ENZYME CATALYZED CROSS ACYLOIN REACTIONS VIA C-C BOND CLEAVAGE

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ENZYME CATALYZED CROSS ACYLOIN REACTIONS VIA C-C BOND CLEAVAGE

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ABSTRACT

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Enzyme-catalysis is an effective tool for asymmetric synthesis under environmentally-friendly conditions, with an increasing number of examples at industrial scale. The optically active enzyme scaffold creates a microenvironment responsible for the chirality generation.

Benzaldehyde lyase (BAL; E.C. 4.1.2.38), is the first and well-known thiamine diphosphate (ThDP)- and Mg^{2+}-dependent enzyme from *Pseudomonas fluorescens* Biovar I with its capability to catalyze various C-C bond cleavage and C-C bond formation. In this work, this enzyme was used for the synthesis of unsymmetrical acyloin products of a functionalized acetaldehyde (benzyloxyacetaldehyde) with (*rac*)-Benzoin derivatives to selectively obtain optically active functionalized acyloin product with promising biological activity and (*S*)-benzoin derivative which remains unreacted in the reaction medium. As a result of this trans-condensation reaction – with benzyloxyacetaldehyde as the acceptor and different derivatives of aromatic benzaldehydes as the donor most of the cases- stereoselective (*R*)-HPP derivatives

were obtained enantioselectively. In the second part of this work, the synthesis of thiamine-amino acid conjugates and asymmetric thiazolium salts have been tried to be used as organocatalysis in benzoin condensation reactions.

Keywords: benzaldehyde lyase, benzoin, biocatalyst, hydroxy propiophenones, thiamine diphosphate, thiazolium salt

ÖZ

ENZİM KATALİZÖRLÜĞÜNDE C-C BAĞI KOPARMA İLE ÇAPRAZ AÇİLOİN TEPKİMELERİ

Bilir, Gökçil

Yüksek Lisans, Kimya Bölümü
Tez Yöneticisi: Yrd. Doç. Dr. Salih Özçubukçu

Aralık 2015, 67 sayfa

Enzim katalizi asimetrik sentezde çevre dostu koşullar altında, endüstride artan sayıda örneğe sahip etkili bir araçtır. Optikçe aktif enzim yapısı, kiralite sağlamak için mikroçevre oluşturmaktan sorumludur. Benzaldehit liyaz enzimi, bilinen ilk tiyamin difosfat ve Mg^{2+} bağımlı *Pseudomonas fluorescens* Biovar I bakterisinden elde edilen, birçok C-C bağı kırma ve oluşturma tepkimelerini katalizleyebilen (enantiyoseçici olarak (*R*)-benzoin, (*R*)-2-hidroksipropiyofenon (HPP) ve hidroksiasetofenon sentezi vb.) bilinen ilk enzimdir. Bu çalışmada, bu enzim bir asetaldehit türevi (benziloksiasetaldehit) ile rasemik benzoin türevlerinin tepkimesi sonucu biyolojik aktivite gösteren optikçe aktif asimetrik açiloin ürün sentezi ve tepkimeye katılmayan (*S*)-benzoin türevinin seçici olarak eldesi için kullanılmıştır. Bu benziloksiasetaldehitin alıcı ve farklı aromatik benzoin türevlerinden elde edilen benzaldehit türevlerinin çoğunlukla verici olarak gerçekleştirdiği trans karboligasyon tepkimesi sonucu (*R*)-HPP türevleri enansiyoseçici olarak elde edilmiştir. Bu çalışmada uygulanan kinetik rezolüsyon ile yüksek biyolojik aktivite gösteren alfa-hidroksi keton türevleri (trans-kondenzasyon ürünü) ve değerli olan (*S*)-benzoin, *rac*-Benzoinden enantioseçici olarak elde edilmiştir. Çalışmanın ikinci kısmında, benzoin

kondenzasyon tepkimelerinde organokatalizör olarak kullanmak amacıyla, tiyamin-amino asit conjugeleri ve asimetrik tiyazol tuzlarının sentezi denenmiştir.

Anahtar Kelimeler: benzaldehit liyaz, benzoin, biyokatalizör, hidroksipropiyofenonlar, tiyamin difosfat, tiyazol tuzu

To my beloved family and Prof. Dr. Ayhan Sıtkı Demir

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BAL: Benzaldehyde Lyase

BFD: Benzoylformate decarboxylase

BnOAc: Benzyloxyacetaldehyde

HPP: Hydroxypropiophenones

IPTG: Isopropyl β-D-1-thiogalactopyranoside

LB: Luria broth

PAC: Phenylacetylcarbinol

PDC: Pyruvate dehydrogenase

ThDP: Thiamine diphosphate

TPP: Thiamine pyrophosphate

CHAPTER 1

INTRODUCTION

1.1 Biotransformation

Biotransformation has been performed since 7000 B.C. for alcohol synthesis from sugar fermentation [1]. Biotransformation can be defined as the use of natural catalysts like isolated enzymes or whole cells to produce the expected products by opening different pathways for the reaction without being consumed or changed during the reaction. Usage of whole cells can provide the system with efficient cofactor regeneration. On the other hand, whole cell systems are mostly limited with the occurrence of side reactions that result in low yield and enantioselectivity [2].

Therefore, the use of enzymes in biocatalysis reactions attracts attention since enzymes offer high chemo-, stereo-, and enantio-selectivity and this property is very important in chiral α-hydroxy ketone synthesis.

Moreover, the use of enzymes is interesting in pharmaceutical industry and production of fine chemicals.

One of the other attractive properties of biocatalysis is that they can be used in mild reaction conditions. These conditions can minimise the side reactions and provide stability of the substrate and product, which occurs at high working temperatures. Furthermore, biocatalysis provides attraction since “natural product” can be used as label in food or cosmetic industry [3].

The most significant effect of biocatalysis still remains in the pharmaceutical sector, since its magnificent regio- and stereo-selective features enable though synthesis to be misled [4].

On the other hand, for enzymatic reactions mild conditions are needed and they are limited to a very narrow range in their process. Enzymes are the proteins that are

labile under harsh conditions. Elevated temperature, pH, and pressure may cause to the deactivation of enzymes [5].

The poor solubility of compounds is another limitation of biocatalysis reactions and it can be solved by adding co-solvents, such as DMSO up to 30% v/v, PEG-400 up to 15% v/v, isopropanol, and MTBE since enzymes can tolerate these co-solvents with this amount. However, these co-solvents cause other problems for technical applications [6].

1.2 Enzymes in Biotransformation Studies

In nature as catalysts derive to increase the rate and co-ordinate the multitude of chemical reactions, it is necessary to develop and maintain life. Several chemical reactions are by far too slow to be useful under conditions common for normal living systems. Compared to the chemical reactions, enzymes succeed up to 1000 fold faster reaction rates than chemical reactions [7].

Enzymes, as catalysts, increase the rate in which a thermodynamic equilibrium is reached, but do not change that equilibrium which means enzymes work reversibly. The acceleration in reaction rate is succeeded by decreasing the energy of activation.

Enzymes link to the substrates by non-covalent interactions on specific surface. They attach to the substrate in the transition state better than the ground state, so it reduces the activation energy. In addition, the chemical potential of specific groups may be intensely changed temporarily compared to aqueous solutions by removal of water in the reactive site upon binding of substrate. These are the both aspects that contribute to the observed phenomenon of high reaction rate.

Enzymes act chemo-, regio-, and enantio- specific way. Substrate binding is prerequisite for enzyme catalysis. It is affected by a chemical compound long enough to a unique chemical potential build in to the system, which explains the type of reaction that will carry out such as hydrolysis, C-C bond formation or oxidation/reduction. The mechanism generally is the same as known from solution chemistry e.g. acid-base catalysis [8]. Since enzymes work on single type of functional group, other functional groups will remain unchanged. The groups that are positioned differently and it will be distinguished owing to the three dimensional structure of the enzyme. Furthermore occurance of any chirality in the substrate leads

chiral molecules from prochiral substrates or kinetic resolution of a racemic substrate [8]. Acceleration of reaction rates and selectivity are significant advantages in biotransformation studies, however Due to their mild reaction conditions, they are also described as green procedures. They work in aqueous environment with appropriate temperature and pH values. Moreover, there are enzymes that can be used for reactions carried out under extreme conditions as well. These classes of enzymes are adventageous for chemoenzymatic reactions in which biocatalysts and chemical reagents are used in a collaborative sequence.

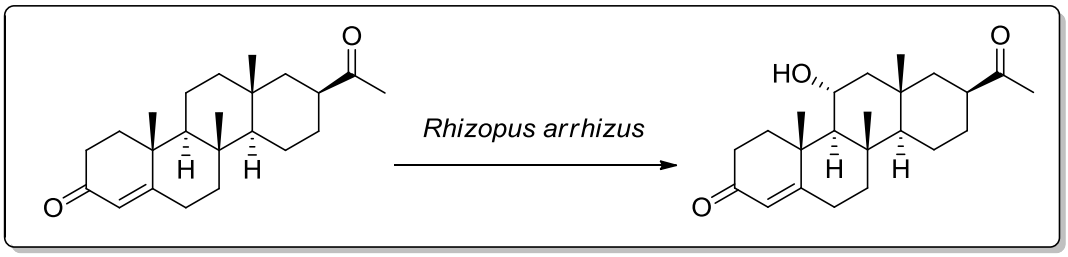
Three types of selectivity that enzymes have [8]:

i. Chemoselectivity

The aim of an enzyme is to act on a particular type of a functional group, other sensitive functionalities that can react to an absolute extent under chemical catalysis and remain untouched.

ii. Regioselectivity and Diastereoselectivity

They may distinguish between functional groups which are chemically occupied in different regions of the same substrate due to their complex three-dimensional structures.
An example of regioselectivity is the hydroxylation of progesterone in the presence of fungus Rhizopus arrhizus (Scheme 1) which happens especially at the position 11 of the hormone [9].



Scheme 1. Progesterone hydroxylation in the presence of *Rhizopus arrhizus* [9].

iii. Enantioselectivity

Almost all enzymes have chiral selectivity. They are also chiral and generally recognise L-amino acids. As a consequence, any kind of chirality present in the substrate molecule is recognized at the time of enzyme-substrate complex formation. Hence a prochiral substrate may be converted into a chiral product and both enantiomers of a racemic substrate may react at different rates, affording a kinetic resolution.

Despite the fact that enantiomers have the same physical and chemical properties, their characteristic features can differ extremely [10]. For instance, (4*S*)-(+)-carvone has caraway odor but (4*R*)-(-)-carvone (Figure 1) has sweet spearmint odor [11].

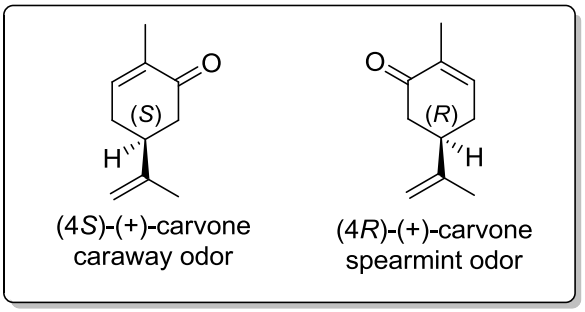


Figure 1. Carvone enantiomers.

These properties collectively include the specificity of an enzyme and represent its the most important feature for asymmetric and selective exploitation.

1.3 Thiamine Diphosphate (ThDP or TPP) as Enzyme Cofactor

Thiamine diphosphate (Figure 2) is the active form of vitamin B_1. This cofactor can be used in a broad range of process and has three subunits. These are the pyrophosphate part, the thiazolium core, and the pyrimidine unit. In the presence of ThDP, the substrate and cofactor bound covalently. Besides, each of the subunits has a specific aspect in enzymatic process.

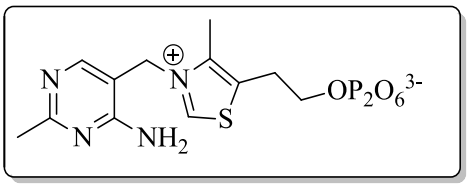


Figure 2. Structure of thiamine diphosphate (ThDP)

The first step of this process is the formation of ylide. C2-carbanion of ThDP works as a nucleophile and it binds to the carbonyl part of the other substrate. There is an active site of enzyme that has a glutamic acid residue and balances the imino tautomer with hydrogen bonding. Moreover, the C2 deprotonation is achieved by ‘’V‘’ confirmation of the cofactor in the presence of bulky residues like methionine, leucine or isoleucine. Hence, the imino group presents next to the C2 of thiazolium ring and it simplifies the abstraction of proton as seen in Figure 3 [12].

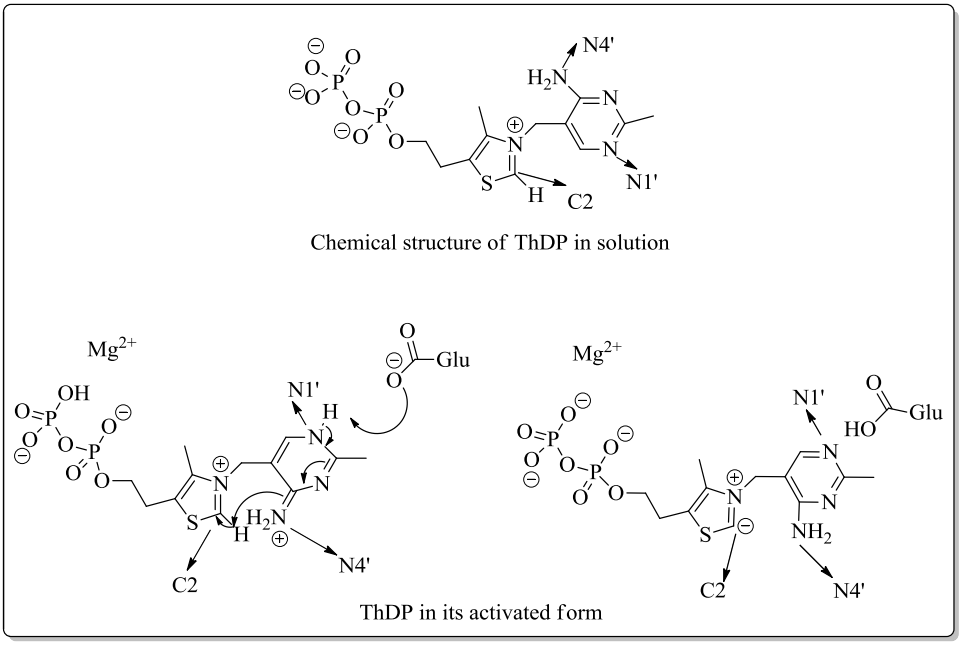
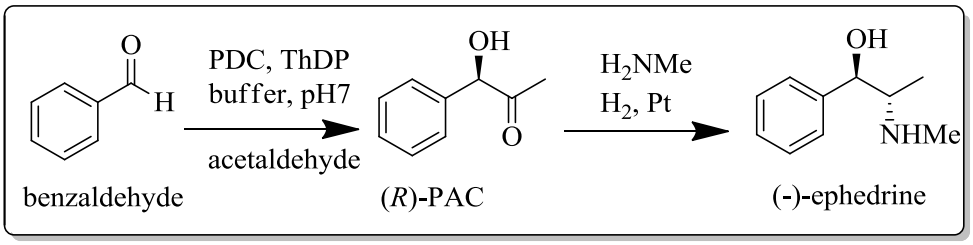


Figure 3. Formation and stabilization of ThDP ylide [12].

1.4 Thiamine Diphosphate Dependent Enzymes

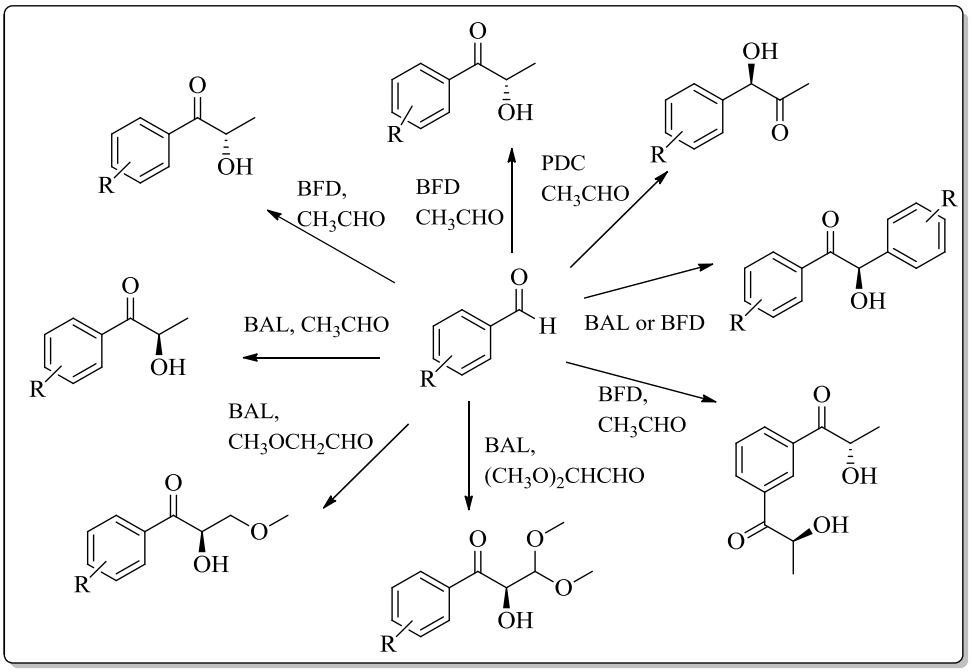
ThDP dependent enzymes were first used for the synthesis of chiral hydroxy ketones in 1921, when the first whole cell biotransformation method was performed. Actually this process is still applied for the synthesis of chiral phenylacetylcarbinol (PAC), the precursor of (-)-ephedrine as shown in Scheme 2 [13].



Scheme 2. Enzymatic synthesis of (*R*)-PAC that is used in the synthesis of (-)-ephedrine [13].

Enzymes that are ThDP-dependence have ability to cleave and form of C-C bonds. Also they catalyze a large spectrum of reactions such as C-N, C-O and C-S bond forming. Starting from aromatic aldehyde using this class of enzyme, a huge variety of different asymmetric products can be formed and they can be used in numerous synthesis.

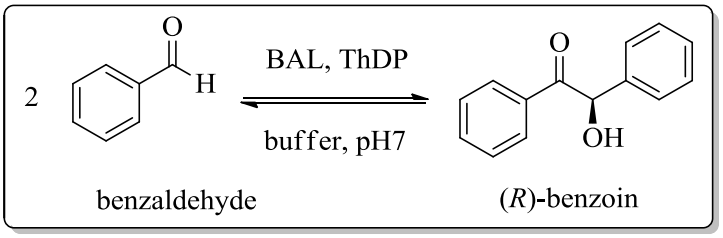
Polarity changing (*umpolung* chemistry) of carbonyl carbon is applied to both acyloin and benzoin condensation reactions. C-C bond formation reaction is catalyzed by various enzymes. These enzymes are benzaldehyde lyase (BAL), acetohydroxyacid synthase (AHAS), phenylpyruvate decarboxylase (PhPDC), benzoylformate decarboxylase (BFD), and pyruvate decarboxylase (PDC). These enzymes are all thiamine pyrophosphate-dependent enzymes and catalyze various benzoin and acyloin reactions as seen in Scheme 3.



Scheme 3. Examples of ThDP-dependent enzyme-catalysed transformations [14].

1.5 Benzaldehyde Lyase (BAL)

First Benzaldehyde Lyase was obtained from *Pseudomonas fluorescens* Biovar I strain found in a cellulose factory and was reported by Gonzales and Vicuna in 1989. They realized that BAL can cleave the acyloin linkage of chiral benzoin with the purpose of using as energy source (Scheme 4). They used benzaldehyde for the condensation reaction [15].

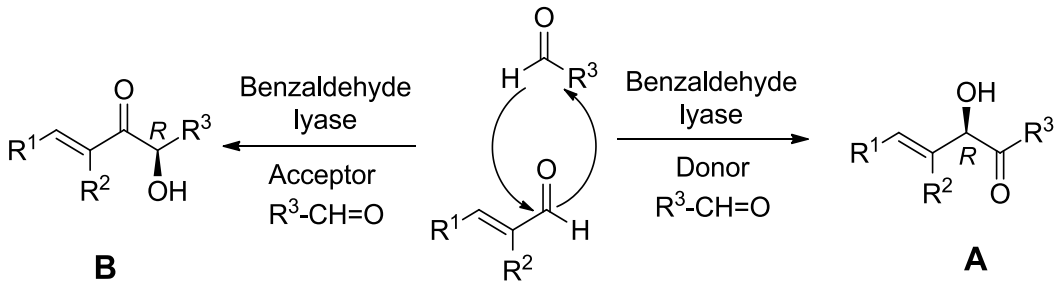


Scheme 4. Cleavage and formation of α-benzoin catalyzed by BAL.

Up to now, only the biochemically characterized benzaldehyde lyase (BAL) was derived from *Pseudomonas fluorescens* strain, which was isolated from wood, showing the ability to grow on lignin-degradation products, such as anisoin (4,4'-dimethoxybenzoin) and benzoin. BAL cleaves these latter compounds to furnish more simple aromatic aldehydes.

Especially the self-ligation of benzaldehyde yields benzoin with high activity and stereoselectivity (e.e. >99), making this enzyme very attractive for industrial processes. For benzoin formation, *o*-, *m*-, and *p*-substituted aromatic aldehydes are extensively accepted as donors.

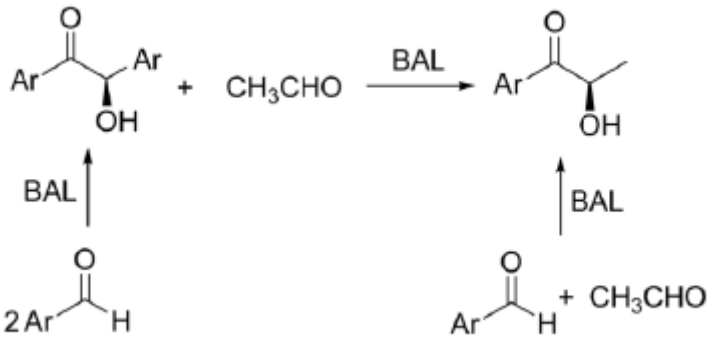
On the acceptor side, formaldehyde, acetaldehyde and their derivatives, such as phenyl-, mono-, or dimethoxyacetaldehyde can be used. The notable synthetic potential of BAL is shown by the regiocomplementary benzoin condensation of α,β-unsaturated aldehydes acting as donor or acceptor, respectively. While large aldehydes acted as donors (product type A), small counterparts served as acceptors leading to isomeric olefinic acyloins B in high *ee*.s (Scheme 5) [8].



