byproducts were formed. But the product **15** was used without any further purification in the next step.

The reaction was done a second time with the reagent but without dichloromethane. Under these conditions the reaction needed also a very long time (9 d) to reach a conversion of 92 %.

The third step was the elimination of the chlorine. (2*S*)-2-Amino-3-chloro-3-(2-chlorophenyl)-2-methylpropanoic acid (**15**) was treated with zinc dust in acetic acid (Scheme 35).

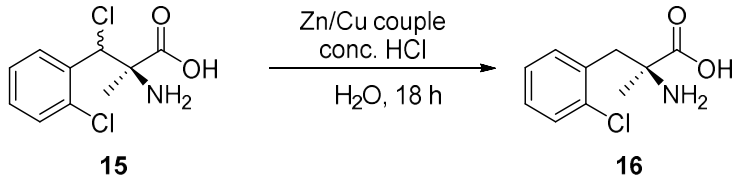


15 **16**

Scheme 35. Synthesis of (*S*)-2-amino-3-(2-chlorophenyl)-2-methylpropanoic acid (**16**)

The product **15** from the previous step was dissolved in 1.2 mL acetic acid. Next 6 mL CH_2Cl_2 and zinc dust (1.5 eq.) were added. After stirring for 3 d at room temperature, no product was formed. Therefore, dichloromethane was removed and 1 mL H_2O added to the reaction mixture. After addition of further zinc dust (8 eq), the reaction was stirred at RT. After another 18 h, full conversion was achieved and the product **16** then purified via reversed-phase flash chromatography. The desired product **16** was isolated in 38 % yield.

The third step was repeated with different conditions, because of moderate yield by the reaction with zinc dust. Therefore the reaction was done with Zn/Cu couple (10 eq.) in HCl at 0 °C (Scheme 36).

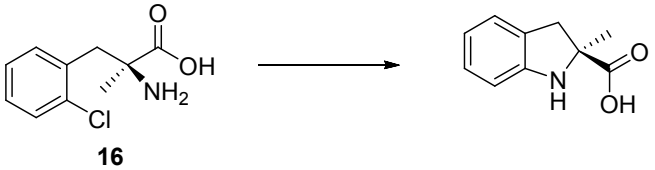


15 **16**

Scheme 36. Synthesis of (*S*)-2-amino-3-(2-chlorophenyl)-2-methylpropanoic acid (**16**)

After 18 h full conversion was achieved and the product **16** was isolated via reversed-phase flash chromatography in 37 % yield.

The next step was the cyclization to generate the desired final product (*S*)-2-methylindoline-2-carboxylic acid. This step turned out to be very challenging (Scheme 37).



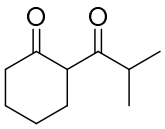
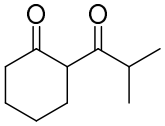
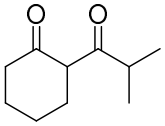
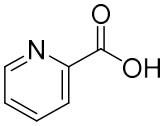
16

Scheme 37. Attempted synthesis of (*S*)-2-methylindoline-2-carboxylic acid

First the reaction was carried out in water in the presence of K_2CO_3 and catalytic amounts of CuCl at 80 °C. Unfortunately, the reaction did not work under these conditions. There was only starting material left.

For the repetition experiments, the conditions for the cyclization were changed. CuCl and K_2CO_3 were replaced by CuI and Cs_2CO_3. The solvent was also changed to aprotic types, different ligands for the Cu-catalysis applied and the reaction carried out at different temperatures (Table 3).

Table 3. Tested conditions for the cyclization to (*S*)-2-methylindoline-2-carboxylic acid

entry	CuI [mol%]	solvent	ligand	temp. [°C]	time [h]	conv. [%]*
1	5	DMF		60	45	traces
2	10	DMF		60	48	no conv
3	10	DMF		80	48	no conv
4	5	DMSO		100	40	no conv.

* Monitored by reversed-phase HPLC

The reaction with CuI, 2-isobutyrylcyclohexan-1-one as ligand and DMF as solvent at 80 °C as well as the reaction with CuI, picolinic acid as ligand and DMSO as solvent at 100 °C did not work. In these cases decomposition of the starting material took place.

The reaction with CuI, 2-isobutyrylcyclohexan-1-one as ligand and DMF as solvent at 60 °C was done with 5 mol% and 10 mol% CuI. In the reaction with 5 mol% CuI traces of the desired product were monitored via HPLC. The reaction was done under the same conditions, but with 10 mol% CuI. In this case no product was monitored via HPLC.

5. Summary and Outlook

5.1 Summary

The aldol addition is a powerful tool for C-C bond formation and aldolases are important enzymes for this reaction. Aldolases accept a very broad range of aldehydes as acceptors and they are very attractive for the synthesis of complex molecules like amino acids. β-Hydroxy-α-amino acids are interesting building blocks for pharmaceutically relevant compounds.

In conclusion 13 α-quaternary β-hydroxy-α-amino acids were isolated. 10 products were synthesized with the enzyme L-threonine aldolase and 3 products with D-threonine aldolase. The yields and the diasteroselectivity with L-threonine aldolase were moderate. Higher yields and diastereoselectivity were obtained with D-threonine aldolase. The isolated products are depicted in (Figure 5).

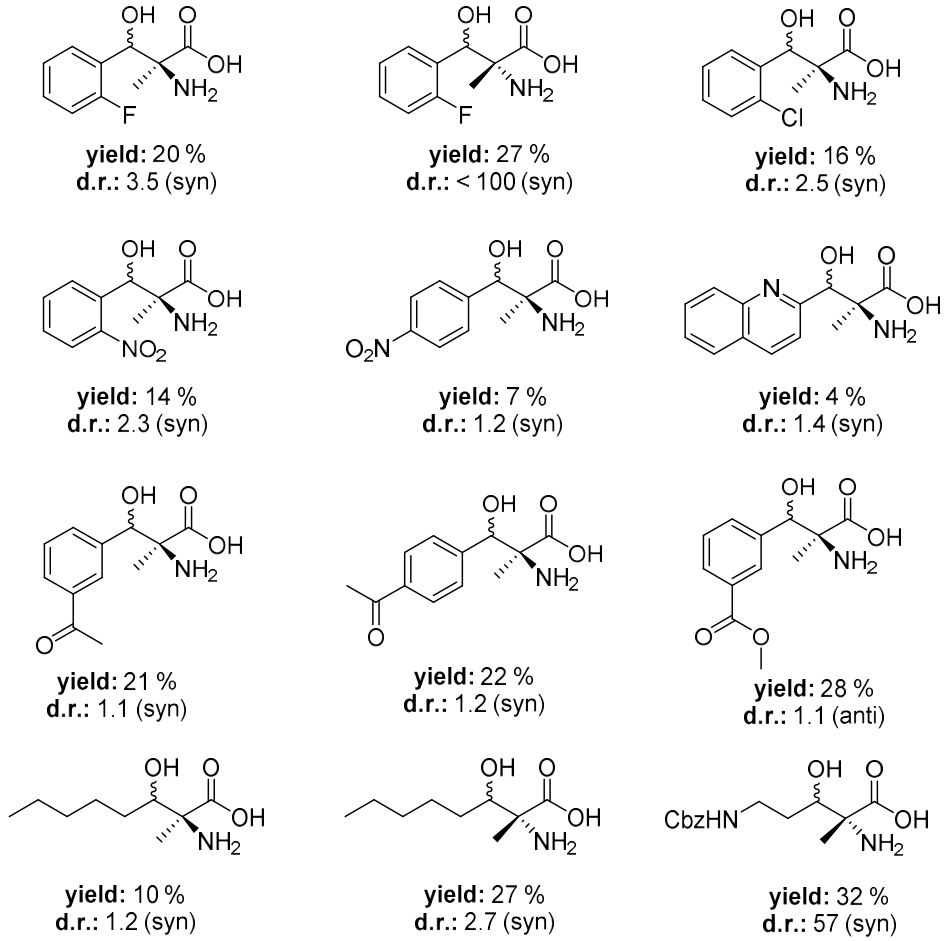
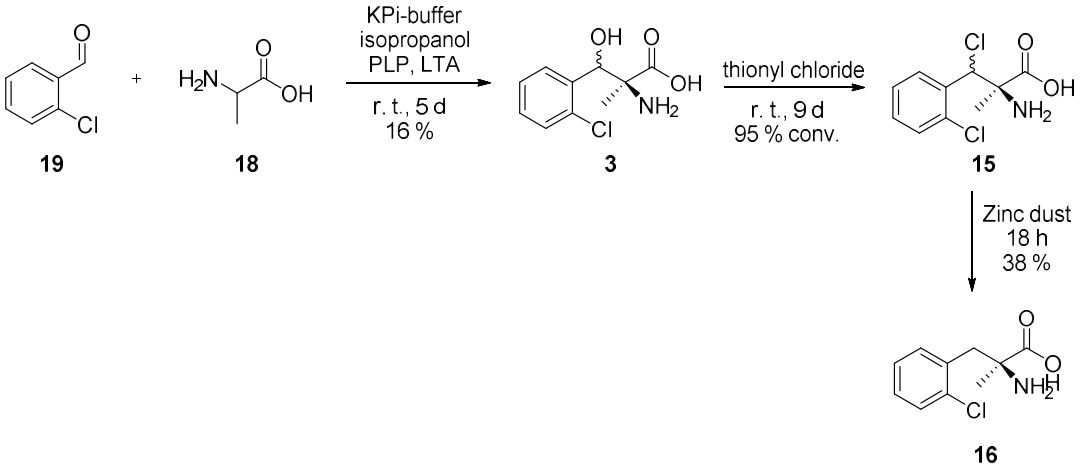


Figure 5. Isolated α-quaternary β-hydroxy-α-amino acids

The best yield (32 %) could be achieved with benzyl (3-oxopropyl)carbamate 28 as acceptor and DTA as enzyme. Also good yields (27 - 28 %) were isolated with methyl 3-formylbenzoate, 2-fluorobenzaldehyde and the aliphatic aldehyde hexanal. The reaction with methyl 3-formylbenzoate was catalyzed by L-threonine aldolase and the reactions with 2-fluorobenzaldehyde and hexanal were catalyzed by D-threonine aldolase. In the case of the reaction with 2-fluorobenzaldehyde as acceptor and DTA a d.r. value >100 was obtained. In contrast (2*S*)-2-amino-3-(2-fluorophenyl)-3-hydroxy-2-methylpropanoic acid (**1**) catalyzed by LTA was isolated in 20 % yield and the product of the reaction with hexanal, alanine and LTA as enzyme was isolated only in 10 % yield. Moreover two new stereogenic centers are formed in one step. The enantiosecificity at the α-position is perfect. In all cases products were isolated in >99 % ee.

The substrate scope of the amino acid donor is small. Only glycine and alanine are accepted for the aldol reaction. But the range of accepted aldehydes is very broad. Aromatic aldehydes with different substituents as well as aliphatic aldehydes are accepted. For aromatic aldehydes many substituents attached to the ring are accepted. For example fluorine, chlorine, nitro-groups, ketones and esters are good substituents.

Furthermore, it was not possible to dehydroxylate α-quaternary β-hydroxy-α-amino acids via BIRCH reduction. Therefore another method for the deoxygenation of α-quaternary β-hydroxy-α-amino acids was developed. This reaction starts from commercially available and simple compounds (Scheme 38). The first step was an enzymatic aldol addition. Then the hydroxyl-group was converted into a chlorine-group. And in the last step the removal of the chlorine-group took place.

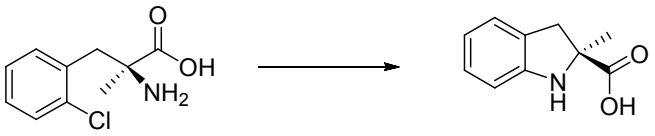


Scheme 38. Deoxygenation of α-quaternary β-hydroxy-α-amino acids

5.2 Outlook

Future work should be dedicated to the improvement of the synthesis of (*S*)-2-methylindoline-2-carboxylic acid. The first three steps worked already, but the yields are moderate. An opportunity for further optimization for the enzymatic aldol condensation would be to reduce the amount of enzyme. Moreover, the second step has a very long reaction time (7 d). One option would be to heat up the reaction mixture. Maybe the reaction time can be reduced under these conditions.

The last step has to be optimized as well (Scheme 39). So far only traces of the desired product were monitored via HPLC. A possible strategy is to do the reaction at 100 °C. But as we saw in our experiments this is impossible for the reaction with these amino acids, because decomposition took place, therefore one has to fund more active metal/ligand complexes



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Scheme 39. Attempted synthesis of (*S*)-2-methylindoline-2-carboxylic acid

6. Experimental section

6.1 General section, materials and methods

6.1.1 General aspects

All experiments were carried out on air, unless noted otherwise. Inert reactions were carried out employing standard Schlenk techniques under an inert atmosphere of argon or nitrogen. Before use the glass apparatuses were dried in oil pump vacuum by heating with a heat-gun, cooled to room temperature and flushed with inert gas. Reagents and solvents were always added under an inert gas counter-stream.

All chemicals and reagents used were purchased from ACROS Organics, Sigma Aldrich, Fluka, Merck, Riedel-de Haën, Sigma-Aldrich, Roth and VWR and were used without further purification unless otherwise mentioned.

The conversions of the described syntheses were monitored by HPLC-MS. 1 µL of the reaction solution was added to 25 µL MeOH, 22 µL dest. H_2O and 2 µL HCOOH. If there was a precipitation the solution was centrifuged with a Heraeus sepatech biofuge A centrifuge and the supernatant was injected.

6.1.2 Solvents

Dimethylsulfoxide was purchased from ACROS Organics and was stored in a brown 1 L bottle. It was used without further purification.

Ethanol used for non-inert conditions was purchased from Merck (stabilized with 1% methylethyl ketone) and was used without further purification.

Isopropanol was purchased from VWR in 25 L plastic bottles and used without further purification.

Methanol was purchased from VWR in 5 L plastic bottles and used without further purification.

***N,N*-Dimethylformamide** was purchased from ACROS Organics as extra dry solvent (<50 ppm water, over 3 Å molecular sieves, AcroSeal®) and was transferred to a dry brown 1 L Schlenk bottle with activated 3 Å molecular sieves and stored under argon atmosphere.

Non dry solvents, i.e. **cyclohexane**, **ethylacetate**, **methanol**, **dichloromethane**, **acetone**, **ethanol** were purchased from VWR, Fisher Scientific or Sigma Aldrich and used without further purification.

6.1.3 Analytical methods

6.1.3.1 Thin layer chromatography

Analytical thin layer chromatography was performed on commercial silica gel plates (TLC aluminium foil, Merck, silica gel 60 F_{254}). The detection occurred by using a UV lamp with λ = 254 nm or λ = 366 nm. Alternatively, a stain reagent was applied and the plates were developed using a stream of hot air.

Ninhydrin: 300.0 mg Ninhydrin, 100 mL n-Butanol, 3 mL AcOH

Developing solvents and R_f-values were stated for each compound in the experimental procedures.

6.1.3.2 Flash chromatography

Flash chromatography was performed using silica gel 60 (particle size 35-70 µm) from ACROS Organics. The mass of silica gel used was the 20-100-fold (w/w) amount of the dry crude product. Sticky and other non-transferable crude products were dissolved in an appropriate amount of the eluent. Amounts of silica gel used, eluents and gradients were stated for each compound.
Most of the compounds were separated via a reversed-phase silica gel flash chromatography using silica gel 60 C18 (particle size 35-70 µm) from Roth. The mass of C18 silica gel used was 60 g for all separation problems. For the regeneration the column was washed with acetonitrile and methanol after each separation.

6.1.3.3 Nuclear magnetic resonance spectroscopy

The described nuclear magnetic resonance spectra were acquired on a Bruker AVANCE III spectrometer (300.36 MHz-^{1}H-NMR, 75.53 MHz-^{13}C-NMR) with autosampler. The residual protonated solvent peak was set as internal standard. ^{13}C-NMR and APT spectra were acquired proton-decoupled. Coupling constants (*J*) are reported as absolute values in Hertz (Hz) and chemical shifts are indicated in parts per million (ppm). Signal multiplicities *J* are abbreviated as s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublet), t (triplet), dt (doublet of triplet), td (triplet of doublet), q (quadruplet) and m (multiplet). Quaternary atoms are denoted as C_q (^{13}C-data) and aromatic protons as CH-Ar (^{1}H-data).

Deuterated solvents for nuclear resonance spectroscopy were purchased from euriso top® (DMSO-d_6, $CDCl_3$) and Deutero® (D_2O).

6.1.3.4 High performance liquid chromatography

Analytical HPLC measurements were performed on three different instruments. Most of the qualitative and quantitative data were acquired on an Agilent 1200 Series. As eluents methanol and water with 0.01% HCOOH as an additive were used.

Analytical HPLC-MS measurements were performed on a Shimadzu Nexera LCMS-2020 system. Detection of the substances was accomplished with a „Shimadzu SPD-M20A Prominence Diode Array Detector“ at a wavelength of λ = 210 nm and with the mass selective detector „Shimadzu LCMS-2020 Liquid Chromatograph Mass Spectrometer” in the positive and/or negative. The separation of the analytes was carried out using a “C-18 reversed-phase” column of the type „Poroshell® 120 EC-C18, 3.0 x 100 mm, 2.7 µm“. Samples were either dissolved in MeCN or MeOH and in the case of undissolved particles the suspension was centrifuged or filtered through syringe filters. The following method was used for the separation:

> **method_1:** 0.0 - 0.5 min 98 % water/ 0.01 % HCOOH and 2 % CH_3CN, 0.5 – 3.5 min linear to 32 % CH_3CN, 3.5 – 4.5 min linear to 100 % CH_3CN, 4.5 – 5.5 min 100 % CH_3CN; 0.7 mL/min, 40 °C.

