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Asymmetric Homogeneous Hydrogenations
Catalyzed by Transition Metal Complexes Containing
Chiral Diphosphine Ligands in a Protein Cavity

A
Thesis
forwarded
to
Department of Applied Biology and Chemical Technology
for
the Degree of Doctor of Philosophy
at
The Hong Kong Polytechnic University

by

Chi-Ching Lin

March, 1999
Declaration

I hereby declare that this thesis summarizes my own research work carried out since my registration for the degree of Doctor of Philosophy in November, 1995, and that it has not been previously included in a thesis, dissertation or report presented to this or any other institution for a degree, diploma, or other qualification.

Chi-Ching Lin

March, 1999
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Chi-Ching Lin

March, 1999
Abstract of thesis entitled “Asymmetric Homogeneous Hydrogenations Catalyzed by Transition Metal Complexes Containing Chiral Diphosphine Ligands in a Protein Cavity”

Submitted by Chi-Ching Lin

For the Degree of Doctor of Philosophy

at The Hong Kong Polytechnic University in March 1999

Catalytic enantioselective reactions have received much attention during the past decade and have played a crucial role in modern synthetic chemistry. Transition-metal catalysts containing various chiral ligands have been extensively studied for this purpose. In addition to the use of chiral metal complexes, one strategy used to design selective catalysts is to incorporate nonspecific achiral catalytic groups into chiral cavities. The general method is to modify a known protein or enzyme at a defined site with a cofactor or new functional group to create a semisynthetic system with novel properties. The significant advantage offered by this strategy is the obviation of the arduous synthesis of the chiral ligands. However, most of these systems are usually limited to those substrates which specifically bind to the native enzymes. Only few systems developed recently based on protein cavity can accommodate a variety of substrates. Since transition metal complexes catalyze a large variety of chemical reactions, the development of new catalysts by introducing a chiral catalytic functionality into the tertiary restricted environment of a protein cavity is of great interest. For the convenient test of this concept, we decided to use a carrier which binds strongly to specific sites of the target protein to introduce the catalyst to the protein cavity in a manner like a “guided missile”. If the combination is properly optimized, it may possess not only better
compatibility with different substrates but also a multiplicity of various catalytic reactions. Moreover, the protein-based catalysts may offer the possibility of easier catalyst recovery and reuse.

In this study, we examined the effect of the interaction of a chiral catalyst with a protein cavity by converting three kinds of diphosphine ligands including Pyrphos, PPM and 5-amino-BINAP to their corresponding biotinylated derivatives and attaching the biotinylated diphosphine Rhodium(I) complexes to the specific binding site of avidin. The catalytic hydrogenations of various olefinic substrates with this new type of catalysts were investigated. The details about the preparation of the rhodium(I) complexes with several biotinylated diphosphine ligands attached to the binding site in avidin, and their applications in aqueous asymmetric hydrogenations have been depicted in this thesis. By introducing the chiral diphosphine-rhodium(I) moiety into the constrained environment of the protein cavity it was found that the enantioselectivity of the system was mainly dominated by the tertiary conformation with the avidin cavity rather than by the coordinated chiral ligands. Besides, the reaction conditions such as temperature, hydrogen pressure, and the pH of the buffered solution were observed to have a significant influence on the resulting enantioselectivity. In summary, the binding of the biotinylated chiral metallic complexes to avidin and the subsequent influence of the avidin environment on the catalyst systems are established. All the results and discussion on this subject in this study gave positive albeit preliminary indications for the potential of the combination of chiral transition-metal complexes and protein cavities.
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ABBREVIATIONS

Pyrphos 3,4-bis(diphenylphosphino)pyrrolidine
BPPM  $N$-(tert-butoxycarbonyl)-4-(diphenylphosphino)-2-
       [diphenylphosphino]methyl]pyrrolidine
PPM  4-(diphenylphosphino)-2-[diphenylphosphino]methyl]-
     pyrrolidine
BINAPO 2,2'-bis(diphenylphosphiny)-1,1'-binaphthyl
BINAP  2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
BINAP-NH$_2$ 5-amino-2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
NHS  $N$-hydroxysuccinimide
COD  cyclooctadiene
cymene 4-isopropyltoluene
ee  enantiomeric excess
DMF  dimethylformamide
Chapter 1
Introduction

In response to an increasing demand for optically active compounds in the area of pharmaceuticals and advanced materials[1], a series of asymmetric syntheses for the preparation of different fine chemicals have received explosive interest during the past decades[2]. Especially noteworthy is the development of homogeneous catalytic asymmetric reactions[3] in which a small amount of chiral auxiliary can induce asymmetry (or occasionally dissymmetry) for a given reaction. The possible applications rely on the selectivity of homogeneous catalysts which are therefore of great interest since they provide simple methods to synthesize complex molecules, involving steps which require enantiocontrol of the transformation. Consequently, with more and more chiral ligands or optically active auxiliaries being developed[4], the past 25 years or so have seen the dramatic evolution of homogeneous catalytic asymmetric reactions.

Asymmetric homogeneous catalysis is indeed the most promising strategy to prepare the optically pure compounds in respect of efficiency and selectivity. Lots of effort in this field hence contributed to several methodologies of entirely new synthetic procedures with the ability to perform stereoselective transformations. The initial results gave low levels of acquired optical purities. Today, many of the reactions proceed with close to full stereochemical control. Thus, by using transition metals in conjunction with chiral ligands, or optically active auxiliaries as inducers of asymmetry, a seemingly unlimited variety of stereochemically specified molecular structures are accessible. The achievements in organometallic chemistry over the past two decades are
remarkable, and numerous examples are available showing truly outstanding performances with regard to stereo-, regio-, and chemoselectivities[4]. In addition to chiral organometallic catalysts, the application of enzymes as catalysts in various asymmetric synthesis has also achieved considerable progress[5]. In fact, the chiral tools for asymmetric synthesis developed so far can be sorted into two main categories. One is the natural catalysts, enzymes; the other includes the artificial catalysts such as chiral metallic complexes and synthetic procedures with optically active auxiliaries. The earlier applications of enzymes in synthetic chemistry are fairly restricted. However, changes in the technology for production or modification of enzymes and for their stabilization and manipulation now make these catalysts practical for wider use in large-scale synthetic organic chemistry. Some recent advances in developing new type enzyme or enzyme-like catalysts will be described in this chapter.

1.1 Enzymes as Catalysts in Synthetic Organic Chemistry

It is generally accepted that one of the remaining challenges in organic chemistry is to induce efficient asymmetric synthesis on a prochiral precursor, much as the enzyme does[6]. In fact, the concept of asymmetric catalysis must come from the first discovery of enzyme in living systems. Enzymes catalyze biochemical reactions stereospecifically. For this reason asymmetric syntheses are very common in nature and are often unidirectional. Consequently, most natural products are optically active as a result of having been constructed by the catalytic action of three-dimensional enzymes. In its simplest terms, the substrate “fits” into the active site of the enzyme in a precise geometric alignment. In early days, practical access to enantiomerically pure compounds
from prochiral precursors was considered possible only by using enzymes, cell cultures, or whole microorganisms. These methods are powerful when used to produce chiral substances, particularly those that occur in nature.

Over the past twenty years, there has been explosive growth in the number of chemical transformations that can be effected using biological systems such as intact microorganisms and isolated enzymes[6]. Enzymes can generally be regarded as well-defined chemical entities because they are structurally equivalent to highly complex polyamides constructed from amino acids. Microorganisms are not that simple. They are best characterized as functional assemblies of various enzymes and other constituents, such as cofactors, capable of cooperatively conducting numerous molecular manipulations in a catalytic cycle. However, the scope of such reactions is limited because many biological production systems exhibit single-handed, lock-and-key specificity. Major drawbacks to the usefulness of enzymes include their limited stability under authentic operating conditions, their propensity for being inhibited by the substrate and/or product, and an occasional requirement for regeneration of extremely expensive cofactors. In order to solve the problem and make enzymes more versatile in organic synthesis and biochemical technology, there has been a constant effort to mimic the catalytic action of enzymes. The key step is to design or modify an enzyme active site by the accessible chemical reactions. It is now possible by changing one or more amino acids on an enzyme to generate a new catalyst. This approach is called site-directed chemical mutation of proteins and enzymes.
1.2. Chemical Mutations and Semisynthetic Enzymes

The first controlled modification of an enzyme was reported independently in 1966 by Koshland's group and Bender's associates[8,9]. They used a series of chemical reactions to change the hydroxyl group of the active site serine residue of subtilisin, a bacterial serine protease, to a sulphhydryl group. This corresponds to the replacement of serine residue by a cysteine. However, the resultant thiosubtilisin was inactive against normal substrates. Since then, the reverse operation was also carried out. The replacement of the active cysteine residue of papain by a serine residue also resulted in an inactive enzyme[10].

Scheme 1.1. The first modification of an enzyme.

The exact reasons for the diminished catalytic activity of these semisynthetic enzymes relative to their natural counterparts are not known. A possible explanation is that in a given enzyme active site environment there is a
requirement that the acidic and basic catalytic groups have just the right
difference in their ionization constants in order to maximize the rates of proton
transfer required for the catalytic act to occur efficiently. In other words, while
the ionization constants of the sulphydryl of cysteine and the imidazole of
histidine may have just the right separation in the active site environment of
papain, this may not be the case in the environment of the active site of
thiosubtilisin.

These site-directed modifications have been useful for the elucidation of
enzyme mechanisms. However, the problem with such chemical modifications
of amino acid residues is that rather drastic conditions have to be used that one
would try to avoid. Also, they are often nonspecific. Alternative approaches
have thus been sought. Here some will be presented in the following.

As early as 1978, Whitesides elaborated an elegant approach to
semisynthetic enzymes by the use of a modified biotin molecule[11,12]. He
constructed an asymmetric hydrogenation catalyst based on embedding an
achiral diphosphine-rhodium(I) moiety at a specific site in a protein. In this
case, the protein tertiary structure provides the chirality required for
enantioselective hydrogenation.

The well-characterized protein avidin, composed of four identical subunits,
each of which binds biotin and many of its derivatives, was used. For this, a
hydroxysuccinimide substituted biotin was converted to a chelating diphosphine
and complexed with rhodium(I) by the following sequence:
Scheme 1.2. Preparation of biotinylated diphosphine-rhodium(I) complex (2).

In this procedure the diphosphine intermediate serves as the basis for the elaboration of a water-soluble rhodium-based homogeneous hydrogenation catalyst. The enantioselectivity of the catalyst was tested by the reduction of \( \alpha \)-acetamidoacrylic acid to N-acetylanaline. The presence of avidin resulted in a definite increase in activity of the catalyst and in the production of the \( S \)-enantiomer (natural amino acid) in 40% excess.
The presence of other enzymes such as lysozyme or carbonic anhydrase had no significant influence on enantioselectivity.

Hence, Whitesides showed that it is possible to carry out in aqueous solution homogeneous hydrogenation using a diphosphinerhodium(I) catalyst associated with a protein. In addition, the chirality of the protein is capable of inducing significant enantioselectivity in the reduction.

An alternative approach for the elucidation of enzyme mechanism is based on modification of the structure of amino acid functional groups that are present in the active site. One way to alter the specificity of the enzymes is to attach organic molecules or organometallic catalysts to their surface. These have produced some interesting changes in reactivity. A leader in this field was the late E. T. Kaiser. His approach is to design a new catalyst that relays the combination of an existing protein-binding site with a chemically reactive coenzyme analog, thus exploiting the binding specificity of the protein or enzyme but expressing the characteristic chemical reactivity of the new covalently attached cofactor[13]. This represents a significant step towards the production of enzyme-like molecules or semisynthetic enzymes by a process that is now called “chemical mutation”.

In this way, flavopapain was prepared by covalent modification of the thiol group of the active site Cys-25 of papain by a flavin derivative. The design was first based on the X-ray diffraction studies of covalent papain inhibitor complexes. By modeling the binding interactions, few flavin-papain complexes were prepared without destruction of the enzyme as a catalytic species (Scheme 1.3).
Scheme 1.3. The flavopapain FP-3 (9) was the first effective semisynthetic analog to be prepared.

An attractive feature of the chemical mutagenesis process is that the wealth of X-ray structural information available for relatively simple enzymes makes possible a considerable degree of flexibility in the choice of the natural system in which the new catalytic group is introduced. Until now, much of the effort has been on the conversion of readily available enzymes of moderate molecular weight that are hydrolytic catalysts into modified enzymes capable of catalyzing other important reactions such as oxidation-reduction, decarboxylation, and transamination.
1.3. Enzyme Models and Proteins as Chiral Cavities

1.3.1. Host–Guest Complexation Chemistry

Enzyme models are generally organic synthetic molecules that contain one or more features present in enzymatic systems. They are smaller and structural simpler than enzymes. Consequently, an enzyme model attempts to mimic some key parameter of enzyme function on a much simpler level. The most representative example is the development of "Host–Guest complexation chemistry", which was named by D. J. Cram.[14-16]

The discovery in 1967 by C. J. Pederson[17,18] that crown ethers have the unique ability to form stable complexes with metal ions and primary alkyl ammonium cations opened new horizons in organic chemistry[19-21]. It is easy to see an analogy between such complexes having a "cavity" to bind the ligand (L) and the active site of an enzyme that recognizes its specific substrate. The size of the macroring can be varied to allow the binding of ligands of different shapes.

\[ \text{A host–guest relationship involves a complementary stereoelectronic arrangement of binding site in host and guest. Therefore, any man-made synthetic host–guest complex must have binding sites (polar and dipolar) and steric barriers located to complement each other's structures. Scheme 1.4 represents some available synthetic host compounds.} \]
Scheme 1.4. Examples of the host molecules.

Cram and others have reported that chiral crown ether complexes have this remarkable property of binding selectively one antipode of amino acid derivatives. And after many trials in the molecular design of chiral crown ethers, a 1,1'-binaphthyl unit incorporated in a macroring by substitution in the 2,2'-positions proves to possess the desirable properties. The naphthalene-containing system, chosen for practical and strategic reasons, imparts rigidity and lipophilicity to conventional cyclic polyethers. The synthesis of such a host is presented in Scheme 1.5.
Scheme 1.5. Synthesis of a chiral host molecule.

This host is chiral and possesses a $C_2$ axis of symmetry, and the dihedral angle between the planes of the two naphthalene rings attached to one another can vary between $60^\circ$ to $120^\circ$. Including a binaphthyl system in a crown ether causes the macrocyclic ring to twist like a helix rather than being planar. Both the $(S,S)$- and $(R,R)$-configurations are optically active, they can be used to resolve racemic primary amine salts and amino esters.
1.3.2. *Cyclodextrins*

Besides these synthetic host compounds, some naturally occurring hosts such as micelles and cyclodextrins have also been extensively studied. As for the application of these chiral host compounds in asymmetric synthesis, one strategy that can be used to design selective reaction catalysts is to incorporate nonspecific achiral catalytic molecules into chiral cavities. A number of cavities ranging from cyclodextrins to spherands have been exploited for this purpose[22].

Being formed of D-glucose units, cyclodextrins are chiral and chiral induction on substrates have been observed with cyclodextrin reactions[23]. A recent application of this is the preparation by Breslow’s Team[24] of a covalently linked coenzyme-cyclodextrin “artificial enzyme”. It consists of a β-cyclodextrin-pyridoxamine that can selectively transaminate phenylpyruvic acid to phenylalanine with a 52% excess of the natural L-enantiomer (Scheme 1.6).
Scheme 1.6. The first artificial enzyme, 20, that combined a coenzyme with a cyclodextrin binding group. It selectively converts 19 to 21.

In this case, the coenzyme, pyridoxamine, was linked to the primary face of β-cyclodextrin through a sulfur atom, as a mimic of the coenzyme–coenzyme combination. The combination in this molecule of an enzyme-like binding site has led to good substrate specificity in transaminations and to some chiral induction. Enzymes that synthesize amino acids by transamination do so with stereoselectivity. Thus in transamination by an artificial enzyme, there has been much interest in learning how to direct the proton addition to a particular face of the developing amino acids. The above example[24] of such an enzyme
mimic afforded amino acids with moderate selectivity, because of the chirality of the cyclodextrin unit. Therefore, more selectivity is expected if the proton is delivered by a chirally mounted basic group, as in an enzyme.

In a study of such transamination with a chirally mounted base, but not involving cyclodextrins, it was found that optically active amino acids could be produced up to 98% selectivity[25]. However, less success has attended attempts to extend this to artificial enzymes based on cyclodextrins. A compound 23 carrying both a pyridoxamine and an ethylenediamine unit attached to β-cyclodextrin on neighboring primary methylene groups was prepared and studied for its ability to form amino acids from keto acids with up to 96% enantioselectivity (Scheme 1.7). Although quite good selectivity was reported[26], it has proven difficult to duplicate these findings. In some alternate approaches, optical induction has indeed been produced with related catalysts 24 but with only 75% ee achieved[27].

Scheme 1.7. Two transaminase mimics, 23 and 24, that produce amino acids with some optical selectivity.
There has been much effort devoted to a variety of other catalytic reactions such as benzoin condensation, oxidation-reduction, and hydrolysis catalyzed by the cyclodextrin-coenzyme systems during the past ten years[28]. Most of these studies are still based on the incorporation of different coenzymes into the parent cyclodextrins. Until 1995, Carofiglio and coworkers reported the enantioselective oxidation of thioanisole in water by hydrogen peroxide catalyzed by Mo(VI) in the presence of β-Cyclodextrin-based ligands[29]. This is the first catalytic system with metal ligands attached to cyclodextrins. They synthesized several β-CD-based ligands as shown in the following (Scheme 1.8):

\[ \text{Scheme 1.8. The synthesis of some β-CD-based ligands.} \]
The authors then applied these β-CD-based ligands to the oxidation of thioanisole by hydrogen peroxide in water in the presence of catalytic amount of Na₂MoO₄. Under these conditions an oxodiperoxomolybdenum complex, MoO(O₂)₃L, which is the real oxidant, was formed. The results of the asymmetric oxidation carried out in different conditions are summarized in Table 1.1.

**Table 1.1.** Asymmetric Oxidation of Thioanisole (0.55 mmol) with H₂O₂ (0.55 mmol) Catalyzed by Na₂MoO₄ (0.025 mmol) in the presence of Various β-CD-based ligands under Heterogeneous (HE), Inverse Phase Transfer (IPT), or Homogeneous (H) Reaction Conditions

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<th>entry</th>
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<th>mmol</th>
<th>reaction condition</th>
<th>T (°C)</th>
<th>yield (%)</th>
<th>% ee (config)</th>
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<td>20</td>
<td>98</td>
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<tr>
<td>11</td>
<td>26</td>
<td>0.025</td>
<td>HE</td>
<td>20</td>
<td>76</td>
<td>24 (R)</td>
</tr>
<tr>
<td>12</td>
<td>27</td>
<td>0.025</td>
<td>HE</td>
<td>20</td>
<td>89</td>
<td>20 (R)</td>
</tr>
<tr>
<td>13</td>
<td>28</td>
<td>0.025</td>
<td>HE</td>
<td>20</td>
<td>90</td>
<td>17 (R)</td>
</tr>
</tbody>
</table>
All the reactions proceeded smoothly at 20 °C, so that after ca. 2 h yields of phenyl methyl sulfoxide ranging from fair to good were obtained. Overoxidation to sulfone was not observed. The enantioselectivities were in the range 17–60 % (R enantiomer) depending on the nature of β-CD-based ligand and on the experimental conditions. In conclusion, the results obtained in this paper are of interest because they are among the first examples of supramolecular catalysis in oxidation reactions. This study also indicated that the supramolecular catalysis can provide good potential in variety of asymmetric reactions simply by modifying the attached ligands and the incorporated metal complexes.

In 1997, Manfred T. Reetz and Siegfried R. Waldvogel reported the first type of supramolecular rhodium(I) catalysts by using β-cyclodextrin-modified diphosphines as ligands[30]. These functions as supramolecular catalysts that lead to exceptionally high substrate selectivities when used in hydrogenations of olefins. Furthermore, they are extremely active and selective when employed in the hydroformylation of olefins in a two-phase system.