R^1	R^2	R^3	Product	e.e. [%]
Ph	H	CH_3	A	80
Ph	CH_3	CH_3	A	>96
-$(CH_2)_4$-		$CH(OMe)_2$	A	93
H	CH_3	3,5-di-MeO-C_6H_3	B	>98
H	CH_3	Ph	B	>98

Scheme 5. Regiocomplementary carboligation of aldehydes catalyzed by BAL.

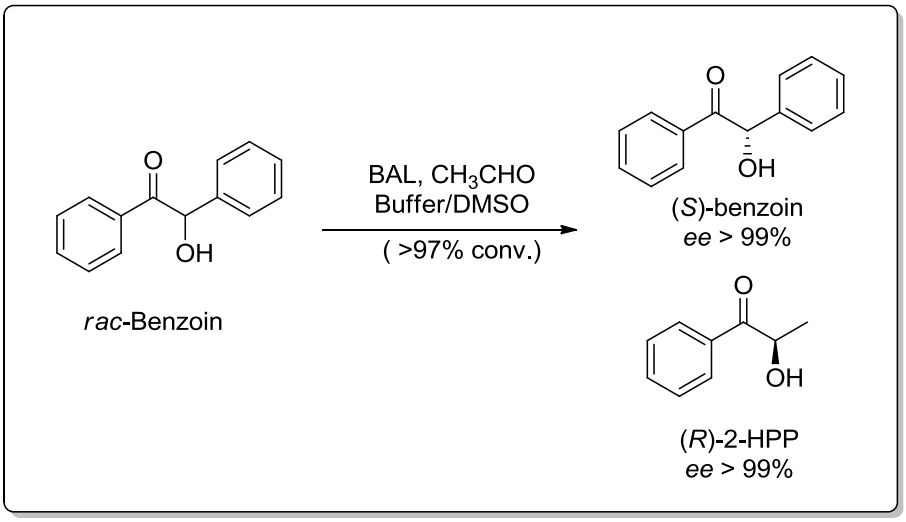
BAL enzyme cleaves the chiral hydroxy ketones -benzoin and anisoin- in a ThDP-dependent reaction. α-2-hydroxypropiophenone derivatives and α-Benzoin were the products of the reaction that were firstly catalyzed by BAL (Scheme 6). After that, benzaldehyde lyase has been used for a large variety of substrates due to their carboligation activity [16].



Scheme 6. General carboligation scheme for benzaldehyde lyase.

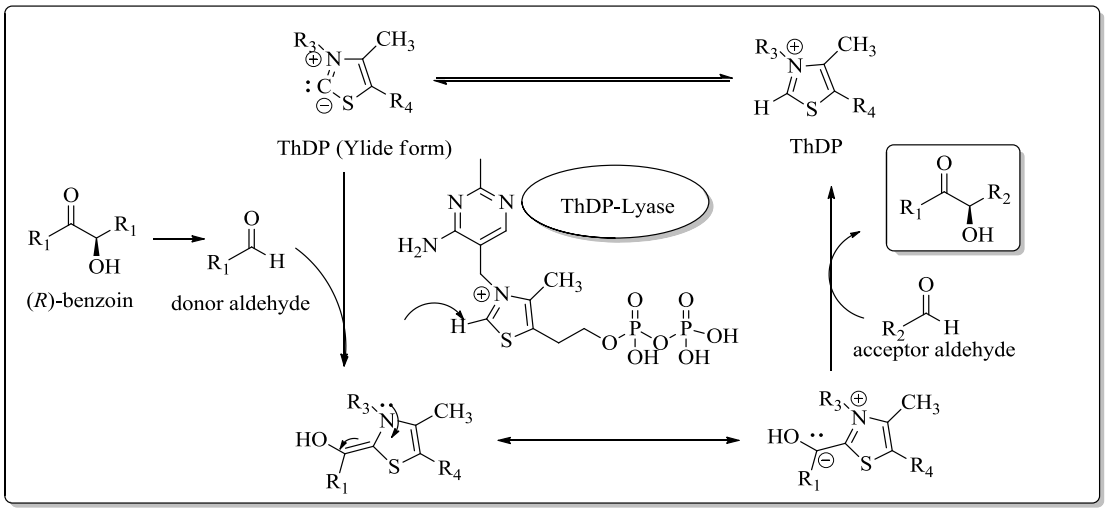
Furthermore, the most featured property of this reaction catalyzed by benzaldehyde lyase was recognized by Demir et. al. [16, 34, 41]. In BAL-catalyzed reaction, only (*R*)-benzoin can be cleaved into benzaldehyde; and (*S*)-benzoin can't be cleaved at all. Therefore BAL is used for the production of chiral α-hydroxy ketones, which are significant class of compounds in natural product and drug synthesis.

Besides that, there is one result where the *rac*-benzoin was used as an aromatic aldehyde source to get a trans-benzoin condenstion product in the presence of acetaldehyde. Then the (*S*)-benzoin that doesn't react in the presence of BAL was released in the medium and the trans benzoin product ((*R*)-2-HPP) was synthesized in very good enantiomeric excess as seen in Scheme 7 [17].



Scheme 7. BAL-catalyzed trans-benzoin condensation.

BAL mechanism is different compared to other thiamine diphosphate-dependent enzymes. Firstly, the cleavage of C-C bonds, that chiral benzoin has, by attacking of the ylide form of ThDP to the carbonyl carbon of benzoin is applied. Also, the nitrogen part of ThDP is very electrophilic, because of that the free benzaldehyde and enamine intermediate are formed by electron rearrangement of ThDP. In addition to the cleavage of C-C bonds, enamine intermediate also catalyzes the synthesis of hydroxy ketones. Furthermore, if there is an acceptor aldehyde in the medium, the enamine intermediate forms the C-C bond between the aldehydes. Thus, this cycle is concluded with the release of formed acyloin, (*R*)-2-HPP analogous, and release of the ThDP cofactor back which is continuing the catalytic cycle for the next molecule (Scheme 8).



Scheme 8. Proposed cycle for ThDP-lyase-catalyzed umpolung carboligation of aldehydes [18].

BAL has a molecular mass of 4 x 58,919 Da. and has homo tetramer of 4 x 563 amino acid residues. Subunits of BAL separately bind to one thiamine diphosphate molecule that attach to Mg^{2+} ion. Its subunit has three domains as shown in Figure 4 and the domains include a central six-stranded parallel *beta*-sheet linked to a various number of *alpha* helices.

The active center is located in the thiazolium ring of thiamine diphosphate, which bound in a deep pocket. Residues from Dom-β make interactions with the diphosphate moiety of thiamine diphosphate and Mg^{2+} ion with C-terminal, while those from Dom-α of a neighboring subunit bind to the pyrimidine ring of ThDP.

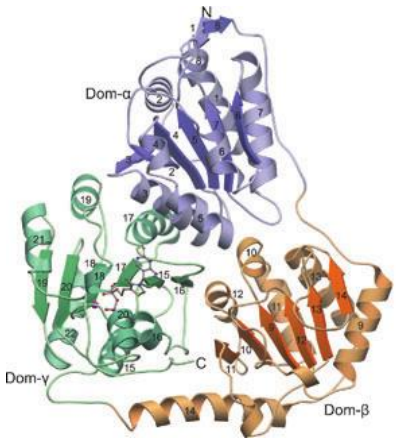
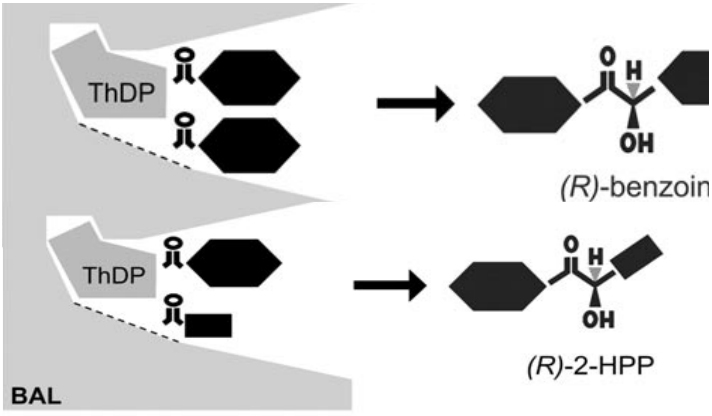


Figure 4. Stereo ribbon plot of BAL [19].

Thiamine diphosphate is placed at the bottom of a narrow channel. So thiazolium ring and the C2 atom of the thiazolium ring channels seperate BAL into two parts.



Scheme 9. Illustrations that show the shape of the binding site for BAL [19].

1.6 Importance of Chiral α-Hydroxy Ketones

Enantiomerically enriched α-hydroxy ketones are significant constituents for the synthesis of pharmaceuticals and fine chemicals. Many of the antidepressants, some inhibitors which are used in Alzheimer's disease treatment, inhibitor of kurasoin A and B and several antitumor antibiotics (olivomycin A, chromomycin A_3, epothilones) includes these types of ketones. Futhermore, many important molecules (amino alcohols, diols, etc) can be obtained from α-hydroxy ketones as seen in Figure 5 [18].

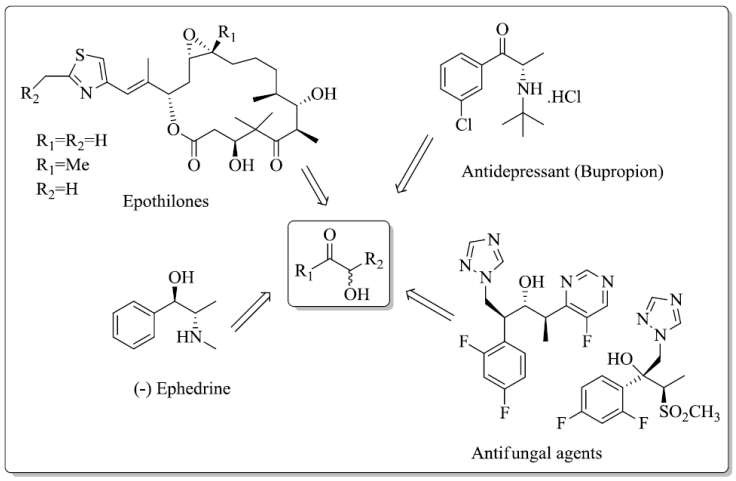
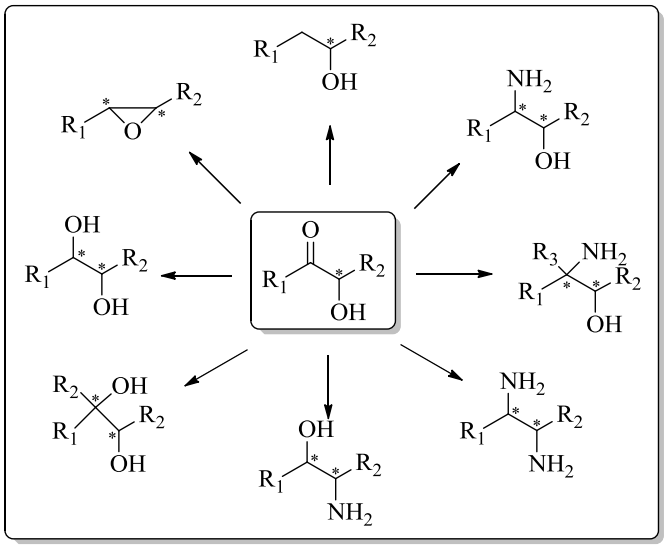


Figure 5. Examples of compounds contains α-hydroxy ketones [18].

Optically active α-hydroxy ketones are indispensable constituents for the asymmetric synthesis of biologically active molecules because they are stereogenic molecules and they have versatile functional groups, which may be easily transformed to other functionalities, e.g. diols, halo or amino derivatives and epoxides.

Various methods have been achieved with the production of the optically active α-hydroxy ketones. For example, the stereoselective oxidation of optically active enolates [22], oxidation of prochiral enolates by using optically active oxaziridines [23], selective oxidation of chiral titanium enolates [24] and asymmetric oxidation of silyl enol ethers [25].

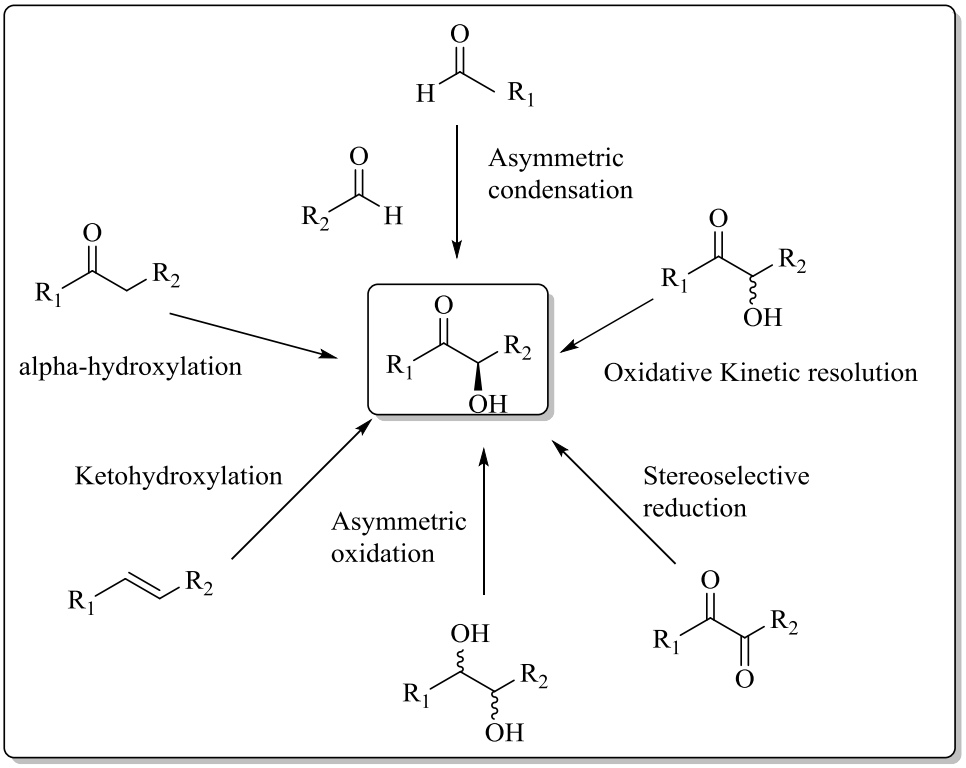


Scheme 10. Hydroxy ketones can be used to synthesize several active compounds [21].

1.7 Chemical asymmetric acyloin synthesis

Multiple chemical processes to obtain asymmetric benzoins are listed below and shown in Scheme 11:

1. α- Hydroxylation
2. Ketohydroxylation
3. Asymmetric condensation
4. Asymmetric oxidation
5. Oxidative kinetic resolution
6. Stereoselective reduction



Scheme 11. Chemical reaction types for the synthesis of acyloins [26].

Possible reported strategies are asymmetric dihydroxylation of ketones and the enantioselective enolate oxidation of ketones [26]. Besides that, ketohydroxylation of olefins, the asymmetric monooxidation of diols, and oxidative kinetic resolution of racemic acyloins are other chemical methodologies. Moreover, organocatalytic approaches can be used to produce acyloins. Proline and alanine mediated oxygenation of ketones and benzoin-type condensation reactions by using chiral thiazolim and triazolium salts can be applied [26].

Nonetheless, although there are useful molecules that have been produced by chemical methods, many chemical steps which are required are uneconomic; also these steps have lack of selectivity and have low yields.

To cope with the problems, biocatalysts which are able to synthesize important chemical products can be used. Thus, with the help of biocatalysts, these desired products can be obtained with high enatio-, regio-, and chemoselectivities and in fact, this is more economical and environmental process [27, 8].

1.8 Advantages and disadvantages of enzymes

If the enzymes are compared with catalysts that are generally used in chemical processes, enzymes are remarkable. They are used for almost all known organic reactions.

Wide ranges of organic reactions are able to occur spontaneously. However, some of them require a certain rate to be catalyzed. Thus, during the process, catalyst lowers the energy barrier as shown in Figure 6.

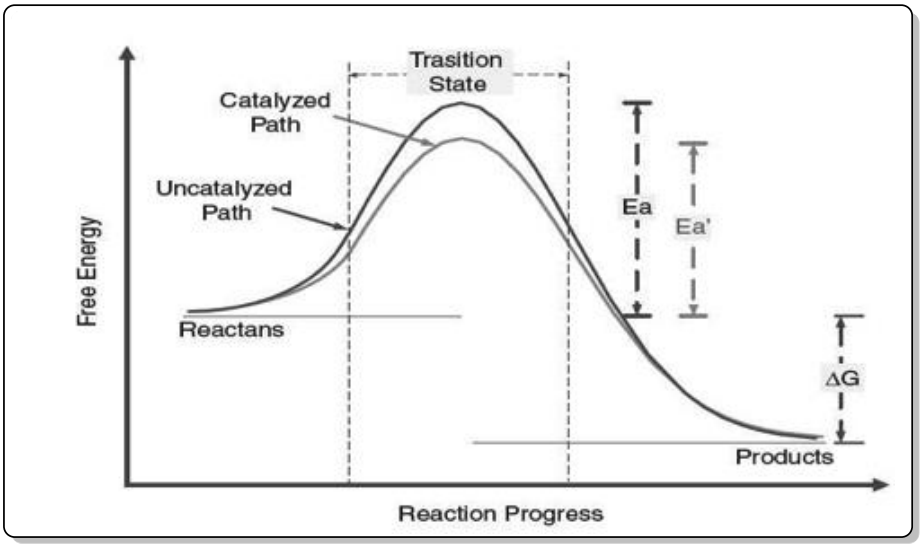


Figure 6. Reaction coordinate diagram for catalyzed and uncatalyzed reactions [8].

Ea is the activation energy of uncatalyzed reaction and Ea' is the activation energy of the catalyzed reaction. Also the free energy change of the reaction is ΔG.

Besides, biocatalysts generally achieve high selectivity up to >99% *ee* with the help of chirality of enzymes. Although this particular feature of enzymes provides chiral synthesis, chemical synthesis generally gives racemic mixtures. This high selectivity of enzyme is very attractive compared to chemical synthesis since it can serve multiple benefits such as minimization of protecting groups or side reactions, easier separation, and fewer environmental problems [8].

Advantages and disadvantages of enzyme-catalyzed reactions are shown in Table 1.

Table 1. Characteristics of biocatalysis.

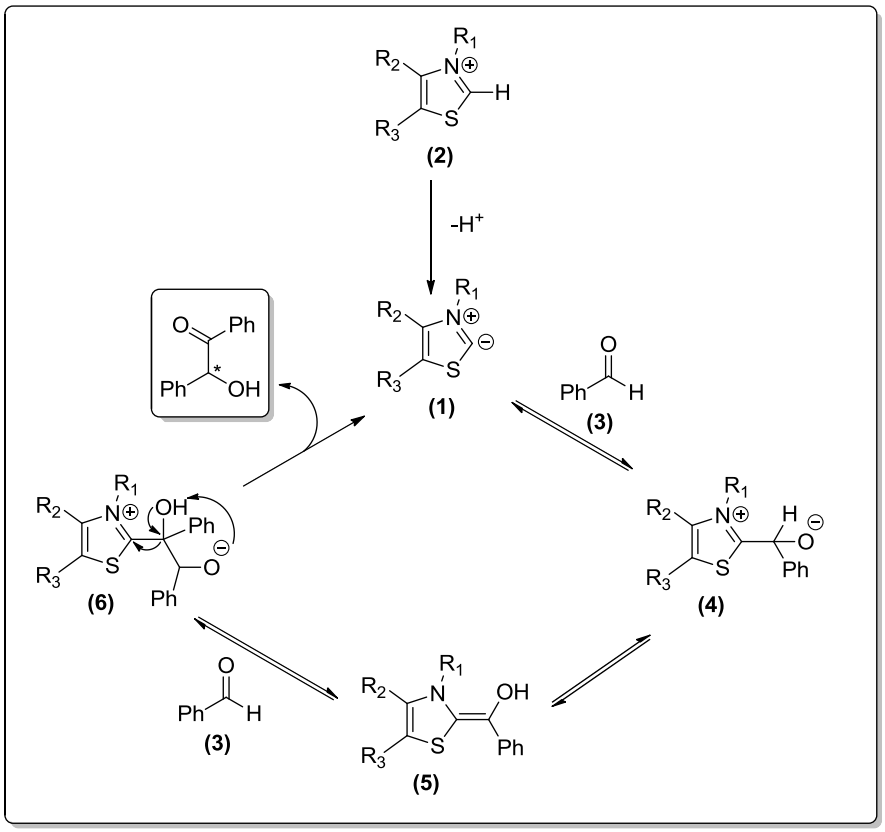
Advantages	Disadvantages
-Enzymes catalyze broad spectrum of reactions and accelerate the rate of reactions -Enzymes are more selective; chemo-selectivity, regio-selectivity, diastereo-selectivity and enantio-selectivity -Enzymes act under mild conditions such as pH range 5-8 and temperature range 20–40°C -Byproducts are low -Environmentally acceptable, they are non-toxic -Large scale production is possible through fermentation -Recycling is possible -Enzymes can be modified to a certain extent	-Availability of enzymes is limited -Protein catalyst stability is limited -Enzymes require co-substrates such as cofactors -Inactivation occur at high temperatures, extreme pH and organic solvents -Inhibition may occur by substrate, product and metal ions -Enzyme can cause allergic reactions

Enzymes are used in industrial area; but, there are well-known restrictions related with biological catalysts. To illustrate, enzymes can work under mild conditions, thus they are not very stable and they may be decomposed by extreme reaction conditions. Nonetheless, enzyme technology has been developed in the last 20 years and these problems have been solved [28].

1.9 Organocatalytic Benzoin Reactions (NHCs as Organocatalysts)

The carbene catalyzed benzoin condensation such as its synthetic, mechanistic and catalytic aspects have been studied extensively [29]. It provides changing the conventional reactivity patterns by following the concept of umpolung (polarity reversal). A significant example of this is *N*-heterocyclic carbene catalyzed benzoin

condensation. This reaction has been the center of the research. A mechanistic model for the reaction was firstly proposed by Breslow in 1958 [30] and it is shown in Scheme 12. In this mechanism, by deprotonation of the thiazolium salt (**2**), the catalytically active species (thiazolin-2-ylidene (**1**)) is formed in situ. Then, the carbene works as a nucleophilic catalyst which attacks to the carbonyl group of the aldehyde to form thiazolium adduct (**4**). After the deprotonation and reprotonation, the active aldehyde in the form of the resonance-stabilised Breslow intermediate forms (**5**). This intermediate reacts again with the carbonyl group of a second aldehyde. At last, benzoin **(7)** is released and the carbene catalyst is regenerated to continue the catalytic cycle for the next molecule.