The measurements for derivatization experiments was performed on an Agilent 1100 Series HPLC system equipped with a temperature controlled column oven. Detection of the substances was accomplished with a Diode Array Detector at a wavelength of λ = 210 nm and λ = 340 nm The separations were carried out on a EC 150/3 Nucleodur® C18 Gravity column (3.0 µm). The following methods were used for the separation:

> **method_2:** 0.0 – 6.0 min 98 % water/ 0.1 % HCOOH and 2 % MeOH linear decrease to 100 % MeOH, 6.0 – 8.0 min 100 % MeOH; 0.7 mL/min, 30 °C.
>
> **method_3:** 0.0 – 11.0 min from 85 % 50mM KOAc (pH 0 6.4) and 15 % CH_3CN liner decrease to 65 % 50mM KOAc (pH 0 6.4) and 35 % CH_3CN, 11.0 – 12.0 min 20 % 50mM KOAc (pH 0 6.4) and 80 % CH_3CN; 0.65 mL/min, 30 °C.

Most of the data were acquired on an Agilent 1200 Series MWD SL UV detector. Signals were detected λ = 210 nm. The separation of the analytes was carried out using a “C-18 reversed-phase” column of the type „Poroshell® 120 EC-C18, 3.0 x 100 mm, 2.7 µm. The following methods were used for performing the separations:

method_4: 0.0 – 6.0 min 98 % water/ 0.1 % HCOOH and 2 % MeOH linear decrease to 68 % water/ 0.1 % HCOOH and 32 % MeOH, 6.0 – 8.0 min linear decrease to 100 % MeOH, 8.0 – 10.0 min 100 % MeOH; 0.7 mL/min, 30 °C.

method_5: 0.0 – 6.0 min 98 % water/ 0.1 % HCOOH and 2 % MeOH linear decrease to 100 % MeOH, 6.0 – 8.0 min 100 % MeOH; 0.7 mL/min, 30 °C.

6.1.3.5 Reversed phase preparative HPLC high performance liquid chromatography

Reversed phase preparative HPLC purifications were run on a Thermo Scientific UltiMate 3000 system. Detection was accomplished with a Dionex UltiMate Diode Array Detector. The separations were carried out on a Macherey-Nagel 125/21 Nucleodur® 100-5 C18EC (125 x 21 mm, 5.0 µm) column. The following method was used:

method_6: 0.0 – 30.0 min 98 % 0.025 % HCOOH and 2 % MeOH linear decrease to 48 % 0.025 % HCOOH and 52 % MeOH, 30.0 – 33.0 min 48 % 0.025 % HCOOH and 52 % MeOH, 33.0 – 34.0 min linear decrease to 100 % MeOH, 34 – 40 min 100 % MeOH; 19 mL/min, 30 °C.

6.1.3.6 High resolution mass spectrometry

The measurement of high resolution mass spectrometry spectra was performed on a “Waters GCT Premier” system after ionization with a MALDI ionization source of a potential of 70 V. All measurements were performed by Ing. Karin Bartl. The corresponding calculated and measured masses are noted for each compound.

6.1.3.7 Determination of the melting point

Melting points were determined using a Mel-Temp® apparatus with integrated microscopical support from Electrothermal in open capillary tubes. The temperature was measured using a mercury thermometer. Melting points were not corrected.

6.1.3.8 Determination of optical rotations

Optical rotations were measured in 1M HCl on a Perkin Elmer 341 polarimeter with a 2 cm cell. Concentration *c* given is in g/100 mL. Each optical rotation measurement was done five times and the mean value is reported.

6.2 Preparation of threonine aldolases

The preparations of the enzymes (L-threonine aldolase and D-threonine alodolase) were done by Kateryna LYPETSKA.

6.2.1 Overexpression of threonine aldolases in Escherichia coli

The vector pEamTA-TA was retransformed into *Escherichia coli* BL21AI cells. This construct contained the *tac* promoter and the LacI repressor, which control the protein expression level. *E. coli* BL21AI cells containing pEamTA-TA plasmids were cultivated on 2xTY media supplemented with 100 mg/mL ampiciline. 100 mL overnight cultures in 300 mL flasks were inoculated with single colonies and grown at 37 °C with shaking. The 330 mL main cultures in baffled 1000 mL flasks were inoculated with 7 ml of the preculture and grown at 37 °C for approximately 3 h to an OD_{600} of 1.5. Temperature was then lowered to 25 °C (LTA) or 16 °C (DTA) and induced with 0.1 mM of β-D-1-thiogalactopyranoside (IPTG). Cultivation was continued overnight and cells harvested by centrifugation for 15 min at 4500 x g. After resuspension of the pellets in sodium phosphate buffer (0.1 M, pH 7) the cells were disrupted by ultrasonic treatment for 7 min. The crude lysate was cleared by centrifugation at 20000 x g for 1 h at 4 °C and the supernatant (cell free extract) was used for the experiments without further purification.[14]

6.2.2 Activity measurement towards threonine

Threonine aldolase activity was measured by a coupled assay with yeast alcohol dehydrogenase (Sigma). In this assay, threonine is converted into glycine and acetaldehyde by the action of TA. Then, by the action of yeast alcohol dehydrogenase, the released acetaldehyde is reduced to ethanol with simultaneous oxidation of NADH to NAD^+. The consumption of NADH is measured spectrophotometrically at 340 nm.

The assay mixture contained 50 mM of L-threonine, 100 mM sodium phosphate buffer (0.1 M, pH 8), 50 µM pyridoxal phosphate, 200 µM NADH, 30 units of yeast alcohol dehydrogenase (Sigma, stock solution can be prepared in 0.1 M sodium phosphate buffer, pH 8), and 50 µl diluted cell-free extract in a final volume of 3 mL. The reactions were started by the addition of 25 to 50 µL of diluted cell free extract (1:1000 for L-TA).

One unit of the enzyme is the amount of enzyme that catalysed the formation of 1 µmol of acetaldehyde (1 µmol of NADH oxidized) per minute at room temperature. The molar extinction coefficient of NADH is 6.2×10^3 M^{-1} cm^{-1} at pH 8.[14]

6.2.3 Activity measurement towards phenylserine

The activity was verified by monitoring the cleavage of DL-*syn*-phenylserine (200 mM) spectrophotometrically at 279 nm (1400 M^{-1} cm^{-1}). The reactions were carried out at 25 °C in sodium phosphate buffer (50 mM, pH 8) containing 50 µM PLP and ca. 5 µM enzyme. One activity unit (U) is defined as the amount of enzyme that catalyzes the formation of 1 µmol of benzaldehyde per minute under these conditions.[14]

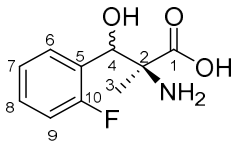
6.3 Experimental procedures and analytical data

6.3.1 General procedure for screenings

10 µL PLP, 625 µL (1000 µmol) donor (alanine or glycine), 100 µL KPi-buffer and 115 µL H_2O for LTA or 65 µL H_2O for DTA were distributed into Eppendorf vials (1.5 mL). Afterwards 50 µL LTA or 100 µL DTA were added to the suspension. A solution of 50 µmol aldehyde in 100 µL DMSO was added to the reaction mixture. The reaction mixture was shaken at 850 rpm in an Eppendorf thermomixer for 24 h at 30 °C for LTA and 18 °C for DTA. After 24 h 50 µL of the reaction mixture were diluted with 450 µL 0.3 M HCl/MeOH (1:1). Afterwards the mixture was centrifuged in an Eppifuge for 3 min at 13,000 rpm. The supernatant was transferred into GC-glass vials and analyzed via analytical HPLC.

For the derivatization of the reaction a stock solution of MPA was prepared. Therefore 7.3 µL MPA were added to 1.4 mL potassium tetraborate-buffer. 25 µL MPA solution, 5 µL sample and 5 µL OPA were distributed into GC-glass vials with inlet. After 1 to 2 h the reaction mixtures were analyzed via HPLC. The methods which were used are stated in section 6.1.3.4.

6.3.2 (*2S*)-2-Amino-3-(2-fluorophenyl)-3-hydroxy-2-methylpropanoic acid (1)



1

In a 100 mL round bottom flask 6.41 g (72.0 mmol, 6.0 eq) DL-alanine (**19**) were dissolved in 40 mL KPi-buffer. Under stirring 2.5 mg (10 µmol) PLP and 4 mL isopropanol were added. The yellow solution was stirred at RT for 15 min. Then 350 µL 2-fluorobenzaldehyde (**18**) and 2.5 mL LTA were added and the solution was stirred at RT for 3 h. After 3 h further 350 µL 2-fluorobenzaldehyde (**18**) were added. After 2 h further 560 µL 2-fluorobenzaldehyde (**18**), 0.8 mL isopropanol and 1.5 mL LTA were added and the suspension was stirred at RT for 6 d. The conversion was monitored via HPLC-MS. 250 mL methanol were added to the yellowish, cloudy suspension and the solution was stirred at RT for 2 h. The colorless precipitate was removed by filtration and washed with methanol (3 x 15 mL). The filtrate was concentrated under reduced pressure, the product

1 was purified via flash chromatography (40 g silica gel, 13 x 2.5 cm, CH_2Cl/methanol/NH_3 = 70:30:5 (fraction 1 – 9), 20:10:1 (fraction 10 – 12), 50 mL fractions).

Yield: 501 mg (2.35 mmol, 20 %), colorless solid.

$C_{10}H_{12}FNO_3$ [213.08 g/mol]

mp = 180 – 191 °C

$\mathbf{R_f}$ = 0.50 (CH_2Cl/MeOH/NH_3 = 10/10/1, UV und Ninhydrin)

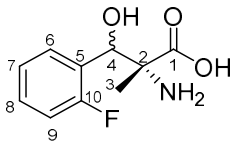
HPLC-MS (method_1): t_{R1} = 1.47 min, t_{R2} = 1.71 min.

^{1}H-NMR (300.36 MHz, D_2O): δ = 7.56 – 7.40 (m, 4H, H-6, H-8), 7.34 – 7.13 (m, 4H, H-7, H-9), 5.54 (s, 1H, H-4, *syn*), 5.34 (s, 1H, H-4, *anti*), 1.60 (s, 3H, H-3, *anti*), 1.42 (s, 3H, H-3, *syn*).

^{13}C-NMR (75.53 MHz, D_2O): δ = 172.8 (C_q, C-1), 171.6 (C_q, C-1), 161.6 (C_q, C-10), 158.3 (C_q, C-10), 131.3 (C-8), 131.2 (C-8), 129.0 (C-6),128.6 (C-6), 125.0 (C-7), 124.6 (C-7), 123.5 (C_q, C-5), 123.4 (C_q, C-5), 115.95 (C-9), 115.8 (C-9), 69.9 (C-4),68.6 (C-4), 64.5 (C_q, C-2), 64.2 (C_q, C-2), 18.5 (C-3), 17.5 (C-3).

HRMS (MALDI-TOF): Calcd. for $C_{10}H_{12}FNO_3H$ $[M+H]^+$: 214.0880; found: 214.0872.

6.3.3 (*2R*)-2-Amino-3-(2-fluorophenyl)-3-hydroxy-2-methylpropanoic acid (2)



2

In a 100 mL round bottom flask 2.67 g (30.0 mmol, 10.0 eq) DL-alanine (**18**) were dissolved in 3 mL KPi-buffer, 21.7 mL dist. H_2O and 3 mL isopropanol. Under stirring 300 µL PLP were added. The yellow solution was stirred at RT for 10 min and cooled to 15 °C. Then 80 µL 2-fluorobenzaldehyde (**17**) and 0.5 mL DTA were added and the solution was stirred at 15 °C. After 3.5 h another 80 µL 2-fluorobenzaldehyde (**17**) were added. The reaction mixture was shaken at 120 rpm at 17.5 – 18.5 °C. After 20 h further 156 µL 2-fluorobenzaldehyde (**17**) and 0.5 mL DTA were added and the suspension was stirred at at 17.5 – 18.5 °C for 3 d whereby the conversion was monitored via HPLC-MS. After adding 0.5 mL formic acid the reaction mixture was heated to 80 °C for 2 h. The resulting precipitate was removed by filtration and washed with H_2O + 0.01 % formic acid (3 x 15

mL). The filtrate was concentrated under reduced pressure and the crude product **2** was purified via flash chromatography (60 g silica gel C18, 10.5 x 3 cm, H_2O/methanol = 98:2 + 0.01 % formic acid (fraction 1 – 3), 90:10 + 0.01 % formic acid (fraction 4 – 10), 100 ml fractions (F1 -2), 50 mL fractions (F3 – 10)).

Yield: 175 mg (821 µmol, 27 %), colorless solid.

$C_{10}H_{12}FNO_3$ [213.08 g/mol]

$[\alpha]_D^{25}$ = 28.8 (c = 0.2 in 1 M HCl)

mp = 220 – 237 °C

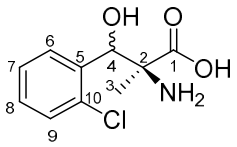
HPLC-MS (method_2): t_{R1} = 3.70 min.

^{1}H-NMR (300.36 MHz, D_2O): δ = 7.57 – 7.44 (m, 4H, H-6, H-8), 7.34 – 7.29 (m, 2H, H-7), 7.24 – 7.18 (m, 2H, H-9), 5.55 (s, 1H, H-4, *syn*), 1.42 (s, 3H, H-3, *syn*).

^{13}C-NMR (75.53 MHz, D_2O): δ = 173.0 (C_q, C-1), 161.6 (C_q, C-10), 158.3 (C_q, C-10), 131.3 (C-8), 131.2 (C-8), 128.6 (C-6), 124.9 (C-7), 123.6 (C_q, C-5), 123.5 (C_q, C-5), 115.9 (C-9), 115.7 (C-9), 68.7 (C-4), 64.6 (C_q, C-2), 17.5 (C-3), 17.1 (C-3).

HRMS (MALDI-TOF): Calcd. for $C_{10}H_{12}FNO_3H$ $[M+H]^+$: 214.0880; found: 214.0878.

6.3.4 (2*S*)-2-Amino-3-(2-chlorophenyl)-3-hydroxy-2-methylpropanoic acid (3)



3

In a 500 mL round bottom flask 19.39 g (218 mmol, 10.0 eq) DL-alanine (**18**) were dissolved in 11 mL KPi-buffer, 157 mL H_2O and 3.8 mL isopropanol. Under stirring 6 mg PLP were added and the yellow solution was stirred at RT for 30 min. Then 5 mL LTA were added. A solution of 3.1 g (21.8 mmol, 1.0 eq) 2-chlorobenzaldehyde (**19**) in 18 mL isopropanol was added under stirring via a syringe pump over 24 h. After addition of another 5 mL LTA the suspension was stirred at RT for 5 d. The conversion was monitored via HPLC-MS. 1 mL formic acid was added and the yellowish, cloudy suspension was heated to 80 °C. After 30 min the suspension was allowed to cool to RT. The suspension was filtrated and the filtrate was extracted with ethyl acetate (1 x 100 mL). The aqueous phase was concentrated by rotary evaporation. The product **3** was purified

via flash chromatography (60 g silica gel C18, 11 x 3 cm, H_2O/methanol = 99:1 + 0.1 % formic acid (fraction 1 – 6), 90:10 + 0.1 % formic acid (fraction 7 – 9), 85:15 + 0.1 % formic acid (fraction 10 – 14), 50 mL fractions).

Yield: 807 mg (3.51 mmol, 16 %), colorless solid.

$C_{10}H_{12}ClNO_3$ [229,05 g/mol]

mp = 195 -212 °C

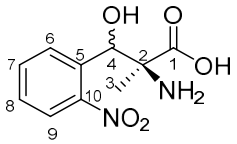
HPLC-MS (method_4): t_{R1} = 4.34 min, t_{R2} = 5.00 min

^{1}H-NMR (300.36 MHz, D_2O): δ = 7.61 – 7.34 (m, 4H, H-Ar), 5.73 (s, 1H, H-4, *syn*), 5.47 (s, 1H, H-4, *anti*), 1.54 (s, 1H, H-3, *anti*), 1.35 (s, 1H, H-3, *syn*).

^{13}C-NMR (75.53 MHz, D_2O): δ = 175.1 (C_q, C-1), 173.4 (C_q, C-1), 135.5 (C_q, C-5), 135.4 (C_q, C-5), 133.3 (C_q, C-10), 132.7 (C_q, C-10), 130.2 (C-Ar), 130.1 (C-Ar), 129.8 (C-Ar), 129.6 (C-Ar), 129.1 (C-Ar), 128.4 (C-Ar), 127.6 (C-Ar), 127.1 (C-Ar), 71.3 (C-4), 70.3 (C-4), 65.7 (C_q, C-2), 65.1 (C_q, C-2), 19.2 (C-3), 18.25 (C-3).

HRMS (MALDI-TOF): Calcd. for $C_{10}H_{12}ClNO_3H$ $[M+H]^+$: 230.0584; found: 230.0585.

6.3.5 (2*S*)-2-Amino-3-hydroxy-2-methyl-3-(2-nitrophenyl)propanoic acid (4)



4

In a 100 mL round bottom flask 6.41 g (72.0 mmol, 6.0 eq) DL-alanine (**18**) were dissolved in 40 mL KPi-buffer. Under stirring 2.6 mg (10 µmol) PLP were added and the yellow solution was stirred at RT for 15 min. Then 1.81 g (12 mmol, 1.0 eq) 2-nitrobenzaldehyde (**20**) were dissolved in 3 mL DMSO. 2.5 mL of the 2-nitrobenzaldehyde/DMSO solution, 4 mL DMSO and 3 mL LTA were added. The suspension was stirred at RT for 18 h. After 18 h further 0.5 mL 2-nitrobenzaldehyde/DMSO solution, 1 mL DMSO and 2.5 mL LTA were added. The suspension was stirred at RT for 6 d. The conversion was monitored via HPLC-MS. 7 mL conc. HCl were added to the yellow, cloudy suspension and the solution was stirred at RT for 30 min. The colorless precipitate was removed by filtration and washed with 0.1 M HCl (2 x 25 mL). After evaporation of the solvent under reduced pressure, the product **4** was purified via flash chromatography (40 g silica gel, 13 x 2.5 cm, CH_2Cl/methanol = 70:30 + 5 % NH_3 (fraction 1 – 9), 20:10 + 5 % NH_3 (fraction 10 –

12), 50 mL fractions). Then a short chromatography was done and therefore the yellow solid was filtered through a pad of silica gel (diameter: 2 cm, height: 2.5 cm) and the filter cake was washed with CH_2Cl/MeOH = 80.20 + 5 % NH_3 (11 x 25 mL fractions). The filtrates of fraction 5 to 10 were collected and the solvent was removed under reduced pressure.