Several β-cyclodextrin-modified diphosphine compounds 32 (Scheme 1.9), 39–41 (Scheme 1.10) and 44 (Scheme 1.11) were prepared in a straightforward manner[31, 32]. These diphosphines were then complexed with bis(1,5-cyclooctadienyl)rhodium(I) tetrafluoroborate [Rh(cod)2]BF4, to afford the corresponding β-CD-modified rhodium catalysts.
Scheme 1.9. Synthesis of diphosphine 32 from tosylated β-CD.

**Scheme 1.11.** Synthesis of the β-CD-modified diphosphines 44.

To establish whether these β-CD-modified rhodium catalysts function as supramolecular catalysts, the alkenes 45 and 46, which possess similar substitution patterns at the double bonds, were subjected to competition experiments (Scheme 1.12). In these experiments, 1:1 mixtures of the two olefins were hydrogenated up to 10 % conversion in a solvent at room temperature in the presence of a catalyst. The ratio of the products 47 and 48, as determined by gas chromatography, was used as a measure of the substrate selectivity. The results are summarized in Table 1.2.

**Scheme 1.12.** Competitive hydrogenation of alkenes 45 and 46.
Table 1.2. Substrate selectivity in the hydrogenation (1 atm H₂) of 45/46 in DMF at 22 °C.

<table>
<thead>
<tr>
<th>Catalyst [a]</th>
<th>47 / 48</th>
</tr>
</thead>
<tbody>
<tr>
<td>[PhN(CH₃PPh₂)₂Rh(cod)]BF₄</td>
<td>50 / 50</td>
</tr>
<tr>
<td>32 / [Rh(cod)]BF₄</td>
<td>68 / 32</td>
</tr>
<tr>
<td>39 / [Rh(cod)]BF₄</td>
<td>74 / 26</td>
</tr>
<tr>
<td>40 / [Rh(cod)]BF₄</td>
<td>71 / 29</td>
</tr>
<tr>
<td>41 / [Rh(cod)]BF₄</td>
<td>66 / 34</td>
</tr>
<tr>
<td>32 / [Rh(cod)]BF₄ [b]</td>
<td>82 / 18</td>
</tr>
<tr>
<td>39 / [Rh(cod)]BF₄ [b]</td>
<td>81 / 19</td>
</tr>
<tr>
<td>44 / [Rh(cod)]BF₄ [b]</td>
<td>87 / 13</td>
</tr>
</tbody>
</table>

[a] 0.5 mol% catalyst. [b] Two-phase system; aqueous phase contains 30% DMF.

According to Table 1.2, no substrate selectivity was achieved in the control experiment with the β-CD-free catalyst [PhN(CH₃PPh₂)₂/Rh(cod)]BF₄ (product ratio 50:50). In contrast, the use of the β-CD-modified rhodium catalyst led to a substantial substrate selectivity: The phenyl-substituted alkene 45 was preferentially converted to form alkane 47. The length of the spacer between the β-CD and the diphosphine group plays a crucial role in these reactions. The author postulated that a recognition step precedes the hydrogenation and furthermore that the phenyl group of 45 preferentially enters the hydrophobic cavity of the β-CD framework[33].

Since the hydroformylation of olefins[34] in the two-phase system H₂O/olefin continues to be a challenge[35,36], it appeared attractive to test the rhodium complexes of the β-CD-modified ligands 32 and 39–41 in this reaction as well. In all cases, unexpectedly high catalyst activities were observed. For instance, in the hydroformylation of 1-octene (49, Scheme 1.13) in the two-phase system H₂O (30% DMF)/olefin with 0.03 mol% of the catalyst...
39/[Rh(cod)]BF₄ at 60 ºC and 100 bar, quantitative conversion was achieved within 18 h with a turnover number (TON) of 3172. The most remarkable feature of the hydroformylation is the chemoselectivities obtained were over 99% in all cases. The results about the activities and regioselectivities of the hydroformylation are listed in Table 1.3.

![Chemical structure](image)

**Scheme 1.13.** Hydroformylation of 1-octene (49).

**Table 1.3.** The regioselectivities and turnover number (TON) of the hydroformylation of 49 in the two-phase system[a].

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>50/51</th>
<th>TON</th>
</tr>
</thead>
<tbody>
<tr>
<td>[PhN(CH₂PPh₂)₂Rh(cod)]BF₄[b]</td>
<td>62 / 38</td>
<td>-</td>
</tr>
<tr>
<td>32 /[Rh(cod)]BF₄</td>
<td>75 / 25</td>
<td>3179</td>
</tr>
<tr>
<td>39 /[Rh(cod)]BF₄[c]</td>
<td>76 / 24</td>
<td>3172</td>
</tr>
<tr>
<td>40 /[Rh(cod)]BF₄</td>
<td>69 / 31</td>
<td>3170</td>
</tr>
<tr>
<td>41 /[Rh(cod)]BF₄</td>
<td>67 / 33</td>
<td>3170</td>
</tr>
</tbody>
</table>

[a] The hydroformylations were carried out in the two-phase system H₂O (30% DMF)/olefin with 0.03 mol% of the catalyst at 60 ºC and 100 bar for 18 h. [b] One-phase system. [c] Same reaction conditions as [a] except at 80 ºC.

The importance of the cyclodextrin substituent in this system is demonstrated by the unexpectedly enhanced regioselectivity from Table 1.3. A possible explanation for this enhanced selectivity is the participation of the host–guest complex, which plays an important role in the phase-transfer...
catalysis (Scheme 1.14). If the water-soluble catalyst is in the region of the phase boundary, the olefin molecules can easily enter the hydrophobic cavity of the $\beta$-cyclodextrin. Since the catalytically active metal center is fixed nearby in space, a rapid entropy-favored reaction takes place. It was found that, based on the experimental results, $\beta$-CD-modified phosphines are amphiphilic ligands that are capable of stabilizing catalytically active rhodium as well as acting as a carrier between the two nonmiscible phases.

\[ \text{organic phase} \]

\[ \text{aqueous phase} \]

**Scheme 1.14.** Mode of action of the host–guest complexes in the phase-transfer and Rh catalysis.

1.3.3. Proteins as Chiral Cavities

Enzymes perform chemical reactions with high specificity and rate enhancement in aqueous media at low temperatures and neutral pH. These features have made these catalysts attractive for a variety of purposes in
medicine and industry. However, the use of naturally occurring enzymes is restricted by their inherent specificity. To circumvent this limitation, the development of artificial enzymes has received considerable attention. One approach for the design of new enzymes is to modify a known protein or enzymes at a defined site with a cofactor or new functional group to create a semisynthetic system with novel properties[37–46]. A number of this constructs have been prepared resulting in a new generation of “enzyme-like” catalysts[47]. However, since most of these systems take advantage of a substrate binding site present in the starting enzyme, their specificities are often limited to structures that bind to that native enzyme. This is a significant impediment for the development of catalysts with new specificities.

The new catalytic systems based on the use of protein cavities that can accommodate a variety of substrates have been developed recently by Distefano and coworkers[48]. They firstly employed adipocyte lipid binding protein (ALBP), which is a small 131 residue protein with a simple architecture that consists of two orthogonal planes of β-sheet secondary structure, as the basic cavity for introducing a pyridoxamine cofactor to perform the selective reductive amination reactions[48a]. The synthesis of an ALBP conjugate (ALBP-PX) containing a pyridoxamine cofactor attached to a thiol within the protein interior is illustrated in Scheme 1.15. The ALBP-PX was found to be capable of reductively aminating a number of alkyl, aryl, and side chain functionalized α-keto acids to α-amino acids with enantioselectivities ranged from 42 to 94 % ee (Table 1.4).
Scheme 1.15. Preparation of ALBP-PX.

Table 1.4. Conversion and enantioselectivities of ALBP-PX reductive amination reactions.

<table>
<thead>
<tr>
<th>product</th>
<th>% conversion[a]</th>
<th>major enantiomer</th>
<th>% ee</th>
</tr>
</thead>
<tbody>
<tr>
<td>alanine (56a)</td>
<td>18 ± 3.6</td>
<td>D</td>
<td>42 ± 5.1</td>
</tr>
<tr>
<td>valine (56b)</td>
<td>28 ± 1.9</td>
<td>L</td>
<td>94 ± 0.2</td>
</tr>
<tr>
<td>leucine (56c)</td>
<td>39 ± 1.2</td>
<td>L</td>
<td>54 ± 0.8</td>
</tr>
<tr>
<td>norvaline (56d)</td>
<td>21 ± 0.6</td>
<td>[b]</td>
<td>[b]</td>
</tr>
<tr>
<td>aminocaprylate</td>
<td>13 ± 1.3</td>
<td>[b]</td>
<td>[b]</td>
</tr>
<tr>
<td>tyrosine (56f)</td>
<td>42 ± 1.5</td>
<td>L</td>
<td>67 ± 7.3</td>
</tr>
<tr>
<td>glutamate (56g)</td>
<td>46 ± 5.6</td>
<td>L</td>
<td>84 ± 2.2</td>
</tr>
</tbody>
</table>

[a] Conversion after 24 h. [b] No ee observed.

From Table 1.4, it can be observed that greater selectivity was obtained in reactions using a bulkier substrate, α-keto isovalerate (54b), which produced
valine (56b), whereas leucine (56c) with its γ-methyl branch is generated in much lower ee. Unbranched keto acid substrates 54d (C5) and 54e (C8) gave comparable levels of conversion to their respective amino acid products, 56d and 56e, but without any selectivity. The ALBP-PX system appears to be quite general; a number of alkyl and aryl α-keto acids as well as side chain functionalized α-keto acids are accepted. This is contrast to results obtained with cyclodextrin based systems where only large substrates bind in the cavity[24,25].

In addition to pyridoxamine cofactor, the ligand 1,10-phenanthroline has also been attached to ALBP cavity to perform the enantioselective hydrolysis of ester and amide substrates by Distefano et al [48b]. The same ligand has earlier been attached to a number of proteins to perform oxidative chemistry by other groups[49]. Distefano et al have attached a phenanthroline ligand to a unique cysteine residue present in the interior of ALBP in order to position a Cu(II) ion within the protein cavity (Scheme 1.16). The resulting semisynthetic metalloprotein, ALBP-Phen-Cu(II) catalyzes the enantioselective hydrolysis of several inactivated amino acid esters; these include methyl, ethyl, and isopropyl esters of the amino acids alanine, tyrosine, and serine. Data for these experiments are tabulated in Table 1.5.

Scheme 1.16. Preparation of ALBP-Phen (58).
Table 1.5. Enantioselectivities and turnovers for the hydrolysis of amino acid esters catalyzed by ALBP-Phen-Cu(II).

\[
\begin{align*}
\text{R-} & \quad \text{N} & \quad \text{O} & \quad \text{O} & \quad \text{H}_2\text{O} & \quad \text{pH 6.1} & \quad 25 \degree\text{C} & \quad \text{ALBP-Phen-Cu(II)} \\
\text{R-NH}_2 & \quad \text{O} & \quad \text{R}' & \quad \text{H}_2\text{O} & \quad \text{R-NH}_2 & \quad \text{O} & \quad \text{R}'-\text{OH} \\
\end{align*}
\]

<table>
<thead>
<tr>
<th>Substrate</th>
<th>temp. (°C)</th>
<th>turnovers (n)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala-OMe (59a)</td>
<td>25</td>
<td>5.5</td>
<td>67 ± 6.0</td>
</tr>
<tr>
<td>Ala-OEt (59b)</td>
<td>25</td>
<td>3.0</td>
<td>40 ± 5.0</td>
</tr>
<tr>
<td>Ala-OiPr (59c)</td>
<td>25</td>
<td>1.3</td>
<td>86 ± 9.0</td>
</tr>
<tr>
<td>Tyr-OMe (61a)</td>
<td>25</td>
<td>7.6</td>
<td>39 ± 4.0</td>
</tr>
<tr>
<td>Tyr-OMe (61a)</td>
<td>15</td>
<td>1.3</td>
<td>79 ± 2.0</td>
</tr>
<tr>
<td>Tyr-OMe (61a)</td>
<td>4</td>
<td>0.7</td>
<td>86 ± 8.0</td>
</tr>
<tr>
<td>Tyr-OEt (61b)</td>
<td>25</td>
<td>0.7</td>
<td>31 ± 11</td>
</tr>
<tr>
<td>Ser-OMe (63)</td>
<td>25</td>
<td>2.3</td>
<td>43 ± 1.3</td>
</tr>
</tbody>
</table>

Hydrolysis reactions were performed with racemic amino acid esters in PIPES buffer, pH 6.1, at 25 °C and monitored by derivatizing the amino acid products with o-phthalaldehyde and N-acetyl-L-cysteine. Reaction of ALBP-Phen-Cu(II) with alanine ethyl ester (59b) over 24 h period gave a linear rate for the production of alanine; after 24 h the conjugate had catalyzed the hydrolysis of 3 equivs of the ester substrate with modest stereoselectivity. The L enantiomer was hydrolyzed 2.3 times faster than the D isomer. Better selectivity was obtained with the bulkier substrate alanine isopropyl ester (59c). In this case the L enantiomer was hydrolyzed 13 times faster than the D isomer with 1.3 equivs of alanine produced in 24 h. The L enantiomer of
tyrosine methyl ester (61a) underwent hydrolysis 2.3 times faster than the D isomer giving 7.6 equivs of tyrosine (62) in 24 h. This selectivity increased at lower temperature but accompanied by a decrease in overall conversion. The enantioselectivity that can be obtained in a kinetic resolution reaction is, in part, controlled by the ratio of the rate of the protein-catalyzed reaction versus the background rate. The selectivity and catalytic properties of ALBP-Phen clearly demonstrate that covalent modification of the ALBP cavity can be used to generate a metal ion-containing hydrolytic catalyst with enzyme-like features.

A novel host–guest system based on another protein cavity has just been prepared recently by Distefano et al. [48c]. In this published report, they described the properties of IFABP-PX60, a catalyst based on a mutant form of intestinal fatty acid binding protein (IFABP), a protein structurally related to ALBP[50]. This new conjugate reductively aminates α-keto glutаратate to glutamic acid with a catalytic efficiency at least 200-fold greater than that of free pyridoxamine. IFABP-PX60 (65), a construct incorporating a pyridoxamine moiety at position 60 of IFABP-V60C) was prepared in a similar manner as previously described for ALBP-PX (Scheme 1.17).

Scheme 1.17. Preparation of IFABP-PX60 (65).
The ability of IFABP-PX60 to perform the reductive amination under catalytic reactions in which the pyridoxamine cofactor is regenerated by the addition of a second amino acid that serves as an amine source as shown below. The results of these experiments are summarized in Table 1.6.

**Table 1.6.** Production of Glutamate in 24 h catalyzed by IFABP-PX60 in the presence of different amino acids.

<table>
<thead>
<tr>
<th>Amino acid added</th>
<th>Turnovers (n)</th>
<th>L-Glu/D-Glu</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>&gt;0.99</td>
<td>5.3 ± 0.03</td>
<td>68 (L)</td>
</tr>
<tr>
<td>Ala (68a)</td>
<td>1.3 ± 0.05</td>
<td>5.7 ± 0.27</td>
<td>70 (L)</td>
</tr>
<tr>
<td>Val (68b)</td>
<td>1.1 ± 0.03</td>
<td>8.5 ± 0.14</td>
<td>79 (L)</td>
</tr>
<tr>
<td>Leu (68c)</td>
<td>1.6 ± 0.11</td>
<td>9.0 ± 0.60</td>
<td>80 (L)</td>
</tr>
<tr>
<td>Pgl (68d)</td>
<td>1.1 ± 0.01</td>
<td>5.1 ± 0.12</td>
<td>67 (L)</td>
</tr>
<tr>
<td>Phe (68e)</td>
<td>3.9 ± 0.15</td>
<td>28 ± 0.10</td>
<td>93 (L)</td>
</tr>
<tr>
<td>Tyr (68f)</td>
<td>4.2 ± 0.07</td>
<td>28 ± 0.11</td>
<td>93 (L)</td>
</tr>
<tr>
<td>D-Tyr (68f)</td>
<td>0.94 ± 0.04</td>
<td>4.7 ± 0.18</td>
<td>65 (L)</td>
</tr>
<tr>
<td>L-Tyr (68f)</td>
<td>3.1 ± 0.09</td>
<td>21 ± 0.24</td>
<td>91 (L)</td>
</tr>
<tr>
<td>Trp (68g)</td>
<td>2.2 ± 0.04</td>
<td>24 ± 0.16</td>
<td>92 (L)</td>
</tr>
<tr>
<td>Dopa (68h)</td>
<td>4.3 ± 0.12</td>
<td>32 ± 0.09</td>
<td>94 (L)</td>
</tr>
</tbody>
</table>

Initial experiments with amino acids bearing alkyl side chains including Ala (68a), Val (68b), and Leu (68c) did produce a small amount of turnover. Addition of Leu produced the highest levels of Glu (1.6 turnovers in 24 h).
More significant levels of turnover were obtained using amino acids possessing aromatic side chains. The addition of Phe (68e), Tyr (68f), Trp (68g), and Dopa (68h) all gave several turnovers (2.2–4.3) in 24 h. This back reaction appears to be stereospecific, since substitution of L-Tyr with D-Tyr results in no conversion beyond what is observed under single turnover conditions. The enantioselectivities obtained in these catalytic reactions are of particular interest. The use of aromatic amino acids as amine sources gives excellent enantioselectivities. Table 1.6 shows values ranging from 91 to 94% ee. These results are quite different than the lower selectivities obtained under single turnover conditions. A possible explanation for this behavior is that IFABP-PX60 possesses racemase activity. Under single turnover conditions, the amino acid product (Glu, 67) can remain bound to the protein where racemization can occur. However, under catalytic conditions, the amino acid used as the amine source can displace the bound amino acid product thus preventing racemization. This model is consistent with the correlation we observe between rate and enantioselectivity. With the alkyl-substituted amino acids, low levels of turnover and low values of ee were obtained; with aromatic amino acids, increased turnover and higher enantioselectivity were observed. Finally, longer incubation times with Phe or Tyr result in continued reaction and many turnovers. We have monitored the IFABP-PX60-catalyzed production of Glu for up to 14 days at which time over 50 turnovers and an ee of 95% were obtained. Clearly, this protein-based catalyst is a stable material.

The rates of transamination using α-keto glutarate and Tyr were examined at a range of keto acid concentrations by the authors in order to clarify the origin of the significant increase in rate observed with IFABP-PX60 compared with free PX. Comparison of the \( k_{\text{cat}} \) values for the protein system versus the free cofactor reveals a modest 3.9-fold increase resulting from performing the
reaction in the protein cavity. More interestingly, a similar comparison of $K_i$ values shows a 52-fold decrease suggesting that the protein conjugate binds $\alpha$-keto glutarate with much greater affinity than free pyridoxamine. Thus, it appears that the accelerated catalysis observed with IFABP-PX60 occurs primarily due to an increase in substrate binding together with a smaller effect on the maximal rate. Evaluation of the improvement in catalytic efficiency indicate that IFABP-PX60 is 200-fold more efficient than free PX. The results described here indicate that a host–guest system based on a fatty acid binding protein can be used to generate systems that mimic three key features of enzymatic catalysis: selectivity, rate enhancement, and turnover.

1.4. Conclusions

The section above presented almost all the published research results on the subject of enzymatic catalysis and biomimetic catalytic reactions. In fact, these studies have opened up a new frontier for the development of different types of artificial biomimetic catalyst systems. However, probably due to the difficulties in constructing the suitable structural framework of these enzymatic catalysts, the advances achieved so far may be limited and less effective as compared with those achieved by transition metal complexes. Nevertheless, these enzymatic catalyst systems still provide some remarkable advantages which are hardly reachable by the traditional metal complex catalysts. First, the catalytic transformations with the enzymatic catalysts are almost all performed in the hydrolytic media. This environmentally friendly feature makes the process more favored for practical industrialization. Second, these macromolecular catalysts indeed offer the possibility of recovery and recycle in the manufacturing process. Third, another significant advantage offered by
these biomimetic catalysts is the obviation of arduous synthetic course of the chiral ligands. The suitable chiral ligands play the dominant roles in the metal-mediated catalytic reactions. Finally, proteins and enzymes are attractive scaffolds for the design of new catalysts because their size allows the formation of a large number of interactions between substrate and catalyst. Additionally, recombinant DNA methods allow protein-based catalysts to be modified in a facile manner either by side-directed mutagenesis or by selection approaches. Although limited outcome has been achieved so far, the rapid advances in the recombinant DNA technology still provide a potential prospect for the development of the more effective and efficient biomimetic catalyst systems for asymmetric catalytic reactions.

