UNIVERSITÀ DEGLI STUDI DI MILANO

Department of Pharmaceutical Sciences (DISFARM)

DOCTORATE SCHOOL IN CHEMICAL SCIENCES AND TECHNOLOGIES

PhD in Pharmaceutical Sciences XXVII cycle

Curriculum: Special Synthesis

New chiral aminophosphine and diamines ligands, corresponding transition metal complexes, their applications in asymmetric synthesis and development of hybrid system (artificial-metalloenzymes).

CHIM/03

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Abstract

Transition metal complexes containing chiral bidentate ligands are robust catalytic system for the preparation of chiral compounds as fine chemicals, fragrances and insecticides. Bifunctional P-ligands, N-ligands or P-N ligands are preferentially used for the preparation of late transition metals complexes (Rh, Ru, Pd, Ir, Pt) to modify their catalytic performance. While the reduction of olefin and carbonyl groups has been widely investigated, the asymmetric reduction of imines is relatively underdeveloped, although enantiopure amines play an important role in the preparation of many products. The asymmetric hydrogenation of cyclic imines is more difficult in comparison to the acyclic analogue because aromatic compounds are more stable, the reaction requires harsher conditions and it suffers from easy deactivation of the catalysts due to the poisoning.

The interest of both academic and industrial research groups has increased in recent years in asymmetric hydrogenation of cyclic and acyclic imines, with a focus on the discovery of catalytic system with excellent enantioselectivity and activity under low hydrogen pressure or alternative source of hydrogen. Moreover, the development of ATH in aqueous media is emerged as a valid alternative to the use of organic solvents for its no toxic, economic and environmental compatible profile.

Since the pioneering work of Noyori and Ikariya groups in 1995, the catalysts of choice in ATH reductions of ketones have been established to be the ruthenium(II) complexes chelating different substituted 1,2-diamines such as DPEN and its derivatives. In particular the monotosylated compounds were revealed as the most efficient ones. All these types of catalysts were based on the presence of ligands forming a five membered ring in coordination to the metal centre. Some examples of symmetric 1,4 diamines and few examples of 1,3-diamines were reported in literature, mainly used as a typical ruthenium complex [(diphosphine)-$RuCl_2$-(diamine)] for hydrogenation of simple aromatic and aliphatic ketones.

In this PhD thesis are reported the synthesis of simple asymmetric monotosylated 1,3-aminophosphine and monotosylated 1,3-diamines, up to now poorly investigated. The evaluation of their catalytic performances and their application for the preparation of artificial hydrogen transferases are also investigated.

Chiral benzyl alcohols, used as synthons for the preparation of bidentate ligands, were obtained by biotransformation. Screening results revealed *Rhodotorula rubra* MIM 147 as an efficient catalytic system for this purpose.

The first generation of this type of ligand was based on chiral 1,3 tosyl aminophosphine compounds able to use for the preparation of chelating six member ring complexes containing Ir(I) and Ru(II). Chelating aminophosphines have a combination of hard (N) and soft (P) Lewis base centers which make these ligands particularly useful in a variety of catalytic

reactions. In fact these precatalysts, in hydrogenation reaction of several prochiral substrates such as ketones, imines and inactivated double bonds, showed a wide activity without stereoselectivity.

Second generation of ligands containing linear and branched 1,3 tosyl diamines in which the tosyl moiety was present in different position, were synthesised to improve stereoselectivity.

In respect to the tosyl aminophosphines: they are more easy to synthesise, to functionalize and are more easy to handle because are not air sensitive. Moreover these compounds are water soluble and act as suitable ligands for hydrogen transfer reaction conditions.

[Ru (*p*-Cymene) (Tsdiamine)] complex (**S-9a**) results the best catalyst for the reduction of acetophenone in water used under ATH conditions (*e.e.*=56%, Yield=97%), revealing the importance of stereogenic centre to be in proximity of the amine involved in the catalytic cycle. The Ts moiety contribute to increase both the reaction conversion and enantioselectivity through a steric and/or an electronic effects. Considering different hydrogen donors, the used of HCOONa was revealed the best choice.

Ligand (**S-9**) was used for the preparation of [IrCp*Tsdiamine] complex (**S-9b**) and applied in the reduction of cyclic imines in ATH reaction conditions.

Substrates were reduced in excellent yield, in aqueous medium even if the enantioselectivity was low. The conformation of chelating six member ring seems to play a primary role for the enantiodiscrimination of the substrate adopting a chair conformation in this condition. Starting from the assumption that the reduction of these substrates is not easy to obtain, the results using this catalyst, encouraged our research to improve the stereoselectivity of the system, and for this reason, transition metal catalysts was applied in the development of artificial metallo enzymes. This hybrid system results from the incorporation of a catalytically competent organometallic moiety within a macromolecule. For this work was exploited the biotin-streptavidine technology. In fact tethering a biotin anchor to a catalyst precursor ensures that, in presence of streptavidine (Sav), the metal moiety is quantitatively incorporated within the host protein.