Yield: 410 mg (1.71 mmol, 14 %), yellow solid.

$C_{10}H_{12}N_2O_5$ [240.07 g/mol]

mp = 158 – 166 °C

$\mathbf{R_f}$ = 0.72 (CH_2Cl/MeOH/NH_3 = 10/10/1, UV und Ninhydrin)

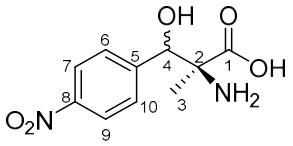
HPLC-MS (method_1): t_{R1} = 2.29 min, t_{R2} = 2.65 min

^{1}H-NMR (300.36 MHz, D_2O): δ = 8.05 (d, $^3J_{HH}$ = 8.2 Hz, 2H, H-9), 7.91 – 7.64 (m, 6H, H-Ar), 6.07 (s, 1H, H-4, *syn*), 5.81 (s, 1H, H-4, *anti*), 1.53 (s, 1H, H-3, *anti*), 1.29 (s, 1H, H-3, *syn*).

^{13}C-NMR (75.53 MHz, D_2O): δ = 174.6 (C_q, C-1), 148.4 (C-6), 133.9 (C-7), 133.3 (C-7), 133.1 (C-Ar), 132.1 (C-Ar), 129.7 (C-Ar), 129.0 (C-Ar), 128.9 (C-Ar), 125.1 (C-Ar), 124.9 (C-Ar), 69.3 (C-4), 68.6 (C-4), 65.4 (C-2), 65.3 (C-2), 20.1 (C-3), 17.6 (C-3).

HRMS (MALDI-TOF): Calcd. for $C_{10}H_{12}N_2O_5H$ $[M+H]^+$: 241.0824; found: 241.0845.

6.3.6 (2*S*)-2-Amino-3-hydroxy-2-methyl-3-(4-nitrophenyl)propanoic acid (5)



5

In a 100 mL round bottom flask 6.41 g (72.0 mmol, 6.0 eq) DL-alanine (**18**) were dissolved in 40 mL KPi-buffer. Under stirring 2.5 mg (10 µmol) PLP were added and the yellow solution was stirred at RT for 15 min. Then 1.81 g (12 mmol, 1.0 eq) 4-nitrobenzaldehyde (**21**) were dissolved in 3 mL DMSO. 2.5 mL of the 4-nitrobenzaldehyde/DMSO solution, 4 mL DMSO and 3 mL LTA were added. The suspension was stirred at RT for 18 h. Then another 0.5 mL 4-nitrobenzaldehyde/DMSO solution, 1 mL DMSO and 2.5 mL LTA were added. The suspension was stirred at RT for 9 d. The conversion was monitored via HPLC-MS. 7 mL conc. HCl were added to the yellowish, cloudy suspension and the solution was stirred at RT for 30 min. The colorless precipitate was removed by filtration

and washed with 1 M HCl (2 x 10 mL). After evaporation of the solvent under reduced pressure, the product **6** was purified via flash chromatography (40 g silica gel, 13 x 2.5 cm, CH_2Cl /methanol/NH_3 = 70:30 + 5 % NH_3 (fraction 1 – 9), 20:10 + 5 % NH_3 (fraction 10 – 12), 50 mL fractions).

Yield: 210 mg (873 µmol, 7 %), yellowish solid.

$C_{10}H_{12}N_2O_5$ [240.07 g/mol]

mp = 167 – 178 °C

$\mathbf{R_f}$ = 0.20 (CH_2Cl/MeOH/NH_3 = 75/25/1, UV und Ninhydrin)

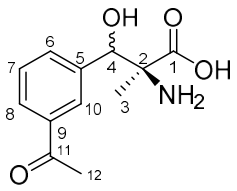
HPLC-MS (method_1): t_{R1} = 2.45 min, t_{R2} = 2.90 min

^{1}H-NMR (300.36 MHz, D_2O): δ = 8.28 – 8.20 (m, 4H, H-7, H-9), 7.67 – 7.58 (m, 4H, H-6, H-10), 5.33 (s, 1H, H-4, *syn*), 5.19 (s, 1H, H-4, *anti*), 1.63 (s, 1H, H-3, *anti*), 1.41 (s, 1H, H-3, *syn*).

^{13}C-NMR (75.53 MHz, D_2O): δ = 172.5 (C_q, C-1), 171.4 (C_q, C-1), 148.1 (C_q, C-8), 148.0 (C_q, C-8), 144.5 (C_q, C-5), 143.7 (C_q, C-5), 128.6 (C-6, C-10), 128.2 (C-6, C-10), 123.9 (C-7, C-9), 123.68 (C-7, C-9), 74.08 (C-4), 73.66 (C-4), 64.38 (C-2), 63.94 (C-2), 19.33 (C-3), 17.83 (C-3).

HRMS (MALDI-TOF): Calcd. for $C_{10}H_{12}N_2O_5H$ $[M+H]^+$: 241.0829; found: 241.0824.

6.3.7 (2*S*)-3-(3-Acetylphenyl)-2-amino-3-hydroxy-2-methylpropanoic acid (6)



6

In a 100 mL round bottom flask 2.67 g (30.0 mmol, 10.0 eq) DL-alanine (**18**) were dissolved in 3 mL KPi-buffer, 21.7 mL H_2O and 2 mL isopropanol. Under stirring 300 µL PLP were added and the yellow solution was stirred at RT for 10 min. Then a solution of 445 mg (3 mmol, 1.0 eq) 3-acetylbenzaldehyde (**22**) in 1 mL isopropanol and 1 mL LTA were added. The suspension was stirred at RT for 18 h. After addition of another 1 mL LTA the suspension was stirred at RT for 3 d. The conversion was monitored via HPLC-MS. 2.5 mL formic acid were added to the yellowish, cloudy suspension and the solution

was stirred at RT for 10 min. After evaporation of the solvent under reduced pressure, the product **6** was purified via flash chromatography (60 g silica gel C18, 10.5 x 3 cm, H_2O/methanol = 98:2 + 0.1 % formic acid (fraction 1 – 5), 90:10 + 0.1 % formic acid (fraction 6 – 10), 50 mL fractions). Then a short chromatography was done and therefore the yellow solid was filtered through a pad of silica gel (diameter: 2 cm, height: 2 cm) and the filter cake was washed with CH_2Cl/MeOH = 80.20 + 5 % NH_3 (11 x 25 mL fractions). The filtrates of fraction 6 to 10 were collected and the solvent was removed under reduced pressure.

Yield: 152 mg (640 µmol, 21 %), yellowish solid.

$C_{12}H_{15}NO_4$ [237.10 g/mol]

mp = 173- 185 °C

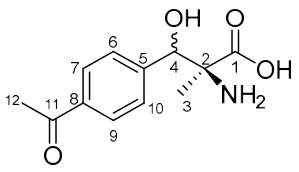
HPLC-MS (method_4): t_{R1} = 3.37 min, t_{R2} = 3.65 min

^{1}H-NMR (300.36 MHz, D_2O): δ = 8.02 – 7.97 (m, 4H, H-Ar), 7.72 – 7.53 (m, 4H, H-Ar), 5.22 (s, 1H, H-4, *syn*), 5.13 (s, 1H, H-4, *anti*), 2.68 (d, $^2J_{HH}$ = 5.5 Hz, 6H, H-12), 1.63 (s, 3H, H-3 *anti*), 1.30 (s, 3H, H-3, *syn*).

^{13}C-NMR (75.53 MHz, D_2O): δ = 203.7 (C_q, C-11), 203.6 (C_q, C-11), 175.2 (C_q, C-1), 174.1 (C_q, C-1), 138.5 (C_q, C-9), 138.2 (C_q, C-9), 136.7 (C_q, C-5) 136.6 (C_q, C-5), 132.7 (C-Ar), 132.2 (C-Ar), 129.1 (C-Ar), 129.0 (C-Ar), 128.8 (C-Ar), 128.8 (C-Ar), 127.1 (C-Ar), 126.7 (C-Ar), 74.6 (C-4), 74.4 (C-4), 65.8 (C_q, C-2), 65.0 (C_q, C2), 26.37 (C-12), 20.1 (C-3), 18.4 (C-3).

HRMS (MALDI-TOF): Calcd. for $C_{12}H_{15}NO_4H$ $[M+H]^+$: 238.1079; found: 238.1078.

6.3.8 (2*S*)-3-(4-Acetylphenyl)-2-amino-3-hydroxy-2-methylpropanoic acid (7)



7

In a 100 mL round bottom flask 2.67 g (30.0 mmol, 10.0 eq) DL-alanine (**18**) were dissolved in 3 mL KPi-buffer, 21.7 mL dist. H_2O and 2 mL isopropanol. Under stirring 300 µL PLP were added. The yellow solution was stirred at RT for 10 min. Then a solution of 445 mg (3 mmol, 1.0 eq) 4-acetylbenzaldehyde (**23**) in 1 mL isopropanol and 1 mL LTA

were added. The suspension was stirred at RT for 18 h. After addition of another 1 mL LTA the suspension was stirred at RT for 4 d. The conversion was monitored via HPLC-MS. 2.5 mL formic acid were added to the yellowish, cloudy suspension and the solution was stirred at RT for 20 min. After evaporation of the solvent under reduced pressure, the product **7** was purified via flash chromatography (60 g silica gel C18, 10.5 x 3 cm, H_2O/methanol = 98:2 + 0.1 % formic acid (fraction 1 – 5), 90:10 + 0.1 % formic acid (fraction 6 – 10), 50 mL fractions).

Yield: 157 mg (662 µmol, 22 %), yellowish solid.

$C_{12}H_{15}NO_4$ [237.10 g/mol]

mp = 172 – 181 °C

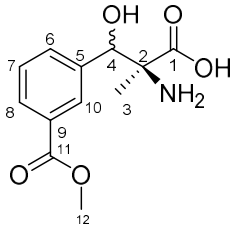
HPLC-MS (method_5): t_{R1} = 3.01 min, t_{R2} = 3.36 min.

^{1}H-NMR (300.36 MHz, D_2O): δ = 8.05 – 7.98 (m, 4H, H-7, H-9), 7.59 – 7.51 (m, 8.2 Hz, 4H, H-6, H-10), 5.22 (s, 1H, H-4, *syn*), 5.12 (s, 1H, H-4, *anti*), 2.7 (s, 3H, H-12, *syn*), 2.6 (s, 3H, H-12, *anti*), 1.63 (s, 3H, H-3, *anti*), 1.31 (s, 3H, H-3, *syn*).

^{13}C-NMR (75.53 MHz, D_2O): δ = 203.7 (C_q, C-11), 203.6 (C_q, C-11), 174.8 (C_q, C-1), 173.7 (C_q, C-1), 143.6 (C_q, C-5), 143.3 (C_q, C-5), 136.8 (C_q, C-8), 136.7 (C_q, C-8), 128.8 (C-7, C-9), 128.7 (C-7, C-9), 127.7 (C-6, C-10), 127.2 (C-6, C-10), 74.5 (C-4), 74.4 (C-4), 65.8 (C_q, C-2), 64.9 (C_q, C-2), 26.4 (C-12), 20.0 (C-3), 18.3 (C-3).

HRMS (MALDI-TOF): Calcd. for $C_{12}H_{15}NO_4H$ $[M+H]^+$: 238.1079; found: 238.1081.

6.3.9 (2*S*)-2-Amino-3-hydroxy-3-[3-(methoxycarbonyl)phenyl]-2-methyl-propanoic acid (8)



8

In a 100 mL round bottom flask 2.67 g (30.0 mmol, 10.0 eq) DL-alanine (**18**) were dissolved in 3 mL KPi-buffer, 21.7 mL H_2O and 2 mL isopropanol. Under stirring 300 µL PLP were added and the yellow solution was stirred at RT for 10 min. Then a solution of 492 mg (3 mmol, 1.0 eq) methyl-3-formylbenzoate (**24**) in 1 mL isopropanol and 1 mL LTA

were added. The suspension was stirred at RT for 18 h. After addition of another 1 mL LTA the suspension was stirred at RT for 4 d. The conversion was monitored via HPLC-MS. 2.5 mL formic acid were added to the yellowish, cloudy suspension and the solution was stirred at RT for 15 min. After evaporation of the solvent under reduced pressure, the product **8** was purified via flash chromatography (60 g silica gel C18, 10.5 x 3 cm, H_2O/methanol = 98:2 + 0.1 % formic acid (fraction 1 – 4), 90:10 + 0.1 % formic acid (fraction 5 – 14), 50 mL fractions).

Yield: 210 mg (829 µmmol, 28 %), colorless solid.

$C_{12}H_{15}NO_5$ [253.10 g/mol]

mp = 175 – 184 °C

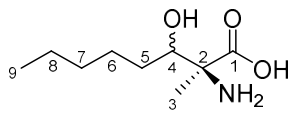
HPLC-MS (method_5): t_{R1} = 4.48 min, t_{R2} = 4.83 min

^{1}H-NMR (300.36 MHz, D_2O): δ = 8.04 – 7.94 (m, 4H, H-Ar), 7.75 – 7.46 (m, 4H, H-Ar), 5.27 (s, 1H, H-4, *syn*), 5.14 (s, 1H, H-4, *anti*), 3.89 (s, 6H, H-12), 1.62 (s, 3H, H-3, *anti*), 1.39 (s, 3H, H-3, *syn*).

^{13}C-NMR (75.53 MHz, D_2O): δ = 172.8 (C_q, C-1), 171.6 (C_q, C-1), 169.0 (C_q, C-11), 165.6 (C_q, C-11), 137.6 (C_q, C-5), 136.8 (C_q, C-5), 132.4 (C_q, C-Ar), 132.0 (C_q, C-Ar), 130.2 (C-Ar), 130.1 (C-Ar), 130.0 (C-Ar), 129.8 (C-Ar), 129.2 (C-Ar), 129.0 (C-Ar), 128.3 (C-Ar), 127.8 (C-Ar), 74.4 (C-4), 74.0 (C-4), 64.4 (C_q, C-2), 64.0 (C_q, C-2), 52.8 (C-12), 19.2 (C-3), 17.9 (C-3).

HRMS (MALDI-TOF): Calcd. for $C_{12}H_{15}NO_5H$ $[M+H]^+$: 254.1028; found: 245.1022.

6.3.10 (2*S*)-2-Amino-3-hydroxy-2-methyloctanoic acid (9)



9

In a 100 mL round bottom flask 2.67 g (30 mmol, 10.0 eq) DL-alanine (**18**) were dissolved in 3 mL KPi-buffer and 23.2 mL dist. H_2O. Under stirring 300 µL PLP and 1.5 mL isopropanol were added. The yellow solution was stirred at RT for 10 min. Then 1 mL LTA was added. 362 µL hexanal (**25**) were added under stirring in 4 portions over 6 h. After 20 h further 1 mL LTA were added. The suspension was stirred at RT for 5 d. The conversion

was monitored via HPLC-MS. The yellow, cloudy suspension was heated to 90 °C. After 45 min the suspension was allowed to cool to RT. The suspension was concentrated by rotary evaporation. The product **9** was purified via flash chromatography (60 g silica gel C18, 10.5 x 3 cm, H_2O/methanol = 98:2 + 0.1 % formic acid (fraction 1 – 2), 96:4 + 0.1 % formic acid (fraction 3), H_2O/acetonitrile 50:50 + 0.1 % formic acid (fraction 4 - 7), 100 mL fractions).

Yield: 57 mg (300 µmol, 10 %), colorless solid.

$C_9H_{19}NO_3$ [189,14 g/mol]

$[\alpha]_D^{25}$ = -32.2 (c = 0.2 in 1 M HCl)

mp = 205 – 245 °C

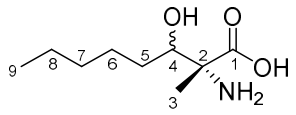
HPLC-MS (method_5): t_{R1} = 3.97 min, t_{R2} = 4.31 min.

^{1}H-NMR (300.36 MHz, D_2O): δ = 3.98 (d, $^3J_{HH}$ = 8.9 Hz, 1H, H-4, *syn*), 3.82 (d, $^3J_{HH}$ = 7.5 Hz, 1H, H-4, *anti*), 1.59 – 1.57 (m, 2H, H-5), 1.56 (s, 3H, H-3, *anti*), 1.48 (s, 3H, H-3, *syn*), 1.29 (m, 12H, H-6, H-7, H-8), 0.85 (s, 3H, H-9), 0.84 (s, 3H, H-9).

^{13}C-NMR (75.53 MHz, D_2O): δ = 173.2 (C_q, C-1), 172.5 (C_q, C-1), 73.3 (C-4), 72.6 (C-4), 64.3 (C_q, C-2), 64.1(C_q, C-2), 30.5 (C-7), 30.4 (C-7), 29.4 (C-5), 25.1 (C-6), 24.8 (C-6), 21.8 (C-8),

HRMS (MALDI-TOF): Calcd. for $C_{29}H_{30}N_2O_5H$ $[M+H]^+$: 190.1443; found: 190.1444.