Scheme 12. Proposed catalytic cycle of the benzoin condensation.

Furthemore, a large variety of thiazolium, imidazolium and triazolium salts have been progressed which has resulted in developed selectivity and yields. After these studies, attention was focused on the development of an asymmetric type of the benzoin condensation. Early studies were about implementing chirality in thiazolium structure. In 1966, Sheehan and Hunneman applied the first example of an asymmetric benzoin condensation using a thiazolium salt as a pre-catalyst (**8**). The

resulting *ee* was 22% [31]. Then, the modification of the thiazolium salt (**9**) improved the *ee* up to 51%, but with very low yields of 6% [32]. Many years later, Rawal developed this protocol and reached enantiomeric excesses up to 48% and with improved yields [33]. In 1993, Lopez Calahorra and co-workers obtained a bisthiazolium salt catalyst (**10**) that obtained benzoin product in 27% *ee* and a yield of 21% as shown in Figure 7 [34].

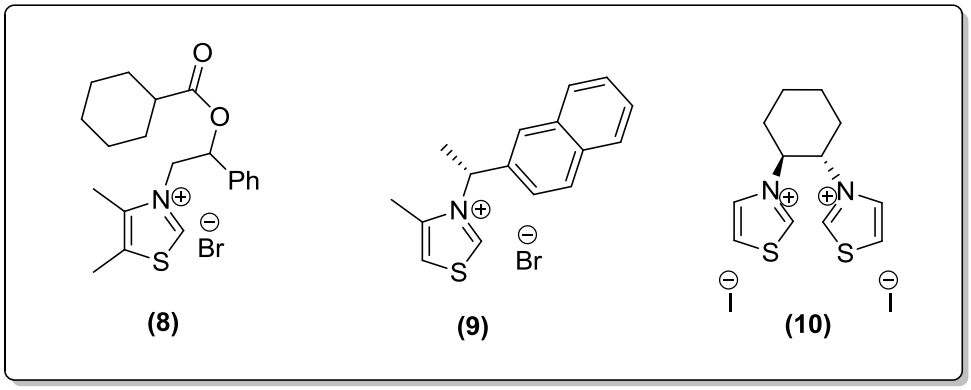


Figure 7. Examples of thiazolium salt catalysts.

High yields and enantioselectivity remained elusive until triazole heterocycles were improved as an alternative core structure.

1.10 Aim of the study

BAL is a valuable tool for chemoenzymatic synthesis as it generates various enantiomerically pure α-hydroxy ketones through aldehyde ligation or by partial decomposition of racemic mixtures [35]. Under the light of this, the aim of this study is to get novel α-hydroxy ketones in the presence of the enzyme, benzaldehyde lyase. This thiamine diphosphate dependent enzyme's substrate profile and its capacity for producing enantiopure benzoin and 2-hydroxypropiophenone derivatives and racemic resolution of benzoins yielding (*S*)-benzoins will be questioned. Therefore, this study can extend the scope of the substrate range of the benzaldehyde lyase catalyzed reactions.

Moreover, the coenzyme thiamine (vitamin B1) as a natural thiazolium salt and it is involved in many enzymatic catalysis [36]. Therefore, synthesis of thiamine-peptide conjugates was the second part of my study. It can also be used as useful

organocatalysts in asymmetric benzoin condensation reactions. This work describes the synthetic efforts to get thiamine-peptide conjugates which can be used in asymmetric benzoin condensation.

In the third part, synthesis of asymmetric thiazolium salts (thiazole-amino acid conjugates) for the asymmetric benzoin condensation was covered. For this purpose amino acids were used as chiral pool. The aim was to use these synthesized organocatalysts in asymmetric benzoin condensation reactions.

CHAPTER 2

RESULTS AND DISCUSSION

2.1 Production of BAL

In this study, the first step was the production of BAL. With this purpose, the recombinant *E.coli* strains were grown on LB (Luria Broth) agar that contains ampicillin and chloramphenicol for overnight in oven (37 °C). Firstly, for the enzyme production, cells were got from LB agars by sterile loop and transferred to sterile LB medium containing amphicillin and chloramphenicol as the recombinant *E.coli* strain is resistant to ampicillin and chloramphenicol. And the second reason is to avoid the production of other bacteria. This medium was inoculated for 12 hours at 37 °C. In precultivation process, growing time range is important because cells begin to die at some point. In this part, Erlenmeyer flask was used for LB (90 mL distilled water + 10 mL growth cell) medium which contains amphicillin and chloramphenicol inoculation ratio 1/1000. It was grown for 6 hours at 37 °C, and then transferred to the production medium containing LB medium. It is shaking with 120 rpm, 37 °C. Four hours after the inoculation of the microorganism, the synthesis of enzyme was initiated with addition of isopropyl-β-D-thiogalacto pyranosid (IPTG). After the induction, the production of enzyme was continued for 12 hours, cell pellets were collected with centrifugation. In order to break the cell walls to release our enzyme, the cell pellets which are taken from -20 °C has been melted to room temperature and sonicated. Finally, water was removed from cells by lyophilization using freeze dryer.

2.2 Activity of BAL Determination

In the second part, the activity of BAL was determined [42]. One unit (U) of activity is defined as the amount of enzyme which catalyzes the formation of 1 μmol benzoin (1.5 mM) from benzaldehyde in potassium phosphate buffer (50 mM, pH 7) that contains $MgSO_4$ (2.5 mM), ThDP (0.15 mM) and DMSO (20%, v/v) in 1 minute at 30 °C.

To accomplish this process, a set of reaction with the same concentration of benzaldehyde was prepared with commercially available benzaldehyde. At appropriate time intervals, samples were withdrawn to measure the amount of benzoin that was formed. Then, the standard curve was drawn with HPLC analysis to measure the activity of BAL (Figure 8). Benzoin concentration has been calculated based on the standard curve obtained by using commercially available benzoin with different concentrations. Therefore, the activity of BAL was calculated as 0.2 U.

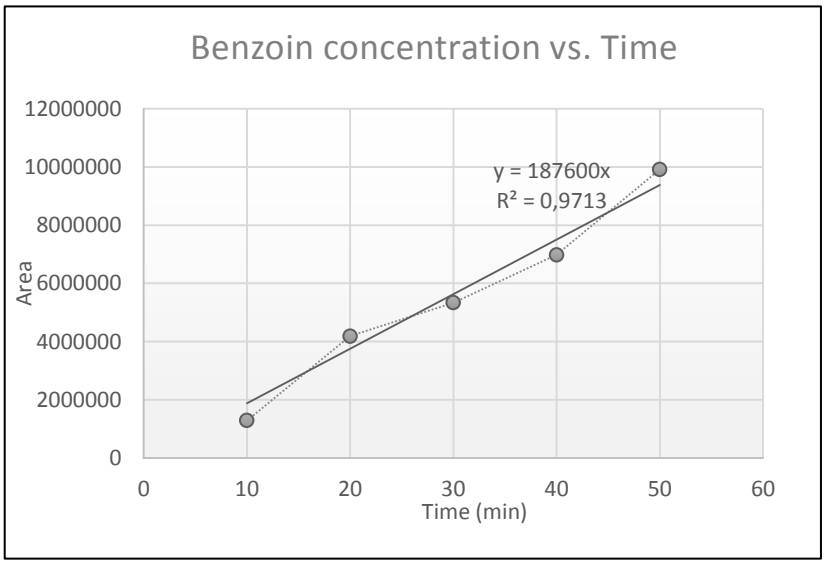
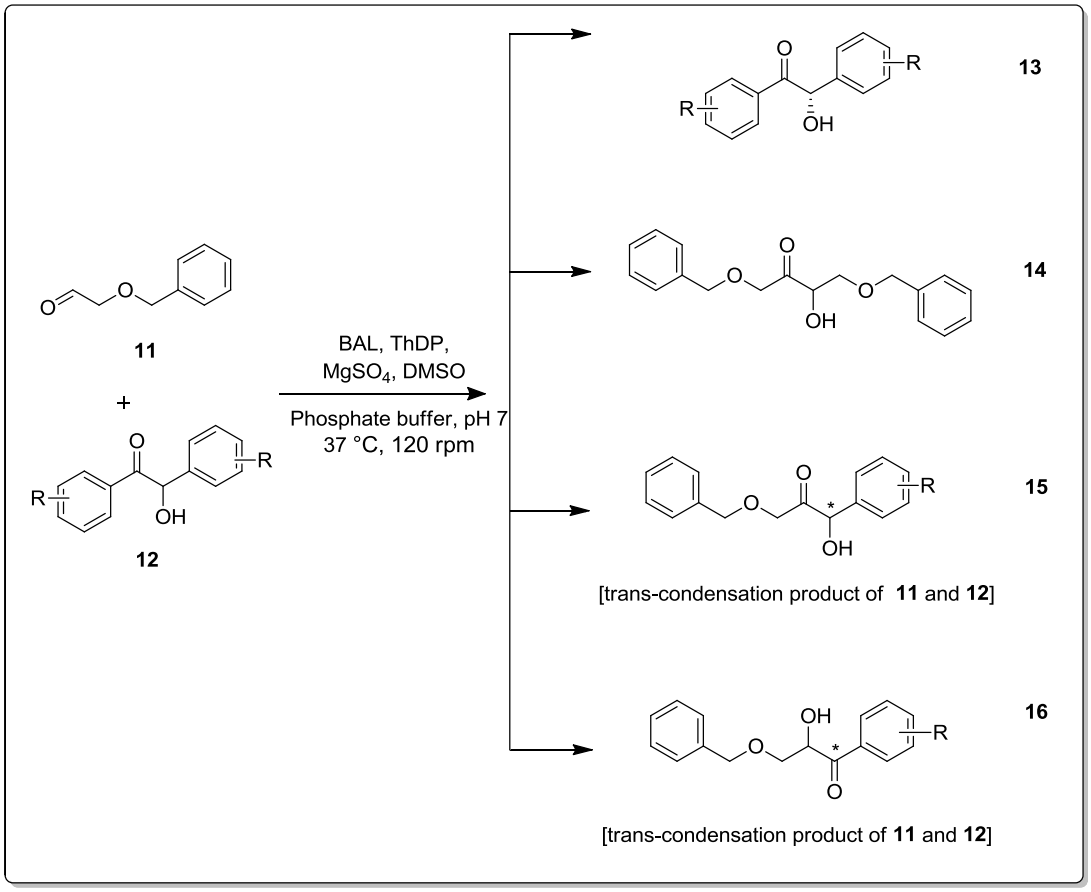


Figure 8. Benzoin calibration curve for the calculation of enzyme activity.

2.3 Enzymatic Trans-Benzoin Condensation using *rac*-Benzoin

In the third part, enzymatic trans-benzoin condensation reactions were performed. According to this coupling reaction, four different possible products can be formed as seen in Scheme 13. One is the enantiomerically enriched self-product, **13**, and the

second one is self-condensation of benzyloxyacetaldehyde, **14** and two trans-condensation products where benzyloxyacetaldehyde (BnOAc) acts as donor or acceptor, **15, 16**. These are the products that were expected to see after the reaction, however at most cases, the major product was the *S*-benzoin, **13**, (that does not react in the presence of BAL) and the minor one was the product where BnOAc acts as acceptor, **16**. Self-condensation product of BnOAc was not observed at all. The other trans-condensation product where BnOAc acts as donor was observed only once.



Scheme 13. Possible products of BAL catalyzed trans-benzoin condensation.

As a test reaction, commercially available *rac*-benzoin is used to see the applicability of this new trans-benzoin condensation reaction. For this purpose, *rac*-benzoin and benzyloxyacetaldehyde were used in the presence of MOPS buffer (50 mM, pH 7) that contains 0.15 mM ThDP and 2.5 mM $MgSO_4$ and DMSO was used as co-solvent. Addition of BAL starts the reaction. Every 24 hours, the reaction was

monitored by TLC. If there was still *rac*-benzoin in the reaction mixture, the same amount of BAL and benzyloxyacetaldehyde were added. This reaction was terminated after 72 h. After the ^{1}H NMR and HPLC analysis, the trans-benzoin condensation products were obtained in moderate yields.

Under the light of this preliminary study, it was decided to expand the scope of this new reaction by using various racemic benzoin derivatives. For that reason, different racemic benzoin products were synthesized based on the literature procedures.

2.4 Synthesis of *Rac*-Benzoin Derivatives

Starting from derivatives of benzaldehyde, *rac*-benzoin derivatives were synthesized by using sodium cyanide as catalyst in the presence of water and ethanol. As seen in Figure 9, these *rac*-benzoin derivatives were synthesized in acceptable yields. Racemic benzoin and 4,4'-dimethoxybenzoin were used from commercial sources.

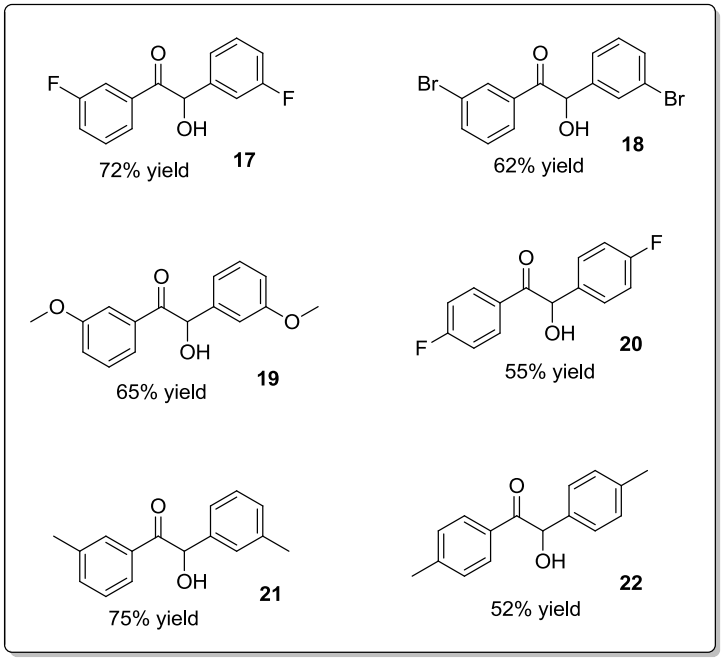


Figure 9. *rac*-Benzoin derivatives that were synthesized.

2.5. Enzymatic Trans-Benzoin Condensation Using Different Benzoin Derivatives.

After the synthesis of *rac*-benzoin derivatives, **17-22**, these benzoins were used in enzymatic trans-benzoin condensation reactions. *Rac*-benzoin derivatives and benzyloxyacetaldehyde were dissolved in DMSO; then MOPS buffer (50 mM, pH 7) that contains ThDP and $MgSO_4$ was added to the solution. With the addition of BAL, the reaction was started (0.2 U) at 37 °C (120 rpm). BAL (0.2 U) was added daily. This process was controlled by TLC. The reaction was stopped after 72 hours and was extracted with diethylether. Then, the combined organic layers washed with brine and dried over $MgSO_4$. The solvent was removed under reduced pressure. At the end, the product was purified by using flash column chromatography technique. However some of the derivatives couldn't be purified. Trans-benzoin products with 3-F, 3-Br, 4-F, 4-Br substituents were observed in NMR, but couldn't be purified by column chromatography since *rac*-Benzoin and the product has very similar polarities. Although different solvent systems were tried, these derivatives were all failed to isolate in pure form.

For other derivatives that can be purified by using column chromatography, **23-26**, enantiomeric excesses were obtained by HPLC which are shown in Figure 10.

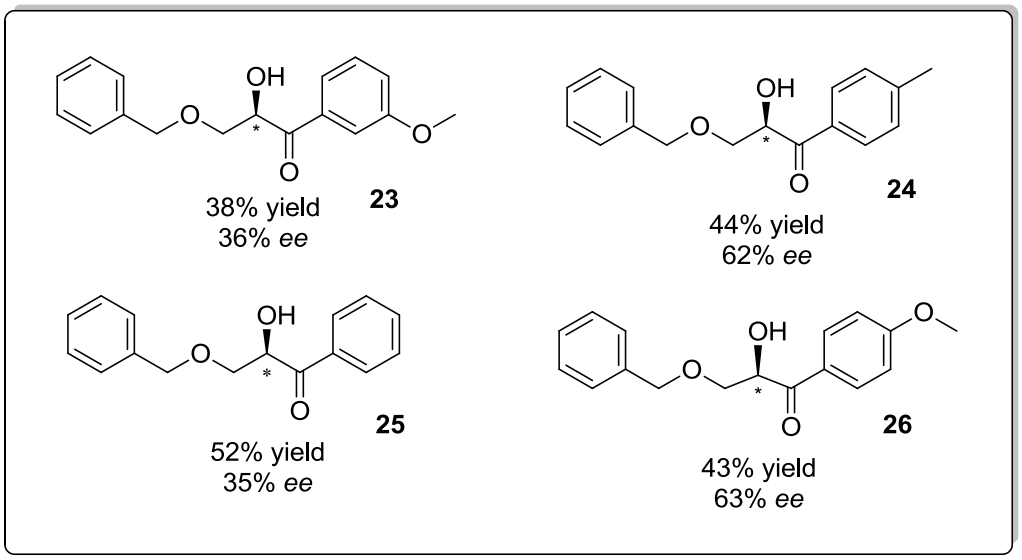


Figure 10. Enzymatic trans benzoin products with their enantiomeric excesses and yields that were synthesized and isolated

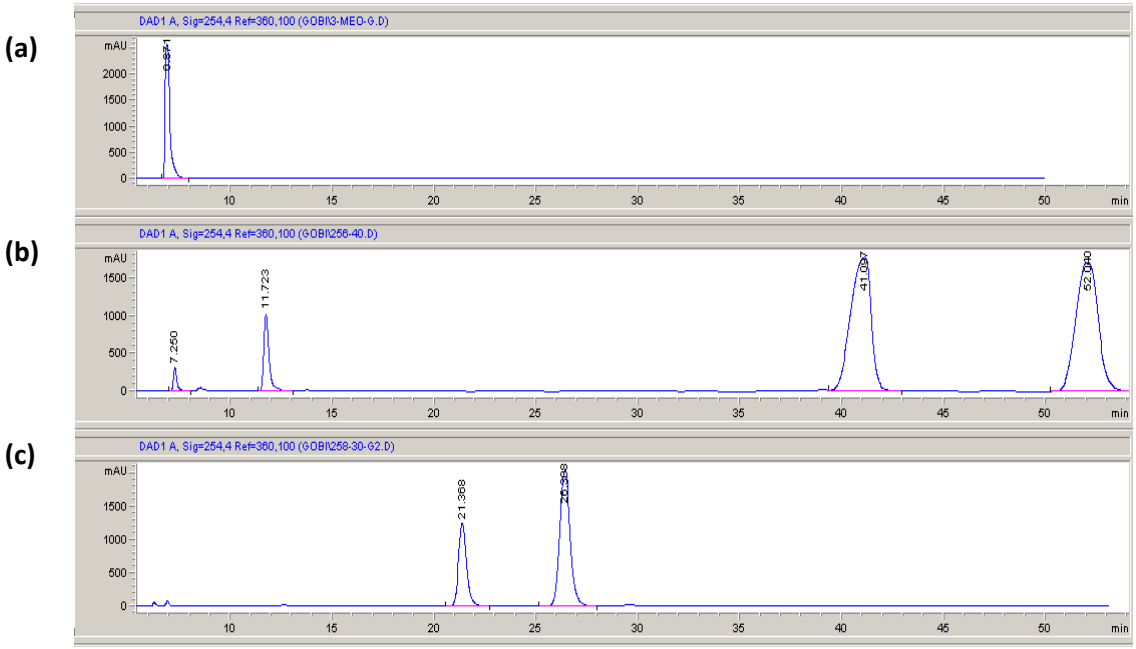


Figure 11. HPLC chromatogram of **(a)** 3-Methoxy benzaldehyde, **(b)** 3,3'dimethoxy benzoin, **19** and **(c)** trans-benzoin condensation product of 3-methoxy benzaldehyde and benzyloxyacetaldehyde, **23** that was isolated.

For the derivatives that couldn't be purified by using column chromatography, **27-29**, enantiomeric excesses were obtained by HPLC through comparing the HPLC results with thiazolium catalysed cross benzoin condensation products. The enantiomeric excess of the derivatives that couldn't be isolated is shown in Figure 12.

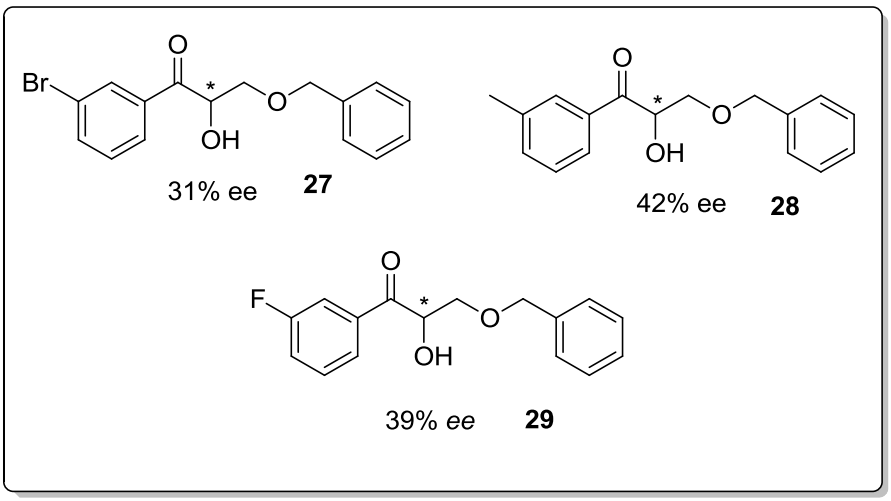


Figure 12. Chiral hydroxy propiophenone derivatives that couldn't be isolated.

Furthermore, in order to be sure about the determination of enantiomeric excesses, the racemic products had to be also synthesized. For this purpose, racemic cross benzoin condensation was performed under various conditions.