Meta and *para* biotinylated aminosulfonamide iridium d^5-pianostool complexes were prepared and their performances were evaluated in combination with Sav wild type and 10 different mutants in position 112. After this chemo-genetic optimization, the *para*-biotinylated aminosulfonamide iridium d^5-pianostool complex (**S-33a**) embedded in combination with Sav S112C allowed to obtain an imine reductase able to reduce dihydroisoquinolines with good activity and enantioselectivity (e.e.=66%,Yield=90%), in aqueous solution using HCOONa as hydrogen donor.

1

INTRODUCTION

An invention requires four Gs to succeed, Geist, Geld, Geduld and Gluck. The first of these is axiomatic. You have got to have a good idea. The second is essential. One needs financial support but I would suggest a proper balance. Too much or too little is inhibitory. For the third you must have patience. Things never move as fast as you would have them. Finally luck is all-important. I suspect that no invention has ever been made without some fortuitous help.

Paul Ehrlich

1.1 ORGANOMETALLICS CHEMISTRY

Organometallics compound, with their metal-carbon bonds, lie at the interface between classical organic and inorganic chemistry in dealing with the interaction between inorganic metal species and organic molecules. The presence of *d* electrons in their valence shell distinguishes the organometallic chemistry of the element of group 3-12 of the periodic table (transition elements), from that of the group 1-2 and12-18, the main groups of periodic table. Transition metal ions can bind ligands (L) to give a coordination compound or *complex* ML_n ($2< n <9$).

Organometallic chemistry is a subfield of coordination chemistry in which the complex contains M-C or M-H bond. Depending on the number of ligands bound to the metal, complexes adopt different geometry (L=6 Octahedral, L=5 square pyramid or trigonal bipyramid, L=4 tetrahedral or square planar). In addition, if ligand contain more than one donor to the metal, we observe a so called chelate effect. An interesting behaviour of complexes is the TRANS effect by which certain ligands L_T, facilitate the departure of a second ligand L, trans to the first, and their replacement or substitution by an external ligand.

TRANS effect series:

$$OH^- < NH_3 < Cl^- < Br^- < CN^-, CO, C_2H_4 < PR_3 < H^-$$

Low ← TRANS effect → high

1.2 CRYSTAL FIELD

Crystal field model provide to understand structure, spectra and magnetisms of transition metal complexes explaining how the *d* orbital of the transition metal are affected by the presence of the ligands. Metal ion isolated contains five *d* orbital *degenerate* (same energy).

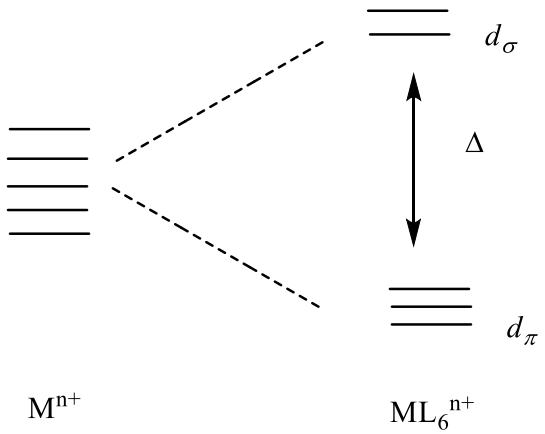


Fig.1

As the ligands L (negative charge) approach the metal from the six octahedral direction, *d* orbitals that point toward the L group are destabilized and move to high energy (d_σ) while those that point away from L decrease in energy (d_π). The magnitude of the difference between this set of energy (*crystal field splitting*, Δ) depends on the effective negative charge and the nature of the ligand.(Fig. 1). Higher Δ leads to stronger L-M. To this value is also linked the *high-spin* or *low-spin* metal configuration. If crystal field splitting is small, electrons may rearrange to give high-spin form (all the unpaired spin are aligned). On the other hand, if Δ becomes large enough, then the energy, gained by dropping from d_σ to the d_π level, will be sufficient to drive the electrons into pairing up. The spin state is correlated to the magnetic moment of the complexes. For example, in the low spin of a d^6 ion, the molecule is *diamagnetic*, so it's very weakly repelled by the magnetic field. Differently the high spin form is *paramagnetic* and in this case it's attracted into the field because there are unpaired electrons. The colors of transition metal complexes often arise from the absorption light that corresponds to the Δ (energy gap). So called *high-field ligands* such as CO give rise to a large Δ, while low-field ligands like H_2O or NH_3, can give a low Δ, and the spin pairing is lost and even the d^6 configuration can become paramagnetic. The general trend shows that π donor

ligand such as halide or H_2O tend to be weakly field while π acceptor ligand like CO is a strong-field ligand.

$$I^- < Br^- < Cl^- < F^- < H_2O < NH_3 < PPh_3 < CO,H$$

← low Δ — High Δ →
← π donor — π acceptor/σ donor →

Other ligand such as NH_3 are good σ donors but are not significant π acceptor. CO in contrast is an example of π acid ligands, which have empty orbitals of the right symmetry to overlap with a filled d_π orbital of the metal. In general π acceptor ligand has a filled orbital that acts as σ donor and an empty orbital that acts as π acceptor.This orbitals are the highest occupied (HOMO) and the lowest unoccupied molecular orbitals (LUMO) of L. This *back bonding* is a key feature of M-L bonds and these ligands (also hydride) are high field.
Ligands such as OR^- and F^- are π donor as a result of the lone pairs that are left when one lone pair has formed the M-L σ bond. The repulsion of two filled π orbitals, destabilize the d_π of the complex decreasing the Δ which corresponds to a weaker L-M bond.