6.3.11 (2*R*)-2-Amino-3-hydroxy-2-methyloctanoic acid (10)



10

In a 100 mL round bottom flask 2.67 g (30 mmol, 10.0 eq) DL-alanine (**18**) were dissolved in 3 mL KPi-buffer and 23.2 mL dist. H_2O. Under stirring 300 µL PLP and 1.5 mL isopropanol were added. The yellow solution was stirred at 15 °C for 15 min. Then 90 µL hexanal (**25**) and 0.5 mL DTA were added. The reaction mixture was stirred at 15 °C. After 3.5 h further 90 µL hexanal (**25**) were added. The reaction mixture was shaken at 120 rpm at 17.5 – 18.5 °C. After 20 h further 182 µL aldehyde **25** and 0.5 mL DTA were added. The suspension was shaken at 17.5 – 18.5 °C for 4 d. The conversion was monitored via HPLC-MS. The suspension was concentrated by rotary evaporation. The product **10** was purified via flash chromatography (60 g silica gel C18, 10.5 x 3 cm, H_2O/methanol = 98:2

+ 0.01 % formic acid (fraction 1 – 2), 85:15 + 0.01 % formic acid (fraction 3 - 8), 80:20 + 0.01 % formic acid (fraction 9 - 16), 100 mL fractions (fraction 1 – 2), 50 mL fractions (fraction 3 – 16)).

Yield: 153 mg (808 µmol, 27 %), colorless solid.

$C_9H_{19}NO_3$ [189.14 g/mol]

mp = 221 – 235 °C

R_f = 0.34 (CH_2Cl/MeOH/NH_3 = 10/10/1, UV und Ninhydrin)

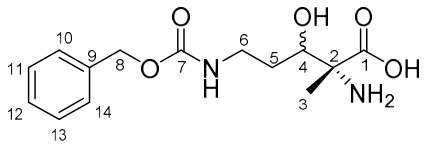
HPLC-MS (method_5): t_{R1} = 4.03 min, t_{R2} = 4.66 min.

^{1}H-NMR (300.36 MHz, D_2O): δ = 3.96 (d, $^3J_{HH}$ = 9.2 Hz, 1H, H-4, *syn*), 1.57 (m, 2H, H-5, *syn*), 1.46 (s, 3H, H-3, *syn*), 1.30 (m, 6H, H-6, H-7, H-8), 0.84 (m, 3H, H-9, *syn*).

^{13}C-NMR (75.53 MHz, D_2O): δ = 173.1 (C_q, C-1), 72.6 (C-4), 64.3 (C_q, C-2), 30.5 (C-7), 29.4 (C-6), 24.8 (C-5), 21.8 (C-8), 16.7 (C-3), 13.2 (C-9).

HRMS (MALDI-TOF): Calcd. for $C_9H_{19}NO_3H$ $[M+H]^+$: 190.1443; found: 190.1443.

6.3.12 (2*R*)-2-Amino-5-(((benzyloxy)carbonyl)amino)-3-hydroxy-2-methyl-pentanoic acid (11)



11

In a 100 mL round bottom flask 1.34 g (15 mmol, 10.0 eq) DL-alanine (**18**) were dissolved in 1.5 mL KPi-buffer and 10.85 mL dist. H_2O. Under stirring 150 µL PLP, 0.5 mL and 30 µL $MnCl_2$ were added. The yellow solution was stirred at 15 °C for 15 min. 0.25 mL DTA were added. Then a solution of 311 mg (1.5 mmol, 1 eq.) benzyl (3-oxopropyl)carbamate (**27**) in 0.5 mL isopropanol was added over a period of 10 h. The reaction mixture was shaken at 120 rpm at 17.5 – 18.5 °C. After 20 h further 0.25 mL DTA were added. The suspension was shaken at 17.5 – 18.5 °C for 2 d. The conversion was monitored via HPLC-MS. 85 mL MeOH were added to the reaction mixture and it was stirred for 18 h. The precipitate was removed by filtration and washed with MeOH (3 x 15 mL). The filtrate was concentrated by rotary evaporation and the product **11** was purified via flash chromatography (60 g silica gel C18, 10.5 x 3 cm, H_2O/methanol = 98:2 + 0.01 % formic

acid (fraction 1 – 2), 90:10 + 0.01 % formic acid (fraction 3 - 15), 85:15 + 0.01 % formic acid (fraction 16 - 28), 100 mL fractions(fraction 1 – 2), 50 mL fractions (fraction 3 – 16)).

Yield: 140 mg (473 µmol, 32 %), colorless solid.

$C_{14}H_{20}N_2O_5$ [296.14 g/mol]

$[\alpha]_D^{25}$ = -36.3 (c = 0.2 in 1 M HCl)

mp = 180 – 193 °C

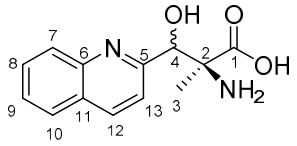
HPLC-MS (method_5): t_{R1} = 4.54 min, t_{R2} = 4.61 min.

^{1}H-NMR (300.36 MHz, D_2O): δ = 7.38 (s, 5H, H-10, H-11, H-12, H-13, H-14), 5.08 (s, 2H, H-8), 4.02 (d, $^3J_{HH}$ = 10.4 Hz, 1H, H-4), 3.28 – 3.17 (m, 2H, H-6), 1.90 – 1.80 (m, 1H, H-5), 1.53 – 1.47 (m, 1H, H-5), 1.42 (s, 3H, H-3).

^{13}C-NMR (75.53 MHz, D_2O): δ = 172.8 (C_q, C-1), 158.5 (C_q, C-7), 136.5 (C_q, C-9), 128.8 (C-Ar), 128.4 (C-Ar), 127.6 (C-Ar), 67.0 (C-4), 66.9 (C-8), 64.2 (C_q, C-2), 37.1 (C-6), 29.8 (C-5), 16.8 (C-3).

HRMS (MALDI-TOF): Calcd. for $C_{14}H_{20}N_2O_5H$ $[M+H]^+$: 297.1451; found: 214.1459.

6.3.13 (2*S*)-2-Amino-3 hydroxy-2-methyl-3-(quinolin-2-yl)propanoic acid (12)



12

In a 100 mL round bottom flask 2.67 g (30 mmol, 10.0 eq) DL-alanine (**18**) were dissolved in 3 mL KPi-buffer and 21.7 mL H_2O. Under stirring 300 µL PLP were added and the yellow solution was stirred at RT for 10 min. Then 1 mL LTA was added. A solution of 472 mg (3.0 mmol, 1.0 eq) quinolone-2-carbaldehyde (**27**) in 2 mL ethanol was added under stirring in 5 portions over 8 h. After 20 h another 1 mL LTA was added. The suspension was stirred at RT for 7 d. The conversion was monitored via HPLC-MS. The brown, cloudy suspension was heated to 80 °C. After 2 h the suspension was allowed to cool to RT. The suspension was transferred to a separating funnel and extracted with ethyl acetate (2 x 20 mL). The aqueous phase was concentrated by rotary evaporation. The product **12** was purified via flash chromatography (60 g silica gel C18, 10.5 x 3 cm, H_2O/methanol = 98:2

+ 0.1 % formic acid (fraction 1 – 3), 80:20 + 0.1 % formic acid (fraction 4 – 20), 75:25 + 0.1 % formic acid (fraction 21 – 25), 50 mL fractions).

Yield: 807 mg (3.51 mmol, 16 %), colorless solid.

$C_{13}H_{14}N_2O_3$ [246.10 g/mol]

mp = 170 – 178 °C

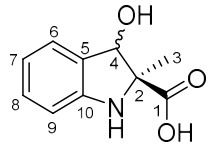
HPLC-MS (method): t_{R1} = 3.57 min, t_{R2} = 4.02 min,

^{1}H-NMR (300.36 MHz, D_2O): δ = 9.06 (d, $^3J_{HH}$ = 8.7 Hz, 1H, H-12), 8.27 (t, J = 9.4 Hz, 2H, H-Ar), 8.15 – 8.06 (m, 2H, H-Ar), 8.02 (d, $^3J_{HH}$ = 8.6 Hz, 1H, H-Ar), 7.91 (d, $^3J_{HH}$ = 7.3 Hz, 1H, H-Ar), 5.61 (s, 1H, H-4), 1.71 (s, 3H, H-3).

^{13}C-NMR (75.53 MHz, D_2O): δ = 170.4 (C_q, C-1), 165.6 (C-2), 154.3 (C-4), 147.5 (C-Ar), 135.8 (C-Ar), 130.5 (C_q, C-11), 129.3 (C-Ar), 128.9 (C-Ar), 120.8 (C-Ar), 120.0 (C-Ar), 72.8 (C_q, C-6), 64.0 (C_q, C-5), 19.5 (C-3).

HRMS (MALDI-TOF): Calcd. for $C_{13}H_{14}N_2O_3H$ $[M+H]^+$: 247.1083; found: 214.1087.

6.3.14 (2*S*)-3-Hydroxy-2-methylindole-2-carboxylic acid (13)



13

A flame dried and N_2 flushed 100 mL three-neck round-bottom flask equipped with magnetic stirring bar, dry-ice condenser, and gas-bubbler was charged with 150 mg (624 µmol, 1 eq.) 2-amino-3-hydroxy-2-methyl-3-(2-nitrophenyl)propanoic acid (**4**). It was cooled to -78°C (dry ice/acetone) and 25 mL NH_3 were condensed into the flask. Afterwards 34 mg Li (4.9 mmol, 7.85 equiv.) were slowly added. It was stirred for 4 h. Then the dark blue suspension was quenched by the addition of 20 mL H_2O. The suspension was concentrated by rotary evaporation. The product **13** was purified via flash chromatography (5 g silica gel, 6.5 x 1 cm, DCM/methanol = 70:30 + 5 % NH_3, 3 mL fractions).

Yield: 11 mg (57 µmol, 9 %), yellow oil.

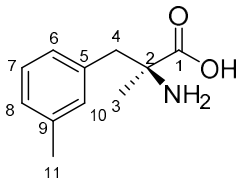
$C_{10}H_{11}NO_3$ [193,26 g/mol]

HPLC-MS (method_5): t_{R1} = 4.44 min, t_{R1} = 4.66 min

^{1}H-NMR (300.36 MHz, D_2O): δ = 7.50 - 7.36 (m, 4H, H-Ar), 7.28 – 7. 20 (m, 2H, H-Ar), 7.05 -6.98 (m, 2H, H-Ar), 5.21 (s, 1H, H-4), 1.43 (s, 3H, H-3, *anti*), 1.33 (s, 3H, H-3, *syn*).

^{13}C-NMR (75.53 MHz, D_2O): δ = 169.2 (C_q, C-1), 165.7 (C_q, C-1), 133.1 (C_q, C-10), 130.9 (C_q, C-10), 129.9 (C_q, C-5), 129.5 (C_q, C-5),125.1 (C-6), 124.8 (C-6), 124.5 (C-7), 116.6 (C-9), 116.1 (C-9), 71.1 (C-4), 68.9 (C-4), 60.2 (C_q, C-2), 18.2 (C-3), 13.8 (C-3).

6.3.15 (S)-2-Amino-2-methyl-3-(m-tolyl)propanoic acid (14)



14

A flame dried and N_2-flushed 100 mL three-neck round-bottom flask equipped with magnetic stirring bar, dry-ice condenser, and gas-bubbler was charged with 100 mg (395 µmol, 1 eq.) (2*S*)-2-amino-3-hydroxy-3-[3-(methoxycarbonyl)phenyl]-2-methyl-propanoic acid (**8**). It was cooled to -78°C (dry ice/ acetone) and 25 mL NH_3 were condensed into the flask. Afterwards 8.5 mg Li (1.2 mmol, 3.1 equiv.) were slowly added. It was stirred for 1 h. Further 27 mg Li (3.9 mmol, 9.9 eq.) were slowly added. Then the dark blue suspension was quenched by the addition of 15 mL H_2O. The suspension was concentrated by rotary evaporation and the residue was dissolved in 1.5 mL water/MeOH (1:2) and 300 µL formic acid. The product **14** was purified via preparative reversed phase HPLC (method_6).

Yield: 8 mg (41 µmol, 11 %), colorless solid.

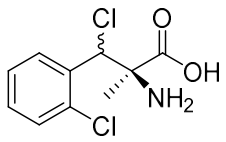
$C_{11}H_{15}NO_2$ [193,11 g/mol]

HPLC-MS (method_4): t_{R1} = 4.17 min

^{1}H-NMR (300.36 MHz, D_2O): δ = 7.0 (t, $^{3}J_{HH}$ = 7.5 Hz, 1H, H-Ar), 7.22 (d, $^{3}J_{HH}$ = 7.4 Hz, 1H, H-Ar), 7.11 – 7.06 (m, 2H, H-Ar), 3. 11 (dd, $^{3}J_{HH}$ = 96.0, Hz, $^{3}J_{HH}$ = 14.2 Hz, 2H, H-4), 2.33 (s3 6H, H-11), 1.55 (s, 3H, H-3).

^{13}C-NMR (75.53 MHz, D_2O): δ = 176.2 (C_q, C-1), 139.1 (C-9), 134.3 (C-5), 130.7 (C-10), 128.9 (C-7), 128.5 (C-8), 127.0 (C-6), 62,2 (C_q, C-2), 42.6 (C-4), 22.4 (C-3), 20.4(C-11).

6.3.16 (2*R*)-2-Amino-3-chloro-3-(2-chlorophenyl)-2-methylpropanoic acid (15)



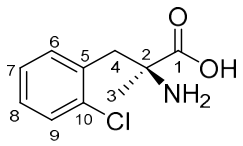
15

In a flame dried 10 mL round-bottom flask with nitrogen inlet 230 mg (1.0 mmol, 1.0 eq) 2-amino-3-(2-chlorophenyl)-3-hydroxy-2-methylpropanoic acid (**3**) were dissolved in 750 µL (10.3 mmol, 10.3 eq) thionyl chloride. The orange/red oil was stirred at RT for 7 d. The conversion was monitored via HPLC-MS. After 95 % conversion the red oil was added dropwise to 1 mL H_2O and 2 mL acetone at 0 °C. The solvent was removed under reduced pressure using an evaporator and a cooling trap. The collecting vessel was filled with 50 mL MeOH/H_2O (1:1) to bind HCl and quench thionyl chloride. The crude product was used without further purification in the next reaction.

$C_{10}H_{11}Cl_2NO_2$ [248.10 g/mol]

HPLC-MS (method_2): t_{R1} = 4.42 min, t_{R2} = 4.51 min.

6.3.17 (*S*)-2-Amino-3-(2-chlorophenyl)-2-methylpropanoic acid (16)



16

In a flame dried 50 mL two-neck round-bottom flask with nitrogen inlet 248 mg (1.0 mmol, 1.0 eq) 2-amino-3-chloro-3-(2-chlorophenyl)-2-methylpropanoic acid (**15**) were dissolved in 1.2 mL (20 mmol, 20 eq) acetic acid and 1 mL dest. H_2O. 100 mg zinc dust were added and the reaction mixture was stirred at RT for 2 d. 490 µL (18 eq) conc. HCl were added to the brown/grey suspension. The product **16** was purified via flash-chromatography (60 g silica gel C18, 10.5 x 3 cm, H_2O/methanol = 98:2 + 0.01 % formic acid (fraction 1 – 10), 90:10 + 0.01 % formic acid (fraction 11 - 21), 85:15 + 0.01 % formic acid (fraction 22 - 30), 50 mL).

Yield: 82 mg (384 µmol, 38 %), colorless solid.

$C_{10}H_{12}ClNO_2$ [213.66 g/mol]

$[\alpha]_D^{25}$ = -6.3 (c = 0.2 in 1 M HCl)

mp = 175 – 196 °C

HPLC-MS (method_2): t_{R1} = 3.37 min, t_{R2} = 3.65 min.

^{1}H-NMR (300.36 MHz, D_2O): δ = 7.49 (m, 1H, H-9), 7.34 (m, 3 H, H-6, H-7, H-8), 3.44 (dd, J = 14.6 Hz , 2 H, H-4), 1.66 (s, 1 H, H-3).

^{13}C-NMR (75.53 MHz, D_2O): δ = 173.2 (C-1), 123.6 (C-5), 132.4 (C-10), 131.0 (C-9), 130.0 (C-8), 129.9 (C-7), 127.5 (C-6), 61.0 (C-2), 39.0 (C-4), 21.4 (C-3).

HRMS (MALDI-TOF): Calcd. for $C_{10}H_{12}ClNO_2H$ $[M+H]^+$: 214.0635; found: 214.0637.

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8. Abbreviations

Analytial methods:

^{13}C-NMR	carbon NMR
^{1}H-NMR	proton NMR
APT	Attached Proton Test
bs	broad singlet
CH_{Ar}	aromatic proton
C_q	quarternary carbon
d	doublet
dd	doublet of doublet
decomp.	decomposition
dt	doublet of triplet
e.e.	enantiomeric excess
ESI	electrospray ionization
eV	electron volt
HH-COSY	proton-proton correlation spectroscopy
HPLC	high performance liquid chromatography
HPLC-MS	high performance liquid chromatography mass spectroscopy
HSQC	heteronuclear single quantum coherence
Hz	Hertz
J	signal multiplicity
m	multiplet
m/z	mass/charge-ratio
M^+	molecule peak
MHz	megahertz
mp	melting point
NMR	nuclear magnetic resonance
opt. rot.	optical rotation

ppm	parts per million
R_f	retention factor
RP-HPLC	reversed phase-HPLC
s	singlet
t	triplet
TLC	thin layer chromatography
t_R	retention time
UV	ultraviolet
δ	chemical shift

Chemical abbreviations:

AcOH	acetic acid
$CDCl_3$	deuterated chloroform
D_2O	deuterium oxide
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
Et	ethyl
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
Me	methyl
MeOH	methanol
SiO_2	silica gel
TFA	trifluoroacetic acid
DTA	*D*-threonine aldolase
LTA	*L*-threonine aldolase

Others:

(v/v)	volume/volume

(v/v/v)	volume/volume/volume
(w/w)	weight/weight
°C	Celsius
Å	Ångström
cm	centimeter
conc.	concentrated
d	day/-s
E	potential / enantioselectivity
EI	electron impact
eq	equivalents
et. al.	*et alii* (lat.: and co-workers)
g	gram
h	hour/-s
H^+	acidic
L	litre
m	meter
M	molar (mol/L)
min	minute/-s
mL	milliliter
mm	millimeter
nm	nanometer
ppm	parts per million
rpm	rounds per minute
U/mL	enzyme unit per millimeter
λ	wavelength
µL	microliter
µm	micrometer
µM	micromolar

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An erster Stelle gilt mein ganz besonderer Dank Prof. Rolf BREINBAUER für die Möglichkeit meine Masterarbeit in seiner Arbeitsgruppe anzufertigen. Außerdem bedanke ich mich für die interessante Themenstellung und für die nette Aufnahme in die Arbeitsgruppe. Vielen Dank für die Unterstützung während meiner Masterarbeit.