2.6 Synthesis of Racemic Cross Benzoin Derivatives

Racemic cross benzoin reactions by using commercially available N-heterocyclic carbene catalyst (3-ethyl-5-(2-hydroxyethyl)-4-methylthiazol-3-ium bromide) was applied to compare the results with the enzymatic trans-benzoin condensation products. With that purpose, cross benzoin condensation procedure from the literature [37] was applied by using the same aldehydes. Benzyloxyacetaldehyde and aromatic benzoin derivatives were dissolved in THF. 3-Ethyl-5-(2-hydroxyethyl)-4-methylthiazol-3-ium bromide (Figure 13) was added as a catalyst with the base Cs_2CO_3. The reaction was stirred at room temperature for nearly 15 hour, and extracted with EtOAc, dried with $MgSO_4$ and removed the solvent under reduced pressure. With some derivatives of aromatic benzaldehyde (3-floro benzaldehyde, 3-bromo benzaldehyde, 3-methyl benzaldehyde and 4-methoxy benzaldehyde), pure hydroxy ketone was obtained after column chromatography.
Unfortunately, with other derivatives of benzaldehyde we did not observe the product.

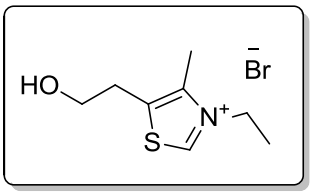
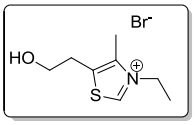


Figure 13. Structure of 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazol-3-ium bromide, **30**.

To get other cross benzoin products, NaCN was used as catalyst. In the literature, the usage of cyanide as catalyst in self and cross benzoin condensation reactions is known [37]. However, no desired product was observed in the reaction between bezaldehydes and benzyloxyacetaldehyde. The racemic cross condensation reaction

was observed only in thiazolium salt catalysed cross benzoin condensation reactions. The reaction conditions to get racemic cross products were summarized in Table 2.

Table 2. The conditions of racemic cross product synthesis.

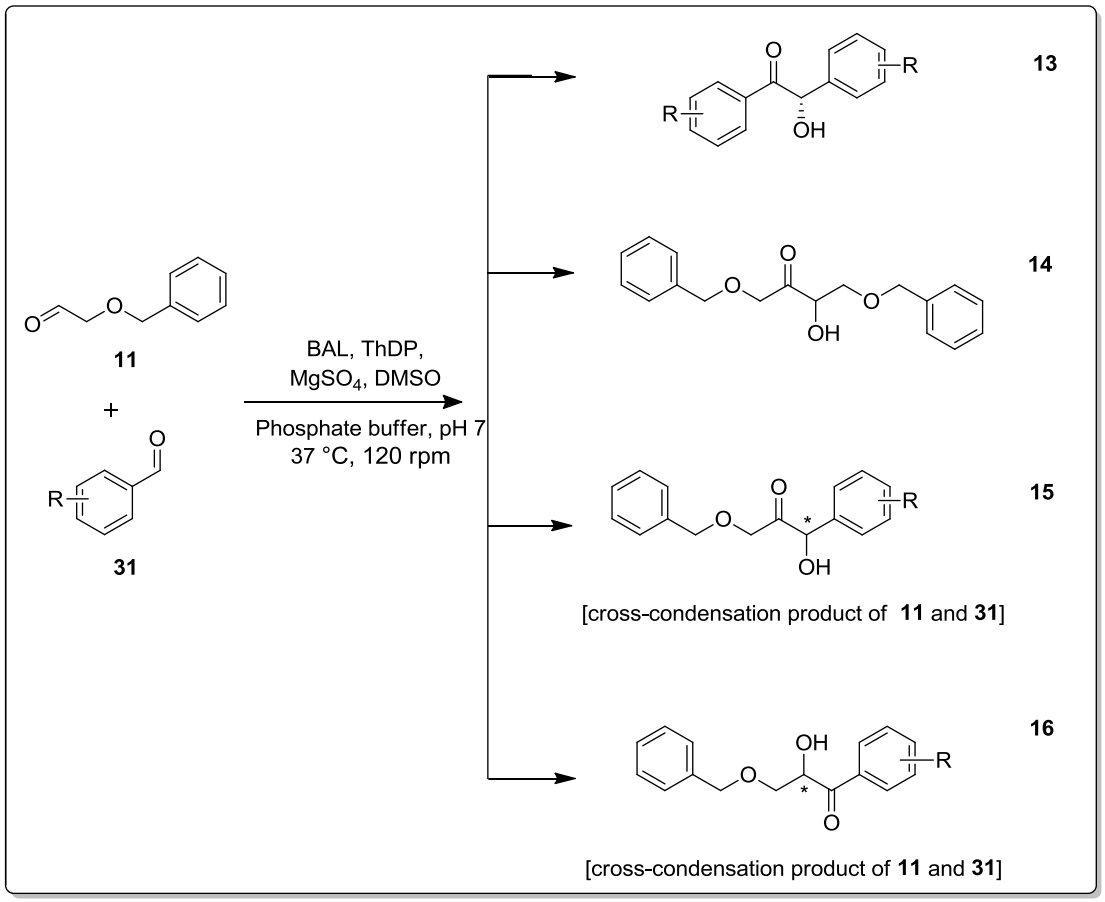
Catalyst	Aromatic Aldehyde (1 eq)	Solvent	Base	Temp.
	Benzaldehyde 3-Fluorobenzaldehyde 4-Fluorobenzaldehyde 3-Bromobenzaldehyde 3-Methoxybenzaldehyde 4-Methoxybenzaldehyde 3-Methylbenzaldehyde	THF	Cs_2CO_3	r.t.
	Benzaldehyde 2-Fluorobenzaldehyde 3-Fluorobenzaldeyhde 4-Methoxybenzaldehyde	EtOH / H_2O	--	75 °C

As a conclusion, only 3-fluoro, 3-bromo, 3-methyl and 4-methoxy derivatives were succesfully synthesized and others were failed to give the desired racemic cross products.

2.7 Enzymatic Cross-Benzoin Condensation

In the literature, there is only one example of enzymatic cross benzoin condensation using benzyloxyacetaldehyde derivative. But in that work, furfural was used as donor aldehyde. Therefore, as a complementary study, enzymatic cross benzoin condensation were tried with benzyloxyacetaldehyde using benzaldehyde derivatives to get enantiomerically enriched cross benzoin products which are basically same as obtained from trans benzoin condensation products.

According to this benzoin condensation reaction, four different possible products can be formed as seen in Scheme 14. One is the enantiomerically enriched self, **13**, and the second one is self-condensation of benzyloxyacetaldehyde, **14** and two cross condensation products where benzyloxyacetaldehyde (BnOAc) acts as donor or acceptor, **15, 16**. These are the expected products in this condensation reaction.



Scheme 14. Possible products of BAL catalyzed cross benzoin condensation.

In this type of condensation reaction, only one example has been worked and 3-fluoro derivative was obtained in poor yields (17%) with moderate enantiomeric excess (50% *ee*) (Figure 14).

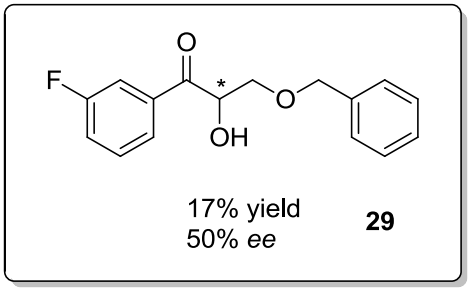


Figure 14. The enzymatic cross-benzoin condensation product.

2.8 Conjugation of Thiamine with Amino Acids

Thiamine (Figure 15) is used as organocatalyst in self-benzoin condensations. However, there are no studies in the literature where some asymmetric thiamine derivatives were used as catalysts in asymmetric self-benzoin condensations. Therefore, it is planned to prepare some thiamine amino acid conjugates to be used as catalyst in this reaction.

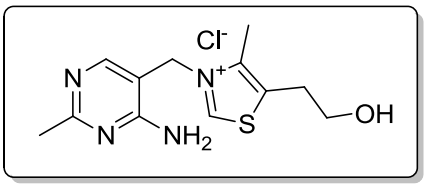
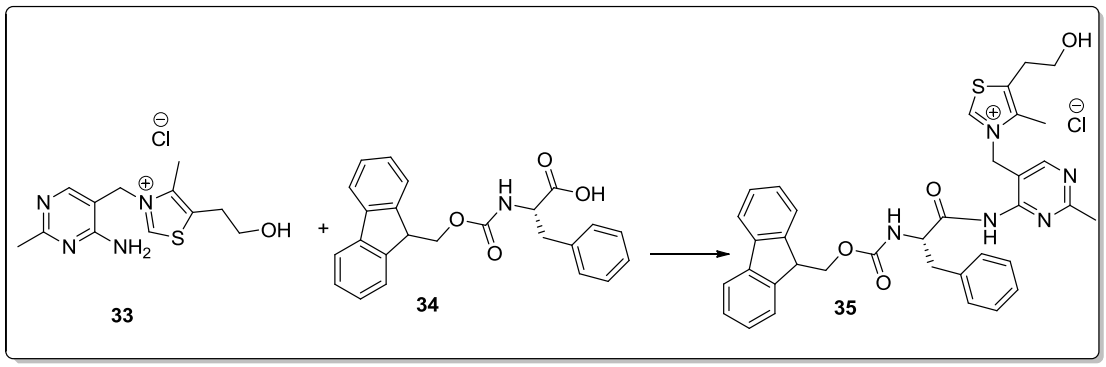


Figure 15. Structure of thiamine, **32**.

In the literature, thiamine is functionalized by a steroid through carboxylic acid using EDC coupling [39]. Therefore, several amino acids are used to conjugate thiamine from NH_2 group using this literature procedure. Conjugation of amino acids with thiamine by using EDC coupling reactions was tried (Scheme 15). Many different amino acid derivatives also some carboxylic acid derivatives, different protecting groups, different base, different coupling agents, different solvents, different temperatures were tried (Table 3), however none of them gave us the product except one case where we observe trace amount of the desired product by using LCMS.



Scheme 15. Example of thiamine-peptide conjugate in the presence of Fmoc-Phe-OH.

Table 3. Methods used for thiamine-peptide conjugates.

Protected Amino acid	Thiamine·HCl	Neutralized Thiamine	EDC·HCl	EDC·IMe	HBTU/ HOBt·H_2O	Solvent	Base	Temp.	Yield %
Fmoc-Phe-OH 1 eq	1.25 eqv	-	1.30 eq	-	-	CH_3CN/ H_2O	DIEA 3 eq	R.T.	No product
Fmoc-Val-OH 1 eq	1.25 eqv	-	1.30 eq	-	-	CH_3CN/ H_2O	DIEA 3 eq	R.T.	No product
Fmoc-Pro-OH 1 eq	1.25 eq	-	1.30 eq	-	-	CH_3CN/ H_2O	DIEA 3 eq	R.T.	No product
Fmoc-Ile-OH 1 eq	1.25 eq	-	1.30 eq	-	-	CH_3CN/ H_2O	DIEA 3 eq	R.T.	No product
Fmoc-Phe-OH 1 eq	1.25 eq	-	-	1.30 eq	-	CH_3CN/ H_2O	DIEA 3 eq	R.T.	No product
Boc-Ala-OH 1 eq	1.25 eq	-	-	-	1.30 eq/ 1.30 eq	DMF	DIEA 3 eq	R.T.	No product
Fmoc-Phe-OH 1 eq	1.25 eq			1.30 eq	-	DMF/ H_2O	DIEA 3 eq	R.T.	No product
Fmoc-Phe-OH 1 eq	1.25 eq	-	-	-	1.30 eq/ 1.30 eq	DMF	DIEA 3 eq	R.T.	3
Fmoc-Phe-OH 1 eq	1.25 eq	-	1.30 eq	-	-	CH_3CN/ H_2O	DIEA 3 eq	70 °C	No product
Fmoc-Phe-Cl 1 eq	1 eqv	-	-	-	-	CH_3CN	DIEA 3 eq	R.T.	No product
Acetic acid 1 eq	-	1.25 eq	1.30 eq	-	-	CH_3CN/ H_2O	-	R.T.	No product
Acetic acid 1 eq	-	1.25 eq		1.30 eq	-	CH_3CN/ H_2O	-	R.T.	No product
Z-Gly-OH 1 eq	-	1.25 eq	-	1.30 eq	-	CH_3CN/ H_2O	-	R.T.	No product
Fmoc-Phe-OH 1 eq	-	1.25 eq	-	1.30 eq	HOBt·H_2O 1.30 eq	THF/ $CHCl_3$	-	R.T.	No product
Fmoc-Phe-Cl 1 eq	1 eq	-	-	-	-	DMF	DIEA 3 eq	70 °C	No product
Fmoc-Phe-Cl 1 eq	1 eq	-	-	-	-	CH_3CN	DIEA 3 eq	70 °C	No product
Fmoc-Phe-OH 1 eq	1.25 eq	-	-	-	1.30 eq/ 1.30 eq		DIEA 3 eq	R.T. (in dark)	No product

On this LC-MS spectrum, the mass chromatogram and spectrum is shown in Figure 16 and 17. Peak at retention time 17.8 minutes indicates the desired product. In the HPLC spectrum, the peak at the retention time 10.4 minutes indicates HOBt.

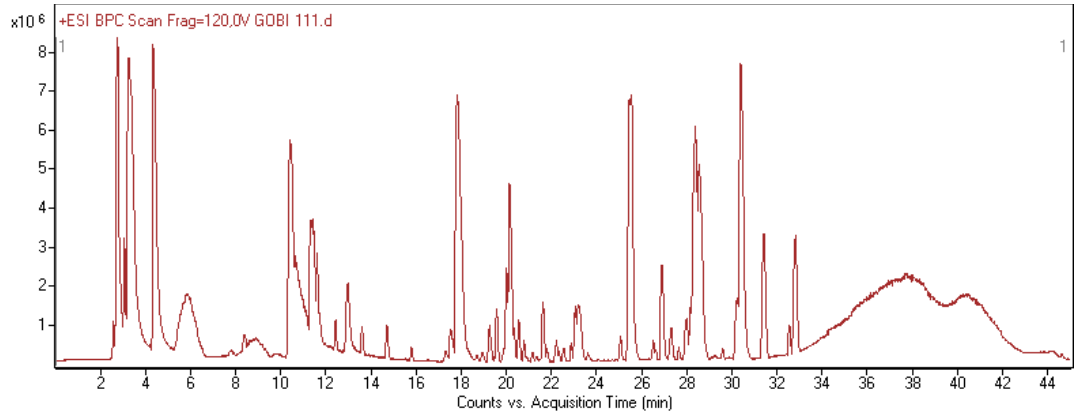


Figure 16. HPLC spectrum of Thiamine-Fmoc-Phe-OH conjugate.

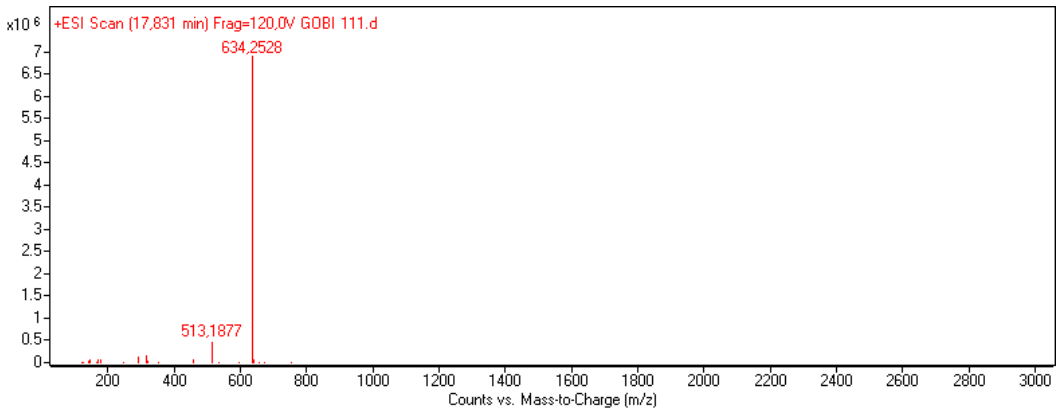
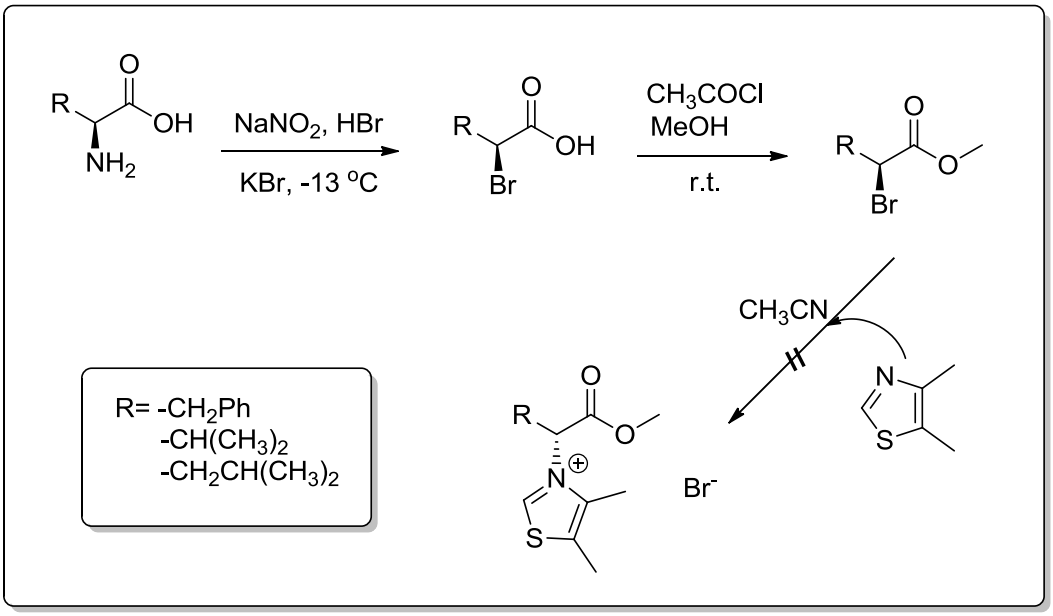


Figure 17. HRMS spectrum of the Thiamine-Fmoc-Phe-OH conjugate.

After all these efforts, we concluded that thiamine is decomposing and it is not suitable for functionalization.

2.9 Synthesis of Thiazolium Based Organocatalysts

After the failure of the conjugation of thiamine with amino acid, it is planned that thiazolium salts of amino acids derivatives can be synthesized (Scheme 16) and used as catalyst in self-benzoin condensation.



Scheme 16. General scheme for the synthesis of asymmetric thiazole catalyst.

For this purpose amino acids were used as chiral pool. Three different amino acids (phenylalanine, valine, and isoleucine) were chosen due to their hydrophobic and non-reactive side chains. Thiazolium based organocatalysis were tried to be synthesized starting from the bromination of the amino acids. This reaction is known in literature and all amino acids derivatives were converted into bromo derivatives without any problem. The second step was the esterification of the bromo derivatives. Typical esterification reactions did not proceed well, therefore esterification via mixed anhydride formation were used by using acetyl chloride. After getting the bromo ester derivatives, 4,5-dimethyl thiazole is used to substitute bromide to get the desired thiazolium salts. However, direct substitution of thiazoles with bromide group at room temperature and at reflux in ethanol or neat conditions did not give the corresponding thiazolium salts. The ability of silver ions to abstract the bromide would facilitate the reaction towards the substitution, therefore, silver

nitrate is used in the reaction and the precipitation of silver bromide is observed. The crude product is identified by ^{1}H-NMR to confirm the formation of thiazolium salts however, the product could not be isolated by simple methods. Addition of KPF_6 is used for anion exchange to precipitate the corresponding thiazolium salts. Precipitation and purification of these salts did not give any product.

As a result, the target thiazolium salts could not be synthesized and consequently, couldn't be tested in the asymmetric self-benzoin condensation.

CHAPTER 3

EXPERIMENTAL

3.1 Materials

Benzaldehyde Lyase was supplied from the Institut für Biotechnologie, Jülich, Germany.

Benzaldehyde and its derivatives were supplied from Sigma Aldrich, Germany.

Amino acids were supplied from CHEM-IMPEX, USA.

Solvents are either in technical or higher grade. They were supplied from Sigma Aldrich when necessary; they were purified and dried with drying agents or by distillation.

3.2 Methods

Nuclear magnetic (^{1}H-NMR and ^{13}C-NMR) spectra were recorded in $CDCl_3$ on Bruker Spectrospin Avance DPX 400 spectrometer. Chemical shifts are given in parts per million (ppm) with TMS as internal reference. ^{1}H and ^{13}C NMR spectra of products which are unknown in literature are given in Appendix A.

Chromatographic separation was performed on glass precoated silica gel purchased from Macherey-Nagel.

Polarimetric measurements were made by Rudolph Scientific Autopol III polarimeter and reported as follows $[\alpha]_D^T$ (*c* in g per mL, solvent).

HPLC chromatograms were recorded on an Agilent 1100 Series. Daicell AD-H chiral column was used with different solvent systems. HPLC chromatograms of chiral products and racemic forms of them were given in Appendix B.

HRMS data were detected on a Agilent 6224 TOF LC/ MS at UNAM, Bilkent University.

Infrared Spectra were recorded on Bruker Alpha Platinum ATR. Band positions were reported in reciprocal centimeters (cm^{-1}).

All reactions were monitored by TLC using precoated slica gel plates (Merk Silica Gel 60 F_{254}), visualized by UV-light. Chromatographic separations were performed by glass precoated silica gel -200 purchased from Macherey-Nagel and column chromatography was performed on silica gel 60 with particle size of 0.063–0.200 mm.

For disrupting a cell wall sonics, vibra cell instrument was used. Shaking of cells was provided by Shaker Infors-HT instrument.

Freeze dryer, Telstar Cryodos instrument was used for lyophilization.

3.3 Production of Benzaldehyde lyase

The cells of *E. coli SG13009/BALHis* that contains the overexpressed enzyme were supplied from Institute of Biotechnology, Research Centre Jülich. Hexahistidine-tagged BAL was obtained from recombinant *E.Coli SG13009* cells.

The recombinant *E.coli* strains were grown on LB agar that contains 100 μg/mL ampicillin and 35 μg/mL chloroamphenicol incubated in oven for overnight at 37 °C. Firstly, to produce our enzyme, cells were taken from LB agars by sterile loop and transported to sterile 10 mL LB medium contains 20 μL ampicillin and 20 μL chloroamphenicol. This medium was inoculated for 12 hours at 37 °C. In precultivation part, growing time range of the bacteria is critical because cells begin to die at some point. In this part 500 mL Erlenmeyer flask was used for 100 mL LB (90 mL distilled water + 10 mL growth cell) medium containing 100 μL ampicillin

and chloroamphenicol inoculation ratio 1/1000. It was incubated and grown for 6 hours at 37 °C, then 100 mL transferred to production medium contains 1500 mL LB medium is shaking with 180 rpm. Four hours after the inoculation of the microorganism, production of enzyme was initiated with addition of isopropyl-β-D-thiogalacto pyranosid (IPTG). After the induction, enzyme production was started and continued for 12 hours, cell pellets were collected by using centrifugation. In order to break the cell walls to release our enzyme, the cell pellets which are taken from -20 °C has been melted to room temperature and sonicated. Finally, removal of water from cells by lyophilization was made with freeze dryer.