1.5. Aims and Objectives of this Project

Asymmetric transition-metal-mediated catalysis with the incorporation of chiral diphosphine ligands has been an active area for the past two decades. There is no doubt that asymmetric homogeneous catalysis is indeed the most promising method to prepare the optically pure compounds so far in respect of efficiency and selectivity, and only one enantiomer could be produced theoretically by precise matching of the structures between metal complex and substrate for ideal intramolecular chirality transfer under suitable kinetics conditions. Lots of efforts hence were made in the field of developing various new chiral ligands in order to fit the demands of different substrates and many significant advances have also been achieved during the past two decades. Nevertheless, most of the chiral metal catalysts suffer from the recovery problem which limits the application into an industrial scale. Some extensions of usefulness of chiral catalysts by attaching the metallic site to an insoluble
polymer were then extensively developed. The main advantage of this modification is the possibility of recovering the optically active metal complex catalyst. One important and challenging problem in polymer-supported catalysis is the proper choice of the polymer matrix and the synthesis of the catalyst site in the matrix. These two factors, which contradict to each other sometimes, involve the catalytic activity of the repeatedly used catalyst and the optical purity of the product. This type of insoluble polymer-supported catalysts may be recovered from the reaction mixture by filtration and reused many times. These advances have made the homogeneous asymmetric catalysis industrialized successfully in some cases. The ultimate variant of this approach was reported as early as 1978 by G. M. Whitesides[11, 12]. He constructed an asymmetric hydrogenation catalyst based on embedding an achiral diphosphine-rhodium(I) moiety at a specific site in a protein. In this case the protein tertiary structure provided the chirality required for enantioselective hydrogenation. In his study, the well-characterized protein avidin, composed of four identical subunits, each of which binds biotin and many of its derivatives, was used. For this, a hydroxysuccinimide substituted biotin was converted to a chelating diphosphine and complexed with rhodium(I) to obtain the biotinylated rhodium(I) complex. In this procedure the diphosphine intermediate serves as the basis for the elaboration of a watersoluble rhodium-based homogeneous catalyst. The enantioselectivity of the catalyst was tested by the reduction of α-acetamidoacrylic acid to N-acetylalanine. The presence of avidin resulted in a definite increase in activity of the catalyst and in the production of the S-enantiomer (natural amino acid) in 40% excess. Hence, this research showed that it is possible to carry out in aqueous solution homogeneous hydrogenation using a diphosphinerhodium(I) catalyst associated with a protein. This example did offer an extraordinarily
attractive method with regard to the easy recovery of the catalyst. In addition, the avidin-biotin system has been extensively explored as a multipurpose tool for the biochemist. Current interest in avidin derives mainly from its applications in biotechnology based on its rapid and almost irreversible binding of any molecule to which biotin can be linked. That is the essential factor for the incorporation of avidin as a chiral cavity in the metal-catalyzed hydrogenations. Because transition metal complexes catalyze a variety of chemical reactions, the type of new catalysts by introducing a chiral catalytic functionality into the tertiary constrained environment of the protein cavity appeals to us. In our initial thought, the combination, as properly optimized, can possess not only the better compatibility of different substrates but also the multiplicity of various catalytic reactions. In this project, we exploit the catalytic reactions using the avidin-containing catalysts, and disclose the preliminary results of hydrogenations catalyzed by the biotinylated diphosphine-rhodium(I) complexes with and without association of avidin.

1.6. Avidin

1.6.1. Introduction

The discovery of the protein avidin resulted from intensive nutritional investigations into the vitamin B complex[51]. Avidin proved to be a minor constituent of egg white that could induce a nutritional deficiency in rats by forming a very stable noncovalent complex with what was subsequently proved to be the B vitamin biotin. The biological role of avidin in egg white appeared to be that of a scavenger, inhibiting bacterial growth. A variant of
lower affinity for biotin has been found in egg yolk, which may be important in regulating the supply of biotin during development[52].

Current interest in avidin derives mainly from its applications in biotechnology based on its rapid and almost irreversible binding of any molecule to which biotin can be linked. The biochemical basis for the very high affinity was first investigated by chemical modification[53]. Interest was further stimulated by the discovery of the coenzyme function of biotin in CO₂ transfer [54] and by the subsequent use of avidin to identify biotinyl enzymes.

During the past period there has been relatively little new biochemical work on the structure and binding properties of avidin. Considerable efforts have been devoted to an X-ray crystallographic approach to the structure, but it proved very difficult to grow acceptable crystals of sufficient size[55], possibly because avidin is a glycoprotein and its carbohydrate is characteristically heterogeneous[56].

1.6.2. General Properties of Avidin

Avidin is a protein originally isolated from chicken egg white. It is also found in the tissues of birds. Avidin, a basic glycoprotein with an isoelectric point of approximately 10, can be crystallized from high salt buffers between pH 5 and 7. Crystallization from buffers near the isoelectric point has not been reported. Avidin is tetrameric with four identical subunits having a combined molecular weight of about 67,000. The reported molecular weight of avidin ranges from 66–69,000 depending on the method of analysis. The avidin monomer contains 128 amino acid residues; the sequence of the monomer is shown in Table 1.7.
Table 1.7. The amino acid sequence of avidin[a].

<table>
<thead>
<tr>
<th></th>
<th>Ala</th>
<th>Arg</th>
<th>Lys</th>
<th>Cys</th>
<th>Ser</th>
<th>Leu</th>
<th>Thr</th>
<th>Gly</th>
<th>Lys</th>
<th>Trp</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
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<td>Gly</td>
<td>Ser</td>
<td>Asn</td>
<td>Met</td>
<td>Thr</td>
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<td>Glu</td>
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<td>Thr</td>
<td>Tyr</td>
<td>Thr</td>
<td>Thr</td>
<td>Ala</td>
<td>Val</td>
<td>Thr</td>
<td>Ala</td>
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<td>Thr</td>
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<td>Asn</td>
<td>Lys</td>
<td>Arg</td>
<td>Thr</td>
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<tr>
<td>61</td>
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<td>Thr</td>
<td>Phe</td>
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<td>71</td>
<td>Lys</td>
<td>Phe</td>
<td>Ser</td>
<td>Glu</td>
<td>Ser</td>
<td>Thr</td>
<td>Thr</td>
<td>Val</td>
<td>Phe</td>
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<tr>
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<td>Cys</td>
<td>Phe</td>
<td>Ile</td>
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<td>Arg</td>
<td>Asn</td>
<td>Gly</td>
<td>Lys</td>
</tr>
<tr>
<td>91</td>
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<td>Val</td>
<td>Leu</td>
<td>Lys</td>
<td>Thr</td>
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<td>Leu</td>
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<td>Asp</td>
<td>Asp</td>
<td>Trp</td>
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<td>Thr</td>
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<td>Phe</td>
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<td>Leu</td>
<td>Arg</td>
<td>Thr</td>
<td>Gln</td>
<td>Lys</td>
<td>Glu</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[a] This information is according to Dayhoff [57].

The main common characteristics of avidin can be summarized briefly in Table 1.8. The avidins are stable tetramers with 2-fold symmetry, the binding sites being arranged in two pairs on opposed faces of the molecule. The stability is greatly enhanced by biotin binding, since the total free energy of binding is about 330 KJ/mol of tetramer. The dissociation constant for biotin is so low that it can be estimated only from the ratio of the rate constants for binding and exchange. The binding is accomplished by a red shift of the tryptophan spectrum and by a decrease in fluorescence, either of which can be used as the basis for quantitative assays[58,59].
Table 1.8. Properties of avidins

<table>
<thead>
<tr>
<th>Property</th>
<th>Avidin egg white</th>
<th>Avidin egg yolk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acid residues</td>
<td>128</td>
<td>–</td>
</tr>
<tr>
<td>Subunit size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>From sequence</td>
<td>15,600</td>
<td>–</td>
</tr>
<tr>
<td>SDS gels</td>
<td>16,400</td>
<td>19,000</td>
</tr>
<tr>
<td>Subunits</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Isoelectric pH</td>
<td>10</td>
<td>4.6</td>
</tr>
<tr>
<td>ε&lt;sub&gt;20&lt;/sub&gt;</td>
<td>24,000</td>
<td>–</td>
</tr>
<tr>
<td>Δε&lt;sub&gt;20&lt;/sub&gt; (+biotin)</td>
<td>24,000</td>
<td>7,000</td>
</tr>
<tr>
<td>Fluorescence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>λ&lt;sub&gt;max&lt;/sub&gt; (nm)</td>
<td>338</td>
<td>–</td>
</tr>
<tr>
<td>τ (nsec)</td>
<td>1.8</td>
<td>–</td>
</tr>
<tr>
<td>K_a biotin (M) (pH 7, 25°C)</td>
<td>0.6 × 10&lt;sup&gt;16&lt;/sup&gt;</td>
<td>1.7 × 10&lt;sup&gt;12&lt;/sup&gt;</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (days)</td>
<td>200</td>
<td>0.07</td>
</tr>
</tbody>
</table>

1.6.3. Relation of Avidins to Proteins of Known Structure

A search of the Protein Information Resource (PIR) database revealed no protein with significant similarity to avidin. A more detailed examination of potentially similar β-structured proteins proved more informative. It is clear that avidin is composed almost entirely of β strands and bends, from analysis of its Roman spectrum[58], from circular dichroism (CD) measurements[59], and from the secondary structure predicted from the sequences[58]. Proteins with known antiparallel β structure fall into two main classes: (1) β sandwiches of 6–10 strands and (2) orthogonal β barrels. Comparison with the sequences of avidins showed no similarity to any of the first class but did reveal a short N-terminal motif common to all members of the second class[60].
Although uncertainties of both the alignment and the secondary structure prediction prevent detailed structural conclusions, it can be said that avidins are probably orthogonal $\beta$ barrels with binding sites enclosed between $\beta$ sheets. Such a deep binding site is consistent both with the very high affinity and with the deep burial of the binding sites, implied by the results with bifunctional ligands[61].

A more recent report[62] based on the analysis of the amide I band and the prediction algorithms suggested that the subunit of avidin has at least five strands of $\beta$ structure. Thus, avidin may be similar to the class of $\beta$ sandwich proteins characteristic of the domains of the immunoglobulins (Epp et al., 1975) and human prealbumin (Blake et al., 1978).

1.6.4. Dissociation Constants for Biotin

The rate constant for biotin has been measured only once ($7 \times 10^{-7}$ M$^{-1}$sec$^{-1}$ for avidin and biotin at pH 5, 25°C), so that values quoted for dissociation constants of other ligands or other conditions, which are based on this value, are only approximate. Direct measurements of the much higher dissociation constants of the D and L isomers of hexylimidazolidone have been compared with those calculated from the rate constants and were found to be higher by a factor of about 4. This could result partly from the rate of binding for the uncharged ligand being lower than that for biotin, since avidin and biotin carry opposite charges at pH 5. Other factors are also involved so that absolute values of most binding constants estimated from rate constants could be in error by an order of magnitude. Some results of recent measurements of the exchange rate are given in Table 1.9
Table 1.9. Dissociation rates of avidin-biotin complexes

<table>
<thead>
<tr>
<th>Rate and half-life</th>
<th>pH</th>
<th></th>
<th></th>
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<tr>
<td></td>
<td>1.7</td>
<td>2.0</td>
<td>3.0</td>
<td>5.0</td>
<td>7.0</td>
<td>9.2</td>
<td>10.5</td>
</tr>
<tr>
<td>Avidin-biotin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k (sec-1 x 10^7)</td>
<td>–</td>
<td>200</td>
<td>9</td>
<td>0.9</td>
<td>0.4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>τ_{1/2} (days)</td>
<td>–</td>
<td>0.4</td>
<td>9</td>
<td>90</td>
<td>200</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

1.6.5. Avidin Polymers

Current knowledge of the spacial relations between the four binding sites of avidin comes from an electron microscopic study of polymers made with bifunctional biotinyldiamine[60]. In the reaction with avidin, at least 9 methylene groups between biotinamides are required to produce polymers, and 12 are required to make them sufficiently stable to resist depolymerization by biotin, implying binding sites of considerable depth (15 Å). A further increase in length of linking chain (19–22 atoms) greatly decreases the length of the polymers, showing that intramolecular bridging occurs and that the shortest path between biotin carboxyls (not necessarily linear) is about 25 Å (Scheme 1.18). Pairs of sites are positioned at opposite ends of the short (40 Å) axis of the avidin molecule, each pairs being located in a depression in the protein surface.
Scheme 1.18. Spacial relations between neighboring binding sites in avidin.

The presence of a depression in the protein surface could account for the observation that the length of linking chain required to produce an effective heterobifunctional reagent can be shorter than that required to give avidin homopolymers. It suggests that a small or flexible second protein can approach more closely to the biotin site than can a second avidin molecule.

The very high affinity and the 2-fold symmetry of the tetramer form the basis of one of the most useful attributes of the avidin–biotin system. The initial binding is almost irreversible, provided that the biotinyl residue is accessible, and the 2-fold symmetry ensures that if the avidin is binding to a surface molecule many outwardly directed biotin sites will remain vacant and can be saturated with a second biotinyl ligand without displacement of the avidin from the labeled site.

1.6.6. Binding Properties

A. Biotin

The early work on avidin showed that the combination with biotin was very firm. In later work[63] the dissociation constant was calculated from the ratio
of the rate constants for the forward and reverse reactions. The rate of
dissociation was determined from the rate of exchange of bound $^{14}$C-labeled
biotin with an excess of unlabeled biotin, by separating free from bound with
CM-cellulose. The rate was insensitive to ionic strength, but increased when
the pH was below 3. The dissociation rate constant of biotin is so low that
reequilibration of biotin after the initial binding can be ignored in any but very
long-term experiments. This enables the forward rate to be measured by
stopping the reaction with biotin-$^{14}$C by using an excess of unlabeled biotin.
The rate constant obtained in this way, $7 \times 10^{-7} \text{ M}^{-1} \text{sec}^{-1}$, was significantly less than
that for a diffusion controlled reaction.

It has been suggested that the stability of the avidin–biotin complex is
decreased at very low ionic strength. The evidence is of two kinds. (1) The
release of biotin on autoclaving the complex was more effective in the absence
of salt; and (2) the combination of avidin with biotin appeared to be less
complete at low ionic strength, when measured by dialysis or by separation of
radioactive avidin–biotin complex by gel filtration. Although the stability of the
avidin–biotin complex to heat is less in the absence of salt, it is unlikely that
the dissociation constant is changed.

B. Stereochemistry of Biotin

Biotin was first isolated and identified as a yeast growth factor in 1935. The
absolute configuration has been determined by X-ray crystallography (Trotter
and Hamilton, 1966) and is shown in Scheme 1.19. There are three asymmetric
centers at C-2, C-3, and C-4. The two rings are fused in the cis configuration
and the valeric acid side chain (C-2) is also cis in relation to the imidazolidone
ring.
Scheme 1.19. The active (+)-form of biotin.

The structure shows that the N-3' of the ureido function is hindered from reaction by the five carbon side chain of valeric acid. The distance between N-3' and C-6 is only 0.28 nm.

C. Relations of Affinity to Structure

Early experiments established that the imidazolidone ring was much more important than the thiophan ring and that modification of the carboxyl group had little effect on the binding to avidin. Later work, showing that biotinyl enzymes are irreversibly inhibited by avidin, implies that covalent attachment of a macromolecule to the carboxyl group of biotin does not affect the binding.

According to the survey on the free energy of binding of hexyl imidazolidone and its analogs [63], an approximately linear decrease in \( \Delta G \) with decreasing chain length was observed when the alkyl chain was shortened. This observation implies that the whole of the alkyl chain was contributing to the binding energy. Much larger free-energy changes followed modification of the ring structure. It seems likely that both NH groups are acting as H-bond donors to acceptor atoms on the protein and that the bond with N-1' is particularly strong.
D. Nature of the Binding Site

Although the strength of the avidin–biotin interaction suggests the possibility of a covalent attachment, most of the evidence favors noncovalent binding. The biotin can be released by 6M guanidine•HCl at pH 1.5 or by autoclaving. The study of analog binding shows extensive noncovalent interaction with all parts of the biotin molecule, and although the sum of the weak interactions is a significantly less than the free energy of binding of biotin, the discrepancy is small enough to be explicable in terms of a slightly better fit of biotin as compared with the analogs. These results combined with those of chemical modification studies suggest that three or four tryptophan residues in each subunit interact directly with the biotin.

1.6.7. Stability of the Avidin–Biotin Complex

The avidin–biotin interaction is the strongest known noncovalent, biological interaction between protein and ligand. The bond formation between biotin and avidin is very rapid and, once formed, is unaffected by wide extremes of pH, temperature, organic solvents and other denaturing agents.

While free avidin is inactivated at 85 ºC[64], the avidin–biotin can withstand brief exposures to 132 ºC. Autoclaving will also break the avidin–biotin interaction[63]. The temperature resistance of the avidin–biotin complex is dependent on the presence of salts[65,66]. Autoclaving studies show that in the absence of salts, approximately 88% of biotin is released from the complex by heating for 10 minutes at 100 ºC. In the presence of salts, however, temperature of 120 ºC for 15 minutes are required for complete dissociation.
This complex is not significantly affected by pH values between 2 and 13 nor by concentrations of guanidine•HCl up to 8 M at neutral pH's. Outside these parameters, the loss of biotin binding activity results from the denaturation of avidin. The dissociation of avidin into subunits has been reported to be largely reversible[63]. The avidin–biotin complex can be precipitated by zinc sulfate without dissociation[67]. However, dissociation of the precipitated complex by treatment with 70% formic acid for 1 hour irreversibly denatures avidin.
Chapter 2
Experimental Section

2.1. General Method

Except as noted, experiments were carried out under a nitrogen atmosphere. The preparation of samples and the setup of reactions were either performed in a nitrogen-filled continuous purge MBRAUN lab master 130 glovebox or using standard Schlenk-type techniques. Column chromatography was carried out on silica gel (Matrex, 60A 70–200 MY from Micon). Analytical thin layer chromatography was performed on Merck silica gel 60 F-254 pre-coated plates. Hydrogenation reactions were performed in a 50 mL stainless-steel autoclave equipped with a glass liner.

2.2. Apparatus

NMR spectra were recorded on a BRUKER Model ADVANCE DPX 400 spectrometer (¹H: 400 MHz, ³¹P: 162 MHz, ¹³C: 101 MHz) using tetramethylsilane for ¹H and ¹³C as an internal standard, 85% of H₃PO₄ in D₂O for ³¹P as an internal standard, and coupling constants were given in hertz. All signals are reported in ppm unit. Optical rotations were determined on a Perkin-Elmer Model 341 polarimeter. Melting points were determined in capillaries using a Electrothermal 9100 or a BUCHI Melting Point B-545
apparatus. Analyses of HPLC were performed on a Hewlett-Packard Model HP 1050 LC equipped with a FID detector as well as interfaced to a HP 1050 Series computer workstation. Mass analyses were performed by a V. G. MICROMASS, or a Fisons VG platform, or a Finnigan Model Mat 95 ST. For high pressure hydrogenation, 50 mL stainless-steel autoclaves were purchased from parr company.

2.3. Chemicals

The commercial reagents were used as received without further purification. All solvents used were dried using standard, published methods and were distilled before use under nitrogen atmosphere. THF, diethyl ether, benzene, toluene and hexane were distilled from sodium/benzophenone. Triethylamine, pyridine, dichloromethane and Dimethylformamide were distilled from calcium hydride.
2.4. Preparation of (R,R)- and (S,S)-3,4-Bis(diphenylphosphino) pyrroldine

2.4.1. (3R,4R)-1-Benzyl-3,4-dihydroxy-2,5-pyrrolidine [(R)-70]

A 500-mL, round-bottomed flask equipped with a Dean—Stark apparatus was charged with L-(+)-Tartaric acid (30g, 0.2 mol), Benzylamine (22ml, 0.2 mol) and xylene (250ml). The mixture was heated and stirred at refluxing temperature until the distilling receiver had 7.2 mL of water. The resulting mixture was cooled, filtered through Celite and washed with Ethanol to afford the crude yellow solid. The filtrate was dissolved in acetone and ethanol to crystallize the desired product. The recrystallization of yellow solid collected from acetone—ethanol gave (3R,4R)-1-Benzyl-3,4-dihydroxy-2,5-pyrrolidine as white crystals. The total isolated yield was 75.5%. mp: 196–198 °C.