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10. Appendix

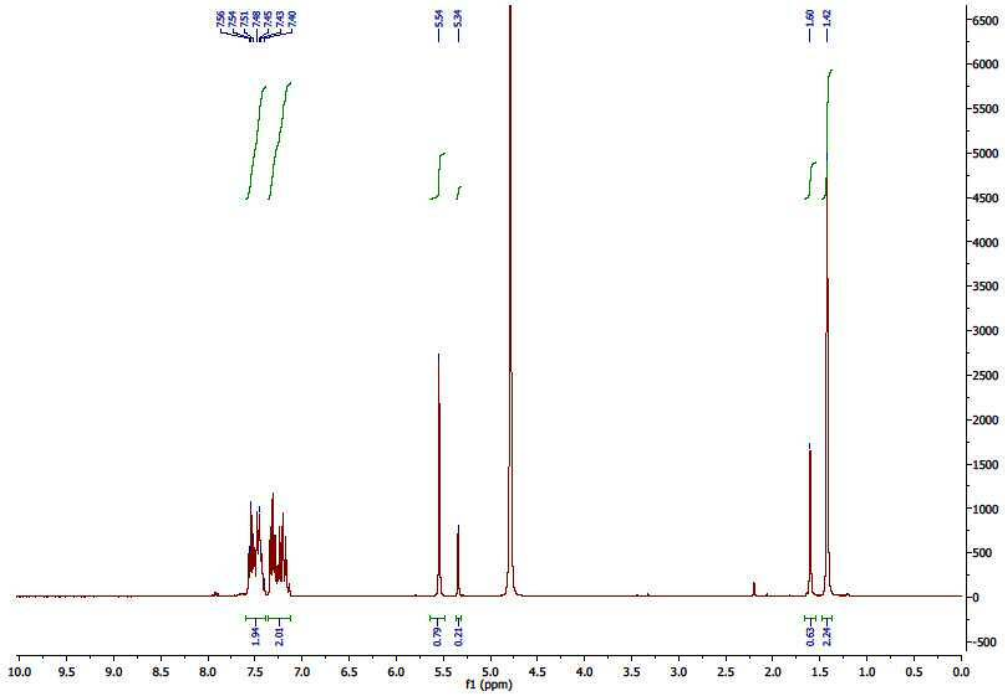

Figure 6. ^{1}H-NMR (2*S*)-2-amino-3-(2-fluorophenyl)-3-hydroxy-2-methylpropanoic acid (**1**)

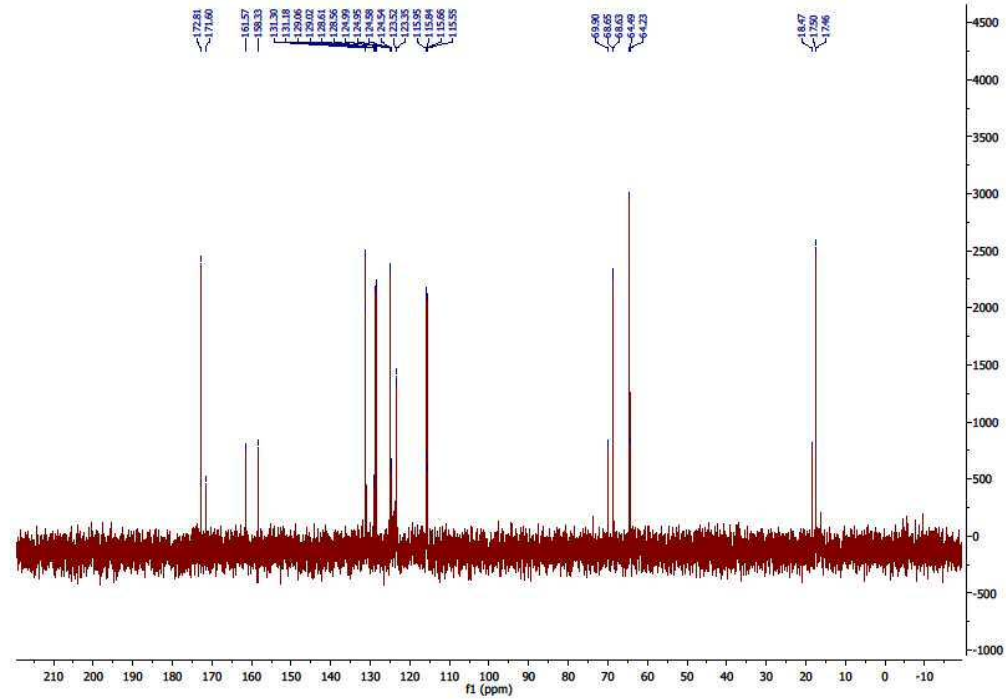

Figure 7. ^{13}C NMR of (2*S*)-2-amino-3-(2-fluorophenyl)-3-hydroxy-2-methylpropanoic acid (**1**)

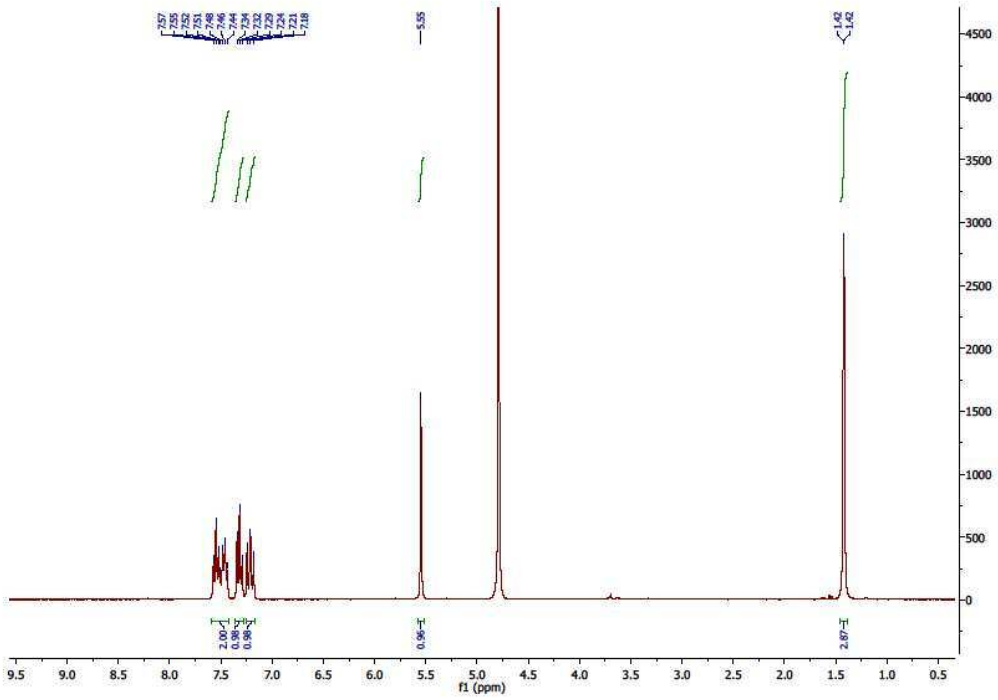

Figure 8. ^{1}H NMR of (2*R*)-2-amino-3-(2-fluorophenyl)-3-hydroxy-2-methylpropanoic acid (**2**)

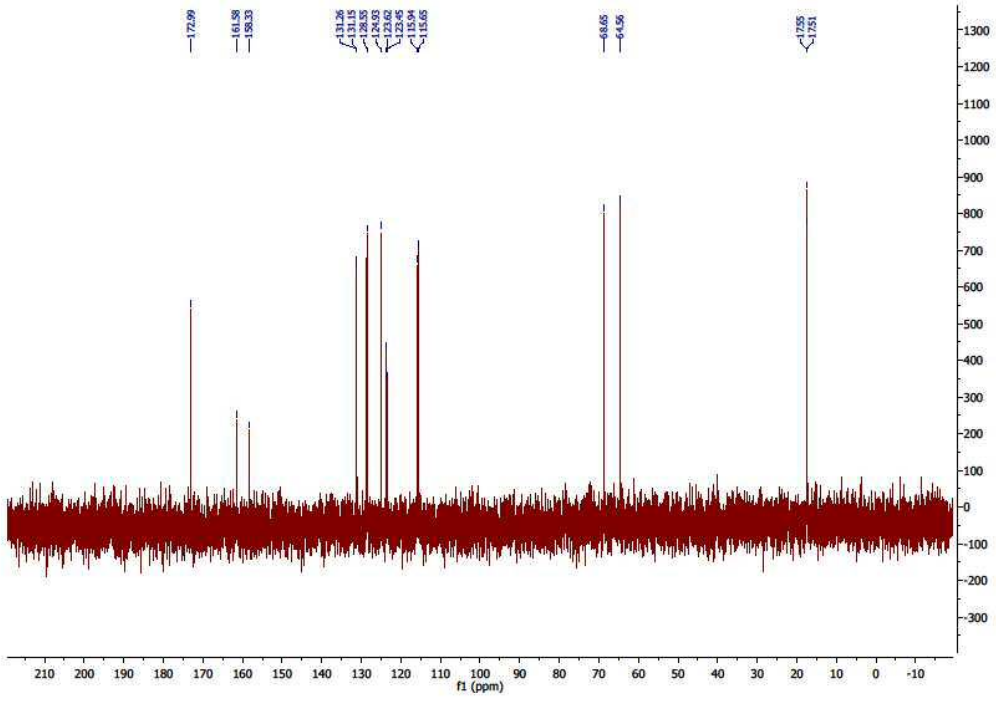

Figure 9. ^{13}C NMR of (2*R*)-2-amino-3-(2-fluorophenyl)-3-hydroxy-2-methylpropanoic acid (**2**)

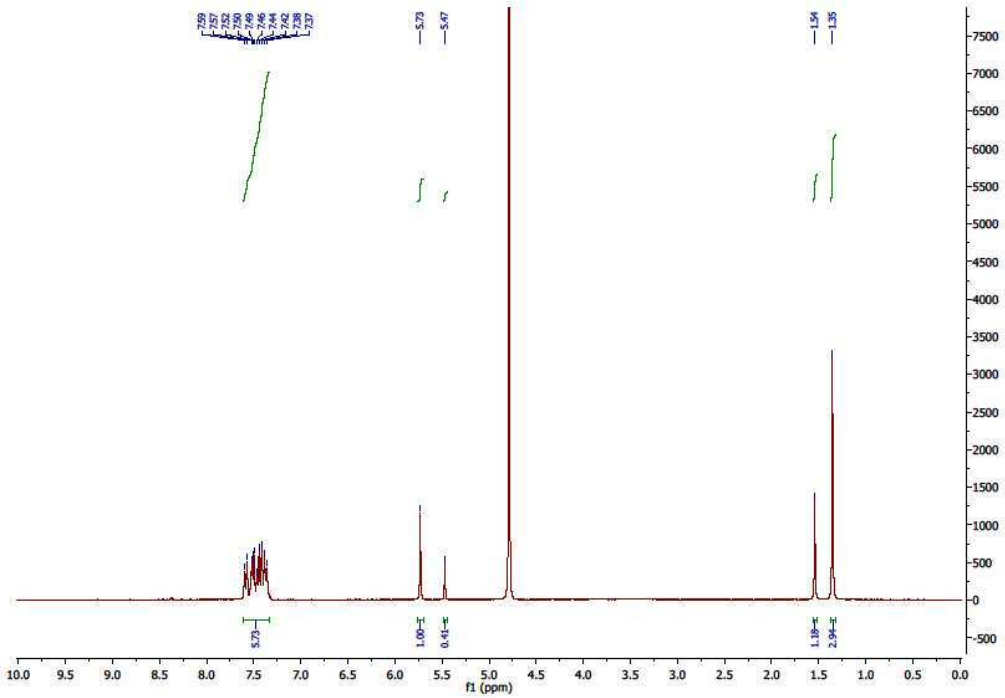

Figure 10. ^{1}H NMR of (2*S*)-2-amino-3-(2-chlorophenyl)-3-hydroxy-2-methylpropanoic acid (**3**)

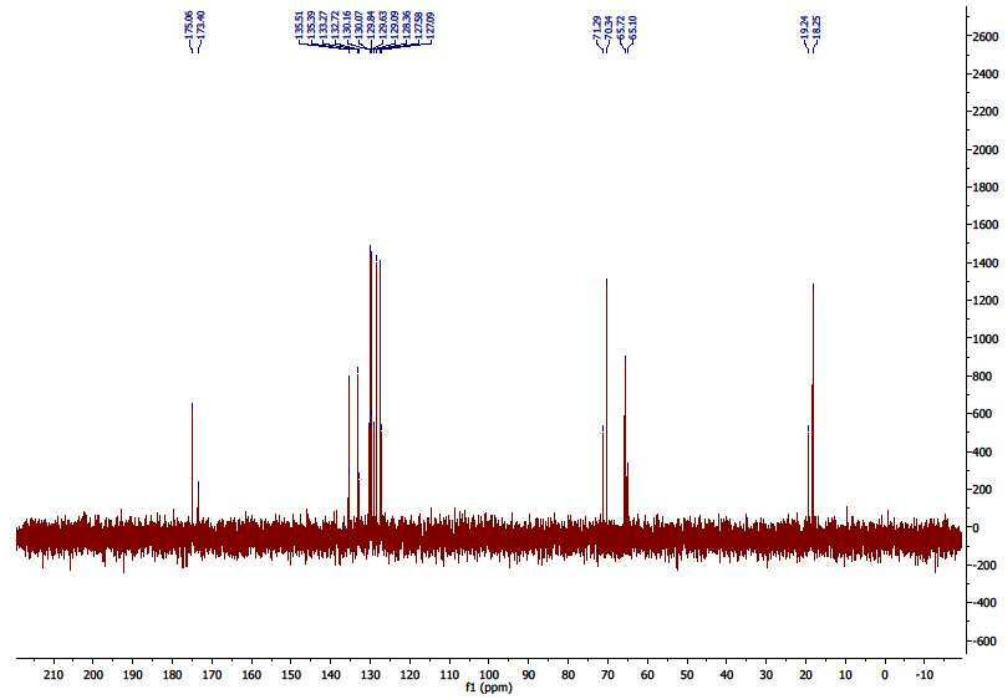

Figure 11. ^{13}C NMR of (2*S*)-2-amino-3-(2-chlorophenyl)-3-hydroxy-2-methylpropanoic acid (**3**)

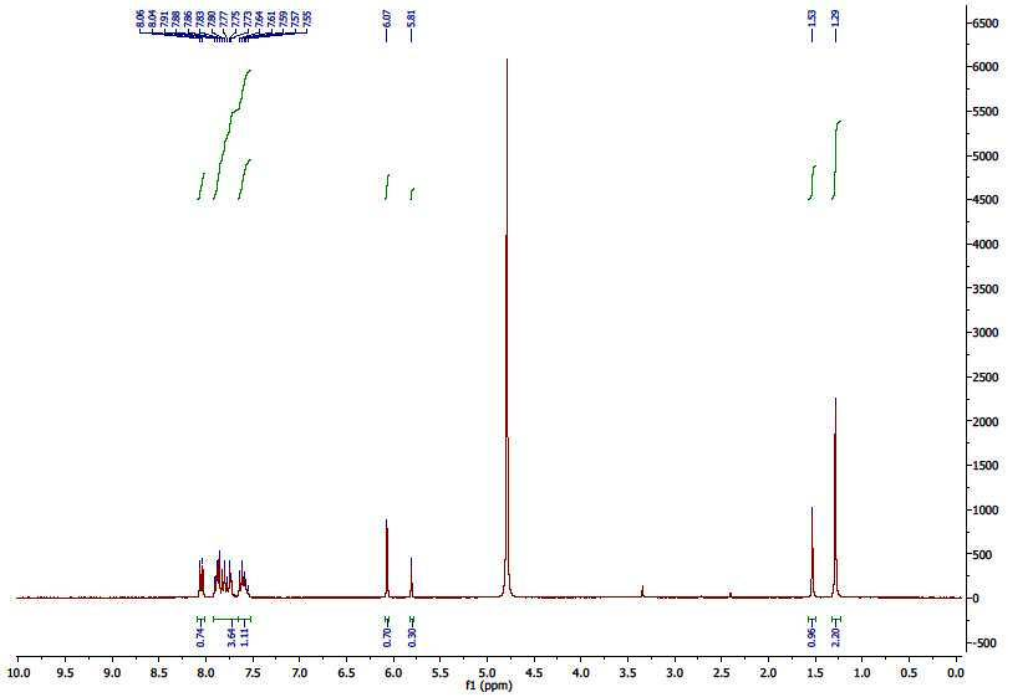

Figure 12. ^{1}H NMR of (2*S*)-2-amino-3-hydroxy-2-methyl-3-(2-nitrophenyl)propanoic acid (**4**)