1.3 TYPES OF LIGANDS

Most ligands form the M-L σ bond by using a lone pair, that is, a pair of electrons that are nonbonding in the free ligand. For ligands such as PR_3 or pyridine these lone pairs are often the HOMO and the most basic electrons in the molecules. Is also possible to find two other types of ligand on the base of their behaviour in organometallic compounds:

π- complexes

Ethylene has no lone pairs, yet it binds strongly to low valence metals. In this case the HOMO is the C=C π bond, and it is these electrons that form the M-L σ bond, hence the term π complex. Arrow "1" represents electron donation from the filled C=C π bond to the empty d_σ orbital on the metal. Arrows "2" represent the back donation from the filled M(d π) orbital to the empty C=C π*.(Fig.2)

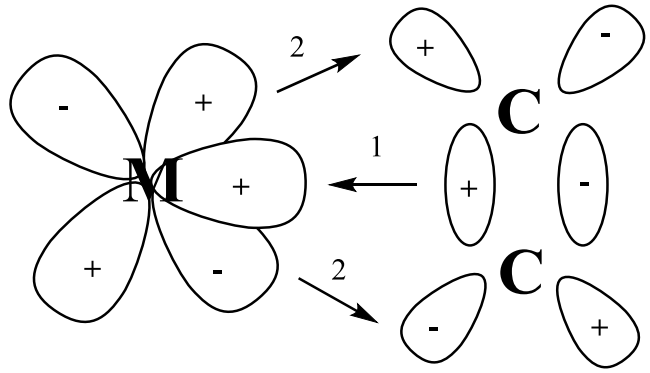


Fig.2 Π-bond donor, ethylene, to a metal. Only one of the four lobes of the d_σ orbital is shown. The + and – signs refer to symmetry.

This type of binding is represented as (η^2-C_2H_4) where η represents the *hapticity* of the ligand, defined as the number of atoms in the ligand bonded to the metal.

σ- complexes

Molecular hydrogen has neither lone pair nor π bond, yet is also binds as an intact molecule to metals in such complexes. The only available electron pair is the H-H σ bond. Arrow 3

represent electron donation from the filled H-H σ bond to the empty d_σ orbital on the metal and 4 represent the back donation from the filled M(d_π) orbital to the empty H-H σ*(Fig.3)

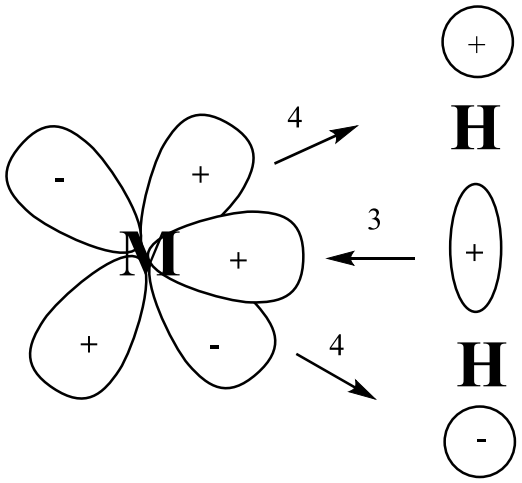


Fig. 3 σ-bond donor, hydrogen, to a metal. Only one of the four lobes of the d_σ orbital is shown. The + and – signs refer to symmetry.

In general, the basicity of electron pairs decreases in the following order: lone pairs > π-bonding pairs > σ- bonding pairs. The usual order of binding ability is the follow:

Lone-pair donor > π donor > σ donor.

Ligands can also classified in *spectator* and *actor* which remain unchanged or undergo some chemical conversion during chemical transformation, respectively. The role of these ligands is to impart solubility, prevent the departure of the metal and influence the electronic and steric properties of the complex. Apparently small changes in spectator ligand can entirely change the chemistry. An interesting example is PPh_3, which is a very useful ligand with thousands of complexes known while, apparently similar ligands, NPh_3 or $P(C_6F_5)_3$, appear to be a very little used ligands. Important aspect are: nature of donor, nature of substituents and steric factor. Moreover, an important role of spectator ligands is to block certain sites, to leave a specific set of sites available for the actor ligands so the desired chemistry can occur.

1.4 PHOSPINE LIGANDS

Tertiary phosphines, PR_3, are important ligands because they constitute one of a few series of ligands in which electronic and steric properties can be altered in a predictable way by varying R. They also stabilize an exceptionally wide variety of ligands of interest to the organometallic chemist as their phosphine complexes $(R_3P)_n$ M-L. Similar to NH_3, phosphines have a lone pair on the central atom that can be donated to a metal. Differently to NH_3, they are also π acid, to an extent that depends on the nature of the R group.