3.4 Activity assay

According to the literature [35], one unit (U) of activity is described as the quantity of enzyme that catalyzes the formation of 1 μmol benzoin (1.5 mM) from benzaldehyde in potassium phosphate buffer (50 mM, pH7) that contains $MgSO_4$ (2.5 mM), ThDP (0.15 mM) and DMSO (20%, v/v) in 1 minute at 30 °C.

To measure the activity of our enzyme, a set of reaction with the same concentration of benzaldehyde was prepared with commercially available benzaldehyde. At appropriate time intervals, samples were withdrawn to measure the amount of benzoin. Then, the standard curve was drawn by using HPLC analysis results to measure the activity of BAL.

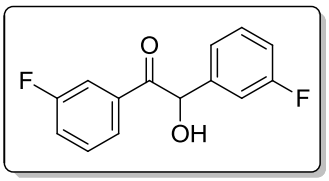
For activity experiment, to a set of 2.5μL benzaldehyde, 0.5 mL DMSO and 1.5 mL phosphate buffer containing ThDP and $MgSO_4$ was added. The process was initiated with the addition of 5 mg crude enzyme. At 10 minute intervals, one sample was withdrawn by adding chloroform and it was centrifuged. Finally, organic layer was collected and analyzed with HPLC to terminate the activity.

3.5 General procedure for racemic synthesis of self-benzoin condensation products [36]

A solution of sodium cyanide (2 mmol, 0.098 g) in H_2O (2 ml) was added to a stirred solution of a benzaldehyde derivative (10 mmol) in EtOH (10 ml). The mixture was

then refluxed. The progress of reaction was monitored by TLC using hexane/ethyl acetate as eluent. The solvent was then removed by evaporation under reduced pressure. The residue was washed with water and diethyl ether. The product was purified by using flash column chromatography technique.

3.5.1 Synthesis of 3,3'-difluoro benzoin (17)

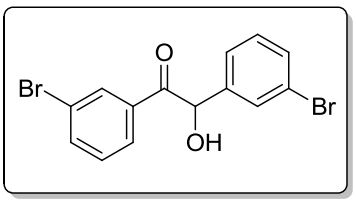


General procedure stated above starting from 3-fluoro benzaldehyde was applied and pure product was obtained after crystallization with ethyl acetate and washed with ethyl acetate (48% yield, white solid).

TLC hexane/ethyl acetate = 80:20 R_f; 0.60

^{1}H NMR (400 MHz, *$CDCl_3$*) δ: 7.67-7.58 (m, 1H), 7.55-7.50 (m, 1H), 7.41-7.29 (m, 2H), 7.08-7.03 (m, 2H), 6.98-6.88 (m, 2H), 5.83 (s, 1H), 4.43 (br s, 1H, OH).

3.5.2 Synthesis of 3,3'-dibromo benzoin (18)

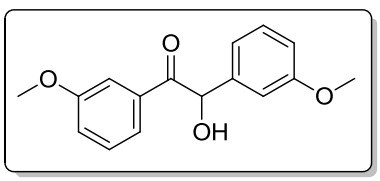


General procedure stated above starting from 3-bromo benzaldehyde was applied and pure product was obtained after crystallization with ethyl acetate and washed with ethyl acetate (60% yield, yellow oil).

TLC hexane/ethyl acetate = 80:20 R_f; 0.75

^{1}H NMR (400 MHz, *$CDCl_3$*) δ: 8.06 (t, *J* = 1.8 Hz, 1H), 7.78 (t, *J* = 7.5 Hz, 1H), 7.66 (dd, *J* = 8.01, 1.0 Hz, 1H), 7.48 (t, *J* = 1.7 Hz, 1H), 7.42 (d, *J* = 7.7 Hz, 1H), 7.31-7.17 (m, 3H), 5.87 (s, 1H), 4.45 (br s, 1H, OH)

3.5.3 Synthesis of 3,3'-dimethoxy benzoin (19)

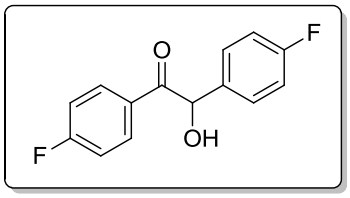


General procedure stated above starting from 3-methoxy benzaldehyde was applied and pure product

was obtained after crystallization with ethyl acetate and washed with ethyl acetate (45% yield, light yellow solid). TLC hexane/ethyl acetate = 80:20 R_f; 0.65

^{1}H NMR (400 MHz, *$CDCl_3$*) δ: 7.50-7.44 (m, 2H), 7.33-7.20 (m, 2H), 7.09-7.02 (m, 1H), 6.92 (d, *J* = 7.6 Hz, 1H), 6.86-6.79 (m, 2H), 5.90 (s, 1H), 4.55 (br s, 1H, OH), 3.79 (s, 3H), 3.76 (s, 3H)

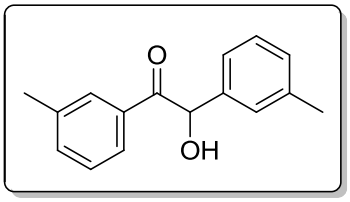
3.5.4 Synthesis of 4,4'-difluoro benzoin (20)



General procedure stated above starting from 4-fluoro benzaldehyde was applied and pure product was obtained after crystallization with ethyl acetate and washed with ethyl acetate (68% yield, light yellow solid). TLC hexane/ethyl acetate = 80:20 R_f; 0.63

^{1}H NMR (400 MHz, *$CDCl_3$*) δ: 8.09-8.03 (m, 1H), 7.89-7.82 (m, 2H), 7.26-7.20 (m, 1H), 7.03-6.89 (m, *4*H), 5.83 (s, 1H)

3.5.5 Synthesis of 3,3'-dimethyl benzoin (21)

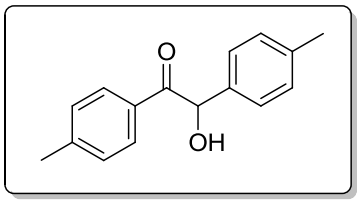


General procedure stated above starting from *m*-tolualdehyde was applied and pure product was obtained after crystallization with ethyl acetate and washed with ethyl acetate (42% yield, yellow oil).

TLC hexane/ethyl acetate = 80:20 R_f; 0.62

^{1}H NMR (400 MHz, *$CDCl_3$*) δ: 7.76 (s, 1H), 7.69 (d, *J* = 7.6 Hz, 1H), 7.32 (d, *J* = 7.6 Hz, 1H), 7.21-7.17 (m, 2H), 7.13 (d, *J* = 5.8 Hz, 2H), 7.06 (t, *J* = 6.4 Hz, 1H), 5.90 (d, *J* = 5.5 Hz, 1H), 4.54 (d, *J* = 6.0 Hz, 1H, OH), 2.34 (s, 3H), 2.29 (s, 3H)

3.5.6 Synthesis of 4,4'-dimethyl benzoin (22)



General procedure stated above starting from *p*-tolualdehyde was applied and pure product was obtained after crystallization with ethyl acetate and

washed with ethyl acetate (55% yield, light yellow oil). TLC hexane/ethyl acetate = 80:20 R_f ; 0.50

^{1}H NMR (400 MHz, *$CDCl_3$) δ*: 7.70 (d, *J* = 8.1 Hz, 2H), 7.07 (d, *J* = 8.0 Hz, 2H), 7.04-6.90 (m, 4H), 5.78 (s, 1H), 2.12 (s, 3H), 2.08 (s, 3H)

3.6 Cross acyloin condensation of aliphatic aldehyde reactions with thiazolium catalyst [38]

This procedure was tried with *N*-hetercyclic carbene catalyst (3-ethyl-5-(2-hydroxyethyl)-4-methylthiazol-3-ium bromide) which is shown in Figure 18.

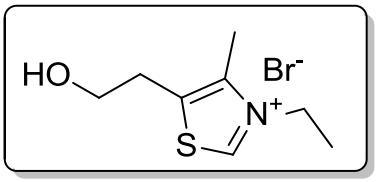
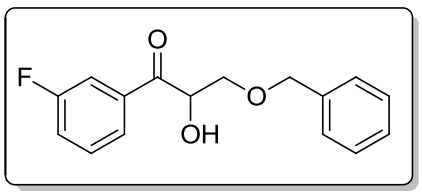


Figure 18. Structure of (3-ethyl-5-(2-hydroxyethyl)-4-methylthiazol-3-ium bromide), **36**.

Firstly, to the derivatives of benzaldehyde (0.3 mmol) and benzyloxyacetaldehyde (0.5 mmol), dry THF (1 mL) and 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazol-3-ium bromide was added as a catalyst (0.05 mmol) was added; subsequently Cs_2CO_3 (0.05 mmol) was transferred into the reaction medium. After 20 hours, the reaction mixture was extracted with EtOAc (10 mL x 3), dried over Na_2SO_4 and solvent was removed under reduced pressure. The residue was afforded after flash column chromatography (EtOAc/Hexane =1:10) as an oil. This procedure was adapted from reference [38].

3.6.1 Synthesis of 3-(benzyloxy)-1-(3-fluorophenyl)-2-hydroxypropan-1-one (37)

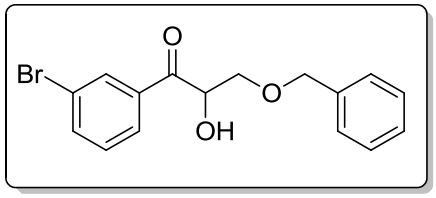


General procedure described above starting from 3-fluorobenzaldehyde and BnOAc, provided pure product obtained as white powder (36% yield).

TLC hexane/ethyl acetate = 90:10 R_f; 0.63

^{1}H NMR (400 MHz, *$CDCl_3$*) δ: 7.83 (d, *J* = 7.7 Hz, 1H), 7.59 (d, *J* = 7.7 Hz, 1H), 7.37 (tt, *J* = 7.9, 5.5 Hz, 2H), 7.27-7.19 (m, 3H), 7.06 (dd, *J* = 7.9, 5.1 Hz, 2H), 5.08 (t, *J* = 3.7 Hz, 1H), 4.45 (d, *J* = 12.3 Hz, 1H), 4.36 (d, *J* = 12.3 Hz, 1H), 3.73 (dd, *J* = 9.8, 3.5 Hz, 1H), 3.69 (dd, *J* = 10.3, 4.2 Hz, 1H)

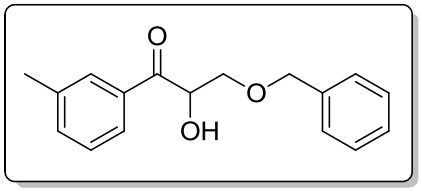
3.6.2 Synthesis of 3-(benzyloxy)-1-(3-bromophenyl)-2-hydroxypropan-1-one (38)



General procedure described above starting from 3-bromobenzaldehyde and BnOAc, provided pure product obtained as yellow powder (21% yield). TLC hexane/ethyl acetate = 90:10 R_f; 0.67

^{1}H NMR (400 MHz, *$CDCl_3$*) δ: 8.18 (t, *J* = 1.7 Hz, 1H), 7.98-7.96 (m, 2H), 7.74-7.70 (m, 1H), 7.32-7.28 (m, 1H), 7.19-7.16 (m, 4H), 5.07 (t, *J* = 3.8 Hz, 1H), 4.44 (d, *J* = 12.3 Hz, 1H), 4.35 (d, *J* = 12.3 Hz, 1H), 3.71 (dd, *J* = 10.3, 3.6 Hz, 1H), 3.69 (dd, *J* = 10.3, 4.1 Hz, 1H)

3.6.3 Synthesis of 3-(benzyloxy)-2-hydroxy-1-(m-tolyl)propan-1-one (39)



General procedure described above starting from 3-methylbenzaldehyde and BnOAc, provided pure product obtained as white oil (23% yield). TLC hexane/ethyl acetate = 90:10 R_f; 0.73

^{1}H NMR (400 MHz, *$CDCl_3$*) δ: 7.68-7.59 (m, 3H), 7.35 (d, *J* = 7.6 Hz, 2H), 7.16 (m, 2H), 7.06 (dd, *J* = 6.8, 2.5 Hz, 2H), 5.13 (t, *J*= 3.8 Hz, 1H), 4.44 (d, *J*= 12.3 Hz, 1H), 4.37 (d, *J*=12.3 Hz, 1H), 3.71 (dd, *J* = 10.3, 3.2 Hz, 1H), 3.67 (dd, *J* = 10.3, 4.4 Hz, 1H), 2.33 (s, 3H)

In Figure 19, listed racemic cross benzoin products were synthesized and isolated by using flash column chromatography technique.

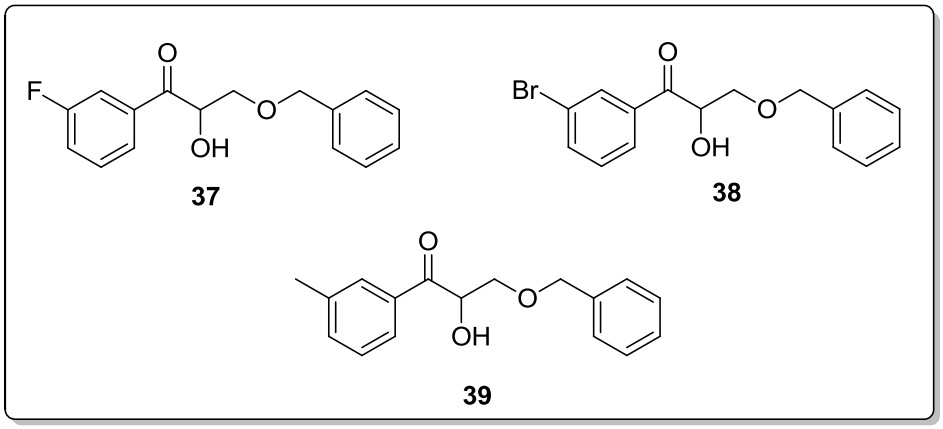
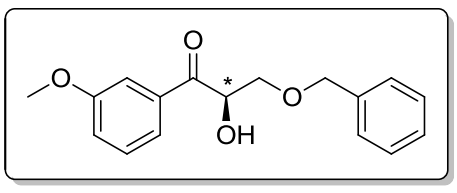


Figure 19. Racemic cross benzoin products that were synthesized.

3.7 General procedure of enzymatic trans-benzoin condensation of benzoin and BnOAc [38]

To a mixture of *rac*-benzoin product derivatives (0.25 mmol) and benzyloxyacetaldehyde (0.25 mmol) in 2.5 mL DMSO (25 vol%) 7.5 mL (75 vol%) MOPS buffer (50 mM, pH 7) that contains 0.15 mM ThDP and 2.5 mM $MgSO_4$ was transferred. The reaction was initiated with the addition of BAL (0.2 U) at 37 °C (120 rpm). Every 24 hours 0.2 U of BAL was added. The reaction was controlled with TLC and concluded after 72 h. The reaction mixture was extracted with diethylether (8 x 40 mL) and the combined organic layers were washed with brine and dried over $MgSO_4$, and the solvent was concentrated in vacuo to yield product which was further purified by using flash column chromatography.

3.7.1 Synthesis of (*R*)-3-(benzyloxy)-2-hydroxy-1-(3-methoxyphenyl) propan-1-one (23)



General procedure described above starting from *m*-anisoin and BnOAc, provided pure

product obtained as white oil (43% yield). TLC hexane/ethyl acetate = 10:1 R_f ; 0.28

^{1}H NMR (400 MHz, *$CDCl_3$*) δ: 7.40-7.35 (m, 2H), 7.32-7.26 (m, 1H), 7.18-7.12 (m, 3H), 7.10-7.02 (m, 3H), 5.10 (br s, 1H), 4.44 (d, *J* = 12.3 Hz, 1H),), 4.36 (d, *J* = 12.3 Hz, 1H), 3.90 (br s, 1H, OH), 3.78 (s, 3H), 3.74 (dd, *J* = 10.3, 3.8 Hz, 1H), 3.68 (dd, *J* = 10.3, 4.4 Hz, 1H)

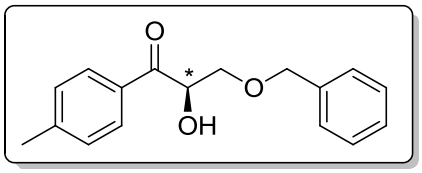
^{13}C NMR (100 MHz, *$CDCl_3$*) δ: 199.5, 159.9, 137.6, 135.3, 129.8, 128.3, 127.6, 127.5, 121.0, 120.5, 112.8, 73.9, 73.4, 72.6, 55.5

IR: 3444, 2959, 2922, 2859, 1684, 1596, 1581, 1452, 1258, 1094, 1014 cm^{-1}

$[\alpha]_D^{22}$= +3.22^o (*c* = 0.026 g/mL, $CHCl_3$)

HRMS $C_{16}H_{16}O_3$ (MNa^+): Calcd 309.1103, found 309.1104

3.7.2 Synthesis of (*R*)-3-(benzyloxy)-2-hydroxy-1-(*p*-tolyl) propan-1-one (24)



General procedure described above starting from 4,4'-dimethyl benzoin and BnOAc, provided pure product obtained as yellow oil 44% yield). TLC hexane/ethyl acetate = 10:1 R_f; 0.21

^{1}H NMR (400 MHz, *$CDCl_3$*) δ: 7.84-7.70 (m, 2H), 7.23-7.14 (m, 5H), 7.07-7.04 (m, 2H), 5.16-5.10 (m, 1H), 4.44 (d, *J* = 12.3 Hz, 1H), 4.38 (d, *J* = 12.3 Hz, 1H), 3.94 (d, *J*= 6.6 Hz, 1H, OH), 3.74 (dd, *J* = 10.3, 3.1 Hz, 1H), 3.66 (dd, *J* = 10.3, 4.5 Hz, 1H), 2.36 (s, 3H)

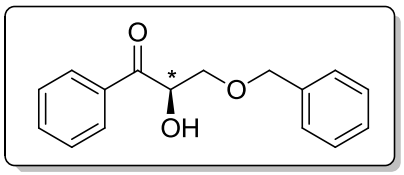
^{13}C NMR (100 MHz, *$CDCl_3$*) δ: 198.0, 144.0, 136.6, 132.9, 130.4, 128.5, 127.7, 127.3, 126.5, 72.6, 72.4, 71.8, 20.7

IR: 3457, 2957, 2919, 2855, 1680, 1606, 1452, 1258, 1093, 1017 cm^{-1}

$[\alpha]_D^{22}$= +6.80^o (*c* = 1.7 x 10^{-3} g/mL, $CHCl_3$)

HRMS $C_{17}H_{18}O_3$ (MNa^+): Calcd 293.1154, found 293.1158

3.7.3 Synthesis of (*R*)-3-(benzyloxy)-2-hydroxy-1-phenylpropan-1-one (25)



General procedure described above starting from commercially available *rac*-benzoin and BnOAc, provided pure product obtained as white powder (24% yield). TLC hexane/ethyl acetate = 50:10 R_f; 0.67

^{1}H NMR (400 MHz, *CDCl$_3$*) δ: 7.92-7.88 (d, *J*= 7.1 Hz, 2H), 7.61 (t, *J* = 7.5 Hz, 1H), 7.48 (t, *J* = 7.8 Hz, 2H), 7.25-7.20 (m, 3H), 7.15-7.09 (m, 2H), 5.24-5.18 (m, 1H), 4.52 (d, *J* = 12.3 Hz, 1H), 4.44 (d, *J* = 12.3 Hz, 1H), 3.96 (d, *J* = 6.5 Hz, 1H, OH), 3.82 (dd, *J* = 10.3, 3.2 Hz, 1H), 3.75 (dd, *J* = 10.3, 4.4 Hz, 1H)

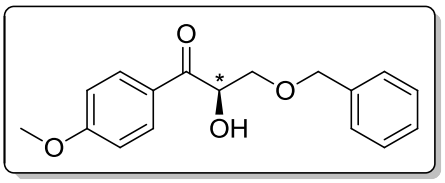
^{13}C NMR (100 MHz, *CDCl$_3$*) δ: 199.5, 137.6, 134.0, 133.9, 128.8, 128.6, 128.3, 127.6, 127.5, 73.8, 73.4, 72.5

IR: 3445, 3017, 2926, 1685, 1618, 1559, 1508, 1496, 1214, 1099 cm^{-1}.

$[\alpha]_D^{22}$= -0.0264^o (*c* = 1.0 x 10^{-4} g/mL, $CHCl_3$)

HRMS $C_{16}H_{16}O_3$ (MNa^+): Calcd 279.0997, found 279.0999

3.7.4 Synthesis of (*R*)-3-(benzyloxy)-2-hydroxy-1-(4-methoxyphenyl) propan-1-one (26)



General procedure described above starting from commercially available *p*-anisoin and BnOAc, provided pure product obtained as white oil (43% yield). TLC hexane/ethyl acetate = 10:1 R_f; 0.24

^{1}H NMR (400 MHz, *CDCl$_3$*) δ: 7.83 (d, *J* = 8.9 Hz, 2H), 7.20-7.15 (m, 3H), 7.09 (dd, *J* = 8.3, 5.9 Hz, 2H), 6.89-6.83 (d, *J*= 8.9 Hz, 2H), 5.10 (br s, 1H), 4.45 (d, *J* = 12.3 Hz, 1H), 4.39 (d, *J* = 12.3 Hz, 1H), 3.94 (br s, 1H, OH), 3.81 (s, 3H), 3.73 (dd, *J*= 9.6, 3.4 Hz, 1H), 3.65 (dd, *J*= 9.6, 4.7 Hz, 1H)

^{13}C NMR (100 MHz, *$CDCl_3$*) δ: 197.7, 164.2, 137.7, 131.0, 129.0, 128.3, 127.5, 126.8, 114.0, 73.4, 73.3, 73.0, 55.6

IR: 3444, 2923, 2856, 1671, 1598, 1572, 1510, 1454, 1256, 1172, 1101, 1025 cm^{-1}

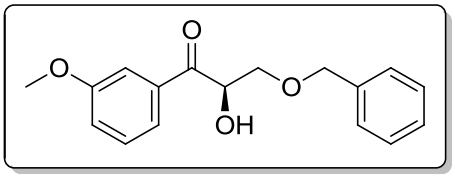
$[\alpha]_D^{22}$= +19.26^o (*c* = 0.0185 g/mL, $CHCl_3$)

HRMS $C_{16}H_{16}O_3$ (MNa^+): Calcd 309.1103, found 309.1106

3.8 General procedure of enzymatic cross benzoin condensation [38]

To a mixture of aromatic benzaldehyde derivatives (0.5 mmol) and benzyloxyacetaldehyde (0.25 mmol) in 2.5 mL DMSO (25 vol%) 7.5 mL (75 vol%) MOPS buffer (50 mM, pH 7) that contains 0.15 mM ThDP and 2.5 mM $MgSO_4$ was transferred. The reaction was initiated with the addition of BAL (0.2 U) at 37 °C (120 rpm). Every 24 hours 0.2 U of BAL was added. The progress of the reaction was with TLC and concluded after 48-72 h. Then, the reaction mixture was extracted with diethylether (8 x 40 mL) and the combined organic layers were washed with brine and dried over $MgSO_4$, and the solvent was concentrated in vacuo to yield product which was further purified by using flash column chromatography.