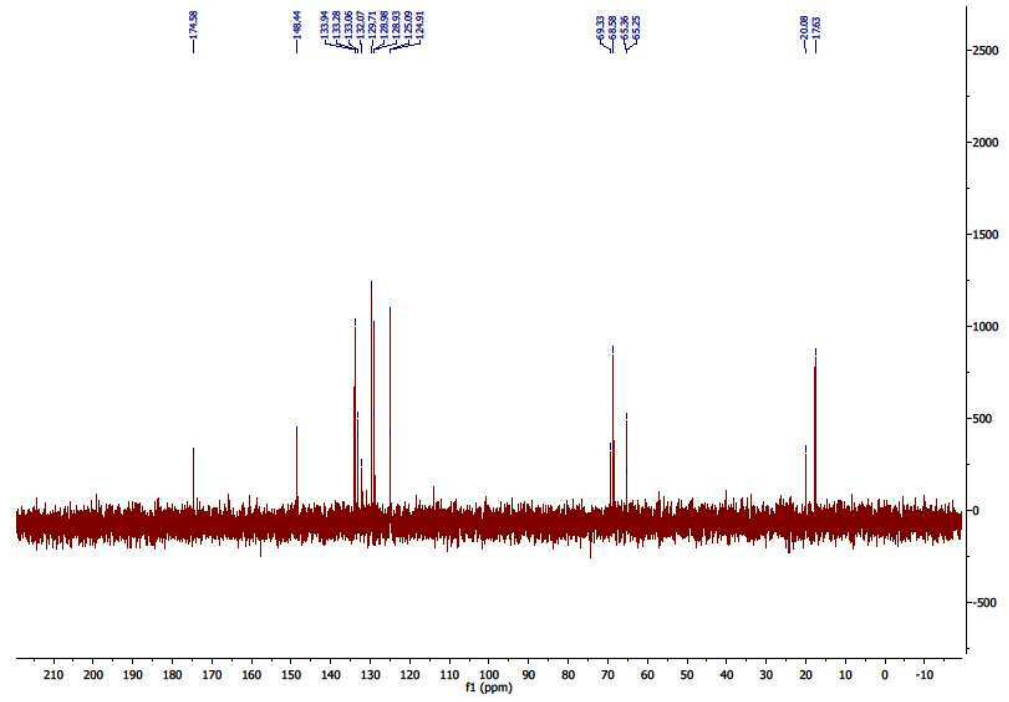

Figure 13. ^{13}C NMR of (2*S*)-2-amino-3-hydroxy-2-methyl-3-(2-nitrophenyl)propanoic acid (**4**)

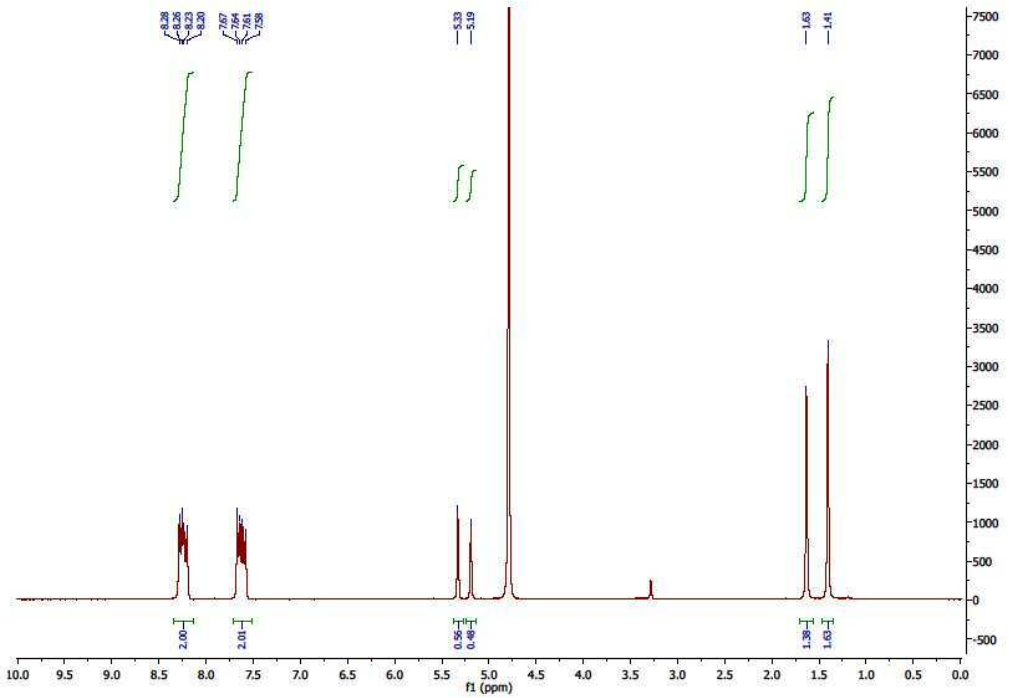

Figure 14. ^{1}H NMR of (2*S*)-2-amino-3-hydroxy-2-methyl-3-(4-nitrophenyl)propanoic acid (**5**)

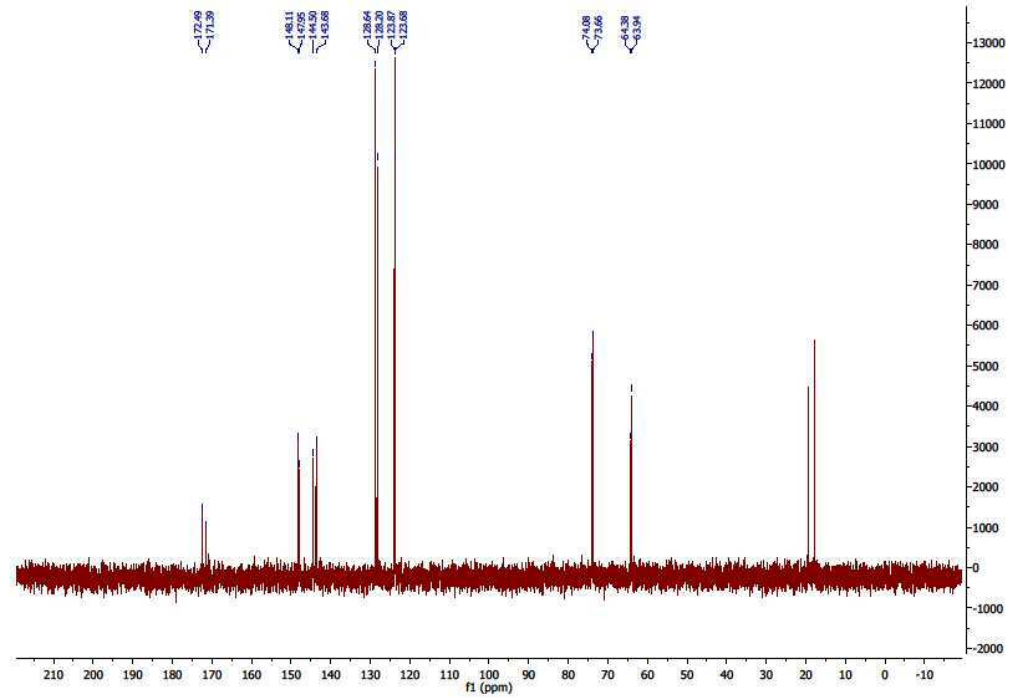

Figure 15. ^{13}C NMR of (2*S*)-2-amino-3-hydroxy-2-methyl-3-(4-nitrophenyl)propanoic acid (**5**)

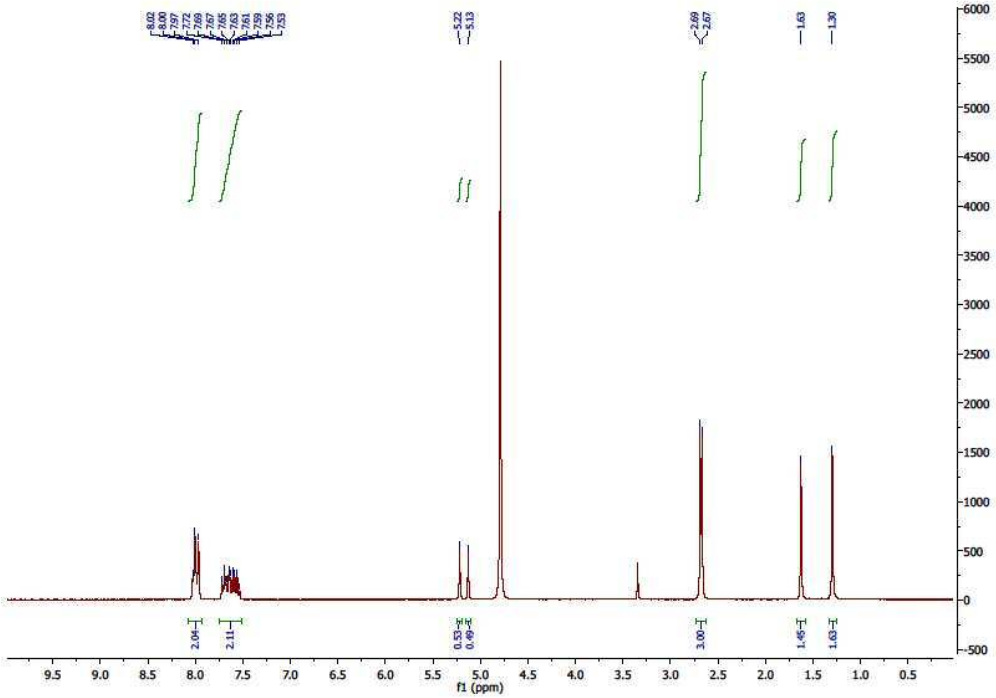

Figure 16. ^{1}H NMR of (2*S*)-3-(3-acetylphenyl)-2-amino-3-hydroxy-2-methylpropanoic acid (**6**)

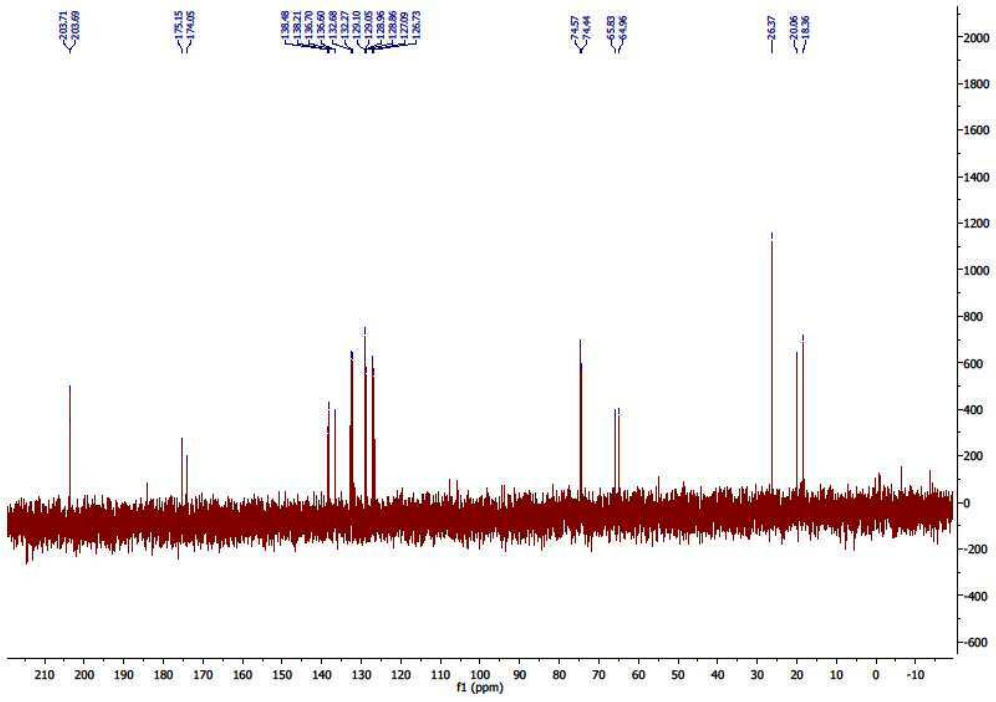

Figure 17. ^{13}C NMR of (2*S*)-3-(3-acetylphenyl)-2-amino-3-hydroxy-2-methylpropanoic acid (**6**)

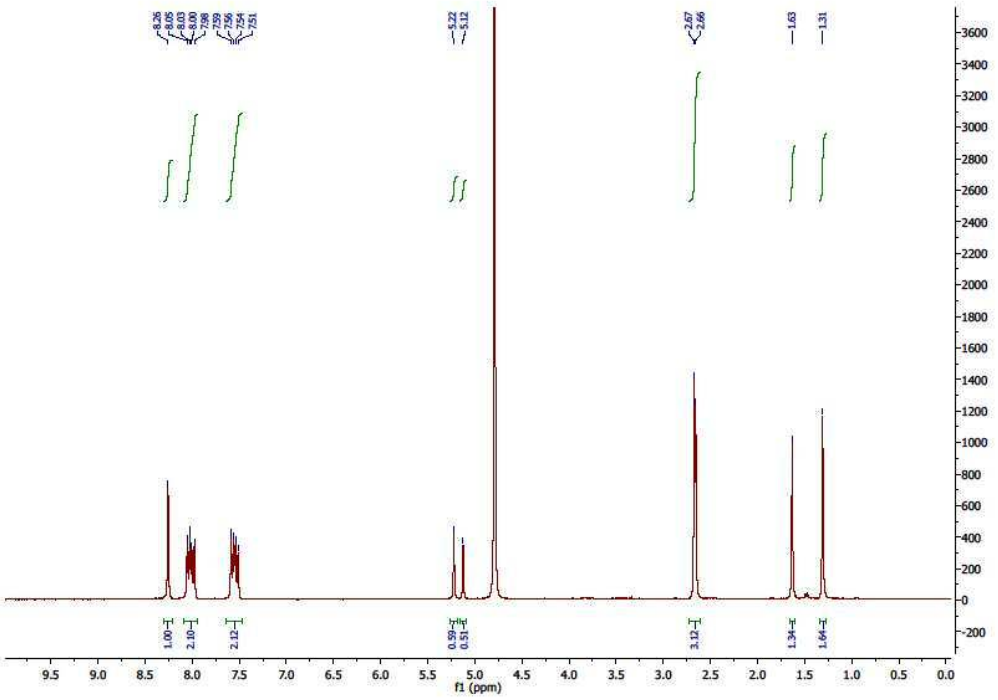

Figure 18. ^{1}H NMR of (2*S*)-3-(4-acetylphenyl)-2-amino-3-hydroxy-2-methylpropanoic acid (**7**)

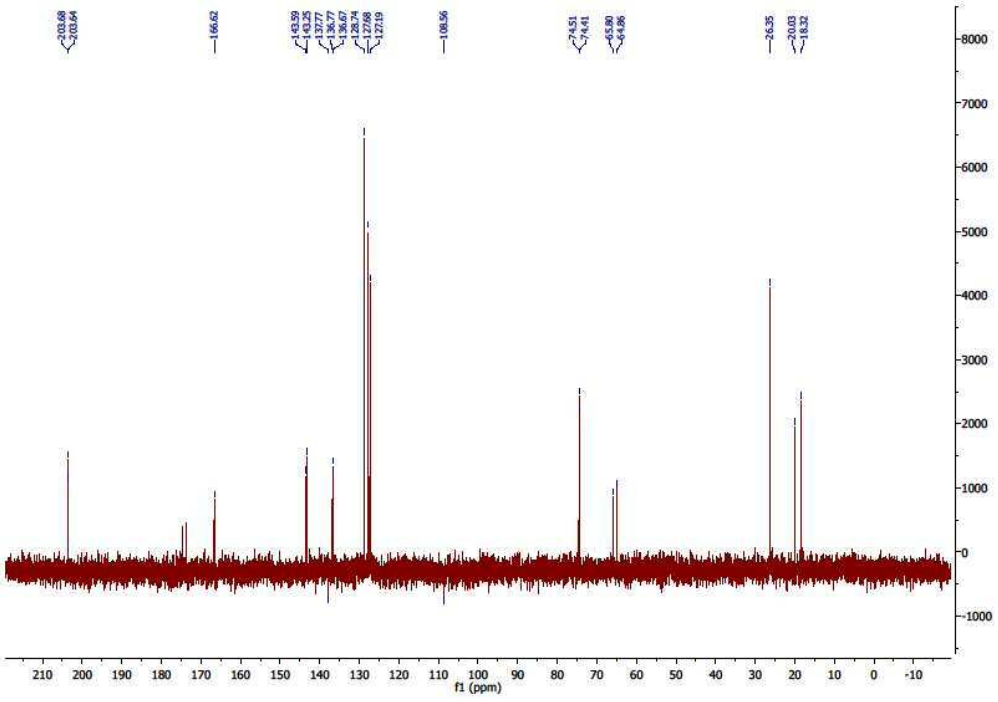

Figure 19. ^{13}C NMR of (2*S*)-3-(4-acetylphenyl)-2-amino-3-hydroxy-2-methylpropanoic acid (**7**)

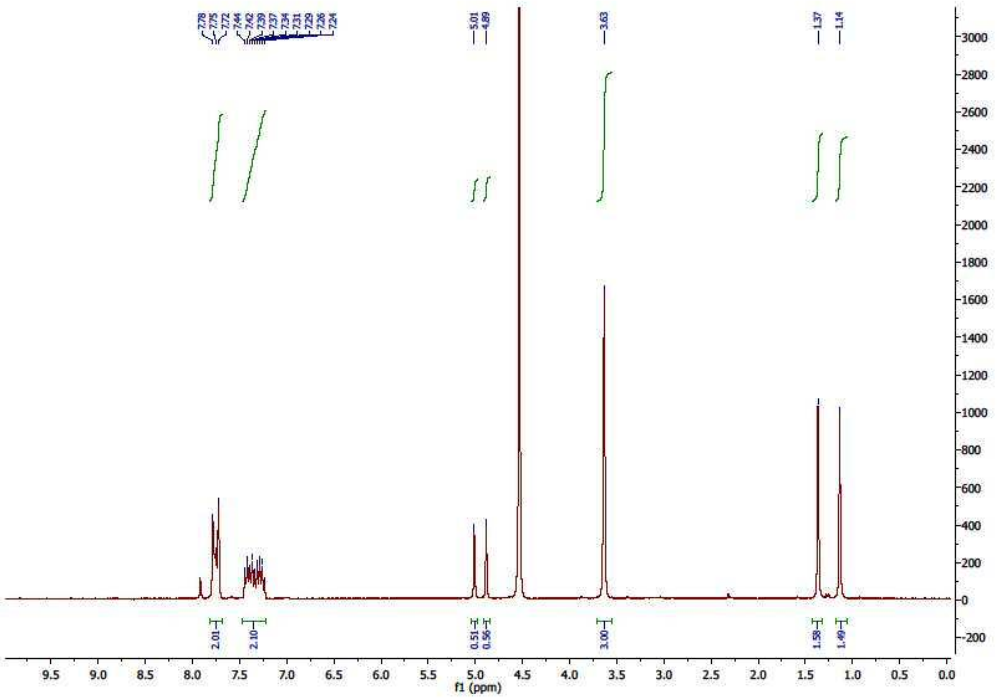

Figure 20. ^{1}H NMR of (2*S*)-2-amino-3-hydroxy-3-[3-(methoxycarbonyl)phenyl]-2-methyl-propanoic acid (**8**)