(3S,4S)-1-Benzyl-3,4-dihydroxy-2,5-pyrrolidine [(S)-70]

The (3S,4S) isomer of 70 was prepared from D-tartaric acid as the starting material in the same manner as described above.
2.4.2. (3S,4S)-1-Benzyl-3,4-pyrrolidindiol [(S)-71]

A 500-mL, three-necked, round-bottomed flask fitted a stirred bar, an addition funnel, and an efficient reflux condenser was charged with (3R,4R)-1-Benzyl-3,4-dihydroxy-2,5-pyrrolidindion (20 g, 90 mmol) and 50mL of THF. A solution of H₂B·THF (180 mL, 1.0 M, 180 mmol) was added slowly at ambient temperature under nitrogen. When the addition was completed, the mixture was stirred and heated at refluxing temperature for 2 h. The mixture was added 100mL (6N) of aqueous Hydrochloric acid at 0 °C, heated at reflux again for 2 h, and then added 24 g of NaOH until the solution became basic. The cooled mixture was diluted with water and extracted with THF. The extracts were dried (Na₂SO₄), concentrated, and chromatographed on a short silica gel column (Toluene–Methanol). The column was eluted with toluene and finally with methanol which removed trans-3,4-dihydroxy-1-benzylpyrrolidine. Recrystallization from CH₂Cl₂–Ether yielded (3S,4S)-1-benzyl-3,4-pyrrolidindiol as colorless crystals.

The analytical data of (S)-71 are as follows:

mp: 100 °C; [α]D²⁰ 32.4° (c = 4.2, methanol); ¹H NMR (400 MHz, CDCl₃) δ : 7.28-7.23 (m, 5H), 4.51 (s, 2H), 4.02 (t, 2H, J = 4.5 Hz), 3.56 (q, 1H, J = 19.8 Hz), 2.88 (dd, 2H, J₁ = 10.0 Hz, J₂ = 6.0 Hz), 2.41 (dd, 2H, J₁ = 10.4 Hz, J₂ = 3.9 Hz) ppm; ¹³C NMR (101 MHz, CDCl₃) δ : 137.2, 129.2, 128.3, 127.4, 78.3, 60.2, 60.1 ppm; HRMS (EI+VE+LMR) for C₇₁H₇₂NO₇: cald 193.1103; found 193.1137.

(3R,4R)-1-Benzyl-3,4-pyrrolidindiol [(R)-71]

The (3R,4R) isomer of 71 was prepared in the same manner as above.
2.4.3. (3S,4S)-1-Benzyl-3,4-Bis(methylsulfonyloxy)pyrrolidine

[(S)-72]

A 100-mL, two-necked, round-bottom flask fitted a stirred bar was placed into an ice bath. To this flask was charged with (3S,4S)-1-Benzyl-3,4-pyrrolidindiol (8.115 g, 42 mmol), dry triethylamine (7mL), and dry CH₂Cl₂ (30mL). The methanesulfonl chloride (7.7mL) was added slowly to above mixture at 0 °C by immersing into an ice bath. The resulting mixture was stirred and allowed to warm to room temperature gradually for 10 h. The mixture was added 10% Hydrochloric acid aq. until the solution became acidic. The resulting mixture was extracted with water (150 mL × 3), neutralized by adding 10% aqueous Sodium hydroxide. Extraction of the basic solution with CH₂Cl₂ (150 mL × 3) was proceeded, and the combined organic layers were dried (MgSO₄), concentrated and chromatographed on a short silica gel column (EA/CH₂Cl₂=1/3). The isolated yield of (3S,4S)-72 was 85.8%.

The analytical data of (S)-72 are as follows:

mp: 56 °C; [α]D 36.4° (c = 2.9, methanol); ¹H NMR (400 MHz, CDCl₃) δ : 7.33–7.26 (m, 5H), 5.14 (m, 2H), 3.66 (s, 2H), 3.12 (dd, 2H, J₁ = 10.7 Hz, J₂ = 6.2 Hz) 3.09 (s, 6H), 2.78 (dd, 2H, J₁ = 11.0 Hz, J₂ = 4.0 Hz) ppm; ¹³C NMR (101 MHz, CDCl₃) δ : 136.7, 128.7, 128.5, 127.6, 82.4, 59.1, 57.8, 38.3 ppm; HRMS (EI+VE+LMR) for C₁₀H₁₂NS₂O₆: cald 349.0654; found 349.0644.

(3R,4R)-1-Benzyl-3,4-Bis(methylsulfonyloxy)pyrrolidine [(R)-72]

The (3R,4R) isomer of 72 was prepared in the same manner as above.
2.4.4. (3S,4S)-3,4-Bis(methylsulfonyloxy)pyrrolidinium-acetate

[(S)-73]

In a 250-mL, one-necked flask was placed (3S,4S)-1-Benzyl-3,4-Bis(methylsulfonyloxy)pyrrolidine (0.5 g, 1.43 mmol), Acetic acid (0.1 mL), Methanol (6.5 mL), water (0.4 mL) and Pd/C (40 mg). The mixture was stirring and purging with 1 atm Hydrogen for 24h. The resulting mixture was filtered through Celite and washed with 50mL of Acetic acid. The filtrate was concentrated under reduce pressure to afford (3S,4S)-3,4-Bis(methylsulfonyloxy)pyrrolidinium-acetate (S)-73 as colorless crystals.

The analytical data of (3S,4S)-73 are as follows:
mp: 129 °C; [α]D 3.1° (c = 3.1, acetic acid); MS (EI): m/z 318 (M+1), 260 (100%), 262.

(3R,4R)-3,4-Bis(methylsulfonyloxy)pyrrolidinium-acetate [(R)-73]

The (3R,4R) isomer of 73 was prepared in the same manner as above.
2.4.5. \((3R,4R)-3,4\text{-bis(diphenylphosphino)}\text{pyrroolidinium-chloride}\) 
\[ ((R)-74) \]

In a 100-mL, two-necked flask fitted with a magnetic stirred bar was charged with 1.37 g (4.31 mmol) of \((3S,4S)-3,4\text{-bis(methylsulfonyloxy)}\text{-pyrroolidinium acetate (S)-73} and 30mL of DMF and then the 0.5 M THF solution of potassium diphenylphosphite (19 mL, 9.5 mmol) was added dropwise to the above mixture at \(-40^\circ\text{C}\) for 8 h. Then the temperature of solution was allowed to raise to \(-10^\circ\text{C} \) and keep stirring for 10 h. The resulting mixture was warm to room temperature and the solvent was evaporated by vacuum pump. The residue was extracted with diethyl ether (50mL $\times$ 2). The organic layers were added 100ml of 2 N Hydrochloric acid and an syrup was formed immediately. The syrup product was then washed by ether (20mL $\times$ 3). The residue was dried in vacuo to give the crude product of \((R)-74\) in 76\% theoretical yield. This crude product without further purification is ready for the directly use for next transformation.

\((3S,4S)-3,4\text{-bis(diphenylphosphino)}\text{pyrroolidinium-chloride [}(S)-74] \)

The \((3S,4S)\) isomer of 74 was prepared in the same manner as above.
2.4.6. (3R,4R)-3,4-bis(diphenylphosphino)pyrrolidine

[(R)-75, (R)-Pyrphos]

A 100-mL flask was charged with (3R,4R)-74 (677.2 mg, 1.64 mmol) dissolving in 10% aqueous NaOH (15 mL). To this solution was added diethyl ether (30 mL) with vigorously stirring for 1 h. Separation of the etheral layer via usual extraction workup, which then dried over Na₂SO₄ and concentrated under reduced pressure gave the desired product of (R)-75 as white solid.

The analytical data of (R)-75 are as follows:

mp: 120 °C; [α]_D^20 154° (c = 3.8, ethanol); ¹H NMR (400 MHz, CDCl₃) δ : 7.30–7.15 (m, 20H), 3.33–3.29 (m, 2H), 2.93–2.82 (m, 4H), 2.24 (s, 1H) ppm; ³¹C NMR (101 MHz, CDCl₃) δ : 133.7, 133.6, 133.5, 133.4, 133.3, 129.0, 128.7, 128.53, 128.50, 128.46, 128.43, 128.40, 50.8, 50.7, 50.6, 40.1, 40.0, 39.9, 39.8 ppm; ³¹P NMR (162 MHz, CDCl₃) δ : -8.48 ppm; HRMS (ESI+VE+LMR) for C₃₆H₃₆NP₂: cald 440.1697; found 440.1722.

(3S,4S)-3,4-bis(diphenylphosphino)pyrrolidine [(S)-75, (S)-Pyrphos]

The (3S,4S) isomer of 75 was prepared in the same manner as above.
2.5. Preparation of \((3R,4R)-\text{Pyrphos-}d\)-Biotinamide [(R)-76]

N-Biotinoxysuccinimide (362mg, 1 mmol) and \((3R,4R)-\text{Pyrphos}\) (424mg, 0.966 mmol) were dissolved in 14mL of DMF. The reaction mixture was stirred at room temperature for 60 h. Then the mixture was slowly diluted with 30mL of degassed water and cooled to 0 °C. The resulting white precipitate was separated by filtration and washed with water (5 mL). The white precipitate was dried in vacuo. The precipitate was reprecipitated by dichloromethane / diethyl ether and washed with diethyl ether to give white solid product (610 mg, 95% theoretical yield).

The analytical data of (R)-76 are as follows:

mp: 123.9 °C; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \& 7.5–7.1 (m, 20H), 5.4 (s, 1H), 5.0 (s, 1H), 4.5 (d, 1H, J = 4.9 Hz), 4.3 (d, 1H, J = 5.2 Hz), 4.0–3.8 (m, 2H), 3.3 (m, 1H), 3.1 (m, 1H), 2.9–2.8 (m, 2H), 2.7 (m, 1H), 2.1 (t, 2H, J = 14.8 Hz), 1.6–1.4 (m, 6H) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \& 171.9, 163.5, 150.0, 134.2, 134.1, 134.0, 113.9, 130.0, 129.8, 129.7, 129.6, 129.3, 129.2, 129.1, 62.2, 60.6, 55.6, 41.1, 37.7, 34.5, 28.7, 25.0 ppm; \(^3\)P NMR (162 MHz, CDCl\(_3\)) \& -13.6 (d, \(J_{pp} = 7.0\) Hz), -13.4 (d, \(J_{pp} = 7.0\) Hz) ppm; HRMS (ESI) for \(\text{C}_{n}\text{H}_{24}\text{SO}_{4}\text{P}_{2}\text{N}_{7}\); cald 666.2473; found 666.2478.

\((3S,4S)-\text{Pyrphos-}d\)-Biotinamide [(S)-76]

The \((3S,4S)\) isomer of 76 was prepared in the same manner as above.
2.6. Preparation of Bis(1,5-cyclooctadiene)rhodium tetrafluoroborate

[Rh(COD)$_2$]BF$_4$, Complex

To a solution of [Rh(COD)Cl]$_2$ (506 mg, 10.26 mmol) in CH$_2$Cl$_2$ (7 mL) was added COD (0.38 mL, 3.10 mmol) followed by a solution of AgBF$_4$ (458 mg, 2.31 mmol) in acetone (3 mL). This resulted in the immediately formation of deep red solution, containing a white precipitate, which was stirred for 2 h and then was filtered through Celite. To the filtrate was added THF (15 mL) and the volume was reduced on a vacuum pump at room temperature to 10 mL. The deep red precipitate so obtained was filtered, washed with diethyl ether and hexane, and finally vacuum-pump-dried. The solid collected was allowed to be recrystallized from CH$_2$Cl$_2$/ether to afford the pure product as red crystals.
2.7. Preparation of [Rh(COD)(S,S)-Pyrphos]BF₄ Complex [77]

[Rh(COD)(R,R)-Pyrphos]BF₄ Complex [78]

(R,R)- or (S,S)-Pyrphos (66mg) and [Rh(COD)₂]BF₄ complex (46.2mg) were dissolved in 3ml of dichloromethane (degassed) and the mixture was stirred at room temperature for 3 h. The mixture was added slowly ether and cooled to 0 °C. The resulting precipitate was separated via filtration and washed with ether to afford the light orange solid. ³¹P NMR (162 MHz, CDCl₃) δ : 33.0 (d, J_{Rh-P} = 148.6 Hz) ppm; HRMS (ESI) for C₂₅H₁₈NP₄Rh: calcd 651.1691; found 651.1698.


[Rh(COD)(R,R)-Pyrphos-Biotinamide]BF₄ Complex [80]

(R,R)- or (S,S)-Pyrphos-Biotinamide (41.2mg) , [Rh(COD)₂]BF₄ complex (21mg) were added to 3ml of dichloromethane (degases). The mixture was stirred at room temperature for 3 h. The mixture was slowly added diethyl ether and cooled to 0 °C. The resulting precipitate was separated via filtration and washed with ether to afford the light yellow solid. ³¹P NMR (162 MHz, CDCl₃) δ : 36.3 (dd, J_{Rh-P} = 117.5 Hz) ppm; HRMS (ESI) for C₃₈H₂₈SO₃P₂N₂Rh: calcd 877.2467; found 877.2471.
2.9. Preparation of \( N-(\text{tert-Butoxycarbonyl})-(2S,4S)-4-(\text{diphenyl}-\text{phosphino})-2-[(\text{diphenylphosphino})\text{methyl}]\text{pyrroldidine} \) (BPPM)

2.9.1. 4-Hydroxy-D-proline Ethyl Ester Hydrochloride [82]

A slurry of 100 g (760 mmol) of 4-hydroxy-D-proline in 600 mL of absolute ethanol was treated with dry hydrogen chloride until homogeneous. The solution was heated to the reflux temperature for 2 h. Upon being cooled in the refrigerator, the product was obtained as white needles which was filtered, washed well with ether, and dried under reduced pressure to yield 141 g (95% theoretical yield) of \( 82 \).

The analytical data of \( 82 \) are as follows:

\( \text{mp: 153–153.5 }^\circ\text{C; } ^1\text{H NMR (400 MHz, DMSO-d}_6\text{)} \delta : 4.20 \text{ (q, 2H, } J = 7.0 \text{ Hz), 1.25 (t, 3H, } J = 7.0 \text{ Hz ppm; } ^{13}\text{C NMR (101 M Hz, D}_2\text{O) } \delta : 177.5, 79.1, 73.8, 68.7, 63.6, 47.1, 24.1 \text{ ppm; IR (KBr) 3320, 1735 cm}^{-1}. \)

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2.9.2. *N*-(tert-Butyloxycarbonyl)-4-hydroxy-L-proline Ethyl Ester [83]

A stirred mixture of 100 g (510 mmol) of 82, 75 mL (540 mmol) of tert-butyloxycarbonyl azide, 150 mL of triethylamine, 500 mL of water, and 500 mL of p-dioxane was heated under nitrogen to 50 °C for 15 h. The mixture was reduced in volume by half on a rotary evaporator and extracted with four ml portions of ether. The combined extracts were washed with brine, dried over magnesium sulfate, and concentrated to a yellow oil under reduced pressure. Distillation of the oil under reduced pressure gave 121.8 g (87%) of 83 as a pale yellow oil.

The analytical data of 83 are as follows:

mp: 126–128 °C; [α]D 20 -69.1° (c = 2.7, ethanol); ¹H NMR (400 MHz, CDCl₃) δ : 4.36–4.34 (m, 1H), 4.29–4.21 (m, 2H), 3.68–3.54 (m, 2H), 2.39–2.28 (m, 1H), 2.11–2.05 (m, 1H), 1.47–1.42 (s, 9H), 1.33–1.29 (m, 3H) ppm; ¹³C NMR (101 MHz, CDCl₃) δ : 173.0, 172.7, 154.3, 153.8, 80.1, 79.9, 69.4, 68.7, 60.9, 58.0, 57.7, 54.4, 39.0, 38.2, 28.3, 28.2, 14.2 ppm; IR (neat) 3450, 1735, 1680 cm⁻¹.
2.9.3. *N*-(*tert*-Butoxycarbonyl)-4-hydroxy-*L*-prolinol [84]

To an ice-cold solution of 50 g (190 mmol) of 83 in 600 mL of tetrahydrofuran was added 15.0 g (690 mmol) of lithium borohydride in one portion. The mixture was stirred at 0 °C for 1 h followed by 15 h at room temperature. The solution was cooled to 0 °C with stirring and 255 mL of water and 100 mL of 1:1 water/concentrated hydrochloric acid were added carefully. The solution was warmed until an organic phase separated. The organic phase was withdrawn, and the aqueous layer was extracted with three 150 mL portions of ethyl acetate. The combined organic layers were washed with 100 mL each of 2N sodium hydroxide, 2N hydrochloric acid, and brine. The organic layers were dried over magnesium sulfate and evaporated under reduced pressure to an oil. The oil was kept under vacuum for overnight and then used without further purification. The chemical yield of 84 was 87% based on the theoretical yield (36.4 g).

The analytical data of 84 are as follows:

\([\alpha]_D^{20} -69.1^\circ \text{ (c } = 2.7, \text{ ethanol) }; \) \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta : 4.37-4.19 \text{ (m, 4H), 4.02 (d, 1H), 3.58-3.28 \text{ (m, 3H), 2.45-2.25 \text{ (m, 1H), 2.13-1.85 \text{ (m, 1H), 1.46-1.40 \text{ (m, 9H), 1.32-1.26 \text{ (m, 2H ppm)}}; } \) } \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta : 156.5, 154.8, 80.2, 68.6, 65.9, 63.5, 58.4, 57.7, 55.4, 54.9, 37.2, 28.4 \text{ ppm}; IR \text{(neat) 3400, 1670, 1420 cm}^{-1}\).
2.9.4. \textit{N-(tert-Butoxycarbonyl)-4-hydroxy-L-prolinol Di-p-toluene sulfonate} [85]

To a stirred solution of 11.3 g (52.0 mmol) of \textit{N-(tert-Butoxycarbonyl)-4-hydroxy-D-prolinol} in 300 ml of dry pyridine at 0 °C under nitrogen was added 29.7 g (156 mmol) of recrystallized p-toluenesulfonil chloride in one portion. The mixture was stored in the refrigerator for 24 hours. The mixture was then cooled to 0 °C, and 600 ml of water was added dropwise with stirring to precipitate a white powder which was filtered, washed with water, and dried under reduced pressure to yield 25.2 g (92%) of crude 85. Recrystallization from absolute ethanol produced pure 85 as white needles.

The analytical data of 85 are as follows:

\textbf{mp}: 105.7 °C; [\alpha]_{D}^{29} -27.1° (c = 0.8, benzene); \textit{\textsuperscript{1}}H NMR (400 MHz, CDCl\textsubscript{3}) \delta : 7.9–
7.3 (m, 8H), 5.2–4.9 (m), 4.4–3.9 (m), 3.8–3.2 (m), 2.5 (s, 6H), 2.2 (m), 1.4 (s, 9H) ppm; \textit{\textsuperscript{13}}C NMR (101 MHz, CDCl\textsubscript{3}) \delta : 150.8, 142.2, 131.1, 130.2, 127.6, 125.4, 79.0, 68.4, 53.4, 51.4, 34.1, 33.5, 27.7, 21.2 ppm; IR (KBr) 1690, 1600, 1450, 1360, 685, 672, 658, 620 cm\textsuperscript{-1}.