3.8.1 Synthesis of (*R*)-3-(benzyloxy)-2-hydroxy-1-(3-methoxyphenyl) propan-1-one (23)



General procedure described above starting from 3-methoxy benzaldehyde and BnOAc, provided pure product obtained as yellowish oil (32% yield). TLC hexane/ethyl acetate = 2:1 R_f; 0.52

^{1}H NMR (400 MHz, *$CDCl_3$*) δ: 7.59-7.50 (m, 1H), 7.40-7.34 (m, 2H), 7.32-7.26 (m, 1H), 7.18-7.12 (m, 3H), 7.10-7.02 (m, 2H), 5.18 (t, *J* =4.1 Hz, 1H), 4.49 (d, *J* = 12.3 Hz, 1H),), 4.42 (d, *J* = 12.3 Hz, 1H), 3.90 (br s, 1H, OH), 3.78 (s, 3H), 3.74 (dd, *J* = 10.3, 3.2 Hz, 1H), 3.68 (dd, *J* = 10.3, 4.4 Hz, 1H)

3.9 General procedure for thiamine-peptide conjugate synthesis [40]

Five different methods were performed in order to get thiamine-peptide conjugates. Afterwards, these would be used as catalyst in benzoin condensation process.

1st method [40]:

A solution of thiamine hydrochloride (253 mg, 0.75 mmol) and Fmoc-*L*-Phe-OH (198 mg, 0.5 mmol and EDC (149 mg, 0.78 mmol) in the presence of DIEA (280 µL, 1.6 mmol) in 10 mL of acetonitrile and 5 mL H_2O were stirred at room temperature for 72 hours. The reaction was ended up 3 days later by monitoring with TLC (MeOH:DCM / 1:9) and extracted with chloroform (2 x 15 mL) organic phase evaporated. Then flash column chromatography was performed. However, no expected product was observed.

2nd method:

A solution of thiamine hydrochloride (253 mg, 0.75 mmol) and Fmoc-*L*-Phe-OH (198 mg, 0.5 mmol and EDC (149 mg, 0.78 mmol) in the presence of DIEA (279 µL, 1.6 mmol) in 10 mL of acetonitrile was stirred at room temperature for 48 hours. The reaction was ended up 2 days later by monitoring with TLC (MeOH:DCM / 1:9)

3rd method:

A solution of thiamine hydrochloride (253 mg, 0.5 mmol) and Fmoc-*L*-Phe-OH (198 mg, 0.5 mmol and EDC (149 mg, 0.78 mmol) in the presence of DIEA (280 µL, 1.6 mmol) in 2 mL of DMF and 1 mL H_2O was stirred at room temperature for 48 hours. The reaction was ended up 2 days later by monitoring with TLC (MeOH:DCM / 1:9).

4th method:

A solution of thiamine hydrochloride (253 mg, 0.5 mmol) and Fmoc-*L*-Phe-OH (198 mg, 0.5 mmol in the presence of DIEA (280 μL, 1.6 mmol) and HBTU (1.6 mL, 0.78 mmol) and HOBt(105.4 mg, 0.78 mmol) in 4 mL of DMF was stirred at room temperature for 48 hours. The reaction was ended up 2 days later by monitoring with TLC (MeOH:DCM / 1:9). The HPLC and LC-MS analysis were done and mass of the desired product was observed.

5th method:

A solution of thiamine hydrochloride (253 mg, 0.5 mmol) and Fmoc-*L*-Phe-OH (197 mg, 0.5 mmol and EDC (149mg, 0.78 mmol) in the presence of DIEA (280 μL, 1.6 mmol) in 10 mL of acetonitrile was stirred at room temperature for 48 hours. The reaction was ended up 2 days later by monitoring with TLC (MeOH:DCM / 1:9), monitored in preparative TLC (1:9 / MeOH:DCM).According to the results of NMR no expected product was observed.

These processes were performed by using different Fmoc-protected amino acids. These are

- Fmoc-Val-OH
- Fmoc-Pro-OH
- Fmoc-Ile-OH

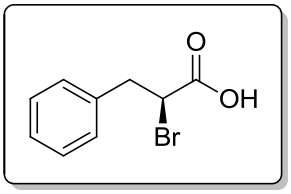
However, no expected product was observed.

3.10 General Procedure for the Synthesis of Asymmetric Thiazole Catalyst [41]

3.10.1 Bromination of amino acid

L-amino acid (12.1 mmol), KBr (4.9 g, 41.2 mmol), HBr (3.5 mL, 48% weight) in 10 mL of H_2O was cooled to -13 °C in an ice-salt water bath with Ar bubbling. $NaNO_2$ (1040 mg, 15 mmol) was added slowly and portion-wise for 45 minutes to avoid raging. The mixture was allowed to cool. The Ar bubbling is stopped and the reaction proceeds for 24 hours. TLC was monitored (1:4 / EtOAc:Hex). The solution was extracted (3 x 20 mL) with diethyl ether and water. After the collection of organic phase, it is dried with $MgSO_4$, which is removed by filtration. The solvent is evaporated to get crude product.

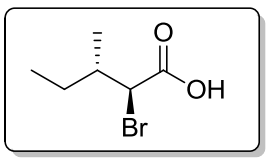
3.10.1.1 Synthesis of (*S*)-2-bromo-3-phenylpropanoic acid (40)



General procedure described above [41] starting from D-Phenylalanine provided crude product obtained. (95% yield). TLC hexane/ethyl acetate = 4:1 R_f; 0.49

^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.50 (br s, 1H, OH), 7.35-7.28 (m, 3H), 7.24-7.22 (m, 2H), 4.42 (t, *J*= 8.1 Hz, 1H), 3.47 (dd, *J* = 14.2, 8.2 Hz, 1H), 3.25 (dd, *J* = 14.2, 7.2 Hz, 1H).

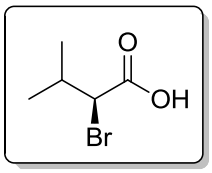
3.10.1.2 Synthesis of (2*S*,3*S*)-2-bromo-3-methylpentanoic acid (41)



General procedure described above [41] starting from L-isoleucine provided crude product obtained. (91% yield). TLC hexane/ethyl acetate = 4:1 R_f; 0.80

^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.80 (br s, 1H, OH), 4.13 (d, *J* = 8.0 Hz, 1H), 2.02-2.08 (m, 1H), 1.82-1.70 (m, *2*H), 1.05 (d, *J* = 6.3 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H)

3.10.1.5 Synthesis of (*S*)-2-bromo-3-methylbutanoic acid (42)



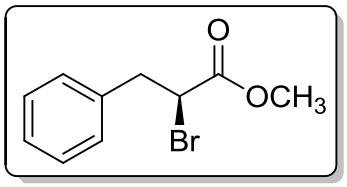
General procedure described above [41] starting from valine provided crude product obtained. (99% yield). TLC hexane/ethyl acetate = 2:1 R_f; 0.81

^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.1 (br s, 1H, OH), 4.19 (d, *J* = 7.6 Hz, 1H), 2.30-2.21 (m, 1H), 1.13 (d, *J* = 6.6 Hz, 3H), 1.09 (d, *J* = 6.7 Hz, 3H)

3.10.2 Esterification of α-bromoacids

The α-bromoacid (5.7 mmol) is treated with solution of acetyl chloride (2.4 mmol, 30 µL/mmol) in methanol (11.5 mL, 2 mL/mmol) for one hour. The solution was cooled to room temperature and concentrated *in vacuo.* Extraction is done with dichloromethane and water (3 x 15 mL). After collection of organic phase, it was dried with $MgSO_4$ which is removed by filtration. The solvent is evaporated under reduced pressure to get crude product. At last, flash column chromatography was performed.

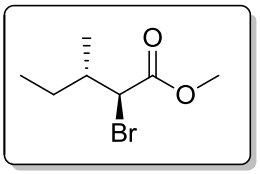
3.10.2.1 Synthesis of (*S*)-methyl 2-bromo-3-phenylpropanoate (43)



General procedure described above starting from (*S*)-2-bromo-3-phenylpropanoic acid provided pure product obtained with flash column chromatography with eluent system 4:1 / EtOAc:Hexane. (55% yield). TLC hexane/ethyl acetate = 4:1 R_f;0.70

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.26-7.14 (m, 3H), 7.13-7.05 (m, 2H), 4.33-4.28 (m, 1H), 3.60 (s, 3H), 3.40-3.30 (m, 1H), 3.18-3.06 (m, 1H)

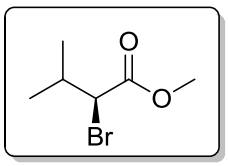
3.10.2.2 Synthesis of (2*S*, 3*S*)-methyl 2-bromo-3-methylpentanoate (44)



General procedure described above starting from (2*S*, 3*S*)-2-bromo-3-methylpentanoic acid provided pure product obtained with flash column chromatography with eluent system 4:1 / EtOAc: Hexane. (57% yield). TLC hexane/ethyl acetate = 4:1 R_f; 0.86

^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.03 (d, *J* = 8.0 Hz, 1H), 3.68 (s, 3H), 2.01-1.89 (m, 1H), 1.73-1.60 (m, 2H), 0.92 (d, *J* = 6.8 Hz, 3H), 0.83 (t, *J* = 7.4 Hz, 3H)

3.10.2.3 Synthesis of (*S*)-methyl 2-bromo-3-methylbutanoate (45)

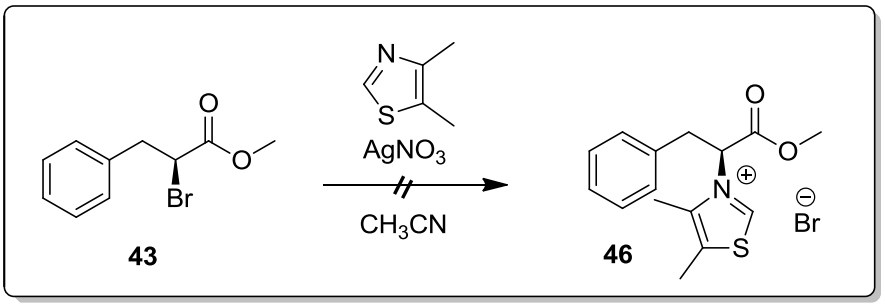


General procedure described above starting from (2*S*,3*S*)-2-bromo-3-methylpentanoic acid provided pure product obtained with flash column chromatography with eluent system 4:1 / EtOAc:Hexane. (45% yield). TLC hexane/ethyl acetate = 4:1 R_f; 0.72

^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.05 (d, *J* = 7.9 Hz, 1H), 3.79 (s, 3H), 2.29-2.18 (m, 1H), 1.10 (d, *J* = 6.7 Hz, 3H), 1.02 (d, *J* = 6.7 Hz, 3H)

3.11 Synthesis of (*S*)-3-(1-methoxy-1-oxo-3-phenylpropan-2-yl)-4,5-dimethylthiazol-3-ium bromide (46)

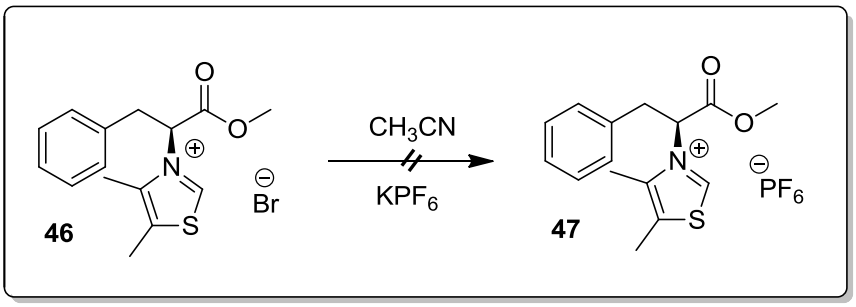
4,5-dimethyl thiazole (116 µL), $AgNO_3$ (85 mg) in 275 µL CH_3CN is added drop wise methyl 2-bromo-3phenylpropanoate (0.5 mmol, 100 mg) and stirred for 24 hours. Precipitate is filtrated and washed with CH_3CN. The solvent is evaporated under reduced pressure. However; based on ^{1}H NMR spectrum, no product was observed.



Scheme 17. Synthesis of (*S*)-3-(1-methoxy-1-oxo-3-phenylpropan-2-yl)-4,5-dimethylthiazol-3-ium bromide.

3.12 Ion exchange in methyl 2-bromo-3phenylpropanoate

Methyl 2-bromo-3-phenylpropanoate (**43**) (0.57 mmol, 159.0 mg) is dissolved in CH_3CN (400 µL), and KPF_6 (0.57 mmol, 105 mg) is added as solid. The reaction mixture is stirred for 24 hours. Precipitate is filtrated and washed with CH_3CN. Filtrate was extracted with EtOAc and water for 3 times. After the collection of organic phase, it is dried with Na_2SO_4 which is removed by filtration. The solvent is evaporated under reduced pressure. In the LC-MS spectrum, the product was observed but ^{1}H-NMR spectrum of that compound was inconclusive. Therefore, it is concluded that the product was not obtained.



Scheme 18. Ion Exchange in (*S*)-3-(1-methoxy-1-oxo-3-phenylpropan-2-yl)-4,5-dimethylthiazol-3-ium bromide.

3.13 HPLC conditions of chiral α-hydroxy ketones

3.13.1 (*R*)-3-(benzyloxy)-2-hydroxy-1-phenylpropan-1-one (25)

Enantiomerically enriched chiral (*R*)-3-(benzyloxy)-2-hydroxy-1-phenylpropan-1-one was obtained in 36% *ee*. The enantiomeric excess was verified by using chiral HPLC analysis (AD-H Column, hexane:*i*-PrOH / 90:10, flow rate 0.75 mL/min, λ= 254 nm) t_R= 19.49 min (minor enantiomer), t_R= 22.75 min (major enantiomer).

3.13.2 (*R*)-3-(benzyloxy)-2-hydroxy-1-(*p*-tolyl) propan-1-one (24)

Enantiomerically enriched chiral (*R*)-3-(benzyloxy)-2-hydroxy-1-(p-tolyl) propan-1-one was obtained in 62% *ee*. The enantiomeric excess was verified by using chiral HPLC analysis (AD-H Column, hexane:*i*-PrOH / 95:5, flow rate 0.75 mL/min, λ= 254 nm), t_R= 31.75 min (minor enantiomer), t_R= 33.46 min (major enantiomer).

3.13.3 (*R*)-3-(benzyloxy)-2-hydroxy-1-(4-methoxyphenyl) propan-1-one (26)

Enantiomerically enriched chiral (*R*)-3-(benzyloxy)-2-hydroxy-1-(4-methoxyphenyl) propan-1-one was obtained in 63% *ee*. The enantiomeric excess was verified by using chiral HPLC analysis (AD-H Column, hexane:*i*-PrOH / 90:10, flow rate 0.75 mL/min, λ= 254 nm), t_R= 33.87 min (minor enantiomer), t_R= 35.76 min (major enantiomer).

3.13.4 (*R*)-3-(benzyloxy)-2-hydroxy-1-(3-methoxyphenyl) propan-1-one (23)

Enantiomerically enriched chiral (*R*)-3-(benzyloxy)-1-(4-fluorophenyl)-2-hydroxypropan-1-one was obtained in 36% *ee*. The enantiomeric excess was verified by using chiral HPLC analysis (AD-H Column, hexane:*i*-PrOH / 90:10, flow rate 0.75 mL/min, λ= 254 nm), t_R= 21.36 min (minor enantiomer), t_R= 26.39 min (major enantiomer).

CHAPTER 4

CONCLUSION

In conclusion, the method described here presents a convenient one-enzyme-catalyzed, highly selective synthesis of (*S*)-Benzoin and (*R*)-2-HPP analogues. The reactions work in organic-aqueous medium, handle the solubility problem with organic substrates, and open the way for large-scale preparation. The products are obtained in moderate yields by using simple, easily available aromatic aldehydes, benzoins and benzyloxyacetaldehyde with moderate enantiomeric excesses.

The desired enzymatic trans-benzoin condensation products of benzyloxyacetaldehyde with benzoin, 4,4'-dimethyl benzoin, 3,3'-dimethoxy benzoin and 4,4'-dimethoxy benzoin was successfully synthesized and isolated with applicable yield and enantiomeric excesses. The trans-benzoin condensation products from 3,3'-difluoro benzoin, 3,3'-dibromo benzoin, 3,3-dimethyl benzoin and 4,4'-difluoro benzoin were synthesized, observed in NMR but couldn't be purified.

The importance of this study comes from that these results expand the aliphatic substrate range of BAL-catalyzed reactions.

Also the synthesis of asymmetric thiazole catalysts and thiamine amino acid conjugated have been tried to be used in benzoin condensation reactions as an alternative catalysts. However, their synthesis has been failed and could not be tried in this reaction.

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APPENDIX A

NMR DATA

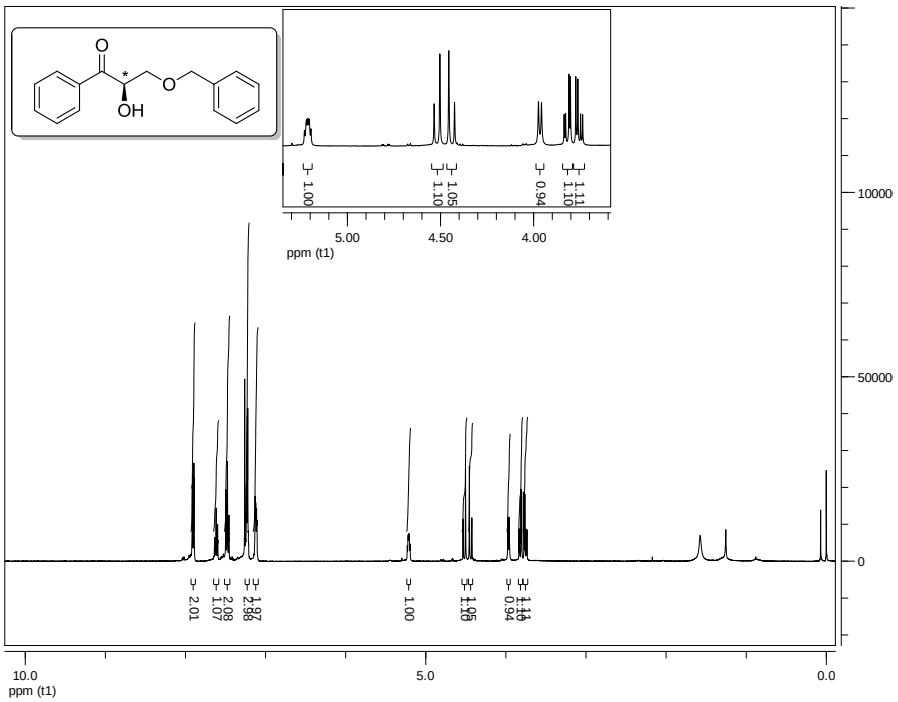

Figure A 1. ^{1}H NMR spectrum of (*R*)-3-(benzyloxy)-2-hydroxy-1-phenylpropan-1-one, **25**.

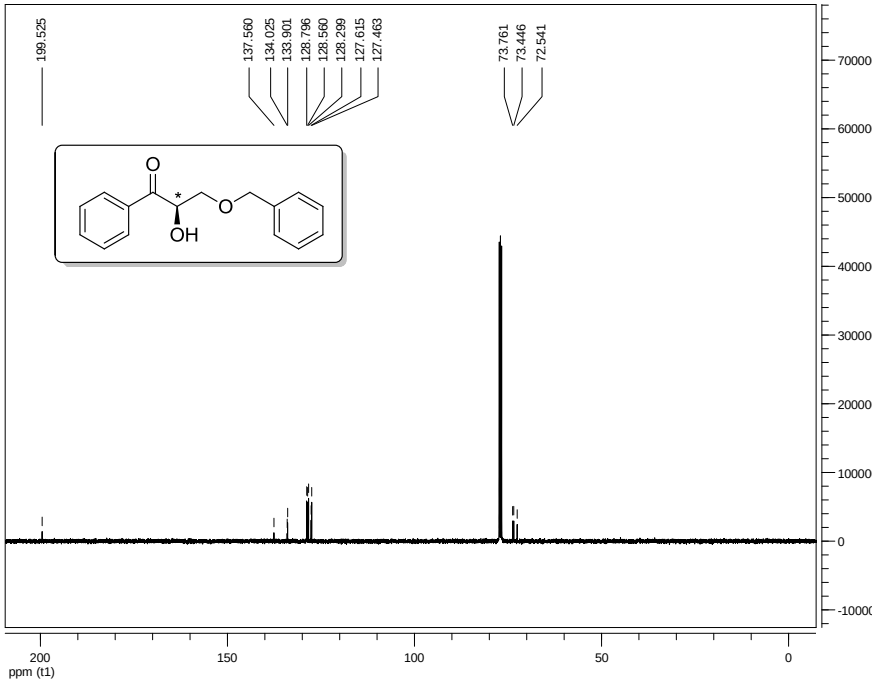

Figure A 2. ^{13}C NMR spectrum of (*R*)-3-(benzyloxy)-2-hydroxy-1-phenylpropan-1-one, **25**.