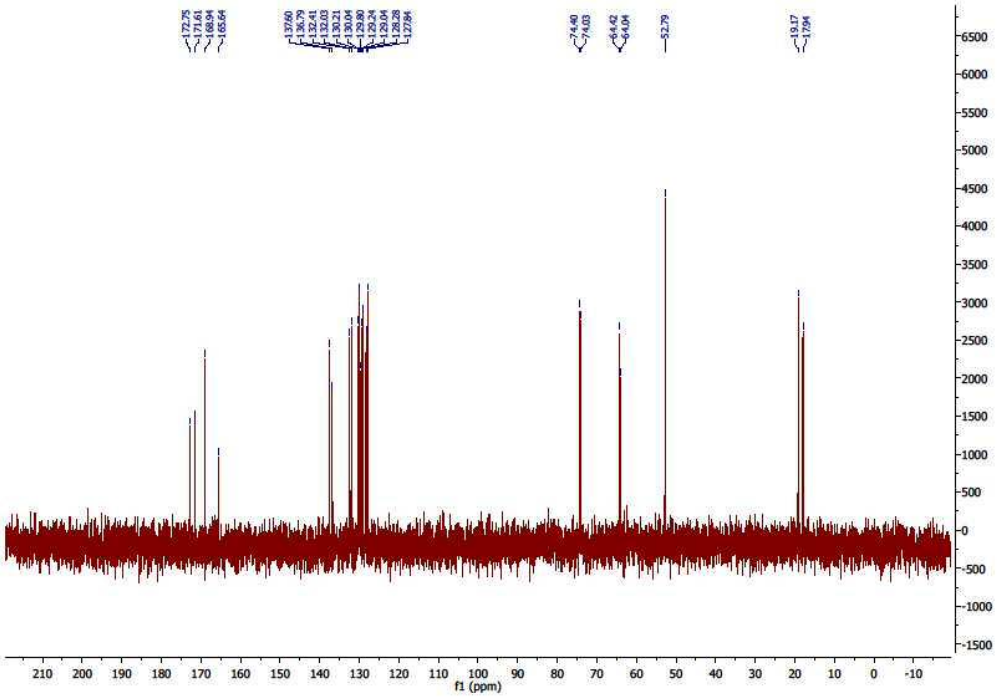

Figure 21. ^{13}C NMR of (2*S*)-2-amino-3-hydroxy-3-[3-(methoxycarbonyl)phenyl]-2-methyl-propanoic acid (**8**)

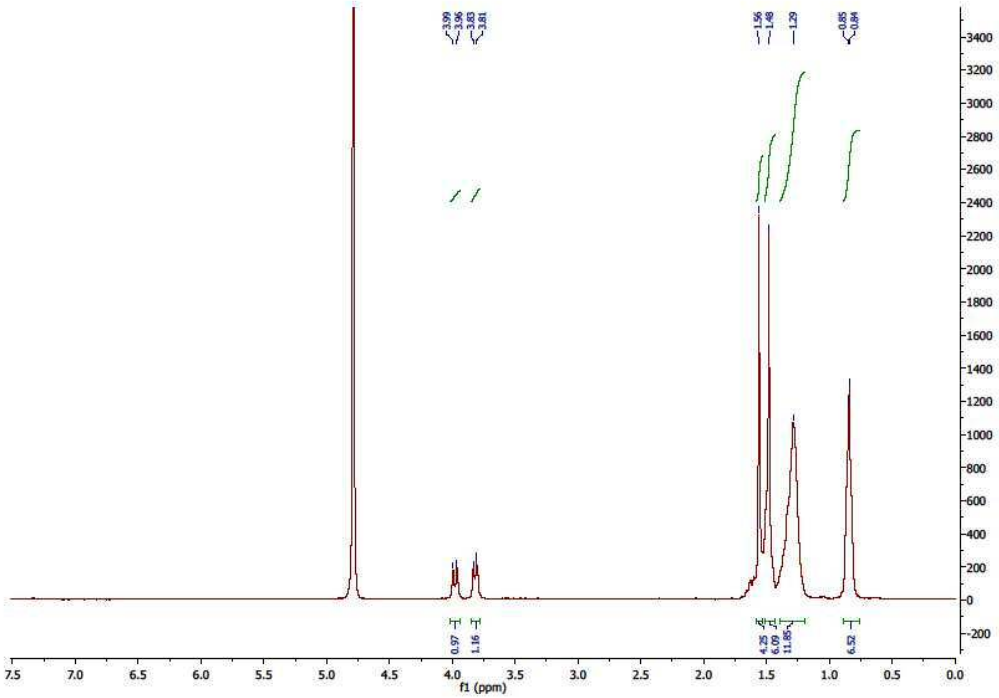

Figure 22. ^{1}H NMR of (2*S*)-2-amino-3-hydroxy-2-methyloctanoic acid (**9**)

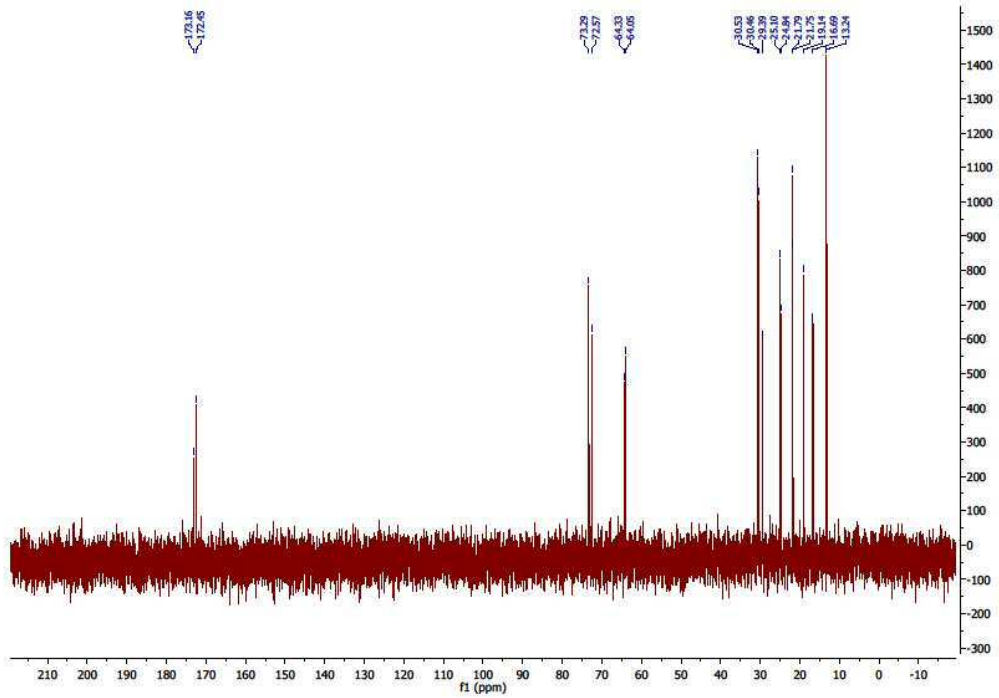

Figure 23. ^{13}C NMR of (2*S*)-2-amino-3-hydroxy-2-methyloctanoic acid (**9**)

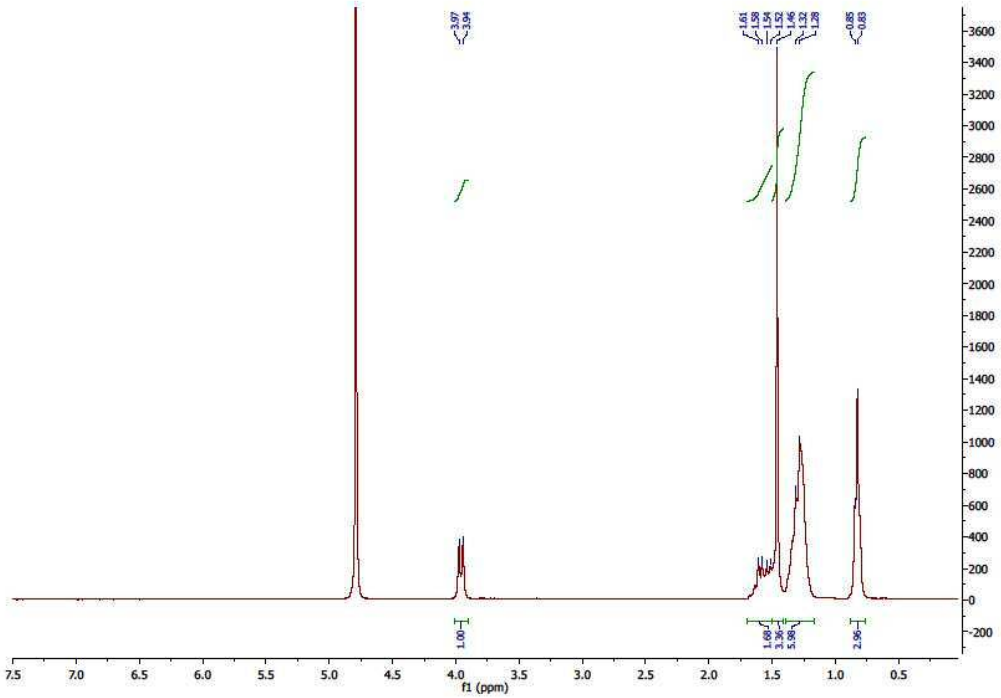

Figure 24. ^{1}H NMR of (2*R*)-2-amino-3-hydroxy-2-methyloctanoic acid (**10**)

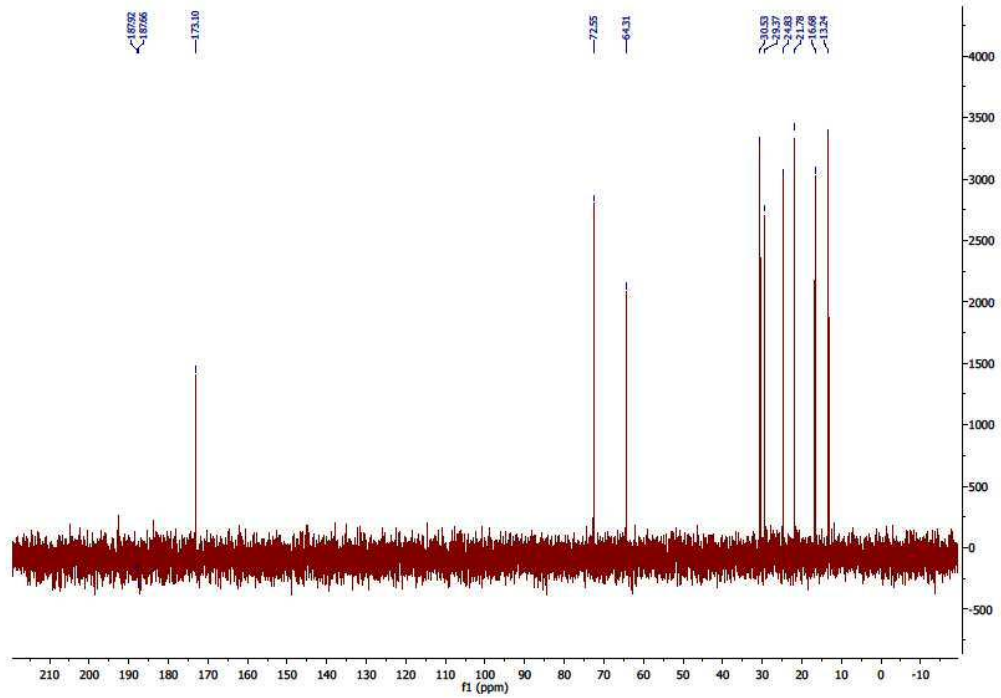

Figure 25. ^{13}C NMR of (2*R*)-2-amino-3-hydroxy-2-methyloctanoic acid (**10**)

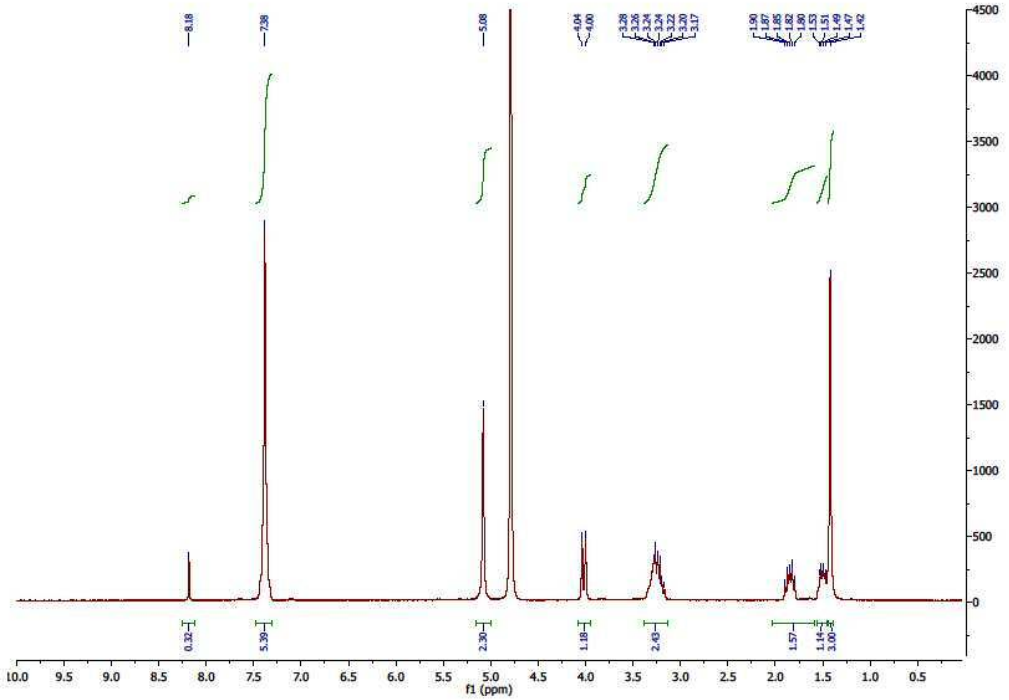

Figure 26. ^{1}H NMR of (2*R*)-2-amino-5-(((benzyloxy)carbonyl)amino)-3-hydroxy-2-methyl-pentanoic acid (**11**)

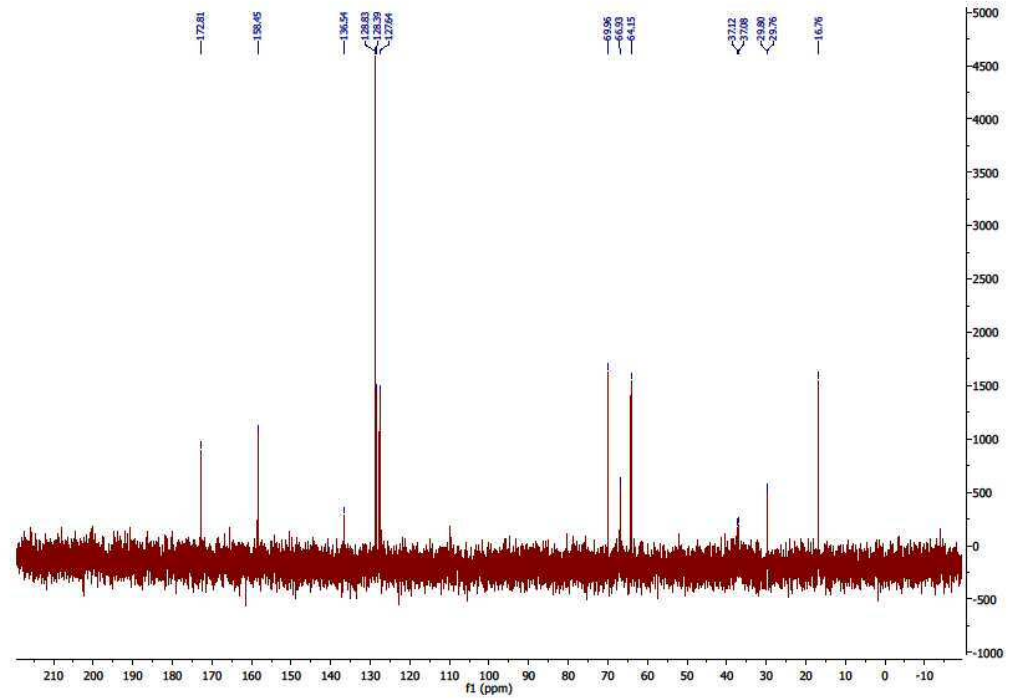

Figure 27. ^{13}C NMR of (2*R*)-2-amino-5-(((benzyloxy)carbonyl)amino)-3-hydroxy-2-methyl-pentanoic acid (**11**)