Low π-acidity-PMe_3 < PAr_3 < $P(OMe)_3$ < $P(OAr)_3$ < PCl_3 < CO -high π-acidity

In other word, phosphines are usually strong σ-donor ligands and only weak π –acceptors and this effect can be increased with electron donating group in the rest R, while electron withdrawing groups in R favour the π-acceptor backbonding. (Fig. 4)

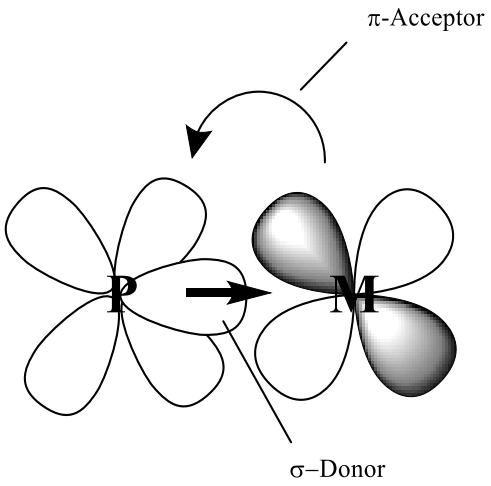


Fig.4

The second important feature of PR_3 as a ligand is the variable steric size, which can be adjusted by changing R. Carbon monoxide is so small that as many can bind as are needed to achive $18e^-$. This is rarely true for phosphines, where only a certain number of phosphines can fit around the metal. The great advantage is that by using bulky PR_3 ligands, we can favour

forming low-coordinate metals or we can leave room for small but weakly bonding ligands, which would be excluded by a direct competition with a smaller ligand such as CO or PMe_3. Thanks to Tolman it is possible to quantify the steric effects of phosphines with its *cone angle*. This is obtained by taking a space-filling model of the $M(PR_3)$ group, folding back the R substituents as far as they will go, and measuring the angle of the cone that will just contain all the ligand, when the apex of the cone is at the metal(Fig. 5)

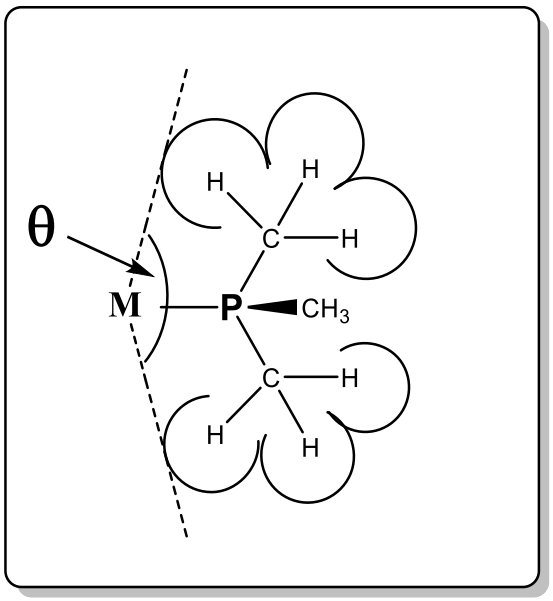


Fig.5

A bulkier ligand (large θ) tends to have a higher dissociation rate than smaller ligands and electron-rich metal-centres tend to accelerate the "oxidative addition", a key-step in the catalytic cycle. Moreover optically pure phosphine ligands are able to provide the chiral information in asymmetric catalytic reactions.

1.5 CATALYSIS

Nowadays catalysis plays a vital role in chemical synthesis, and in particular efficient organometallics catalyst provides a logical basis for molecular science and related technologies.[1,2] Although the control of stereochemistry[3-17] is a major concern of modern organic synthesis, reactivity and productivity are also important in making reactions practical and efficient.[18] These important attribute can be achieved only by designing suitable molecular catalyst and reaction conditions through a deep understanding of the catalytic cycle.

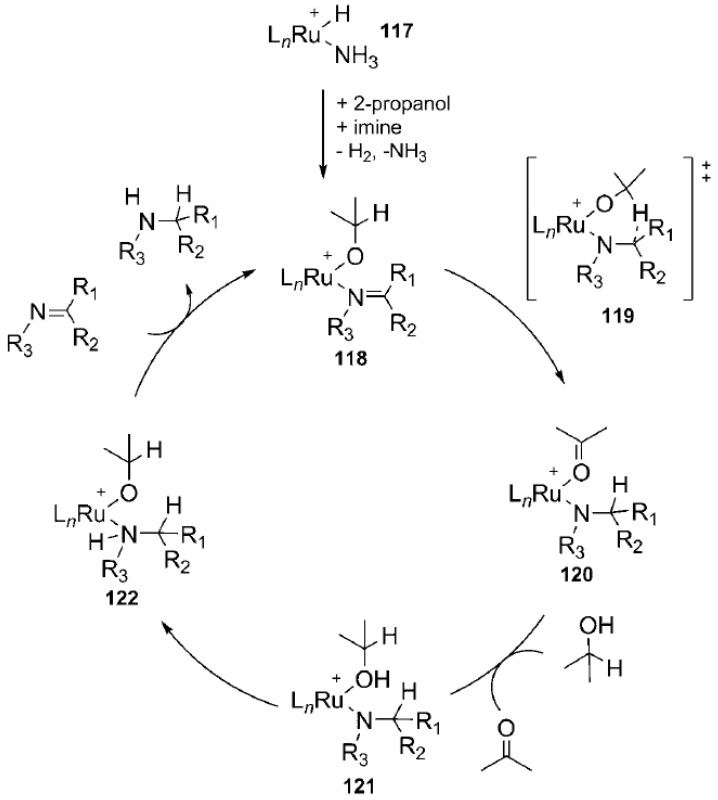


Fig.6. Mechanisms of transfer hydrogenation of imines.