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2.9.5 \(N-(\text{tert}-\text{Butoxycarbonyl})-(2S,4S)-4-(\text{diphenylphosphino})-2-[(\text{diphenyl-phosphino})\text{methyl}]\text{pyrrolidine}\ [86, \text{BPPM}]

In a 250-mL, two-necked flask fitted with a magnetic stirred bar was charged with 5.0 g (9.45 mmol) of \(N-(\text{tert}-\text{Butoxycarbonyl})-4\)-hydroxy-L-prolinol Dip-toluenesulfonate and 50mL of dry THF and then the 0.5 M THF solution of potassium diphenylphosphite (41 mL, 20.8 mmol) was added dropwise over 20 min to the above mixture at 0 °C. Then the temperature of solution was allowed to raise to room temperature and keep stirring for 10 h. The solution was treated with methanol to destroy the excess anion and then filtered through Celite. The solvent of the collected filtrate was concentrated under reduced pressure. The remaining oil was crystallized from 50 mL of absolute ethanol after standing in the refrigerator for 48 h. The reaction yielded 3.96 g of white crystalline 86.

The analytical data of 86 are as follows:

mp: 105.9 °C; \([\alpha]_D^{24} -40.1^\circ (c = 2.4, \text{benzene})\); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta : 7.53-7.26 (m, 20H), 3.84 (br, 2H), 3.23-3.15 (m, 1H), 2.74-2.72 (m, 1H), 2.11-2.05 (m, 2H), 1.41 (s, 9H) \) ppm; \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta : 153.9, 133.3, 133.3, 133.1, 132.9, 129.0, 128.6, 128.55, 128.51, 128.48, 128.4, 93.2, 35.3, 35.2, 28.6 \) ppm; \(^{31}\)P NMR (162 MHz, CDCl\(_3\)) \(\delta : -4.3, -5.2 \) ppm; IR (KBr) 1680, 1480, 1435, 1395, 1175, 1125, 1100, 740, 695 \(\text{cm}^{-1}\); HRMS (ESI) for \(\text{C}_{36}\text{H}_{38}\text{NO}_2\text{P}_4\)_\(_2\); calcd 553.2299, found 553.2306.
2.10. Preparation of \((2S,4S)-4-(\text{Diphenylphosphino})-2-(\text{diphenyl-phosphino})\text{methyl}\)pyrrolidine [87, PPM]

To a 50 mL of ice-cold trifluoroacetic acid under nitrogen was added 6.0 g (10.9 mmol) of 86 in one portion. The mixture was stirred at 0 °C for 30 min, and the trifluoroacetic acid was removed under reduced pressure. The residue was taken up in 50 mL of dichloromethane and washed with 50 mL of water, two 50-mL portions of 2 N sodium hydroxide, and two 25-mL of brine. The organic layer was dried over potassium carbonate and evaporated under reduced pressure to yield 4.75 g (96%) of 87 as a pale yellow solid.

The analytical data of 87 are as follows:

mp: 74.2–75.8 °C; \([\alpha]_D^2 -15.9^\circ\) (c = 1.4, benzene); \(^1\text{H NMR (400 MHz, CDCl}_3\) \(\delta: 7.43–7.26\) (m, 20H), 3.10–2.96 (m, 3H), 2.80–2.78 (m, 1H), 2.43–2.40 (m, 1H), 2.39–2.14 (m, 2H), 1.35 (br, 1H) ppm; \(^{13}\text{C NMR (101 MHz, CDCl}_3\) \(\delta: 138.5, 138.1, 133.3, 133.1, 133.0, 132.9, 132.8, 132.76, 132.70, 132.6, 128.7, 128.6, 128.5, 128.47, 128.44, 128.40, 128.3, 93.2, 93.1, 58.0, 57.9, 57.8, 50.8, 50.5, 38.9, 36.8, 36.7, 35.3, 35.1 ppm; \(^3\text{P NMR (162 MHz, CDCl}_3\) \(\delta: -0.6, -17.2\) ppm; IR (KBr) 1480, 1430, 740, 695 cm\(^{-1}\); HRMS (ESI) for C\(^{29}\)H\(^{29}\)NP\(^{15}\): cald 453.1775, found 453.1792
2.11. Preparation of (2S,4S)-PPM-d-Biotinamide [88]

\(N\)-Biotinoxysuccinimide (362mg, 1 mmol) and (2S,4S)-PPM (443mg, 0.978 mmol) were dissolved in 14mL of degassed DMF. The reaction mixture was stirred at room temperature for 60 h. Then the mixture was slowly diluted with 30mL of degassed water and cooled to 0 °C. The resulting white precipitate was separated by filtration and washed with water (5 mL). The white precipitate was dried in vacuo. The precipitate was reprecipitated by dichloromethane / ether and washed with diethyl ether to give white solid product (638 mg, 96\% theoretical yield).

The analytical data are as follows:
\(^1\text{H} \text{NMR} (400 \text{ MHz}, \text{CDCl}_3) \delta : \text{ppm}; \ ^{13}\text{C} \text{NMR} (101 \text{ MHz}, \text{CDCl}_3) \delta : \text{ppm}; \ ^{31}\text{P} \text{NMR} (162 \text{ MHz}, \text{CDCl}_3) \delta : -8.5, -8.7, -21.6, -22.2 \text{ ppm}; \text{HRMS (ESI) for C}_{10}\text{H}_{14}\text{N}_3\text{SO}_2\text{P}_2: cald 679.2551; found 679.2576.

2.12. Preparation of (Rh(COD)[(S,S)-PPM])BF₄ Complex [89]

(S,S)-PPM (30 mg) and [Rh(COD)]BF₄ complex (24.4 mg) were dissolved in 3mL of dichloromethane (degassed) and the mixture was stirred at room temperature for 3 h. The mixture was added slowly ether and cooled to 0 °C. The resulting precipitate was separated via filtration and washed with ether to afford the light orange solid. \(^{31}\text{P} \text{NMR} (162 \text{ MHz}, \text{CDCl}_3) \delta : 46.0 (dd, J_{Rh,P} = 113.6 \text{ Hz}, J_{\rho,\rho} = 37.6 \text{ Hz}), 35.9 (dd, J_{Rh,P} = 113.6 \text{ Hz}, J_{\rho,\rho} = 37.6 \text{ Hz}) \text{ ppm}; \text{HRMS (ESI) for C}_{12}\text{H}_{24}\text{NP}_2\text{Rh: cald 664.1769; found 664.1782.}
2.13. Preparation of \((\text{Rh(COD)})(\text{(S,S)-PPM-biotin})\) \text{BF}_3, \text{Complex [90]}

\((\text{S,S})\)-PPM-biotinamide (45.2 mg), \([\text{Rh(COD)}]_2\) \text{BF}_3, \text{complex (24.0 mg)} were dissolved in 3mL of dichloromethane (degassed). The mixture was stirred at room temperature for 3 h. The mixture was slowly added ether and cooled to 0 °C. The resulting precipitate was separated via filtration and washed with ether to afford the light yellow solid. \(^{31}\text{P} \text{NMR (162 MHz, CDCl}_3\)) \(\delta : \text{ppm;}\) HRMS (ESI) for \(\text{C}_{16}\text{H}_{28}\text{N}_2\text{SO}_3\text{P}_3\text{Rh}\): calcd 890.2545; found 890.2552.

2.14. Preparation of \((\text{Rh(COD)})(\text{(S,S)-BPPM})\) \text{BF}_3, \text{Complex [91]}

\((\text{S,S})\)-BPPM (40.0 mg) and \([\text{Rh(COD)}]_2\) \text{BF}_3, \text{complex (26.8 mg)} were dissolved in 3mL of dichloromethane (degassed) and the mixture was stirred at room temperature for 3 h. The mixture was added slowly ether and cooled to 0 °C. The resulting precipitate was separated via filtration and washed with ether to afford the light orange solid. \(^{31}\text{P} \text{NMR (162 MHz, CDCl}_3\)) \(\delta : 41.1 \text{ (dd, } J_{\text{Rh-P}} = 147.2 \text{ Hz, } J_{\text{pp}} = 35.5 \text{ Hz})\), 14.4 (dd, \(J_{\text{Rh-P}} = 147.2 \text{ Hz, } J_{\text{pp}} = 35.5 \text{ Hz}) \text{ ppm;}\) HRMS (ESI) for \(\text{C}_{26}\text{H}_{38}\text{NO}_3\text{P}_3\text{Rh}\): calcd 764.2293; found 764.2304.
2.15. Preparation of (R)-5-amino-BINAP

2.15.1. (R)-5-nitro-BINAP [93]

In a 100-mL flask equipped with a thermometer, a stirring bar and an addition funnel was charged with 20 mL of acetic anhydride. After cooling to -30 to -40 °C, 0.5 mL (12 mmol) of fuming nitric acid was slowly added. To this mixture was added two drops of 98% sulfuric acid. Then 500 mg (0.76 mmol) of (R)-BINAPO was added prontwise to the resulting system at -30 to -40 °C, and the reaction mixture was stirred for another 6 h at this temperature. The mixture was poured into 100 mL of cold water with stirring. The precipitate was collected by filtration, washed with water, and dried in vacuo to give the crude product as a yellow solid. Further purification by column chromatography on silica gel with eluent of Hexane/EA (1:1) to afford the pure 93 as pale yellow solid. The isolated yield of 93 was 58%.

The analytical data of (R)-93 are as follows:

mp: 390 °C (decomposed); \(^1^H\) NMR (400 MHz, CDCl\(_3\)) \(\delta: 8.67\) (d, 1H, \(J = 9.0\) Hz), 8.07 (d, 1H, \(J = 7.5\) Hz), 7.73–7.51 (m, 15H), 7.35–7.28 (m, 8H), 7.07 (d, 1H, 8.5 Hz), 6.72 (t, 1H, \(J = 8.0\) Hz), 6.46 (d, 1H, \(J = 4.0\) Hz), 6.33–6.29 (m, 1H), 5.68–5.64 (m, 1H), 5.52 (d, 1H, \(J = 5.0\) Hz) ppm; \(^{13}^C\) NMR (101 MHz, CDCl\(_3\)) \(\delta: 146.11, 143.42, 141.08, 137.09, 134.74, 134.62, 134.42, 132.46, 132.41, 132.35, 132.29, 132.18, 132.09, 131.97, 131.87, 131.79, 137.61, 131.50, 130.77, 130.49, 129.86, 129.73, 129.49, 129.10, 128.98, 128.59, 128.49, 128.37, 128.24, 126.57, 126.05, 125.61, 123.87, 123.50, 123.38 ppm; \(^3^P\) NMR (162 MHz, CDCl\(_3\)) \(\delta: 27.18, 27.54\) ppm; MS (FAB+VE+LMR): \([M+H]^+ = 700.2\).
2.15.2. (R)-5-amino-BINAPO [94]

In a 50 mL autoclave with a glass liner was charged with 250 mg (0.366 mmol) of 93, 20 mg of 10% Pd/C, 5 mL of ethanol. The autoclave was then pressurized with hydrogen to 50 atm and stirred at room temperature for 20 h. The catalyst was removed by filtration through Celite and the filtrate was concentrated under reduced pressure to give 227 mg (98% yield) of (R)-94 as pale yellow solid.

The analytical data of (R)-94 are as follows:
mp: 380 °C (decomposed); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\): 7.83–7.68 (m, 7H), 7.48–7.22 (m, 19H), 6.78 (s, 2H), 6.61 (d, 2H, J = 4.6 Hz), 6.28 (t, 1H, J = 4.6 Hz), 4.13 (s, 2H) ppm; \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\): 142.00, 135.14, 134.10, 133.96, 132.58, 132.50, 132.40, 132.13, 132.04, 131.96, 131.04, 130.85, 128.17, 128.06, 128.02, 127.94, 127.90, 127.82, 127.71, 127.62, 127.18, 127.09, 126.93, 126.26, 125.73, 124.24, 120.45, 120.32, 118.50, 111.15, 99.35 ppm; \(^3\)P NMR (162 MHz, CDCl\(_3\)) \(\delta\): 27.94, 27.56 ppm.
2.15.3. \((R)\)-5-amino-BINAP \([95]\)

A 100 mL three-necked flask equipped with a thermometer and a reflux condenser, which is connected to a nitrogen inlet tube and a bubbler through a three-way stopcock, was charged with 1.0 g (1.46 mmol) of 94. The flask was flushed with nitrogen and to this was added dry toluene (60 mL), triethylamine (2.0 mL, 14.38 mmol), and trichlorosilane (1.2 mL, 11.89 mmol). The mixture was stirred and heated at 100 °C for 1 h, at 110 °C for 1 h, and finally at refluxing temperature for 40 h. After cooling to room temperature, 30% aqueous NaOH (20 mL) was added carefully to the mixture. The organic layer was separated, washed with water, dried over Na₂SO₄, and concentrated under reduced pressure. The residue was recrystallized in methanol/dichloromethane to give a white solid of \((R)\)-95 (893 mg, 94% yield).

The analytical data of \((R)\)-95 are as follows:

mp: 380 °C (decomposed); \([\alpha]_D^22 +224.0^\circ\) (c = 0.05, benzene); \(^1\)H NMR (400 MHz, CDCl₃) δ: 7.90–7.81 (m, 3H), 7.46–7.34 (m, 3H), 7.20–6.78 (m, 23H), 6.68 (d, 1H, J

= 7.3 Hz), 6.38 (d, 1H, J = 8.4 Hz), 4.14 (s, 2H) ppm; \(^13\)C NMR (101 MHz, CDCl₃) δ :

146.15, 145.85, 145.72, 145.55, 141.92, 138.07, 137.95, 137.89, 137.77, 137.47, 137.34, 135.64, 135.55, 135.14, 135.06, 134.26, 134.10, 134.07, 133.89, 133.38, 133.34, 133.19, 133.04, 133.00, 132.86, 132.71, 132.67, 130.65, 129.46, 128.36, 128.25, 127.97, 127.64, 127.60, 127.52, 127.47, 126.47, 126.29, 125.69, 123.37, 120.95, 119.05, 110.72 ppm; \(^3\)P NMR (162 MHz, CDCl₃) δ :

-16.6 (q, J = 9.23 Hz) ppm; MS (ESI): [M+H]⁺ = 637.9.

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2.16. Preparation of (R)-BINAP-\textit{d}-biotinamide [96]

\textit{N}-Biotinoxy succinimide (362 mg, 1 mmol) and (R)-BINAP-NH\textsubscript{2} (623 mg, 0.978 mmol) were dissolved in 14mL of degassed DMF. The reaction mixture was stirred at room temperature for 60 h. Then the mixture was slowly diluted with 30mL of degassed water and cooled to 0 °C. The resulting white precipitate was separated by filtration and washed with water (5 mL). The white precipitate was dried in vacuo. The precipitate was reprecipitated by dichloromethane / diethyl ether and washed with diethyl ether to give white solid product (827 mg, 98% theoretical yield).

The analytical data of 96 are as follows:

\textsuperscript{31}P NMR (162 MHz, CDCl\textsubscript{3}) \textit{δ} : -14.9 ppm; HRMS (ESI) for C\textsubscript{24}H\textsubscript{34}N\textsubscript{2}SO\textsubscript{2}P\textsubscript{2}: calcd 863.2864, found 863.2878.
2.17. Preparation of (Ru([R]-5-amino-BINAP)(cymene)Cl)Cl Complex [97]

A 25-mL Schlenk tube was charged with [Ru(cymene)Cl]₂ (30.6 mg, 0.05 mmol), and (R)-BINAP-NH₂ 95 (70 mg, 0.11 mmol) in 5 mL of CH₂Cl₂ (degassed) under a nitrogen atmosphere. The resulting mixture was stirred at room temperature for 2 h. After partial evaporation of solvent, the residue was diluted slowly with diethyl ether for the precipitation of complex 97. The precipitate was collected through filtration to afford the desired complex 97 in 95.7% yield (90.3 mg). ³¹P NMR (162 MHz, CDCl₃) δ : 41.5 (dd, J₁ = 62.3 Hz, J₂ = 96.7 Hz), 25.5 (dd, J₁ = 62.8 Hz, J₂ = 132.9 Hz) ppm.

2.18. Preparation of (Ru([R]-BINAP-d-biotinamide)(cymene)Cl)Cl Complex [98]

A 25-mL Schlenk tube was charged with [Ru(cymene)Cl]₂ (30.6 mg, 0.05 mmol), and (R)-BINAP-biotin 96 (95 mg, 0.11 mmol) in 5 mL of CH₂Cl₂ (degassed) under a nitrogen atmosphere. The resulting mixture was stirred at room temperature for 2 h. After partial evaporation of solvent, the residue was diluted slowly with diethyl ether for the precipitation of complex 98. The precipitate was collected through filtration to afford the desired complex 98 in 96.9% yield (113.4 mg). ³¹P NMR (162 MHz, CDCl₃) δ : 41.5 (m), 25.3 (m) ppm.

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2.19. A typical procedure for the hydrogenation of 2-acetamidocinnamic acid

In a nitrogen atmosphere glovebox, a stainless steel reactor was charged with 2-acetamidocinnamic acid (1.0 mg, 4.87 μmol) in the presence or absence of avidin (2.5 mg, 27.75 units, binds 0.1126 μmol of biotin) followed by the addition of the 0.1 M Na₂HPO₄ buffered solution (1.0 mL, pH 7.0), and the catalyst solution in methanol (40 μL of a 2.5 mM catalyst solution, 0.1 μmol). The reactor was closed and stirred at ambient temperature for 30 min to ensure the complete association of biotin with avidin. The reactor was pressurized to the pre-determined pressure of H₂ and the resulting mixture was allowed to stir at ambient temperature for 2 to 4 days. The reaction mixture was worked up by addition of 2N aqueous HCl solution and filtered through Celite to remove the resulting precipitate. The filtrate was concentrated under reduced pressure and extracted with chloroform. The collected chloroform layer was dried over MgSO₄ and concentrated to give a crude solution. The level of conversion and the ee of the hydrogenated products were determined from the solution after converting the product to its methyl ester derivative by GC analyses using the following conditions: Chrompack Chiralsil-L-Val capillary column, 25 m length, 0.25 mm inner diameter, nitrogen as carrier gas (15 psi), 180 °C. Retention time for 2-acetamidocinnamic acid methyl ester, min; R form of methyl 2-acetamido-3-phenylpropanoate, 4.740 min; S form, 5.025 min.
2.20. A typical procedure for the hydrogenation of 2-acetamidoacrylic acid

In a nitrogen atmosphere glovebox, a stainless steel reactor was charged with 2-acetamidoacrylic acid (1.0 mg, 7.74 μmmol) in the presence or absence of avidin (2.5 mg, 27.75 units, binds 0.1126 μmmol of biotin) followed by the addition of the 0.1 M Na₂HPO₄ buffered solution (1.0 mL, pH 7.0), and the catalyst solution in methanol (40 μL of a 2.5 mM catalyst solution, 0.1 μmmol). The reactor was closed and stirred at ambient temperature for 30 min to ensure the complete association of biotin with avidin. The reactor was pressurized to the pre-determined pressure of H₂ and the resulting mixture was allowed to stir at ambient temperature for 2 to 4 days. The reaction mixture was worked up by addition of 2N aqueous HCl solution and filtered through Celite to remove the resulting precipitate. The filtrate was concentrated under reduced pressure and extracted with chloroform. The collected chloroform layer was dried over MgSO₄ and concentrated to give a crude solution. The level of conversion and the ee of the hydrogenated products were determined from the solution after converting the product to its methyl ester derivative by GC analyses using the following conditions: Chrompack Chiralsil-DEX CB capillary column, 25 m length, 0.25 mm inner diameter, nitrogen as carrier gas (15 psi), 115 °C. Retention time for 2-acetamidoacrylic acid methyl ester, 3.96 min; R form of acetamidopropanoic acid methyl ester, 7.460 min; S form, 7.195 min.
2.21. A typical procedure for the hydrogenation of itaconic acid

In a nitrogen atmosphere glovebox, a stainless steel reactor was charged with itaconic acid (1.0 mg, 7.7 µmol) in the presence or absence of avidin (2.5 mg, 27.75 units, binds 0.1126 µmol of biotin) followed by the addition of the 0.1 M Na₂HPO₄ buffered solution (1.0 mL, pH 7.0), and the catalyst solution in methanol (40 µL of a 2.5 mM catalyst solution, 0.1 µmol). The reactor was closed and stirred at ambient temperature for 30 min to ensure the complete association of biotin with avidin. The reactor was pressurized to the predetermined pressure of H₂ and the resulting mixture was allowed to stir at ambient temperature for 2 to 7 days. The reaction mixture was worked up by addition of 2N aqueous HCl solution and filtered through Celite to remove the resulting precipitate. The filtrate was concentrated under reduced pressure and extracted with chloroform. The collected chloroform layer was dried over MgSO₄ and concentrated to give a crude solution. The level of conversion and the ee of the hydrogenated products were determined from the crude solution by GC analyses using the following conditions: Chrompack CP-chiralsil-DEX CB capillary column, 50 m length, 0.25 mm inner diameter, nitrogen as carrier gas (15 psi), 130 °C. Retention time for itaconic acid, 10.7 min; R form of methylsuccinic acid, 15.6 min; S form, 15.9 min.
2.22. A typical procedure for the hydrogenation of tiglic acid