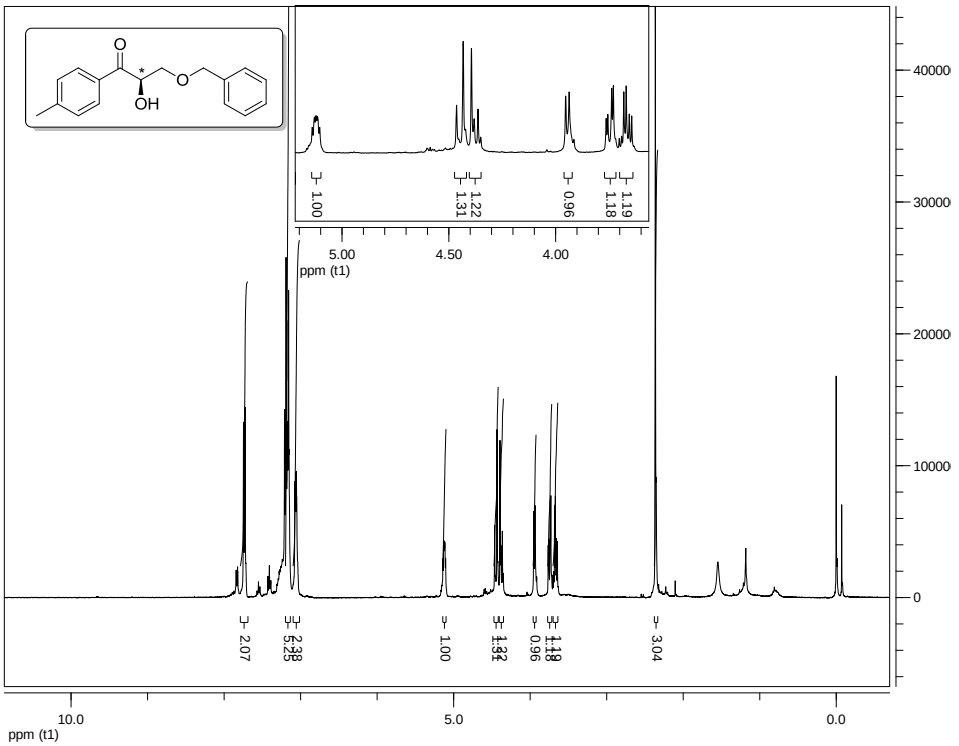

Figure A 3. ^{1}H NMR spectrum of (*R*)-3-(benzyloxy)-2-hydroxy-1-(*p*-tolyl) propan-1-one, **24**.

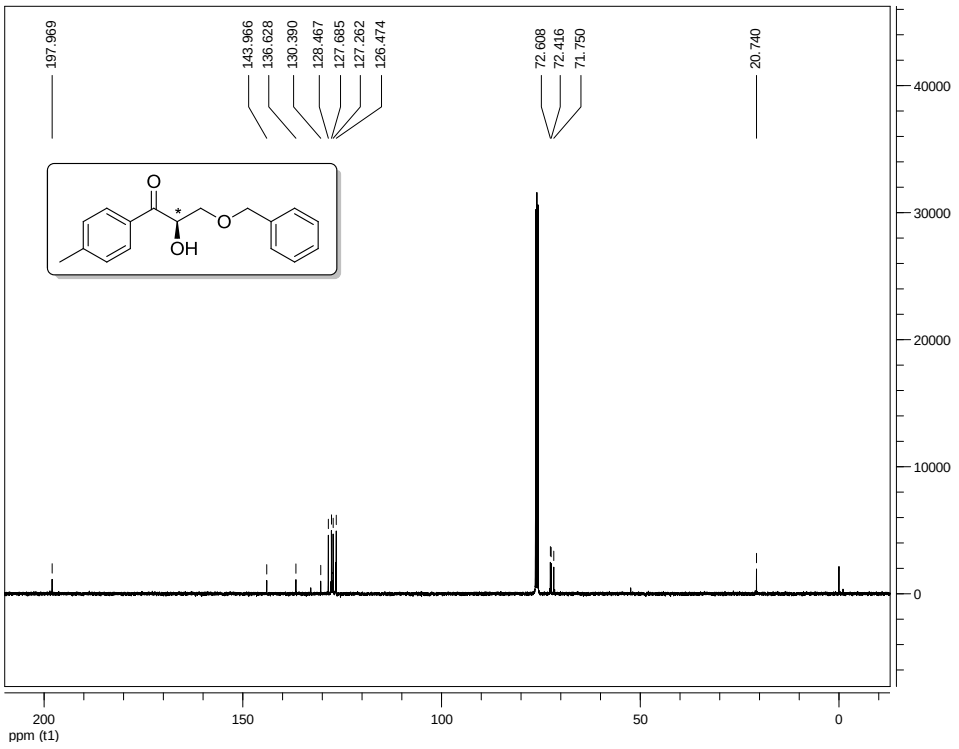

Figure A 4. ^{13}C NMR spectrum of (*R*)-3-(benzyloxy)-2-hydroxy-1-(p-tolyl) propan-1-one, **24**.

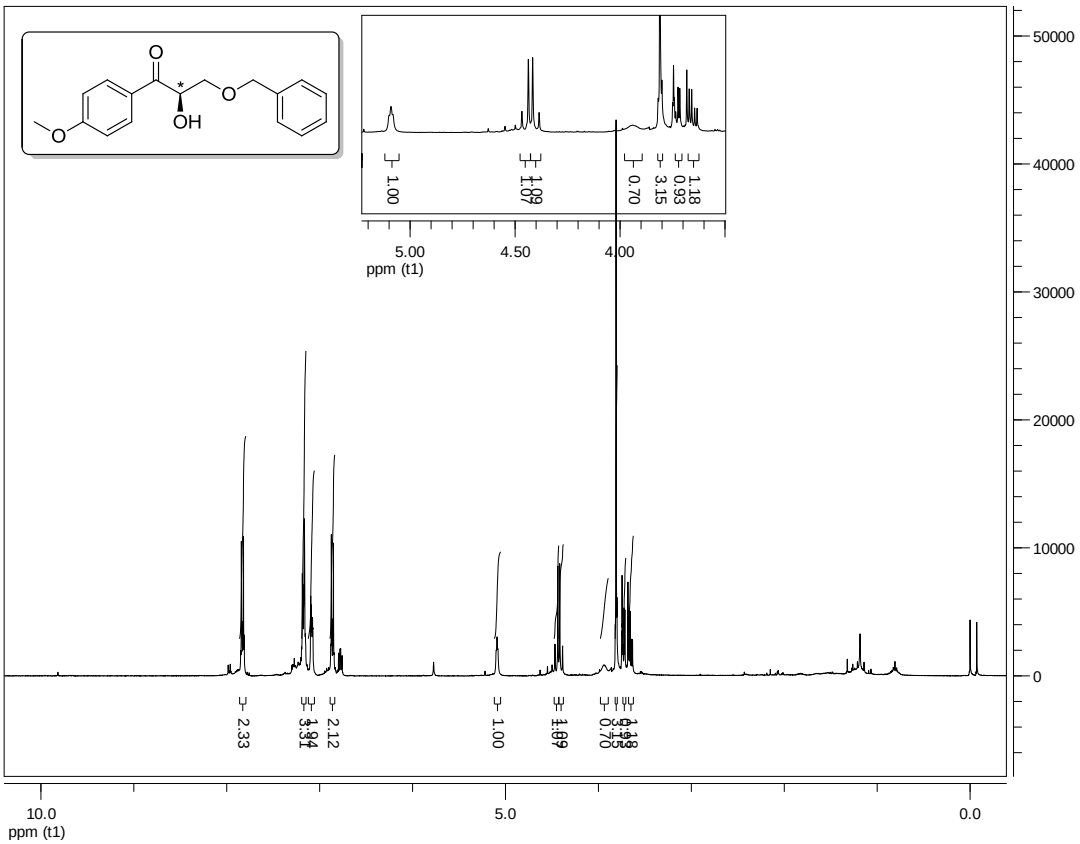

Figure A 5. ^{1}H NMR spectrum of (*R*)-3-(benzyloxy)-2-hydroxy-1-(4-methoxyphenyl) propan-1-one, **26**.

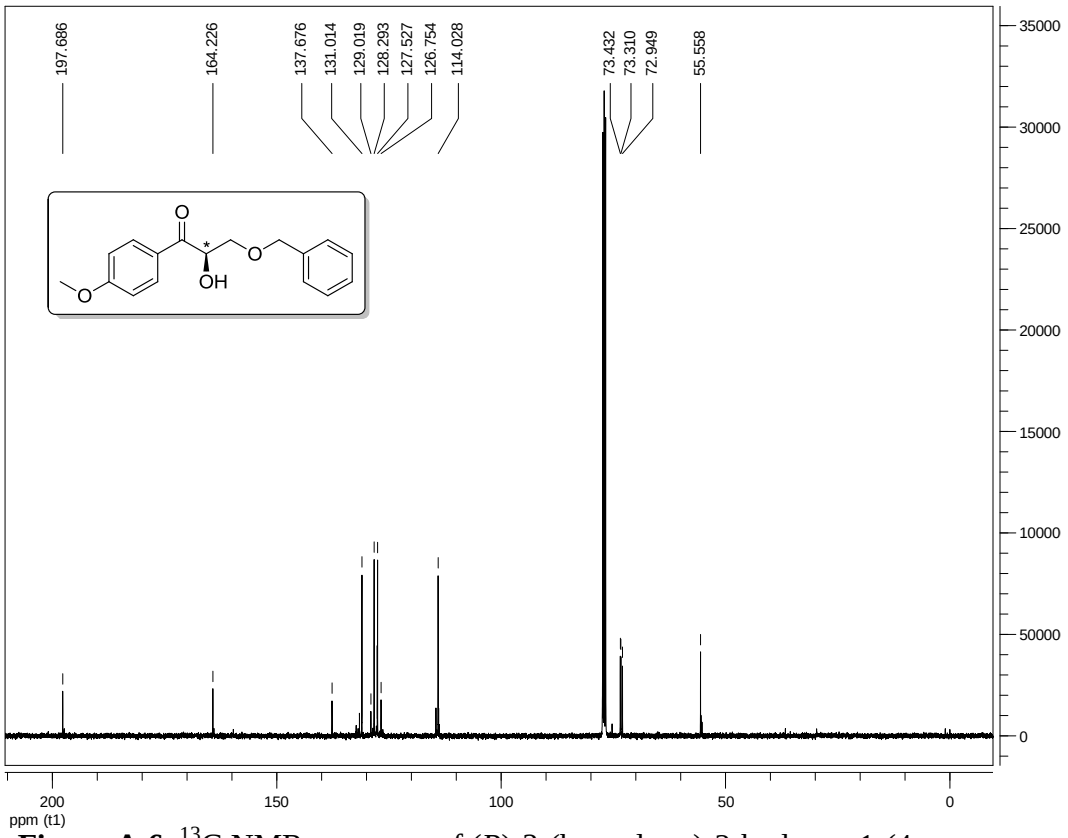

Figure A 6. ^{13}C NMR spectrum of (*R*)-3-(benzyloxy)-2-hydroxy-1-(4-methoxyphenyl) propan-1-one, **26**.

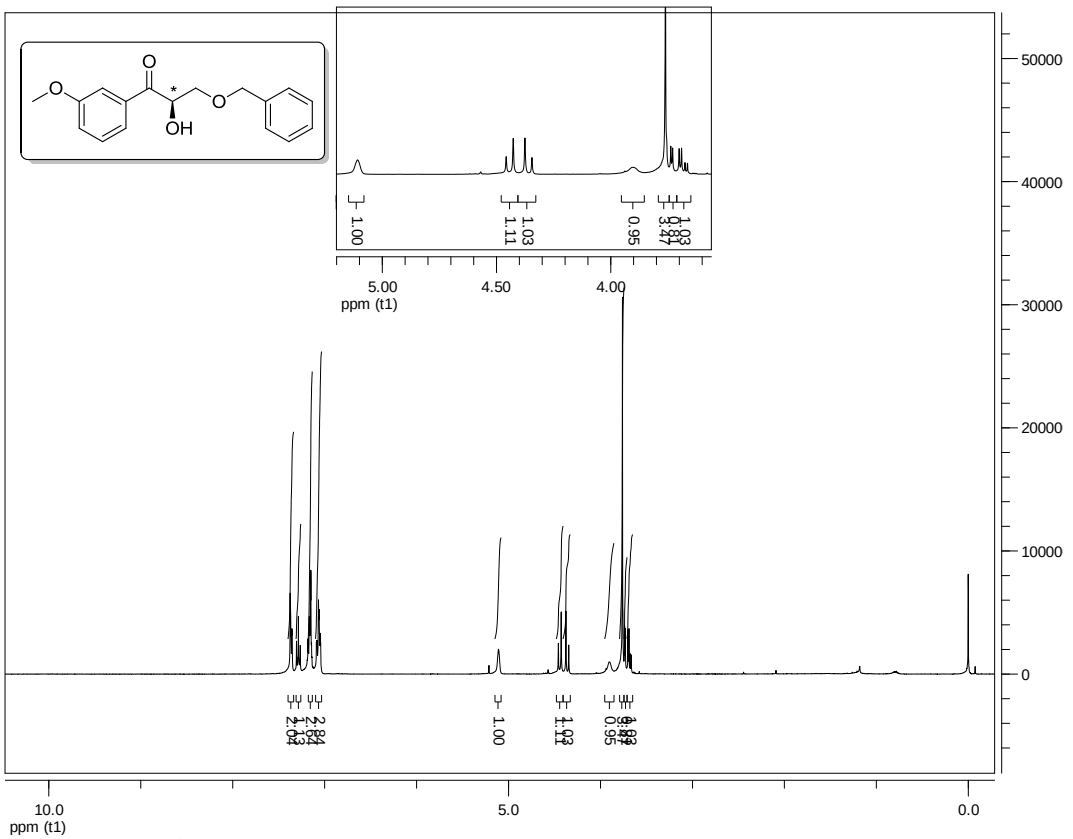

Figure A 7. ^{1}H NMR spectrum of (*R*)-3-(benzyloxy)-2-hydroxy-1-(3-methoxyphenyl) propan-1-one, **23**.

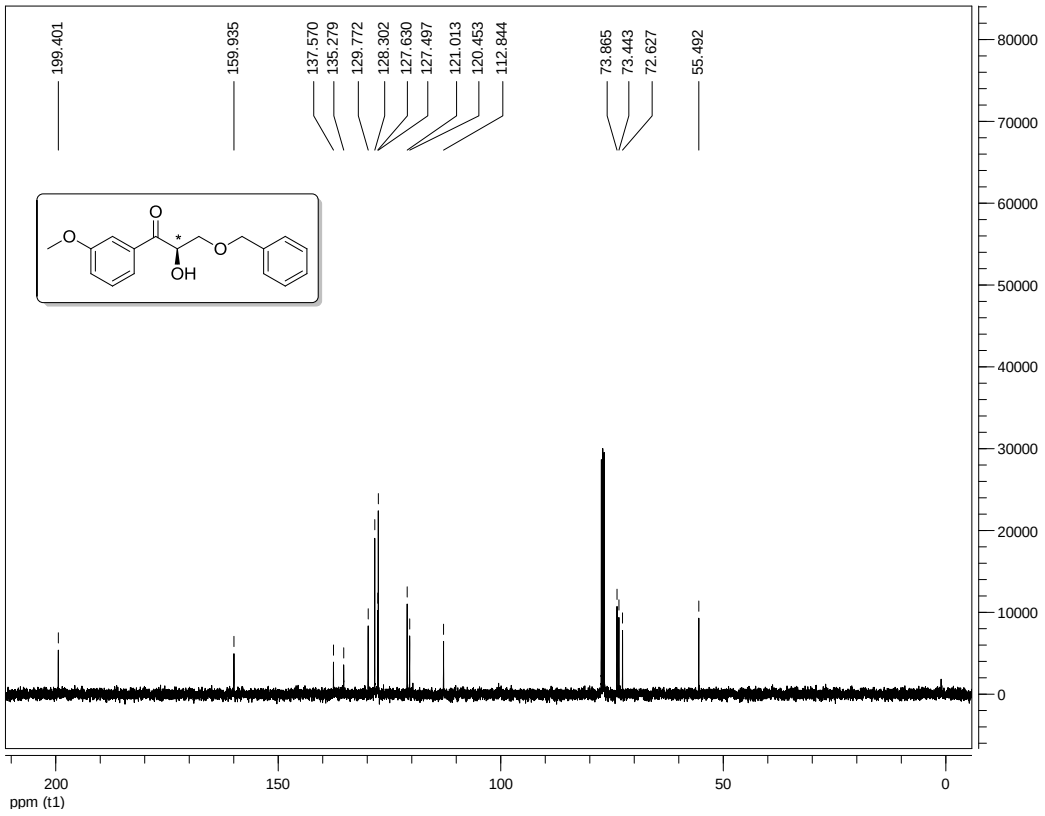

Figure A 8. ^{13}C NMR spectrum of (*R*)-3-(benzyloxy)-2-hydroxy-1-(3-methoxyphenyl) propan-1-one, **23**.

APPENDIX B

HPLC DATA

(a)

(b)

(c)

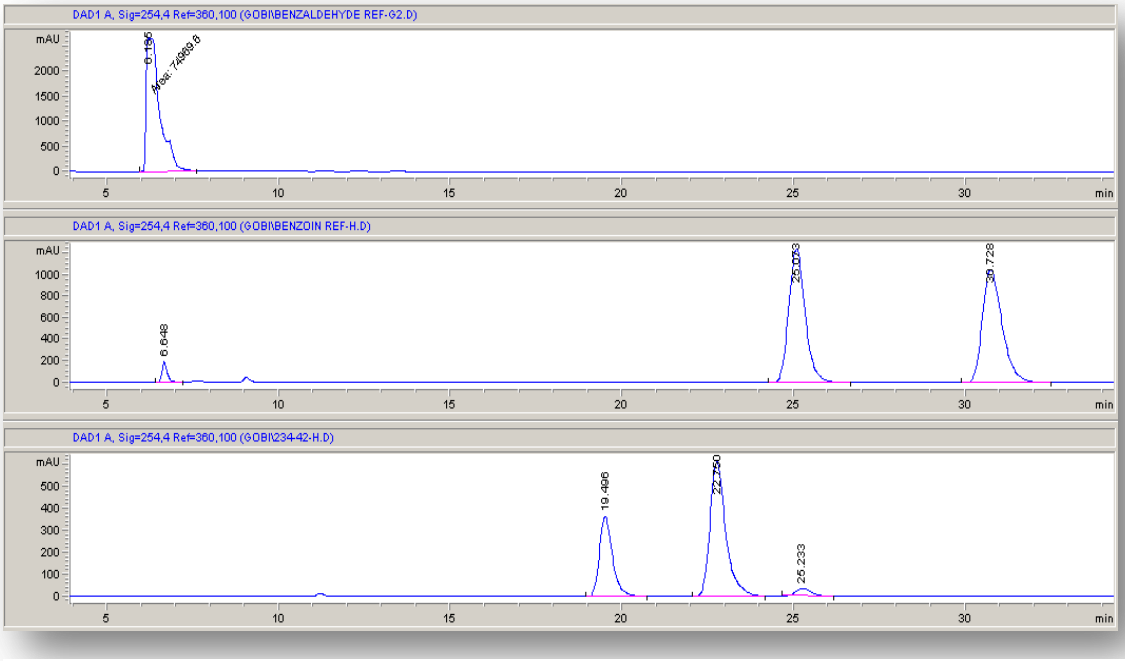

Figure B 1. HPLC chromatogram of **(a)** benzaldehyde **(b)** benzoin and **(c)** enzymatic trans-benzoin condensation product of benzaldehyde and benzyloxyacetaldehyde that was isolated, **25**.

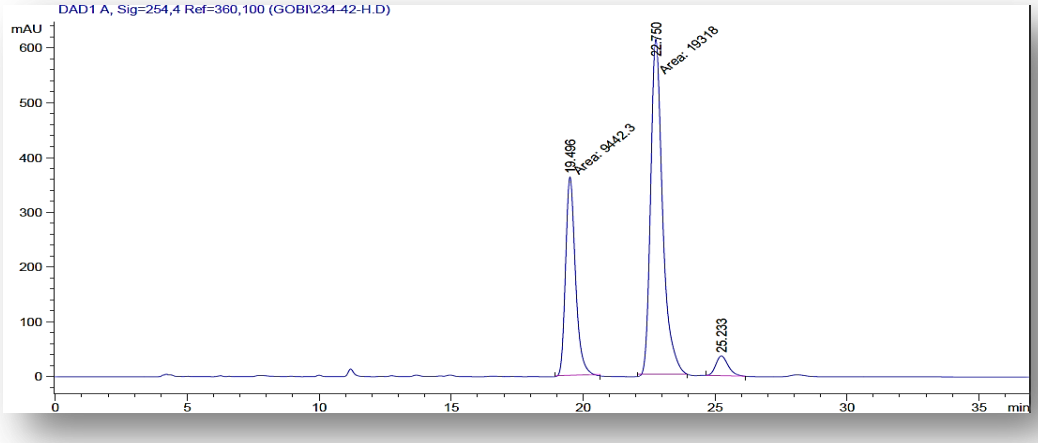

Figure B 2. HPLC chromatogram of (*R*)-3-(benzyloxy)-2-hydroxy-1-phenylpropan-1-one, **25**.

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.496	MM	0.4339	9442.30273	362.70013	31.5302
2	22.750	MM	0.5254	1.93180e4	612.82727	64.5075

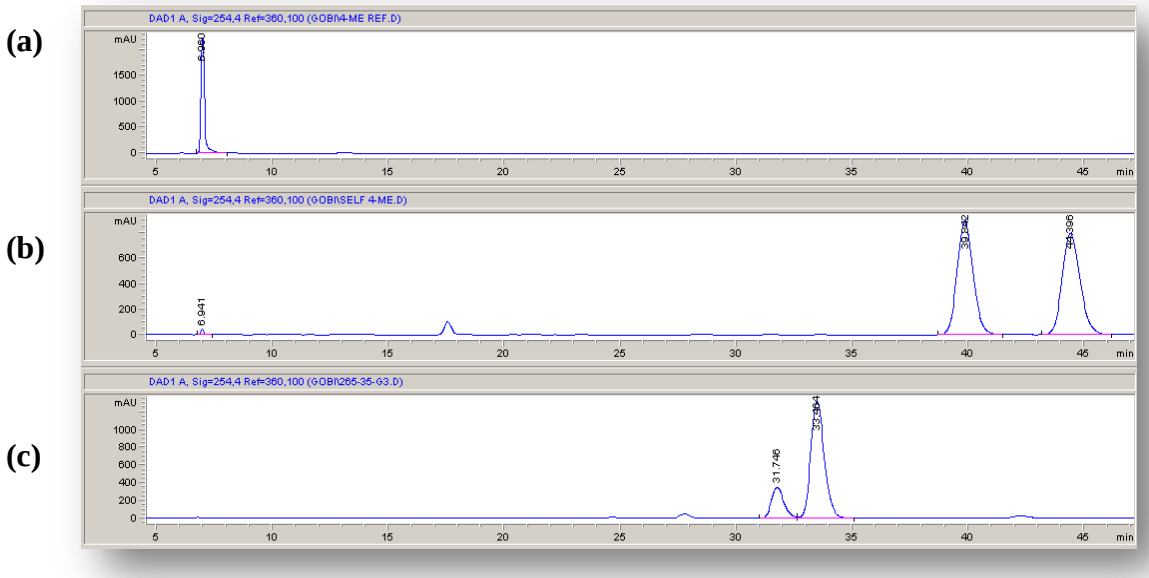

Figure B 3. HPLC chromatogram of **(a)** 4-Methyl benzaldehyde **(b)** 4,4'-dimethyl benzoin, **22** and **(c)** enzymatic trans-benzoin condensation product of 4,4'-dimethyl benzoin and benzyloxyacetaldehyde that was isolated, **24**.