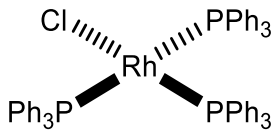
Yamamoto *et al*. proposed a catalytic cycle for the transfer hydrogenation of imines catalyzed by the cationic mono hydride complex **117**, based on deuterium-labelling experiments.[19] (Fig.6) The catalytic cycle starts with the formation of the imine-coordinated isopropoxide complex **118** by the reaction of the cationic ruthenium hydride **117** with 2-propanol in the presence of imine. Hydrogen transfer from the isopropoxide to the coordinated imine carbon through cyclic transition state **119** gives the acetone coordinated amido complex **120**. Substitution of the coordinated acetone by 2-propanol gives the intermediate **122**. A second proton transfer from 2-propanol to the nitrogen generates **123.** Replacement of the amine with the incoming imine liberates the hydrogenation product, regenerating **118**.

1.6 OVERVIEW IN HOMOGENEOUS ASYMMETRIC CATALYSIS

Chemical catalysis could be divided in two main branches: Homogeneous and Heterogeneous catalysis, (catalyst and reagent are present in the same phase or in different phase respectively). These are very different catalytic tools widely used in industry to perform a very large range of reactions.[20-24] While heterogeneous catalysis is the first methodology used on large scale (Haber–Bosch process for the preparation of ammonia applied by BASF using an inexpensive iron catalyst in 1909), the youngest Homogeneous catalysis was born in 1966 when Wilkinson presented the first homogeneus catalyst for the hydrogenation of alkenes (**1**,Fig.7). [25]

Since that date this field became very active and many research groups started to investigate different type of reaction like hydroformylation, carbonylation, hydrocianation, oligomerisation of alkens and hydrogenation including the Asymmetric ones.[26-27]

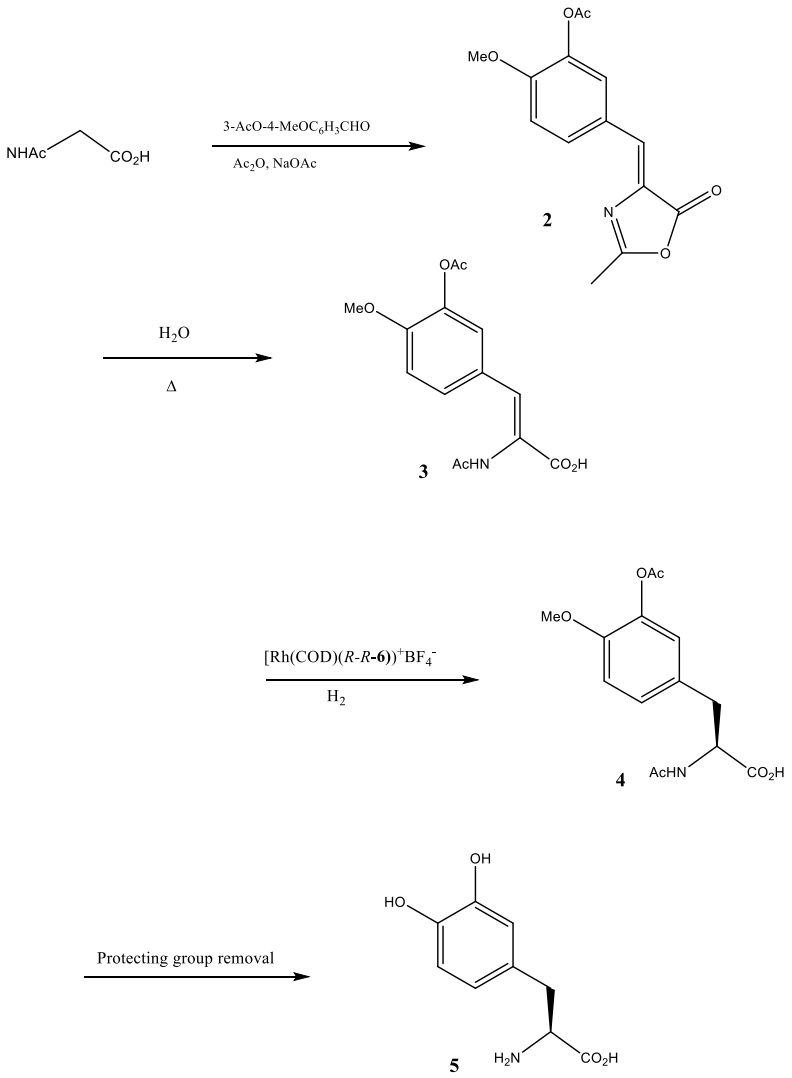
Asymmetric Hydrogenation (AH), provides to introduce a stereogenic centre into a product used in the life sciences industries starting from prochiral compounds containing C=C, C=O or C=N moiety using chiral transition metal complexes. Bifunctional P-ligands, N-ligands or P-N ligands are preferentially used for the preparation of late transition metals complexes (Rh, Ru, Pd, Ir, Pt) to modify their catalytic performance.