In a nitrogen atmosphere glovebox, a stainless steel reactor was charged with tiglic acid (1.0 mg, 7.7 μmmol) in the presence or absence of avidin (2.5 mg, 27.75 units, binds 0.1126 μmmol of biotin) followed by the addition of the 0.1 M Na₂HPO₄ buffered solution (1.0 mL, pH 7.0), and the catalyst solution in methanol (40 μL of a 2.5 mM catalyst solution, 0.1 μmmol). The reactor was closed and stirred at ambient temperature for 30 min to ensure the complete association of biotin with avidin. The reactor was pressurized to the predetermined pressure of H₂ and the resulting mixture was allowed to stir at ambient temperature for 2 to 7 days. The reaction mixture was worked up by addition of 2N aqueous HCl solution and filtered through Celite to remove the resulting precipitate. The filtrate was concentrated under reduced pressure and extracted with chloroform. The collected chloroform layer was dried over MgSO₄ and concentrated to give a crude solution. The level of conversion and the ee of the hydrogenated product were determined from the crude solution by GC analyses using the following conditions: Chrompack CP-chiralsil-DEX CB capillary column, 25 m length, 0.25 mm inner diameter, nitrogen as carrier gas (15 psi), 110 °C. Retention time for tiglic acid, 8.847 min; R form of 2-methylbutyric acid, 7.370 min; S form, 6.935 min.
2.23. A typical procedure for the hydrogenation of 2-(6'-methoxy-2'-naphthyl)propenoic acid

In a nitrogen atmosphere glovebox, a stainless steel reactor was charged with sodium 2-(6'-methoxy-2'-naphthyl)propenate (2.5 mg, 10.0 μmmol) in the presence or absence of avidin (2.5 mg, 27.75 units, binds 0.1126 μmmol of biotin) followed by the addition of the 0.1 M Na₂HPO₄ buffered solution (1.0 mL, pH 7.0), and the catalyst solution in methanol (40 μL of a 2.5 mM catalyst solution, 0.1 μmmol). The reactor was closed and stirred at ambient temperature for 30 min to ensure the complete association of biotin with avidin. The reactor was pressurized to the pre-determined pressure of H₂ and the resulting mixture was allowed to stir at ambient temperature for 10 h. The reaction mixture was worked up by addition of 2N aqueous HCl solution and filtered through Celite to remove the resulting precipitate. The filtrate was concentrated under reduced pressure and extracted with chloroform. The collected chloroform layer was dried over MgSO₄ and concentrated to give a crude solution. The level of conversion and the ee of the hydrogenated product were determined from the crude solution by HPLC analyses using the following conditions: SUMICHIRAL OA-2500 column; 6 mM NH₄OAc of MeOH solution; flow rate = 1.2 mL/min; tᵣ of (R)-naproxen = 14.63 min; tᵣ of (S)-naproxen = 17.33 min; tᵣ of 2-(6'-methoxy-2'-naphthyl)propenoic acid = 14.82 min.
Chapter 3
Synthesis and Application of Various Biotinylated Chiral Diphosphine Ligands in Homogeneous Hydrogenation Reactions in the Presence of Avidin

3.1. Preparation of (R,R)- and (S,S)-Pyrphos Ligands and Their Biotinylated Derivatives

We started with the preparation of nitrogen-containing heterocyclic diphosphines, because the most commonly used biotinylation reagents are N-hydroxysuccinimide (NHS) esters of biotin, which are found to be reactive toward various amine compounds. Several different NHS esters of biotin are commercially available, with varying properties and spacer arm length. The C" symmetric 3,4-bis(diphenylphosphino)pyrrolidine ligands, abbreviated as Pyrphos, are chosen to serve as the standard diphosphine ligands for the construction of the biotinylated chiral diphosphines. The synthetic methods mostly followed the published procedures [68] but with a little modifications. The complete synthetic routes are outlined as Scheme 3.1.

Starting from either enantiomer of tartaric acid, we synthesized the corresponding homochiral N-benzyl 3,4-dihydroxypyrrroloidinediones 70 with 75% chemical yield. Reduction of 70 by borane-THF complex gave the respective enantiopure 3,4-dihydroxypyrrolidines 71 with 81% yield. The following mesylated compound 72 was obtained via the general procedures of mesylation by treating mesylchloride in the presence of triethylamine. Hydrogenation reaction with palladium on charcoal in the presence of acetic acid led to the ammonium acetate salt product 73. The desired diphosphate was
attained by treating 73 with potassium diphenylphosphite in DMF followed by addition of 2 N aqueous hydrochloric acid to give the product 74 as a salt.

\[
\text{L(+)-Tartaric acid} \quad 69 \quad \xrightarrow{\text{xylene, reflux 10h}} \quad 70 \quad (75\%)
\]

\[
72 \quad (85\%)
\]

\[
71 \quad (81\%)
\]

\[
1\text{atm} \text{H}_2, \text{Pd/C} \quad \xrightarrow{\text{HOAc/H}_2\text{O/MeOH, rt, 6h}}
\]

\[
73 \quad (90\%)
\]

\[
74 \quad (76\%)
\]

**Scheme 3.1.** Synthetic Routes of Pyrophos ligand.

This type of heterocyclic diphosphine compounds [68–70] has been extensively studied for the purpose of developing the polymer-supported ligands. Similarly, the amine functionality on these heterocyclic diphosphines could be used for connection of the diphosphine ligands and biotin. Besides,
both two optical forms of Pyrphos ligands were prepared and converted to their corresponding (+)-biotinylated derivatives in order to observe the real influence caused by the tertiary structure within the avidin cavity. The preparation of biotin-NHS reagent and the biotinylation reaction of Pyrphos ligands are shown in Scheme 3.2.

**Scheme 3.2.** Synthetic details of the biotinylated Pyrphos ligand.
3.2. Hydrogenation Reactions of Dehydro α-Amino Acids Catalyzed by Biotinylated Pyrphos-Rhodium(I) Complex with and without Association of Avidin

The rhodium(I) catalysts used for the hydrogenation were prepared by the complexation of bis(1,5-cyclooctadiene)rhodium(I) tetrafluoroborate, [Rh(COD)$_2$]BF$_4$, with Pyrphos ligand and its biotinylated derivative. Four catalysts, as shown in Scheme 3.3, were then applied to catalyze the hydrogenation of (Z)-acetamido-3-phenylacrylic acid in a 0.1 M, pH 7 sodium phosphate solution or methanol or the mixture of each other.

![Chemical structures](image)

**Scheme 3.3.** The catalysts prepared for the hydrogenation of dehydro α-amino acids and α,β-unsaturated carboxylic acids.

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The first trial of the hydrogenation with these catalysts was carried out in three different solvent systems under 1000 psi H₂ at ambient temperature. It was found that 1 mg of the substrate, (Z)-acetamido-3-phenylacrylic acid, can dissolve gradually in 1 mL of the sodium phosphate buffered solution after stirring for 10 minutes. Whereas the substrate concentration larger than 1 mg per mL buffer was found to be barely homogeneous. Also, the solubility would increase largely as adding some methanol as a cosolvent. The results of the hydrogenation are summarized in Table 3.1.

**Table 3.1.** Hydrogenations of (Z)-acetamido-3-phenylacrylic acid catalyzed by complexes 77, 78 and 79, 80 with and without avidin.¹

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Solvent</th>
<th>ee (%)⁵</th>
<th>Config¹</th>
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</thead>
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</tr>
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<td>R</td>
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<td>80</td>
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<td>avidin+80¹</td>
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<td>11.1</td>
<td>S</td>
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a). The hydrogenations were carried out at 22 °C in different solvent systems under 1000 psi H₂ with S/C=50 (molar ratio) for 72 h. b). The ee values and conversions were determined by GC analyses with a 25m Chrompack chiralsil-L-Val capillary column. c). The absolute configurations were determined by comparing the optical rotations with literature values. d). 1.1 eq. avidin based on the biotinylated Pyrophos-Rh(I) complex was added.

According to the Table 3.1, it is obvious that the resulting ee values appeared to be largely dependent on the solvent used. It is notable that the incorporation of biotin with Pyrophos-Rh(I) complex leads to some effect on enantioselectivity of the hydrogenation carried out in methanol with the preference of R chirality (entry 1, 4 and 10, 13). In addition, the hydrogenation reactions carried out in the mixture solvent of methanol/buffer gave the generally poorer enantioselectivities. Unexpectedly, the hydrogenation reactions catalyzed by catalyst 79 with and without association of avidin in the mixture solvent led to the hydrogenated products with the inverse chiral preference (entry 5, 8). The real origin resulted in this event remains unclear, but one thing can be sure is that the chirality of d-biotin contributes something to the alteration on the spacial conformations of the Pyrophos ligand. This effect seems to be the dominant factor responsible for the enantioselectivity as proceeding in the mixture solvent, whereas the effect appeared less effective in the hydrogenation reactions in pure methanol or buffered solution (entry 4, 6 and 13, 15). As for the hydrogenation reactions catalyzed by the avidin-containing catalysts, there are some noticeable features can be found in the Table 3.1. Clearly, the introduction of avidin as a chiral cavity did cause certain influence on the enantioselectivity. The extent of influence appeared to be relative to the solvent systems. It is rational for this observation because the conformation of avidin is mainly driven by the protein folding process which is
sensitive to the solvent system. However, the incorporation of avidin did not promote the enantioselectivity nor increase the reaction rate in the hydrogenation reactions (entry 7–9 and 16–18) tabulated in Table 3.1. More apparent variation on the enantioselectivity was observed in the reactions performed in buffered solution (entry 15, 18). This may be due to the better swelling structure of avidin molecule in aqueous media than that in organic solvents. More swelling structure of avidin molecule means that more interactions between substrate and catalyst should be involved in the hydrogenation process. Nevertheless, no significant alteration on reactivity of the biotinylated Pyrphos-Rh(1) complex before and after associating with avidin was observed in this experiment.

In general, the hydrogenation reactions carried out in buffered solution appeared to be much inactive than those in methanol. Although this hydrogenation of (Z)-acetamido-3-phenylacrylic acid could be proceeded homogeneously in aqueous solution, the rate appeared to be very slow (conversion < 10%) as compared with that in methanol (> 99%). The poor solubility of (Z)-acetamido-3-phenylacrylic acid in buffer should be taken into account for this consequence. Therefore we chose 2-acetamidoacrylic acid as the substrate to perform the hydrogenation reactions with these same catalysts under similar conditions. The results are summarized in Table 3.2.
Table 3.2. Hydrogenations of acetamidoacrylic acid catalyzed by complexes 77, 78 and 79, 80 with and without avidin.

<table>
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<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Solvent</th>
<th>ee (%)</th>
<th>Config</th>
<th>Conv. (%)</th>
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a). The hydrogenations were carried out at 22 °C in different solvent systems under 1000 psi H₂ with S/C=80 (molar ratio) for 48 h. b). The ee values and conversions were determined by GC analyses with a 25m Chrompack Chiralsil-DEX CB capillary column. c). The absolute configurations were determined by comparing the optical rotations with literature values. d). 1.1 eq. avidin based on the biotinylated Pyrophos-Rh(I) complex was added.
From the data in Table 3.2, the incorporation of biotin with Pyrphos-Rh(I) complex (catalyst 78 and 80) led to some effects on enantioselectivity of the hydrogenation in methanol with the preference of R chirality (entry 1, 4 and 10, 13), which are consistent with the results in Table 3.1. That phenomenon probably results from the approach of the two cis-fused rings of biotin to Rh atom by bending the five carbon side chain, which is likely due to intramolecular hydrogen bonding. Moreover, the association of avidin with biotin-Pyrphos-Rh(I) complex does also cause various conformational differences around the Rh atom in different solvents and that accounts for the dramatic drops of ee values. However, the influence of avidin on enantioselectivity, contrary to our expectation, does not accord with the same chiral preference. The consequence also contradicted the hypothesis that avidin is conformationally stable under such conditions. In fact, almost all naturally occurring proteins fold in water because of the molecular recognition phenomenon that depends on the cooperative action of many weak nonbonding interaction. An important feature of protein folding is that the overall structure is determined by the sequence of the protein. And the difference in sequence gives rise to differences in the nature of the secondary structure (the regions of α-helix and β-sheet) and the tertiary structure (the overall folding pattern). That is to say, avidin should fold into its unique three-dimensional structure in water as that is characteristic for each protein. Nevertheless, protein folding has a strong temperature dependence. Based on the premise, the hydrogenations catalyzed by avidin-containing catalysts at different temperatures were carried out for the purpose of comparison. Besides, the hydrogenations in pH 6 buffered solution were also carried out in order to examine if the pH could affect the enantioselectivity of the hydrogenation. The
temperature and pH effects on the enantioselectivity are summarized in Table 3.3.

**Table 3.3.** The temperature and pH effects on the enantioselectivity of the hydrogenation of α-acetamidoacrylic acid.*

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<th>Entry</th>
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<th>Temp. (°C)</th>
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<th>ee (%)</th>
<th>Config*</th>
<th>Conv. (%)</th>
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<td>S</td>
<td>6.3</td>
</tr>
<tr>
<td>10*</td>
<td>avidin+80</td>
<td>7.0</td>
<td>0°C</td>
<td>4</td>
<td>5.1</td>
<td>S</td>
<td>6.8</td>
</tr>
<tr>
<td>11</td>
<td>avidin+80</td>
<td>6.0</td>
<td>0°C</td>
<td>4</td>
<td>17.3</td>
<td>S</td>
<td>51.7</td>
</tr>
<tr>
<td>12</td>
<td>avidin+80</td>
<td>7.0</td>
<td>50°C</td>
<td>2</td>
<td>21.5</td>
<td>S</td>
<td>&gt;99</td>
</tr>
</tbody>
</table>

a). The hydrogenations were carried out in a 0.1 M NaH₂PO₄ buffered solution under 1000 psi H₂ with S/C=80 (molar ratio) for 2–4 days. b). The ee values and conversions were determined by GC analyses with a 25m Chrompack Chiralsil-DEX CB capillary column. c). The absolute configurations were determined by comparing the optical rotations with literature values. d). 1.1 eq. avidin based on the biotinylated Pyrphos-Rh(I) complex was added. e) 2.0 eq. avidin based on the biotinylated Pyrphos-Rh(I) complex was added.

From the data in Table 3.3, it can be found that the enantioselectivities were very dependent on temperature. The trend of ee values was driven to the same preference of chirality as the temperature was lowered. These results also demonstrated that the globular protein, avidin, was aggregated to a tertiary
structure which was stable enough to rearrange the spacial array of the metal complex into a fixed conformation if the temperature was as lower as 0 °C. In addition, the solutions (entry 4, 10) containing 0.5 equiv. of Rh(I) catalyst per avidin subunit showed the similar enantioselectivity with those containing 1.0 equiv. That may reflect the four biotin-binding sites of the avidin tetramer are equivalent and noninteracting under such conditions.

3.3. Hydrogenation Reactions of Itaconic Acid and Tiglic Acid Catalyzed by Biotinylated Pyrphos-Rhodium(I) Complex with and without Association of Avidin

In order to proceed the hydrogenation reactions in the hydrolytic media, the choice of substrate became the essential factor for consideration. In addition to the dehydro α-amino acids, the type of α, β-unsaturated carboxylic acids appears to be the suitable candidate for selection of substrate on account of their good solubility in the aqueous solution. In addition, according to the literature, the Pyrphos-Rh(I) complex was found to be poor catalyst for the hydrogenation of α,β-unsaturated carboxylic acids. Thus we chose two kinds of α, β-unsaturated carboxylic acids, itaconic acid and tiglic acid, for the hydrogenation with this avidin-based catalyst system.

The catalysts prepared for this study as shown in Scheme 3.3 were also used to catalyze the hydrogenation of itaconic acid. The initial results of the hydrogenation are summarized in Table 3.4.
Table 3.4. Hydrogenations of itaconic acid catalyzed by complexes 77, 78 and 79, 80 with and without avidin under different hydrogen pressure. a.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Pressure of H₂ (psi)</th>
<th>ee (%)</th>
<th>Config.</th>
<th>Conv. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>77</td>
<td>1000</td>
<td>1.1</td>
<td>S</td>
<td>94.3</td>
</tr>
<tr>
<td>2</td>
<td>77</td>
<td>100</td>
<td>6.5</td>
<td>S</td>
<td>96.7</td>
</tr>
<tr>
<td>3</td>
<td>77</td>
<td>15</td>
<td>7.9</td>
<td>S</td>
<td>23.0</td>
</tr>
<tr>
<td>4</td>
<td>79</td>
<td>1000</td>
<td>21.4</td>
<td>S</td>
<td>98.2</td>
</tr>
<tr>
<td>5</td>
<td>79</td>
<td>100</td>
<td>11.1</td>
<td>S</td>
<td>74.0</td>
</tr>
<tr>
<td>6</td>
<td>79</td>
<td>15</td>
<td>25.5</td>
<td>S</td>
<td>51.6</td>
</tr>
<tr>
<td>7</td>
<td>avidin+79</td>
<td>1000</td>
<td>6.0</td>
<td>S</td>
<td>86.6</td>
</tr>
<tr>
<td>8</td>
<td>avidin+79i</td>
<td>100</td>
<td>44.2</td>
<td>R</td>
<td>&gt;99</td>
</tr>
<tr>
<td>9</td>
<td>avidin+79i</td>
<td>15</td>
<td>48.1</td>
<td>R</td>
<td>40.2</td>
</tr>
<tr>
<td>10</td>
<td>78</td>
<td>1000</td>
<td>1.3</td>
<td>R</td>
<td>96.1</td>
</tr>
<tr>
<td>11</td>
<td>78</td>
<td>100</td>
<td>2.2</td>
<td>R</td>
<td>80.5</td>
</tr>
<tr>
<td>12</td>
<td>78</td>
<td>15</td>
<td>11.8</td>
<td>R</td>
<td>6.4</td>
</tr>
<tr>
<td>13</td>
<td>80</td>
<td>1000</td>
<td>15.4</td>
<td>R</td>
<td>&gt;99</td>
</tr>
<tr>
<td>14</td>
<td>80</td>
<td>100</td>
<td>3.2</td>
<td>R</td>
<td>56.2</td>
</tr>
<tr>
<td>15</td>
<td>80</td>
<td>15</td>
<td>28.6</td>
<td>R</td>
<td>11.8</td>
</tr>
<tr>
<td>16</td>
<td>avidin+80</td>
<td>1000</td>
<td>36.8</td>
<td>S</td>
<td>&gt;99</td>
</tr>
<tr>
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<td>avidin+80i</td>
<td>100</td>
<td>15.5</td>
<td>S</td>
<td>&gt;99</td>
</tr>
<tr>
<td>18</td>
<td>avidin+80i</td>
<td>15</td>
<td>11.2</td>
<td>R</td>
<td>7.6</td>
</tr>
</tbody>
</table>

a). The hydrogenations were carried out at 22 °C in a 0.1 M NaH₂PO₄ buffered solution (pH 7.0) with S/C=80 (molar ratio) for 48 h. b). The ee values and conversions were determined by GC analyses with a 50m Chrompack Chiralsil-DEX CB capillary column. c). The absolute configurations were determined by comparing the optical rotations with literature values. d). 1.1 eq. avidin based on the biotinylated Pyrphos-Rh(I) complex was added.