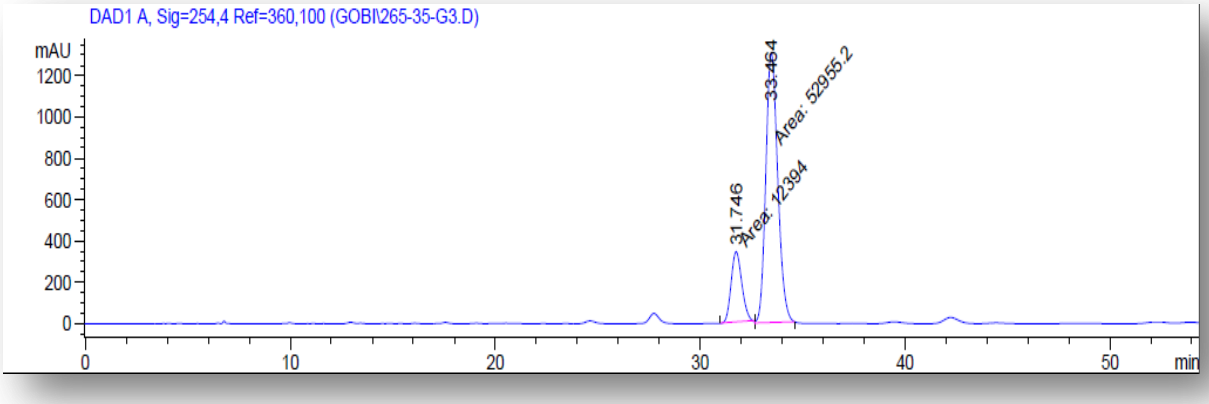

Figure B 4. HPLC chromatogram of 3-(benzyloxy)-2-hydroxy-1-(*p*-tolyl) propan-1-one, **24**.

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	31.746	MM	0.6051	1.23940e4	341.36044	18.9658
2	33.464	MM	0.6750	5.29552e4	1307.53540	81.0342

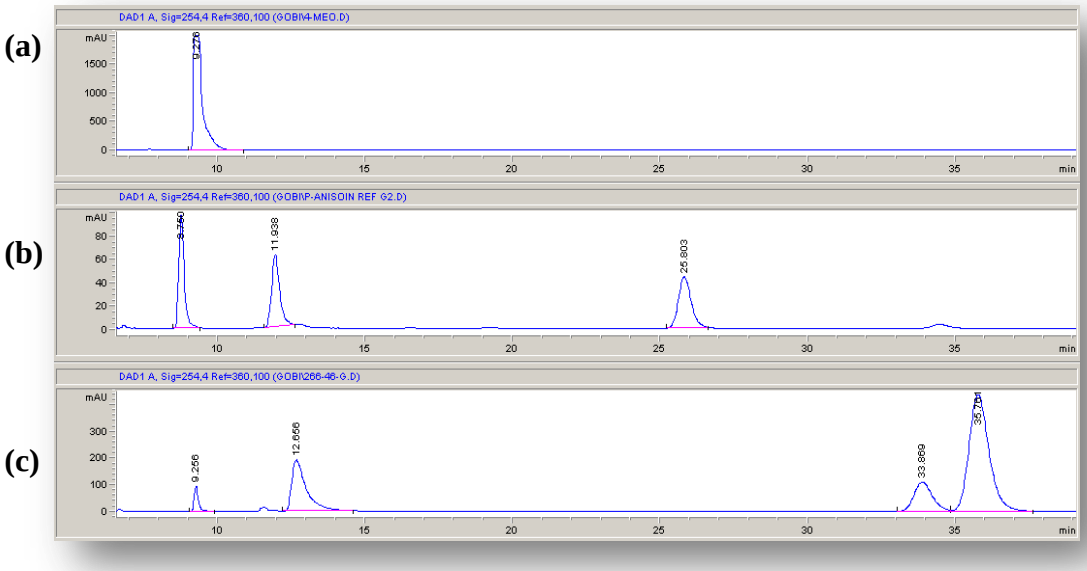

Figure B 5. HPLC chromatogram of **(a)** 4-Methoxy benzaldehyde **(b)** 4,4'-dimethoxy benzoin and **(c)** enzymatic trans-benzoin condensation product of 4,4'-dimethoxy benzoin and benzyloxyacetaldehyde that was isolated, **26**.

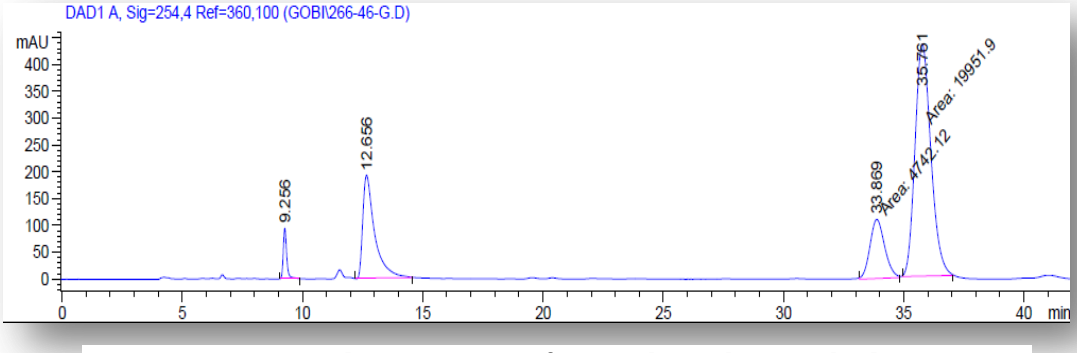

Figure B 6. HPLC chromatogram of (*R*)-3-(benzyloxy)-2-hydroxy-1-(4-methoxyphenyl) propan-1-one, **26**.

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.256	BB	0.1532	947.67426	94.33492	2.9343
2	12.656	BB	0.4991	6654.51953	192.60542	20.6047
3	33.869	MM	0.7135	4742.11816	110.77235	14.6832
4	35.761	MM	0.7674	1.99519e4	433.29465	61.7778

(a)

(b)

(c)

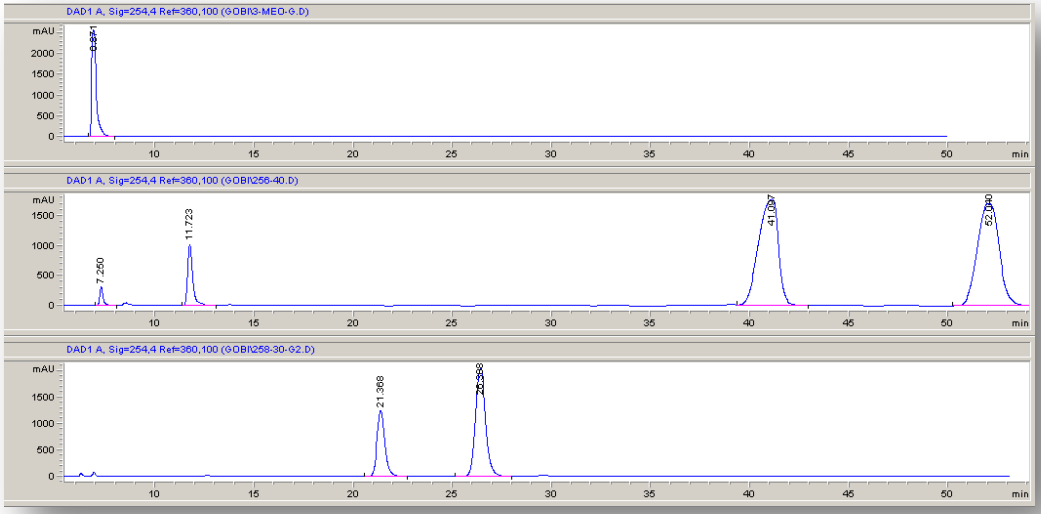

Figure B 7. HPLC chromatogram of **(a)** 3-Methoxy benzaldehyde **(b)** 3,3'-dimethoxy benzoin, **19** and **(c)** enzymatic trans-benzoin condensation product of 3-Methoxy benzaldehyde and benzyloxyacetaldehyde that was isolated, **23**.

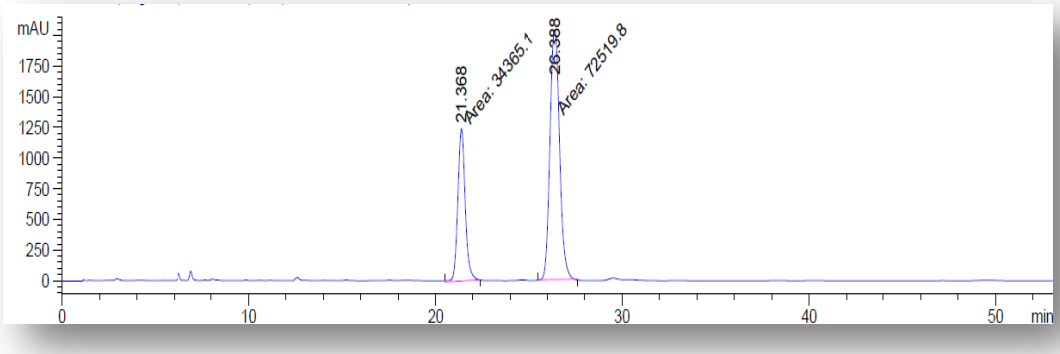

Figure B 8. HPLC chromatogram of (*R*)-3-(benzyloxy)-2-hydroxy-1-(3-methoxyphenyl) propan-1-one, **23**.

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	21.368	MM	0.4609	3.43651e4	1242.68433	32.1515
2	26.388	MM	0.5935	7.25198e4	2036.60022	67.8485

(a)

(b)

(c)

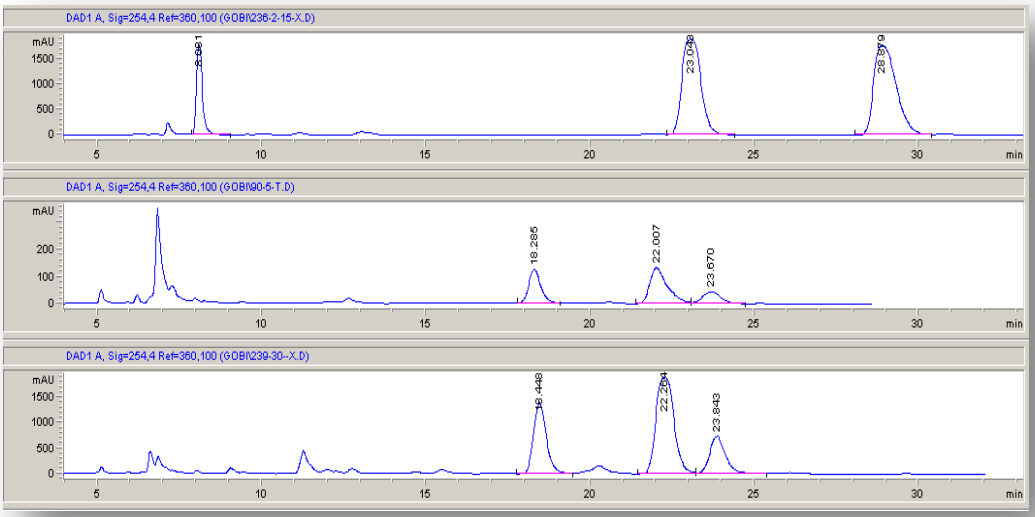

Figure B 9. HPLC chromatogram of **(a)** 3,3'-dibromo benzoin, **18** **(b)** Thiazolium salt catalysed racemic cross benzoin condensation product (crude), **38** and **(c)** enzymatic trans-benzoin condensation product of 3,3'-dibromo benzoin and benzyloxyacetaldehyde (crude), **27**.

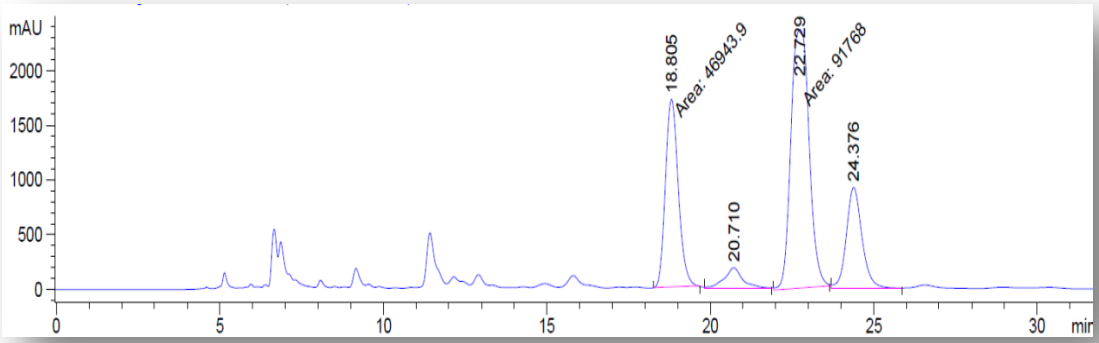

Figure B 10. HPLC chromatogram of (*R*)-3-(benzyloxy)-2-hydroxy-1-(3-bromophenyl) propan-1-one, **27**.

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	18.805	MM	0.4560	4.69439e4	1715.75085	26.2735
2	20.710	VV	0.5930	8420.26074	196.07637	4.7126
3	22.729	MM	0.6458	9.17680e4	2368.24609	51.3607
4	24.376	VB	0.5164	3.15416e4	928.41412	17.6532

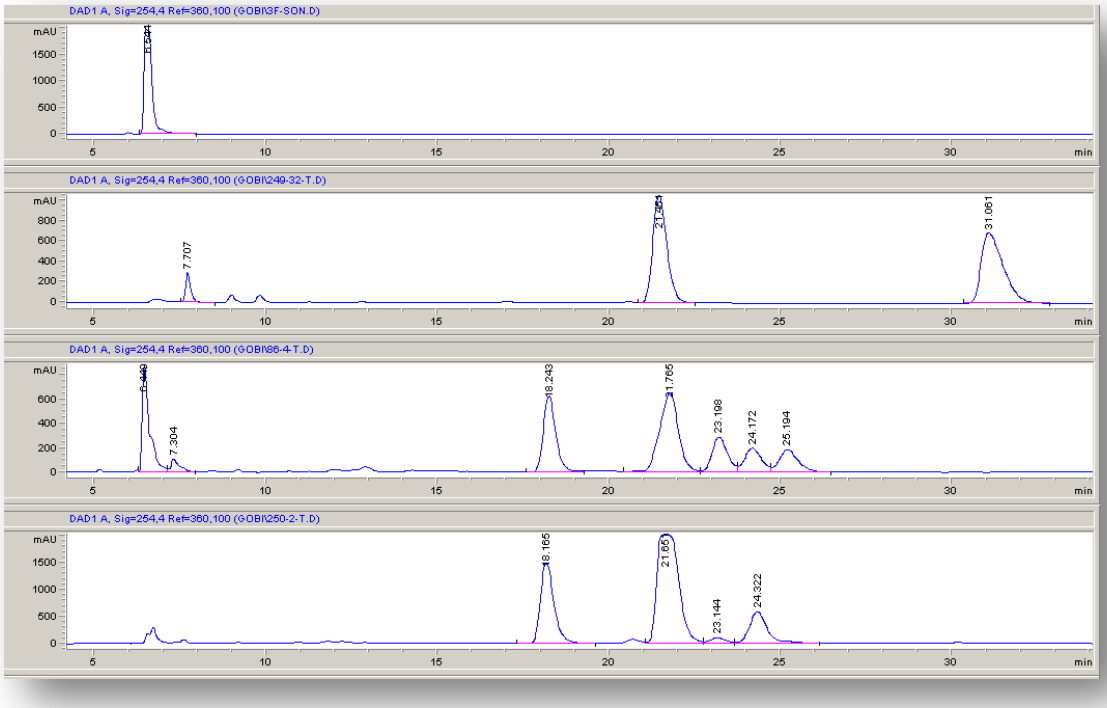

Figure B 11. HPLC chromatogram of (a) 3-fluoro benzaldehyde (b) 3,3'-difluoro benzoin, **17** (c) Thiazolium salt catalysed racemic cross benzoin condensation product (crude), **37** and (d) enzymatic trans-benzoin condensation product of 3,3'-difluoro benzoin and benzyloxyacetaldehyde (crude), **29**.

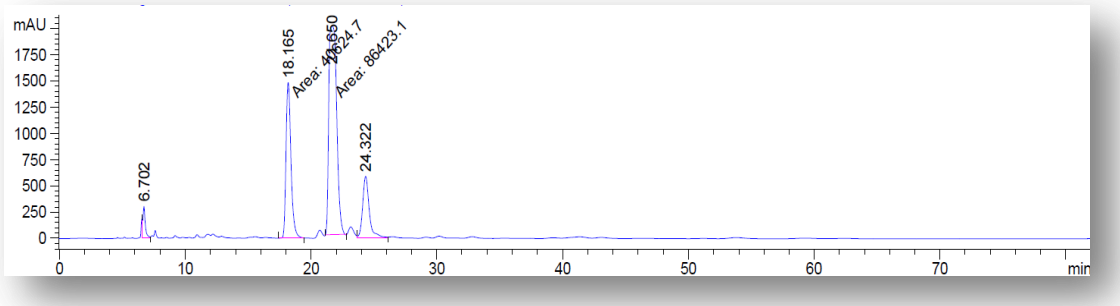

Figure B 12. HPLC chromatogram of crude 3-(benzyloxy)-1-(3-fluorophenyl)-2-hydroxypropan-1-one, **29**.

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	18.165	VB	0.4223	4.06802e4	1481.68689	25.6932
2	20.680	BV	0.4071	2052.81909	77.99625	1.2965
3	21.651	VV	0.5297	8.99186e4	2026.18958	56.7916
4	23.144	VV	0.4958	3587.38843	109.06020	2.2658
5	24.322	VB	0.5560	2.20917e4	591.16241	13.9529

(a)

(b)

(c)

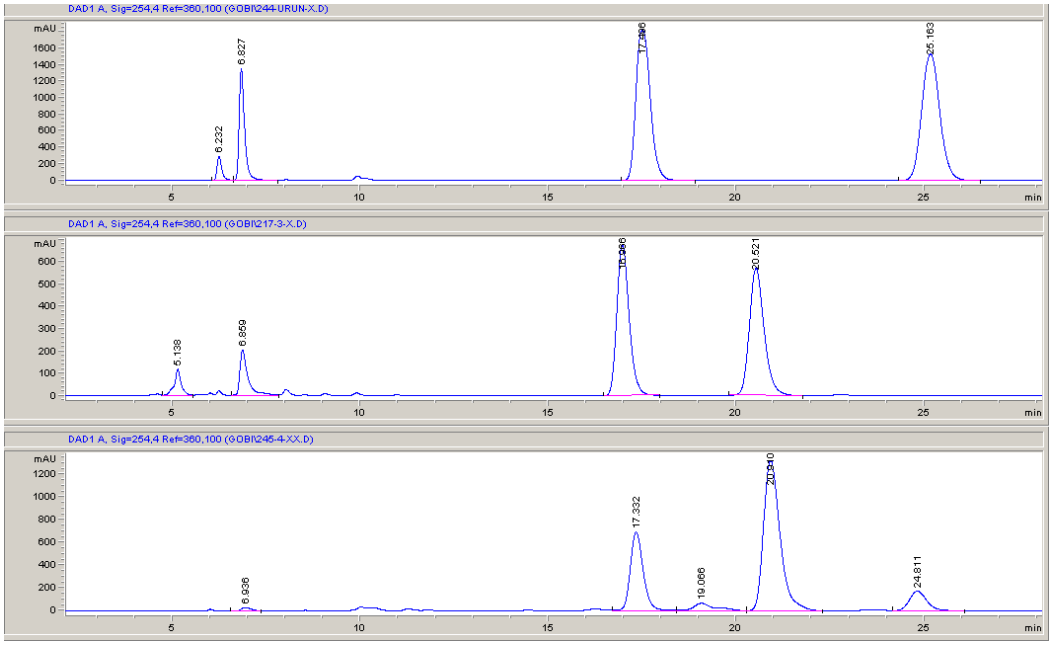

Figure B 13. HPLC chromatogram of **(a)** 3,3'-dimethyl benzoin, **21** **(b)** Thiazolium salt catalysed racemic cross benzoin condensation product (crude), **39** and **(c)** enzymatic trans-benzoin condensation product of 3,3'-dimethyl benzoin and benzyloxyacetaldehyde (crude), **28**.

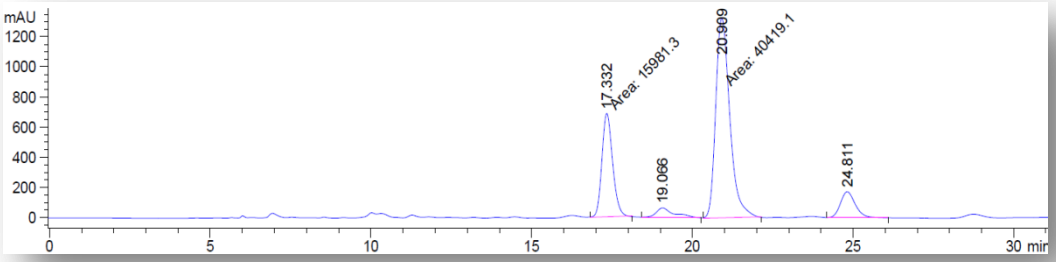

Figure B 14. HPLC chromatogram of crude 3-(benzyloxy)-1-(3-methylphenyl)-2-hydroxypropan-1-one, **28**.

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.332	VV	0.3700	1.68088e4	695.41553	25.4077
2	19.066	VV	0.5925	2890.04370	68.18220	4.3685
3	20.910	VV	0.4726	4.07462e4	1325.66089	61.5905