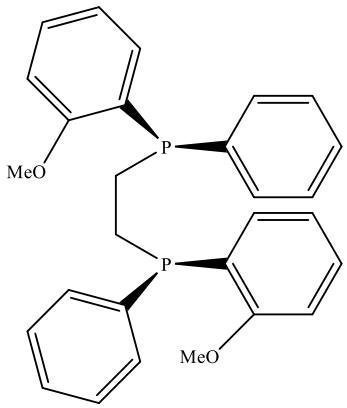
1

Fig.7. Rh pre-catalyst developed by Wilkinson for the reduction of olefin.

Historically, the first industrial application of Asymmetric Homogeneous catalysis was the manufacture of L-DOPA with 94 % *e.e.* (**5**, Scheme 1) used as drug in Parkinson's disease. The stereogenic centre was introduced by asymmetric hydrogenation of olefin performed by enantiopure biphosphane ligand DIPAMP (Fig.8) also known as Knowles catalyst.[11,28]



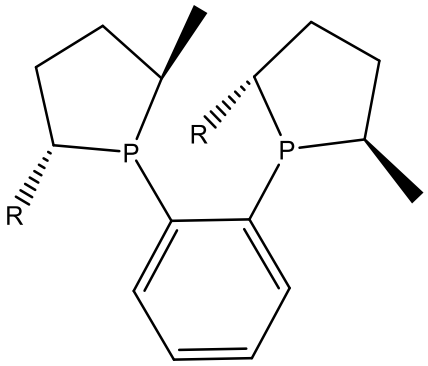
Scheme 1. Industrial process for the synthesis of L-DOPA (**5**).



[(*R,R*)-**6**]

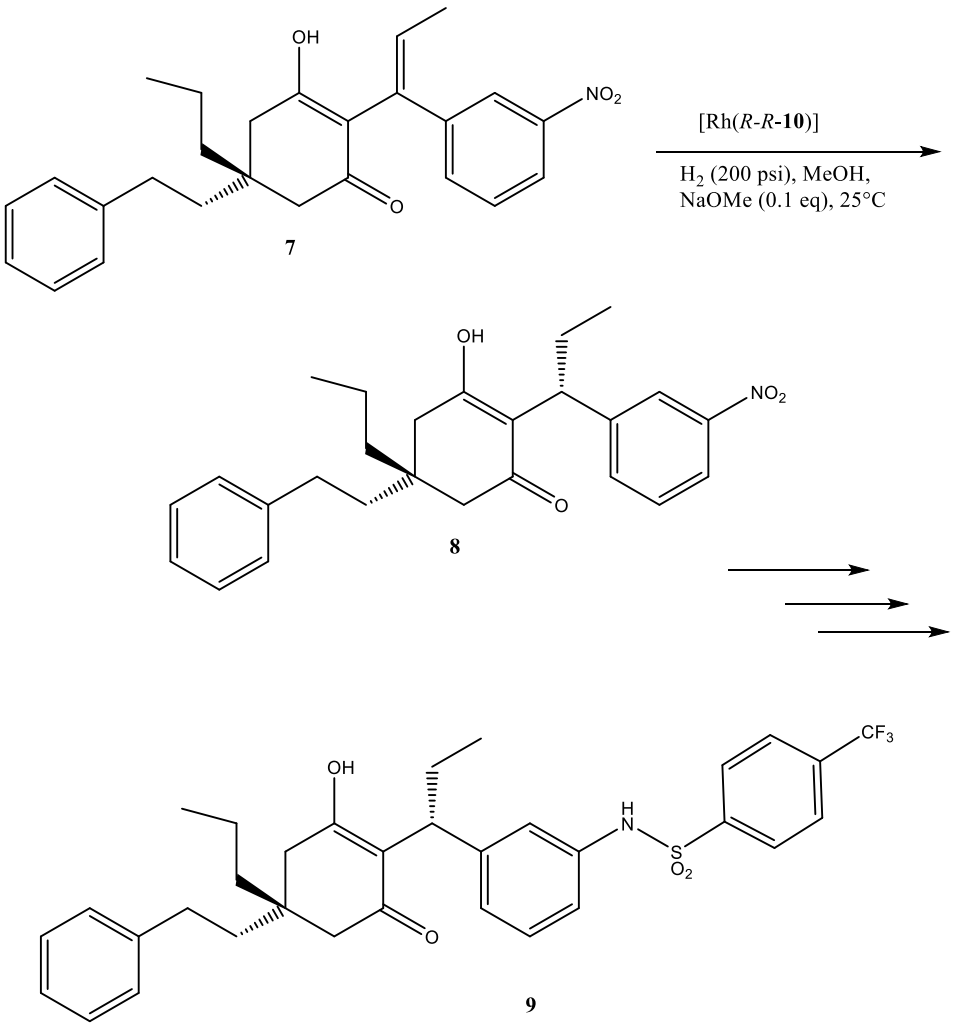
Fig. 8 [Rh(*R,R*)-DIPAMP)] used for asymmetric hydrogenation of dehydro amino acids.