From the data in Table 3.4, it is obvious that the incorporation of biotin with Pyrphos-Rh(I) complex had certain effect on enantioselectivity. This effect is not surprising because biotin itself is chiral. At this stage, it is unclear how the biotin affected the enantioselectivity of the catalyst. The enhancement of the
resulting ee values caused by 79 and 80 is apparently not consistent with the same chiral preference (entry 4-6, and 13-15). The change of enantioselectivity for catalyst 79 and 80 in the presence of avidin is obvious and supports our hypothesis. The enhancement of enantioselectivity by introducing avidin to the catalyst 80 system was most significant. It is also of interest to note that the introduction of avidin induced the reverse chiral preference of the catalyst while maintaining comparable conversion rates. This avidin-based catalyst system does not accelerate the rate of hydrogenated production relative to free biotinylated Pyrphos-Rh(I) complex on the basis of the experimental results. This is probably due to both the lack of specific interactions between the substrate and conjugate of avidin and biotinylated Pyrphos-Rh(I), and the absence of rate-accelerating functional groups within the cavity. The low affinity exhibited by the avidin-based system for substrate also indicates that the hydrogenated product is predominately free (unbound) and suggests that product release is not a problem in this system and that catalytic turnover should be possible. The most remarkable feature in Table 3.4 is that the consistence of chiral preference achieved by the association of avidin with 79 and 80 under 15 psi H₂ (entry 9, 18). This phenomenon implied that the peripheral asymmetric environment within the avidin cavity was the dominant factor responsible for the enantioselectivity. These results also revealed the possibility of the Pyrphos-Rh(I) complex encapsulated completely within the globular tertiary structure of avidin. Because protein folding process involves many complicated kinetic and thermodynamic variables, it is possible that the ensuing conformation through protein folding depends on the temperature as well as pH value of buffer. To evaluate these factors we also altered these two variables in the catalytic hydrogenation so as to explore the matching.
conformation of avidin. The effect of the pH values of the buffered solutions on the enantioselectivity of the catalyst is summarized in Table 3.5.

**Table 3.5.** The effect of the pH values of the buffered solutions on enantioselectivity of the catalyst in the hydrogenation of itaconic acid.\(^a\)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Pressure of (H_2) (psi)</th>
<th>pH of Buffer</th>
<th>ee(^b) (%)</th>
<th>Config.</th>
<th>Conv.(^b) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>1000</td>
<td>6</td>
<td>18.1</td>
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<td>15</td>
<td>6</td>
<td>3.1</td>
<td>R</td>
<td>47.7</td>
</tr>
<tr>
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<td></td>
<td>1000</td>
<td>7</td>
<td>6.0</td>
<td>S</td>
<td>86.6</td>
</tr>
<tr>
<td>5</td>
<td>avidin+79</td>
<td>100</td>
<td>7</td>
<td>44.2</td>
<td>R</td>
<td>&gt;99</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>15</td>
<td>7</td>
<td>48.1</td>
<td>R</td>
<td>40.2</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>1000</td>
<td>8</td>
<td>30.4</td>
<td>R</td>
<td>98.9</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>100</td>
<td>8</td>
<td>20.4</td>
<td>R</td>
<td>77.9</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>15</td>
<td>8</td>
<td>17.0</td>
<td>R</td>
<td>64.3</td>
</tr>
<tr>
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<td></td>
<td>1000</td>
<td>6</td>
<td>16.9</td>
<td>S</td>
<td>&gt;99</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>100</td>
<td>6</td>
<td>12.4</td>
<td>S</td>
<td>&gt;99</td>
</tr>
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<td>15</td>
<td>6</td>
<td>5.1</td>
<td>S</td>
<td>59.1</td>
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<td>1000</td>
<td>7</td>
<td>36.8</td>
<td>S</td>
<td>&gt;99</td>
</tr>
<tr>
<td>14</td>
<td>avidin+80</td>
<td>100</td>
<td>7</td>
<td>15.5</td>
<td>S</td>
<td>&gt;99</td>
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<td>7</td>
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<td></td>
<td>1000</td>
<td>8</td>
<td>18.4</td>
<td>S</td>
<td>71.7</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>100</td>
<td>8</td>
<td>2.9</td>
<td>S</td>
<td>49.1</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>15</td>
<td>8</td>
<td>3.3</td>
<td>S</td>
<td>46.5</td>
</tr>
</tbody>
</table>

\(^a\) The hydrogenations were carried out at 22 °C in a 0.1 M NaH\(_2\)PO\(_4\) buffered solution with S/C=80 (molar ratio) for 48 h. \(^b\) The determinations of ee values and conversions were the same as described in Table 2.4. \(^c\) 1.1 eq. of avidin based on the biotinylated Pyrophos-Rh(I) complex was added.
It can be found from Table 3.5 that the reactions carried out in various buffered solutions with different pH values resulted products in different ee values. The tendency of the produced enantioselectivities seemed to be desultory as using pH 6 or pH 8 buffered solutions as solvent systems. The pH 7 buffered solution gave the best results. It was reported that the half time of avidin–biotin complex can be as long as 200 days at 25 °C in pH 7 salt solution. But the half time decreased dramatically to 0.4 day as the pH dropped to 2. These measurements illustrate the stability of the avidin–biotin complex has a strong pH dependence. Therefore the use of severe pH buffers should be avoided. According to the results of Table 3.5, the only thing we can find is the ee values (entry 4, 13 and 6, 15) were driven to the same preference of chirality in pH 7 buffered solutions. The better ee values achieved so far were carried out under 1000 psi H₂ with avidin+80 as catalyst (entry 13) and under 1 atm H₂ with avidin+79 (entry 6) as catalyst, respectively. In addition, the reaction temperature was also found to have a profound effect on the product ee's (Table 3.6).
Table 3.6. The temperature effect on enantioselectivity in the hydrogenation of itaconic acid.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Pressure (psi)</th>
<th>Temp. (°C)</th>
<th>ee (%)</th>
<th>Config.</th>
<th>Conv. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1\textsuperscript{a}</td>
<td>avidin+79</td>
<td>1000</td>
<td>0</td>
<td>32.4</td>
<td>R</td>
<td>94.9</td>
</tr>
<tr>
<td>2\textsuperscript{b}</td>
<td>avidin+79</td>
<td>100</td>
<td>0</td>
<td>21.4</td>
<td>R</td>
<td>75.8</td>
</tr>
<tr>
<td>3\textsuperscript{b}</td>
<td>avidin+79</td>
<td>15</td>
<td>0</td>
<td>1.5</td>
<td>S</td>
<td>62.3</td>
</tr>
<tr>
<td>4\textsuperscript{b}</td>
<td>avidin+79</td>
<td>15</td>
<td>40</td>
<td>20.3</td>
<td>S</td>
<td>82.0</td>
</tr>
<tr>
<td>5\textsuperscript{b}</td>
<td>avidin+80</td>
<td>1000</td>
<td>40</td>
<td>8.6</td>
<td>S</td>
<td>89.4</td>
</tr>
<tr>
<td>6\textsuperscript{c}</td>
<td>avidin+80</td>
<td>1000</td>
<td>0</td>
<td>12.0</td>
<td>S</td>
<td>46.1</td>
</tr>
<tr>
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<td>0</td>
<td>36.1</td>
<td>R</td>
<td>32.7</td>
</tr>
<tr>
<td>8\textsuperscript{e}</td>
<td>avidin+80</td>
<td>15</td>
<td>0</td>
<td>9.6</td>
<td>S</td>
<td>87.8</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The hydrogenations were carried out at 22 °C in a 0.1 M NaH$_2$PO$_4$ buffered solution (pH 7.0) with S/C=400 (molar ratio) for 2 days.  
\textsuperscript{b} The hydrogenations were carried out with S/C=80 (molar ratio) for 4 days.  
\textsuperscript{c} The hydrogenations were carried out with S/C=80 (molar ratio) for 7 days.  
\textsuperscript{d} 1.1 eq. avidin was added based on the biotinylated Pyrophos-Rh(I) complex.  
\textsuperscript{e} The determinations of ee values and conversions were the same as described above.

It is observed from Table 3.6 that under 15 psi of hydrogen pressure, the same avidin-based catalyst may change its enantioselectivity to the extent of having an opposite chiral preference as proceeding at various temperatures (entry 7, 8). These results indicate that the structure of avidin binding site varied significantly with temperature and the influence of the cavity structure on enantioselectivity was greater than that of the coordinating chiral diphosphine ligand. One possible explanation is that the size of avidin binding site may be too tight for the accommodation of the attached Pyrophos-Rh(I) complex. Different magnitude of distortion on the chiral Pyrophos ligand produced the discrepancy of ee values in the catalytic reactions.
From the data depicted in Table 3.4–3.6, the hydrogenation reactions of itaconic acid catalyzed by the biotinylated Pyrphos-rhodium(I) catalyst system in avidin cavity were proven to be feasible in aqueous media accompanying with moderately enhanced enantioselectivity. Furthermore, the hydrogenation rates of itaconic acid appeared to be much faster than those of dehydro α-amino acids under the similar conditions. We are then interested in examining the hydrogenation of another α,β-unsaturated carboxylic acid, tiglic acid, as the substrate with the same catalyst system. The preliminary results of the hydrogenation of tiglic acid are shown in Table 3.7.

Table 3.7. Hydrogenations of tiglic acid catalyzed by complexes 77–80 with and without avidin.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>ee (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Config&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Conv. (%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>77</td>
<td>1.4</td>
<td>S</td>
<td>49.6</td>
</tr>
<tr>
<td>2</td>
<td>79</td>
<td>2.4</td>
<td>S</td>
<td>2.6</td>
</tr>
<tr>
<td>3</td>
<td>avidin+79&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.3</td>
<td>S</td>
<td>0.8</td>
</tr>
<tr>
<td>4</td>
<td>78</td>
<td>&lt;1</td>
<td>–</td>
<td>29.8</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>2.0</td>
<td>S</td>
<td>3.5</td>
</tr>
<tr>
<td>6</td>
<td>avidin+80&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.1</td>
<td>R</td>
<td>1.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> The hydrogenations were carried out at 22 °C in a 0.1 M NaH₂PO₄ buffered solution (pH 7.0) with S/C=100 (molar ratio) for 72 h. <sup>b</sup> The ee values and conversions were determined by GC analyses with a 50m Chrompack Chiralsil-DEX CB capillary column. <sup>c</sup> The absolute configurations were determined by comparing the optical rotations with literature values. <sup>d</sup> 1.1 eq. avidin based on the biotinylated Pyrphos-Rh(I) complex was added.

Unexpectedly, the hydrogenation rates of tiglic acid were found to be considerably slower than those of itaconic acid. Conversions less than 10% were obtained in the cases using the catalyst systems of 79, and 80 with or without association of avidin after reacting for 3 days. Moreover, the resulting
enantioselectivities appeared to be quite poor even after introducing the avidin cavity. Comparison of the rates between the catalyst systems with and without association of avidin cavity (entry 2, 3 and 5, 6) also demonstrates that there should be no substantial interaction between the substrate and the avidin cavity. From the data collected so far, this interaction seems not significantly involved in the hydrogenation process because similar reactivities were observed in the catalyst systems in the presence or absence of avidin. If there is a strong interaction between the substrate and avidin molecule, the catalytic hydrogenation reactions would be suppressed owing to the inhibition of the substrate from coordinating to the Rh atom. Under these circumstances, significant alteration of reactivity should be observed. Fortunately, this situation did not occur in our system. That is to say, it is likely that the avidin cavity was primarily a shape-selective pocket that only controlled the stereospecificity of the hydrogenation reaction. The produced enantioselectivity of this hydrogenation reaction was a consequence resulted from the entire asymmetric environment which is controlled both by the structure within avidin cavity and by the coordinated chiral diphosphine ligand around the metal atom. Nevertheless, the effect of the tertiary structure within avidin cavity appeared to be more dominant than the coordinated chiral ligand on the basis of above experimental results.

Besides, the hydrogenation rates of different substrates with this avidin-based catalyst system seemed to be relevant to the electronic and steric properties of the substrates. It was found that the substrate with the structure of bigger size was usually less reactive toward the hydrogenated process than its analogue with smaller structure. For example, the reactivity of 2-acetamidoacrylic acid was much better than that of 2-acetamidocinnamic acid under the same reaction conditions. As for the analogous substrates with
similar sizes, the reaction rate was found to be mainly dominated by the electronic effect of the substrate. The substrate with more electron-withdrawing groups adjacent to the C=C bond was more reactive. That accounts for the large alteration of reactivities between the itaconic acid and tiglic acid.

3.4. Preparation of Rhodium(I) Complexes with PPM Ligand and Its Biotinylated Derivative

In addition to the $C_2$-symmetric Pyrphos ligand, we also interested in the non-$C_2$-symmetric analogous $N$-heterocyclic diphosphine compounds for construction of this type of avidin-based Rh(I)-diphosphine catalyst system. Our initial efforts then focused on the preparation of 4-((diphenylphosphino)-2-(diphenylphosphino-methyl)-1-pyrrolidine ligand, abbreviated as PPM. This type of ligands was first developed by K. Achiwa in 1976[69] and proven to give the highly effective rhodium(I) catalysts for the hydrogenation reactions of dehydro $\alpha$-amino acid esters and other olefinic substrates. Also, this type of ligands has been modified into the polymer-attached chiral ligands and proven to be capable of recovery by simple filtration[71]. The total synthesis of PPM ligand was outlined as following Scheme 3.4.

This (2S,4S)-PPM ligand was synthesized from L-hydroxyproline as the starting material. Conventional esterification and butoxycarbonylation treatments of L-hydroxyproline (81) gave in a high yield the Boc-ester (83), which was then converted by reduction of the ester group with 1 molar equivalent of lithium borohydride in THF followed by tosylation with tosyl chloride in triethylamine to the ditosylate (85) in as high as 92% yield.
Subsequent reaction of 85 with potassium diphenylphosphite (KPPH$_2$) afforded the diphosphine (86) in good yield. Further treatment of 86 with an excess of trifluoroacetic acid at 0 °C for 3 hours gave the aminobisphosphine 87 as a viscous oil in 96% yield.

Scheme 3.4. The synthetic routes of PPM ligand.
Accordingly, the biotinylation of PPM ligand was proceeded by treatment of PPM and biotin-NHS reagent in DMF for 60 hours. After evaporation of DMF, the corresponding biotinylated PPM ligand can be obtained from the filtrate by addition of dichloromethane. The subsequent complexation reactions of [Rh(COD)$_2$]BF$_4$ with aminobisphosphine 87 and its biotinylated derivative 88 gave the catalyst 89 and 90, respectively, as shown in Scheme 3.5.

\[
\begin{align*}
\text{87} & \quad \xrightarrow{\text{biotin-NHS, DMF}} \quad \text{88, (S,S)-PPM-biotin} \\
\text{87} & \quad \xrightarrow{[\text{Rh(COD)}_2]BF_4} \quad [\text{Rh(COD)}_2]BF_4
\end{align*}
\]

\[
\begin{align*}
[\text{Rh(COD)}_{S,S}-\text{PPM}]BF_4 & \quad \text{89} \\
[\text{Rh(COD)}_{S,S}-\text{PPM-biotin}]BF_4 & \quad \text{90}
\end{align*}
\]

**Scheme 3.5.** Preparation of rhodium(I) complexes with PPM and biotinylated PPM ligands.
Actually, our initial plan was to prepare all the four stereoisomers of the PPM ligand for a more detailed investigation on the relationship of chirality between the coordinated ligand and the avidin cavity. However, we encountered much hardship in separating these stereoisomers during the synthetic process. This difficulty made us fail to isolate the other optically pure PPM compounds for further transformations. At this stage, we only can obtain the optically pure (2S,4S)-PPM ligand for the subsequent study on the catalytic hydrogenation reactions with the avidin-based Rh(I)-PPM catalyst system. The details will be depicted in next section.
3.5. Hydrogenation Reactions of Dehydro α-Amino Acids Catalyzed by Biotinylated PPM-Rhodium(I) Complex with and without Association of Avidin

Similarly, the (Z)-acetamido-3-phenylacrylic acid was then applied to the hydrogenation reaction under the same conditions as previously described. The results of produced enantioselectivities in different solvent systems are tabulated in Table 3.8.

**Table 3.8.** Hydrogenation reactions of (Z)-acetamido-3-phenylacrylic acid catalyzed by complex 89, and 90 with or without association of avidin.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Solvent</th>
<th>ee</th>
<th>Config</th>
<th>Conv.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>89</td>
<td>MeOH</td>
<td>10.1</td>
<td>S</td>
<td>1.3</td>
</tr>
<tr>
<td>2</td>
<td>89</td>
<td>MeOH/Buffer (v/v=1/1)</td>
<td>16.8</td>
<td>S</td>
<td>3.1</td>
</tr>
<tr>
<td>3</td>
<td>89</td>
<td>Buffer</td>
<td>4.3</td>
<td>R</td>
<td>1.1</td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>MeOH</td>
<td>55.4</td>
<td>R</td>
<td>90.9</td>
</tr>
<tr>
<td>5</td>
<td>90</td>
<td>MeOH/Buffer (v/v=1/1)</td>
<td>86.5</td>
<td>R</td>
<td>98.9</td>
</tr>
<tr>
<td>6</td>
<td>90</td>
<td>Buffer</td>
<td>72.8</td>
<td>R</td>
<td>40.6</td>
</tr>
<tr>
<td>7</td>
<td>avidin+90</td>
<td>MeOH</td>
<td>83.6</td>
<td>R</td>
<td>99.3</td>
</tr>
<tr>
<td>8</td>
<td>avidin+90</td>
<td>MeOH/Buffer (v/v=1/1)</td>
<td>87.4</td>
<td>R</td>
<td>99.7</td>
</tr>
<tr>
<td>9</td>
<td>avidin+90</td>
<td>Buffer</td>
<td>11.3</td>
<td>R</td>
<td>53.4</td>
</tr>
</tbody>
</table>

*a*. The hydrogenations were carried out at 22 °C in different solvent systems under 1000 psi H₂ with S/C=50 (molar ratio) for 48 h.  
*b*. The ee values and conversions were determined by GC analyses with a 25m Chrompack chiralalil-L-Val capillary column.  
*c*. The absolute configurations were determined by comparing the optical rotations with literature values.  
*d*. 1.1 eq. avidin based on the biotinylated PPM-Rh(I) complex was added.

From the data of Table 3.8, it is worth noting that the reactivity of the rhodium(I)-biotin-PPM catalyst 90 appeared to be more satisfactory as
compared with that of rhodium(I)-PPM catalyst 89 under the same hydrogenation conditions. In addition, the introduction of d-biotin did cause some effect on the ensuing enantioselectivity of the hydrogenation with the preference of R chirality. An analogous result has been observed from previous experimental data in Table 3.1. Interestingly, the fact that the enhancement on enantioselectivity between entry 4 and 7 revealed the incorporated avidin can affect the stereospecificity of the hydrogenation even carried out in methanol. The extent of influence contributed by avidin on stereospecificity was found to be fairly dependent on the solvent system used. The best ee value in Table 2.8 was obtained with the catalyst 90 associated with avidin in the 1:1 methanol-buffer solvent system (entry 8). However, only few effect was contributed by the avidin in this kind of solvent system owing to the similar ee values observed in entry 5 and 8. Clearly, the most apparent effect caused by avidin on enantioselectivity was observed in the hydrogenations carried out in pure buffered solution (entry 6 and 9). However, destructive enantioselectivity was attained after introducing avidin with catalyst 90.

The activity of the biotinylated PPM-Rh(I) catalyst appeared to be much better than the biotinylated Pyrphos-Rh(I) catalyst for the catalytic hydrogenation reactions. Besides, comparable reaction rates were found in the hydrogenations with biotinylated PPM-Rh(I) catalyst no matter in the presence or absence of avidin in the buffered solution, whereas poorer reactivity was observed in the case of avidin–biotinylated Pyrphos-Rh(I) catalyst system in comparison with that without avidin. The exact reason for this phenomenon remains unclear at this stage, however it is supposed to have something to do with the difference of steric structures arisen in the buffered solution between Pyrphos and PPM ligands.
The same hydrogenation reactions except the change of hydrogen pressure from 1000 psi to 15 psi were carried out and summarized in Table 3.9.