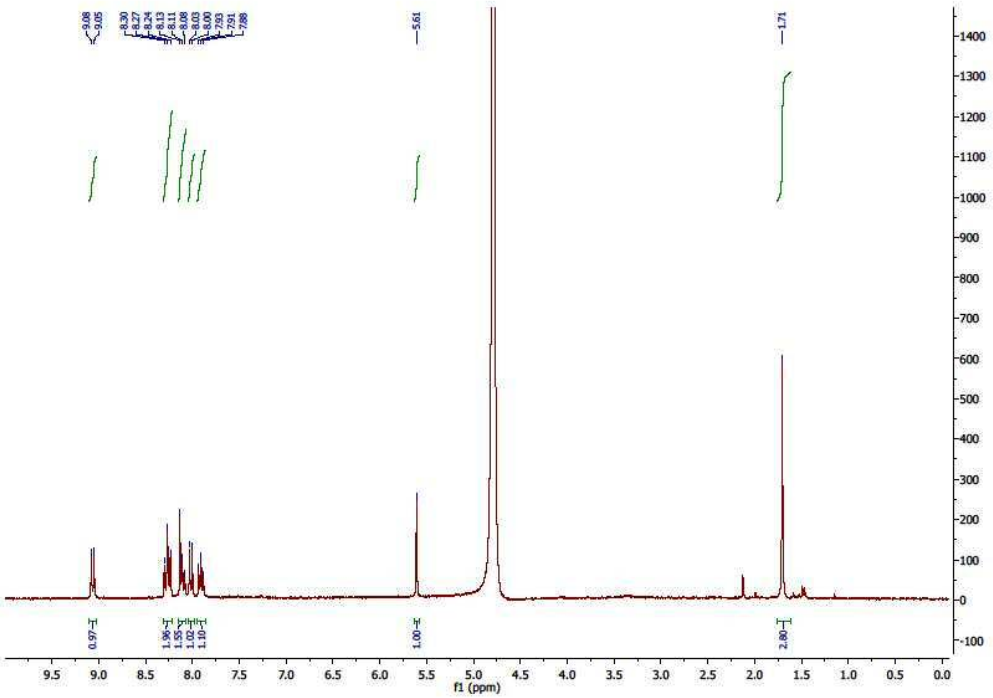

Figure 28. ^{1}H NMR of (2*S*)-2-amino-3 hydroxy-2-methyl-3-(quinolin-2-yl)propanoic acid (**12**)

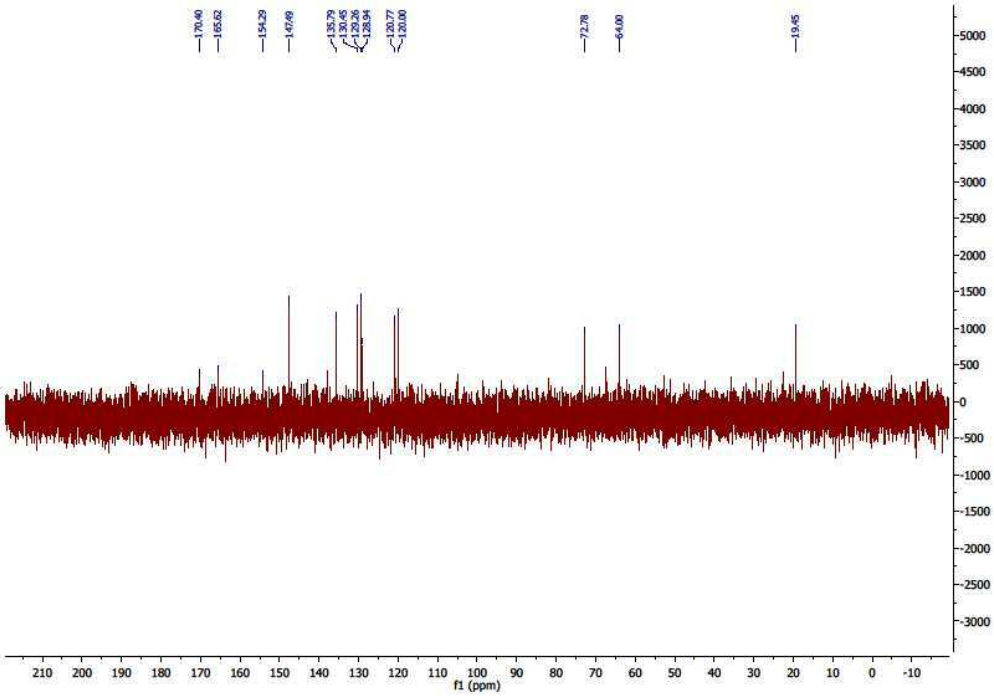

Figure 29. ^{13}C NMR of (2*S*)-2-amino-3 hydroxy-2-methyl-3-(quinolin-2-yl)propanoic acid (**12**)

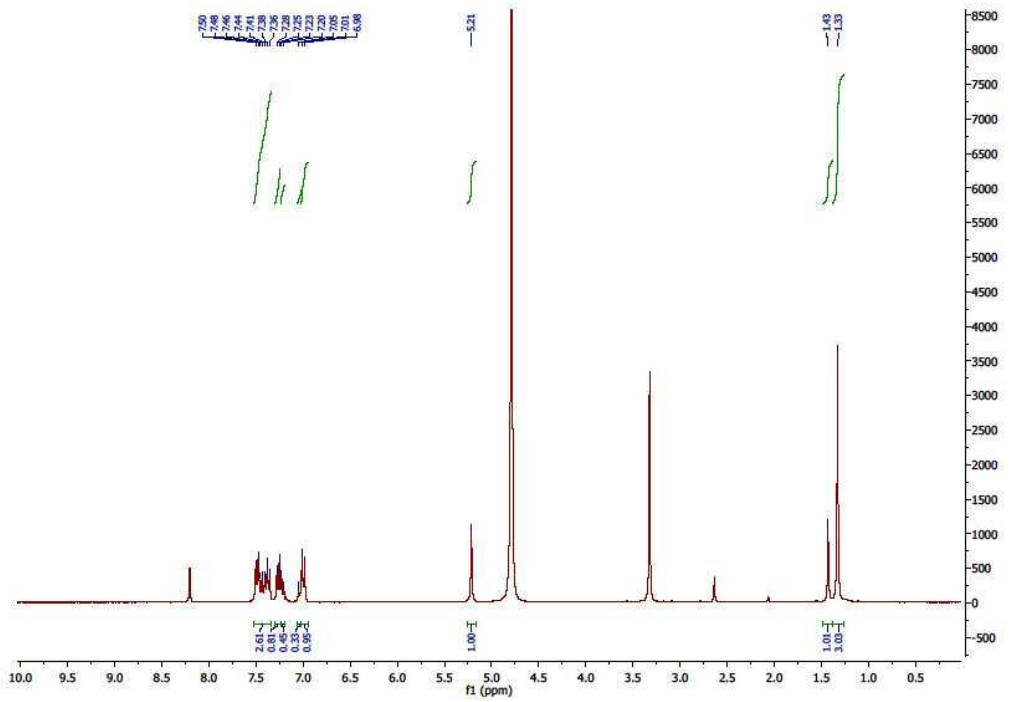

Figure 30. ^{1}H NMR of (2*S*)-3-hydroxy-2-methylindole-2-carboxylic acid (**13**)

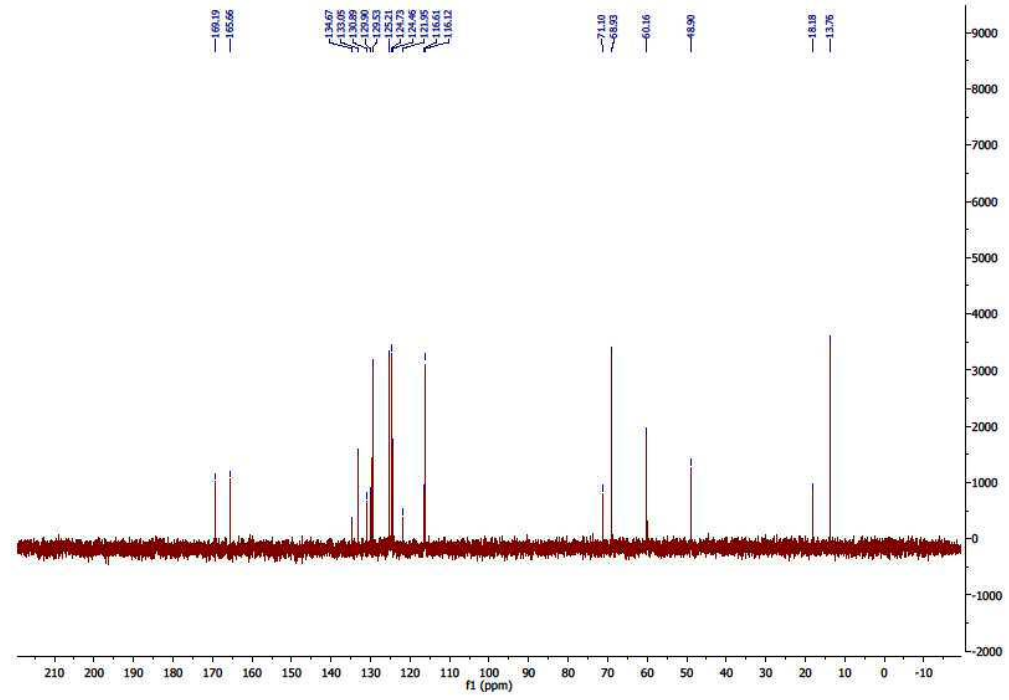

Figure 31. ^{13}C NMR of (2*S*)-3-hydroxy-2-methylindole-2-carboxylic acid (**13**)

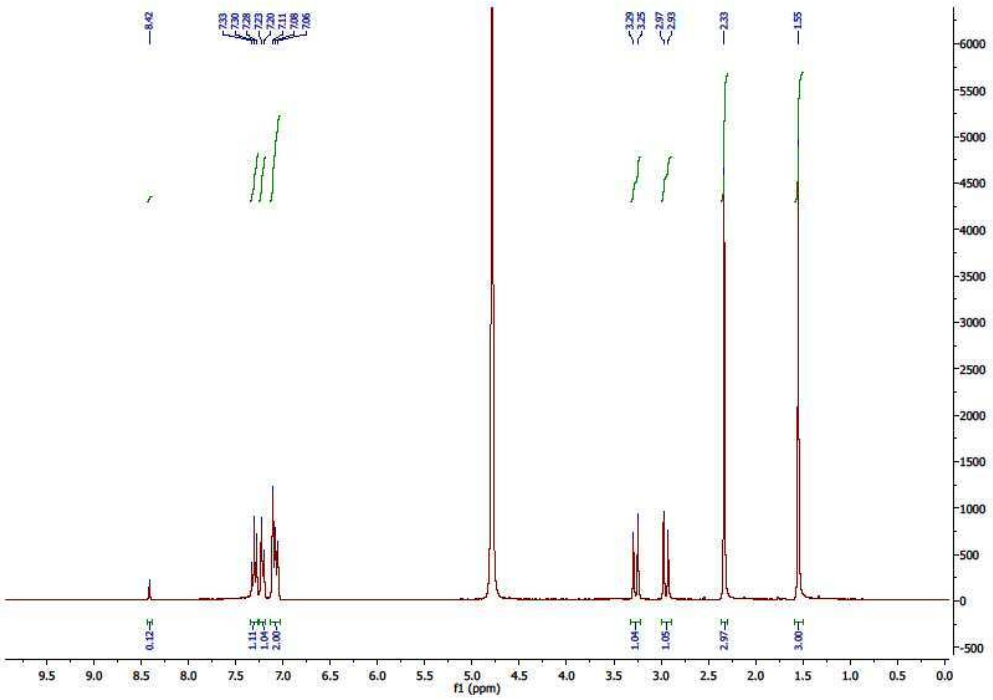

Figure 32. ^{1}H NMR of (*S*)-2-amino-2-methyl-3-(m-tolyl)propanoic acid (**14**)

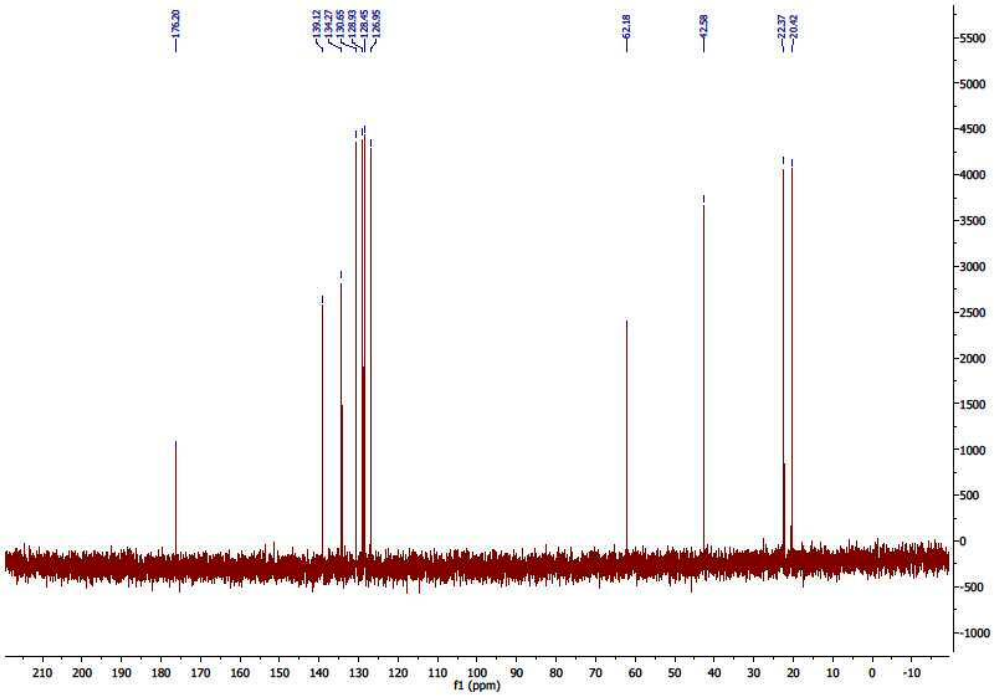

Figure 33. ^{13}C NMR of (*S*)-2-amino-2-methyl-3-(m-tolyl)propanoic acid (**14**)

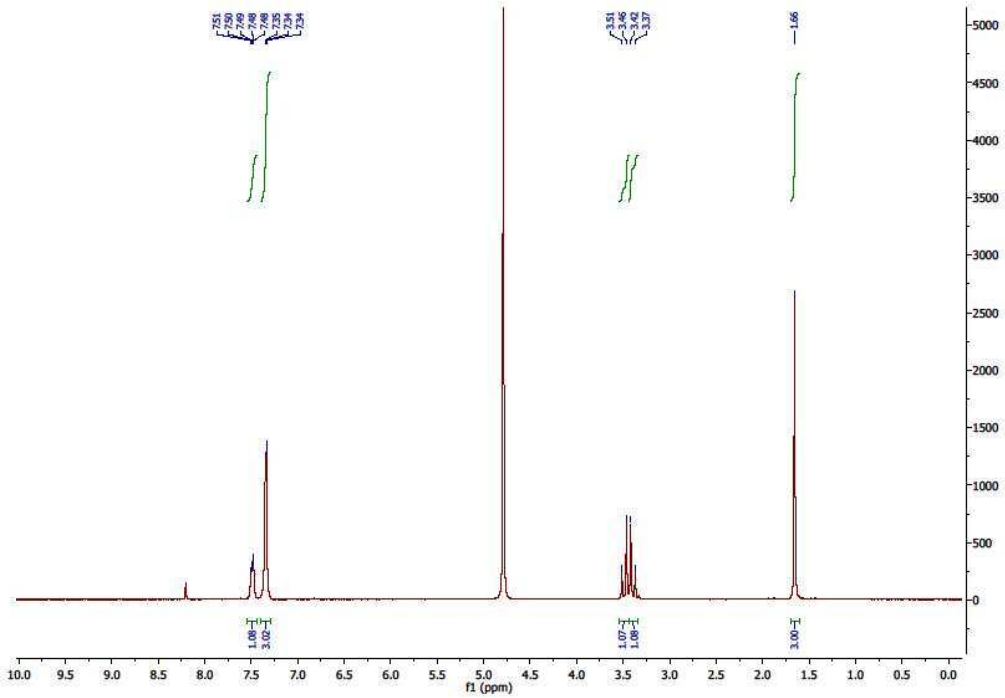

Figure 34. ^{1}H NMR of (*S*)-2-amino-3-(2-chlorophenyl)-2-methylpropanoic acid (**16**)

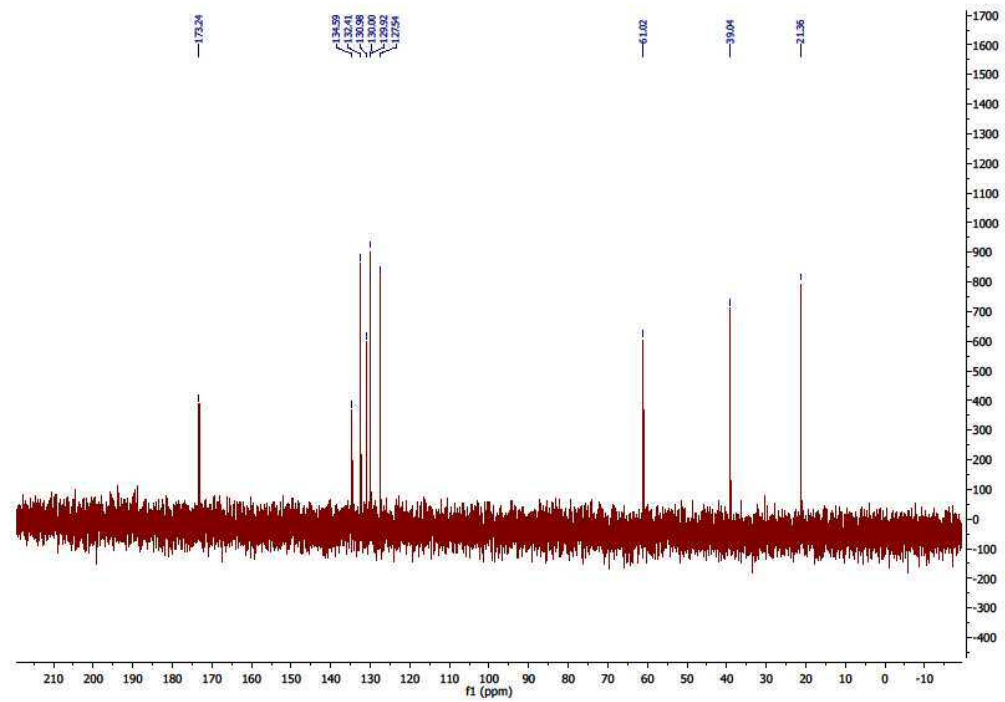

Figure 35. ^{13}C NMR of (*S*)-2-amino-3-(2-chlorophenyl)-2-methylpropanoic acid (**16**)