Another example in which Rhodium is applied in the asymmetric reduction of carbon double bond is shown in the synthesis of Tipanavir, drug used in the treatment of HIV (**9**, Scheme 2).[29] In this compound, one of the key stereogenic centre can be accessed with Rh complex-(*R,R*-Me-DuPhos) **10** (Fig. 9) and its use leads to the formation of compound **9** in >94% *e.e.*



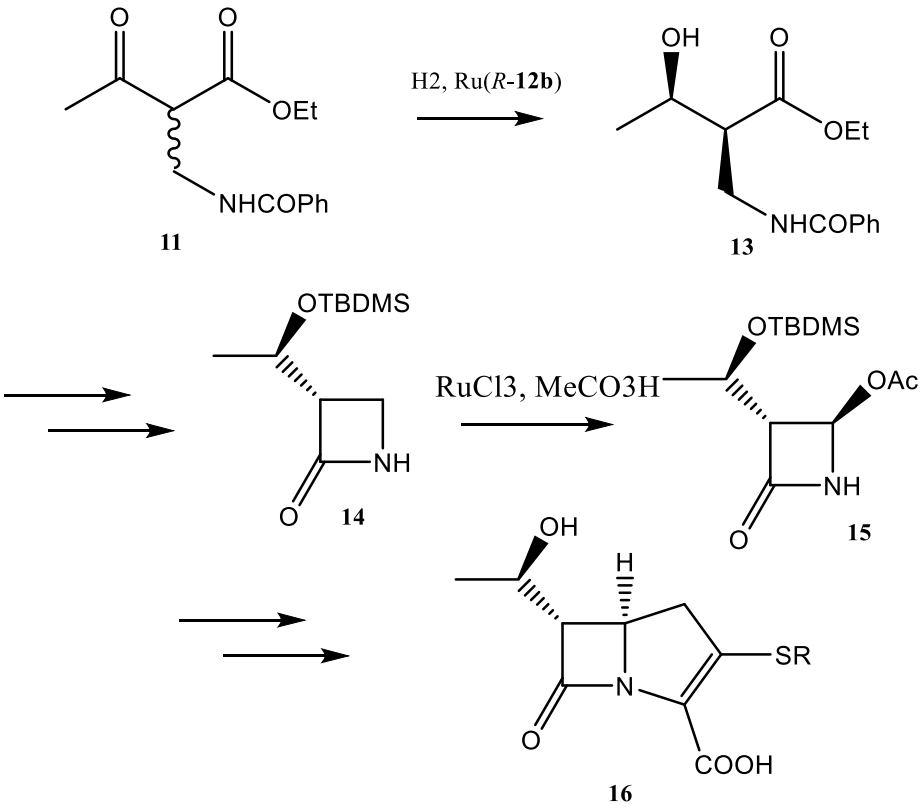
10 = Me[(*R,R*)-Me-DuPhos]

Fig.9 C2-symmetric bis(phospholanes) for the enantioselective hydrogenation of α-amino acid derivatives.

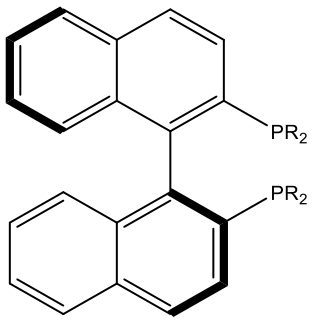


Scheme 2 Industrial process for the Synthesis of Tipanavir (**9**).

Ruthenium based catalysts are a very powerful tools to asymmetrically reduce carbonyl compound such as beta keto esters or ketons.[13] An example of application is the synthesis of intermediate **13**, for the manufacture of carbapenem antibiotics (Scheme 3). The α-substituted beta keto ester is asymmetrically reduced, with concomitant dynamic kinetic resolution, by Ru-Xyl-BINAP (Fig.10) system, synthesised by Noyori in 1980.[30]



Scheme 3 Large scale application for the Azetidinone synthesis.



12a R= Ph [(R)-BINAP]
b R= 3,5-Me_2C6H5 [(R)-Xyl-BINAP]

Fig.10 Atropoisomeric chiral Diphosphines used in asymmetric hydrogenation of beta-keto esters.

All the catalysts mentioned above contain a chiral bifunctional P-ligand able to reduce in very enantioselective manner C=C and C=O bonds under Hydrogenation condition.