**Table 3.9.** Hydrogenation reactions of (Z)-acetamido-3-phenylacrylic acid catalyzed by complex 89, and 90 with or without association of avidin.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Solvent</th>
<th>ee</th>
<th>Config.</th>
<th>Conv.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>89</td>
<td>MeOH</td>
<td>1.3</td>
<td>S</td>
<td>1.8</td>
</tr>
<tr>
<td>2</td>
<td>89</td>
<td>MeOH/Buffer (v/v=1/1)</td>
<td>–</td>
<td>–</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>3</td>
<td>89</td>
<td>Buffer</td>
<td>–</td>
<td>–</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>MeOH</td>
<td>92.8</td>
<td>R</td>
<td>38.1</td>
</tr>
<tr>
<td>5</td>
<td>90</td>
<td>MeOH/Buffer (v/v=1/1)</td>
<td>83.4</td>
<td>R</td>
<td>11.6</td>
</tr>
<tr>
<td>6</td>
<td>90</td>
<td>Buffer</td>
<td>79.1</td>
<td>R</td>
<td>2.8</td>
</tr>
<tr>
<td>7</td>
<td>avidin+90(^d)</td>
<td>MeOH</td>
<td>94.0</td>
<td>R</td>
<td>33.9</td>
</tr>
<tr>
<td>8</td>
<td>avidin+90(^d)</td>
<td>MeOH/Buffer (v/v=1/1)</td>
<td>82.1</td>
<td>R</td>
<td>4.5</td>
</tr>
<tr>
<td>9</td>
<td>avidin+90(^d)</td>
<td>Buffer</td>
<td>45.8</td>
<td>R</td>
<td>7.0</td>
</tr>
</tbody>
</table>

\(^a\) The hydrogenations were carried out at 22 °C in different solvent systems under 15 psi H\(_2\) with S/C=50 (molar ratio) for 48 h. \(^b\) The ee values and conversions were determined by GC analyses with a 25m Chrompack chirasil-L-Val capillary column. \(^c\) The absolute configurations were determined by comparing the optical rotations with literature values. \(^d\) 1.1 eq. avidin based on the biotinylated PPM-Rh(I) complex was added.

According to the data of Table 3.8 and 3.9, it can be found that the resulting chiral preference of enantioselectivities of the same hydrogenation reactions seemed not to be sensitive to the hydrogen pressure applied. Under low hydrogen pressure, higher enantioselectivities were obtained as using complex 90 as the catalyst. The trend of ee values shown in entry 4–6, however, was observed to have a inverse relationship in comparison with those data collected under 1000 psi H\(_2\) (entry 4–6 of Table 3.8). That is to say, higher ee was obtained in the pure MeOH under low hydrogen pressure; lower ee was
obtained in the buffer solution under high hydrogen pressure. The common feature between Table 3.8 and 3.9 is that decrement of ee was found in the case of introducing avidin into the catalyst \textbf{90} in the buffered solution. Besides, the change of ee values after introducing avidin as the chiral cavity was observed to be more apparent in the hydrogenations carried out under high hydrogen pressure (compared entry 9 of Table 3.8 and entry 9 of Table 3.9). This event may be due to the difference of the appeared spacial conformations (protein folding) of avidin under the different pressures.

Similarly, the catalysts \textbf{89} and \textbf{90} were also applied to catalyze the hydrogenation reactions of 2-acetamidoacrylic acid in the buffered solution of pH 7. The effect of hydrogen pressure on the resulting enantioselectivity has been screened and the results are shown in Table 3.10.
Table 3.10. The hydrogenation of 2-acetamidoacrylic acid catalyzed by 89 and 90 with and without association of avidin under different hydrogen pressure.a

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Pressure of H₂ (psi)</th>
<th>ee (^b) (%)</th>
<th>Config (^c)</th>
<th>Conv. (^b) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>89</td>
<td>1000</td>
<td>~0</td>
<td>-</td>
<td>8.8</td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>1000</td>
<td>3.0</td>
<td>S</td>
<td>72.6</td>
</tr>
<tr>
<td>3</td>
<td>avidin+90</td>
<td>1000</td>
<td>26.7</td>
<td>S</td>
<td>18.5</td>
</tr>
<tr>
<td>4</td>
<td>89</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>5</td>
<td>90</td>
<td>100</td>
<td>65.0</td>
<td>R</td>
<td>99.2</td>
</tr>
<tr>
<td>6</td>
<td>avidin+90</td>
<td>100</td>
<td>2.9</td>
<td>S</td>
<td>38.7</td>
</tr>
<tr>
<td>7</td>
<td>89</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>8</td>
<td>90</td>
<td>15</td>
<td>82.6</td>
<td>R</td>
<td>44.8</td>
</tr>
<tr>
<td>9</td>
<td>avidin+90</td>
<td>15</td>
<td>23.9</td>
<td>R</td>
<td>19.3</td>
</tr>
</tbody>
</table>

a). The hydrogenations were carried out at 22 °C under different pressure of H₂ with S/C=80 (molar ratio) for 36 h. b). The ee values and conversions were determined by GC analyses with a 25m Chrompack CP-chiralsil-DEX CB capillary column. c). The absolute configurations were determined by comparing the optical rotations with literature values. d). 1.1 eq. avidin based on the biotinylated PPM-Rh(I) complex was added.

Accordingly, the reactivity of PPM-Rh(I) complex was also found to be quite low for the hydrogenation of 2-acetamidoacrylic acid in the buffered solution. After reacting for 36 h at room temperature, conversions less than 10% were obtained even under the condition of 1000 psi H₂. In addition, a racemic result was observed in the hydrogenation catalyzed by the PPM-Rh(I) complex in the buffered solution (entry 1). It is notable that the poor reactivity of this type of PPM-Rh(I) complex was found to be promoted after introducing the biotin moiety. This is likely due to the solubility of the biotinylated PPM-Rh(I) complex was much better than the PPM-Rh(I) complex in aqueous media. In the biotinylated PPM-Rh(I) catalyst system, the ee values appeared to be fairly
sensitive to the hydrogen pressure applied. Also, almost racemic result was observed when the reaction was carried out under 1000 psi \( H_2 \) (entry 2). The best ee value of 82.6\% in Table 3.10 was obtained under 15 psi \( H_2 \) (entry 8). The change of enantioselectivity for the biotinylated PPM-Rh(I) catalyst in the presence of avidin is clearly observed and verified once again the hypothesis that the avidin cavity is indeed capable of introducing certain selectivity for the hydrogenation reaction. The most interesting event is that the same avidin-based biotinylated PPM-Rh(I) catalyst may change its enantioselectivity to the extent of having an opposite chiral preference when the reaction was carried out at different hydrogen pressures (entry 3, 6, and 9). However, only the destructive results on enantioselectivity have been attained for the avidin-based catalyst system. The biotinylated PPM-Rh(I) catalyst appeared to give better ee values as compared with the one after introducing avidin as the cavity. Therefore, we are interested in exploring if the chiral biotin moiety can contribute some levels of enantioselectivity for this hydrogenation reaction. The analogous BPPM-Rh(I) catalyst was prepared for the purpose of comparison. The preparation of the BPPM-Rh(I) catalyst (91) was shown in the following scheme.

![Scheme 3.6: The preparation of BPPM-Rh(I) catalyst (91).](image)

**Scheme 3.6.** The preparation of BPPM-Rh(I) catalyst (91).
The hydrogenations of 2-acetamidoacrylic acid catalyzed by the BPPM-Rh(I) complex were carried out under the same conditions as described in Table 3.10. The comparison data were listed in the Table 3.11.

**Table 3.11.** The hydrogenations of 2-acetamidoacrylic acid with biotinylated PPM-Rh(I) (90) and BPPM-Rh(I) (91) catalysts under different pressures.a

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Pressure of H₂ (psi)</th>
<th>ee b</th>
<th>Config. c</th>
<th>Conv. d (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>1000</td>
<td>3.0</td>
<td>S</td>
<td>72.6</td>
</tr>
<tr>
<td>2</td>
<td>91</td>
<td>1000</td>
<td>32.0</td>
<td>S</td>
<td>98.7</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>100</td>
<td>65.0</td>
<td>R</td>
<td>99.2</td>
</tr>
<tr>
<td>4</td>
<td>91</td>
<td>100</td>
<td>52.0</td>
<td>R</td>
<td>98.9</td>
</tr>
<tr>
<td>5</td>
<td>90</td>
<td>15</td>
<td>82.6</td>
<td>R</td>
<td>44.8</td>
</tr>
<tr>
<td>6</td>
<td>91</td>
<td>15</td>
<td>89.1</td>
<td>R</td>
<td>99.3</td>
</tr>
</tbody>
</table>

a). The hydrogenations were carried out at 22 °C under different pressure of H₂ with S/C=80 (molar ratio) for 36 h. b). The ee values and conversions were determined by GC analyses with a 25m Chrompack CP-chiralsil-DEX CB capillary column. c). The absolute configurations were determined by comparing the optical rotations with literature values.

The data shown in above table revealed that the introduction of an electron-withdrawing substituent onto the nitrogen atom of PPM ligand can not only accelerate the reactivity but also increase remarkably the enantioselectivity. Clearly, the trend of chiral preferences caused by catalyst 90 and 91 is found to be parallel with each other, and the resulting ee values are also comparable. That means the biotin moiety seems not a contributive group for enantioselectivity in view of the similar ee values collected by catalyst 90 and 91. Nevertheless, by taking advantage of the rapid and irreversible association
process between the biotin and avidin, the combination of biotin moiety and nitrogen-containing diphosphine ligand remains an alternative approach for the recovery of the chiral metallic catalyst.

3.6. Preparation of Ruthenium(II) Complexes with 5-amino-BINAP and Its Biotinylated Derivative

Axially dissymmetric 1,1'-binaphthyl moiety has proven to be a desirable asymmetry-inducing unit because of its structural rigidity and simplicity, resistance to racemization, and effectiveness of chiral recognition. Due to the restricted rotation around the 1,1'-bond, 1,1'-binaphthyl molecules are chiral and a series of corresponding derivative compounds have been developed for applications in asymmetric catalytic reactions. Among the derivatives of 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl, the functionalized BINAP containing amino groups has attracted us much attention owing to the extension of applicability offered by this type of compounds[72]. Thus we have paid much effort on the preparation and purification of 5-amino- and 5,5'-amino-2,2'-bis(diphenylphosphino)-1,1'-binaphthyl ligands[73]. Also, we are interested in exploiting the BINAP type of ligands in the application of hydrogenations catalyzed by the avidin-based metallic catalyst system. The first target compound, BINAP-NH₂, was then synthesized from BINAPO as the starting material according to the following Scheme 3.7.
Scheme 3.7. Synthetic routes on the preparation of BINAP-NH₂ ligand.

The nitration of (R)-BINAPO (92) led to the corresponding di- and mono-nitro-substituted BINAP compounds on the 5 position with almost quantitative chemical yield. The pure mono-nitro-BINAPO (93) can be obtained by the column chromatography through silica gel with 58% isolated yield. The reduction of 93 in the presence of palladium on charcoal in ethanol under 50 atmosphere of hydrogen gave the (R)-5-amino-BINAPO (94) in high yield. Further reduction of 94 by the treatment of trichlorosilane and triethylamine in refluxing toluene afforded the desired product of BINAP-NH₂ (95).

The biotinylation of the BINAP-NH₂ ligand (95) was proceeded in the same manner as described in previous section. The obtained BINAP-NH₂ ligand (95)
and its biotinylated derivative (96) were converted to their corresponding Ru(II) complexes 97 and 98, respectively, via complexation with [Ru(cymene)Cl2]. The synthesis of the two complexes are shown in Scheme 3.8.

![Chemical Structures](image)

**Scheme 3.8.** The synthesis of Ru(II) complexes with BINAP-NH₂ (95) and biotinylated BINAP (96) ligands.

It is known that the Ru(II)-BINAP type of complexes showed excellent enantioselectivity on the hydrogenation of α, β-unsaturated carboxylic acids. These two Ru(II) complexes, 97 and 98, were then used to catalyze the hydrogenation of 2-(6'-methoxy-2'-naphthyl)propenoic acid which its respective saturated product is famous as an antiflammatory drug.

Due to the poor solubility of 2-(6'-methoxy-2'-naphthyl)propenic acid in aqueous solution, this substrate has been converted to its corresponding sodium salt before proceeding the aqueous hydrogenation reactions. The preliminary results of the hydrogenation reactions carried out in methanol and the pH 7 buffered solution are listed in Table 3.12.

**Table 3.12.** The hydrogenation of 2-(6'-methoxy-2'-naphthyl)propenic acid catalyzed by complex 97 and 98 with and without association of avidin.*

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Solvent</th>
<th>ee (%)b</th>
<th>Configc</th>
<th>Conv. (%)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>97</td>
<td>MeOH</td>
<td>86.5</td>
<td>R</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>98</td>
<td>MeOH</td>
<td>78.3</td>
<td>R</td>
<td>61.9</td>
</tr>
<tr>
<td>3</td>
<td>avidin+98d</td>
<td>MeOH</td>
<td>71.6</td>
<td>R</td>
<td>75.1</td>
</tr>
<tr>
<td>4</td>
<td>97</td>
<td>Buffer</td>
<td>16.6</td>
<td>R</td>
<td>17.7</td>
</tr>
<tr>
<td>5</td>
<td>98</td>
<td>Buffer</td>
<td>20.6</td>
<td>R</td>
<td>15.0</td>
</tr>
<tr>
<td>6</td>
<td>avidin+98d</td>
<td>Buffer</td>
<td>6.4</td>
<td>S</td>
<td>32.5</td>
</tr>
</tbody>
</table>

a). The hydrogenations were carried out at 22 °C in different solvents under 1000 psi H\(_2\) with S/C=100 (molar ratio) for 10 h.  
b). The ee values and conversions were determined by HPLC analyses with a SUMICHIRAL OA-2500 column.  
c). The absolute configurations were determined by comparing the optical rotations with literature values.  
d). 1.1 eq. avidin based on the biotinylated BINAP-Ru(II) complex was added.

It was found that the solvent system does play a crucial role in the determination of resulting enantioselectivity of this hydrogenation. The hydrogenations catalyzed by the same catalyst in different solvents resulted in
the products with different ee values. Poorer ee values were observed in the hydrogenations proceeded in the buffered solution. Moreover, the rates of the hydrogenations carried out in buffered solutions (entry 1–3) appeared to be quite slower than those carried out in methanol (entry 4–6) under the same conditions. In general, the aqueous hydrogenation reactions of 2-(6'-methoxy-2'-naphthyl)propenyl acid catalyzed by the biotinylated BINAP-Ru(II) system in the presence or absence of avidin led to unsatisfactory enantioselectivities and conversion rates.
Chapter 4
Conclusions

Catalytic enantioselective reactions have received much attention during the past three decades and have played a crucial role in modern synthetic chemistry. Transition metal catalysts containing various chiral ligands have been extensively studied for this purpose. In addition to the use of chiral metal complexes, one strategy used to design selective catalysts is to incorporate nonspecific achiral catalytic groups into chiral cavities. The general method is to modify a known protein or enzyme at a defined site with a cofactor or new functional group to create a semisynthetic system with novel properties. The significant advantage offered by this strategy is the obviation of the arduous synthesis of the chiral ligands. However, most of these systems are usually limited to those substrates which specifically bind to the native enzymes. Only few systems developed recently based on protein cavity can accommodate a variety of substrates. Since transition metal complexes catalyze a large variety of chemical reactions, the development of new catalysts by introducing a chiral catalytic functionality into the tertiary restricted environment of a protein cavity is of great interest. For the convenient test of this concept, we decided to use a carrier which binds strongly to specific sites of the target protein to introduce the catalyst to the protein cavity in a manner like a "guided missile". If the combination is properly optimized, it may possess not only better compatibility with different substrates but also a multiplicity of various catalytic reactions. Moreover, the protein-based catalysts may offer the possibility of easier catalyst recovery and reuse.
In this study, we have examined the effect of the interaction between a chiral metal catalyst and a protein cavity by converting several diphosphine ligands into their biotinylated derivatives and attaching the biotinylated diphosphine metal complexes to the specific binding site of avidin. The experimental results proved that the catalytic hydrogenation reactions of various olefinic substrates can be proceeded smoothly with the new type of avidin-based catalyst systems in the aqueous media. Besides, the introduced avidin did result in significant influence on the enantioselectivity of the hydrogenation reactions. Optimization of the avidin-based catalyst systems by altering different reaction conditions such as temperature, hydrogen pressure, and pH values of the buffered solutions has been made but no satisfactory results focused on the issue of accurate manipulation for these catalyst systems has been reached so far.

There is no detailed information about the size of avidin binding site for biotin, the only information in literature is the depth of the binding site (15 Å). In addition, since the definite information concerning the secondary and tertiary structure of avidin is not sufficient, it was difficult to point out the exact stereochemistry within the binding site after the incorporation of the chiral diphosphine-Rh(I) complex moiety. Nevertheless, the binding of the biotinylated chiral metallic complex to avidin and the subsequent influence of the avidin environment on the enantioselectivity of the catalyst is established. Owing to the complex interactions involved in protein folding, the precise control on this avidin-based catalyst system is proven to be difficult. This also indicates that a key requirement for constructing this type of catalyst system is the availability of a suitable protein cavity. The fact that the introduction of the biotinylated diphosphine-transition metal moiety into avidin did not
substantially alter the reactivity of the metal complex revealed that the cavity was supposed to be primarily a shape-selective pocket that only controlled the stereoselectivity of the hydrogenation reaction. This also means that the releasing of the hydrogenated product was not a problem and the catalytic turnovers are expectable in the hydrogenation reactions. In fact, the observed turnovers of the hydrogenation reactions catalyzed by our avidin-based catalyst systems are much higher than other developed catalytic reactions based on the protein cavities[48]. Nevertheless, some modifications can be made to this protein–biotinylated diphosphine–transition metal catalyst system for further improvements on enantioselectivity: (1) Alteration on the length of valeric acid side chain of biotin. It was mentioned previously that modification of the carboxylic group of biotin had little effect on the binding to avidin. In addition, the total synthesis of biotin was accomplished now. It is hence possible to modify the length of spacer arm between chiral diphosphine ligand and avidin cavity. Because it was found from experiments that the stereochemistry within the avidin binding site plays the most crucial role in determining the enantioselectivity, the change on the surrounding environment of the introduced metal complex should have certain effects on the resulting enantioselectivity. That is to say, the different locations of chiral metal complex within the cavity should lead to different enantioselectivities. (2) More flexible chiral diphosphine ligands. There is no doubt that the matching of chirality between the chiral diphosphine and protein cavity could result in constructive selectivity. However, it was found to be rather difficult to fulfill this goal from the work done in this thesis. That reflects that the chiral ligands used in this work are perhaps too rigid to adapt their conformations into the suitable templates. Thus, the use of more flexible chiral diphosphine ligands may offer
a different opportunity for achieving higher selectivity. (3) Different protein cavities. In addition to avidin, biotinyl compounds are able to associate with other proteins such as streptavidin[63]. The goal of varying the protein is also to find the better match for the linked chiral metal complex. The replacement of protein cavity in the catalyst system is an alternative approach for improvements.

Although the naturally occurring proteins were proven to be utilized as the effective chiral pockets in various catalytic reactions, the reaction conditions obviously limited the use of certain type of proteins due to protein denaturation. This accounts for the intensive exploration grown in the area of artificial enzymes and enzymatic mimics in the past several years. This kind of semisynthetic enzymatic mimics, generally organic synthetic molecules, is more adaptable and endurable to the change of reaction conditions than the natural enzymes. The rapid development in this type of artificial enzymes has formed an individual pioneering field which is called "enzyme models". The major advantages present in the "enzyme models" systems are the easy manipulation of chemical modification, and the endurance of repeated use. These advantages appear to be almost out of reach of the natural proteins due to the inherent limitation. Therefore, the prospective potential of applications of enzyme models in asymmetric catalysis is fairly expectable.

In summary, the avidin–biotinylated diphosphine–transition metal systems developed in this study gave positive albeit preliminary indications for the potential of the combination of chiral transition-metal complexes and protein cavities. In our opinion, natural proteins will be more versatile as the chiral cavities attached by another catalytic groups if the information concerning the entire stereochemistry have been fully collected, even though it seems to be
barely reachable in terms of current technology. However, with the rapid progress focused on numerous mutagenesis and protein folding studies, the use of the transition-metal complexes attached to specific sites in proteins on selective catalytic reactions is expected to offer more positive potentials in the future.
References


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APPENDIX I

$^{1}H$, $^{13}C$, and $^{31}P$ NMR Spectra

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Appendix II

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