1.7 IMINES REDUCTION BY HOMOGENEOUS CATALYSIS

While the reduction of olefin and carbonyl group have been widely investigated,[24] the asymmetrical reduction of imines, is relatively underdeveloped, although enantiopure amines play an important role in the preparation of many product.[31] The interest of both academic and industrial research groups in asymmetric hydrogenation of cyclic and acyclic imines (Fig.11) has increased in recent years, with a focus on the discovery of catalytic system with excellent enantioselectivity and activity under low hydrogen pressure or alternative source of hydrogen.[32]

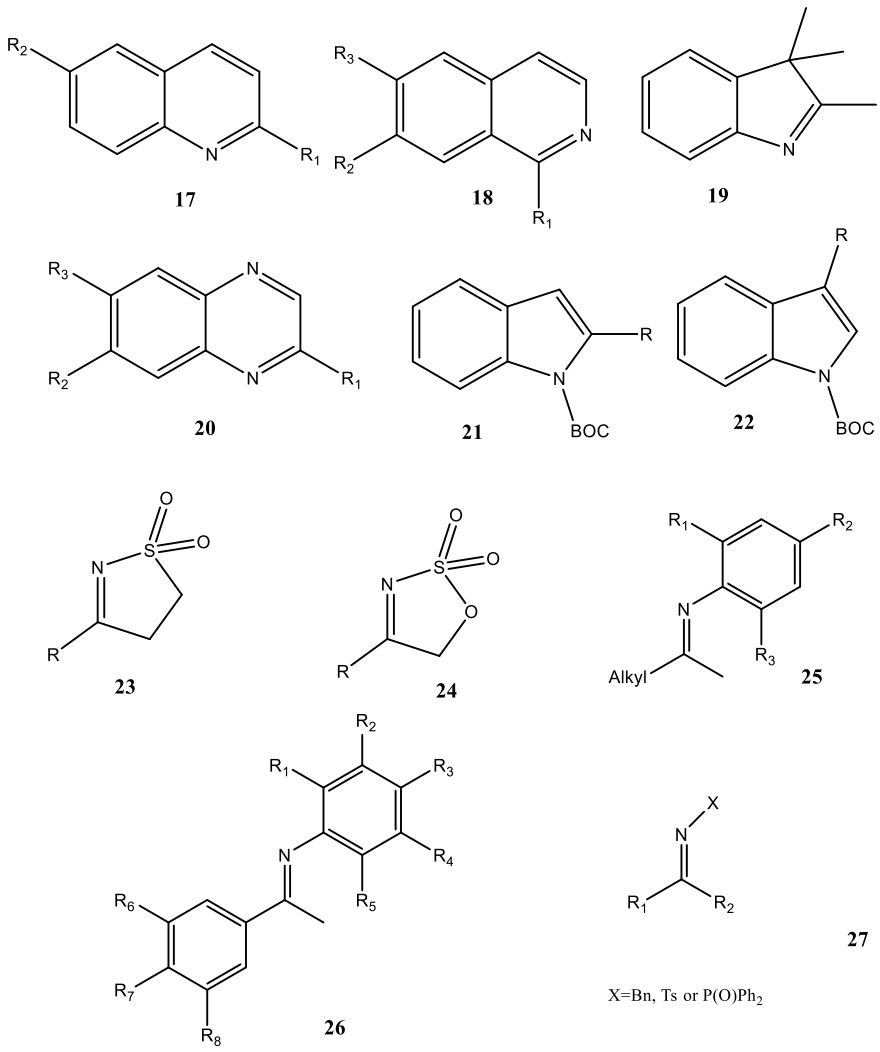


Fig 11 Example of Cyclic and Acyclic imines studied in asymmetric Hydrogenation.

The Asymmetric Hydrogenation of cyclic imines is more difficult compared to the acyclic analogue because aromatic compounds are more stable, the reaction requires harsher conditions and it suffers from easy deactivation of the catalysts due to poisoning. To modify the intrinsic catalytic performance of late transition metals (Rh, Ru, Pd, Ir, Pt) preferentially bifunctional P-ligands, N-ligands or P-N ligands are used for the preparation of complexes. Considering the use or not of Hydrogen gaseous as reductant it's possible to divide the reactions into 2 different classes: Hydrogenation and Transfer Hydrogenation of imines.

1.8 ASYMMETRIC HYDROGENATION OF IMINES

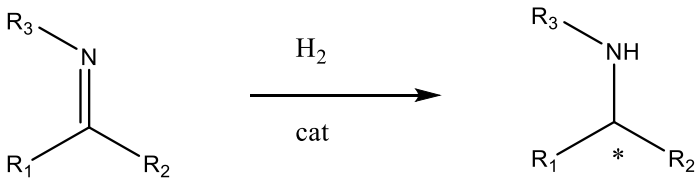


Fig. 12 General reaction of enantioselective hydrogenation of imine.

1.8.1 PHOSPHOROUS - PHOSPHOROUS LIGANDS

Iridium is the most efficient metal used for the formation of chiral amines with high to excellent enantioselectivities under mild conditions.[33]

Ligands **28**-**30** reported in Fig.13 were the first chiral diphosphines used in 1990 by Blaser to prepare complexes with $[Ir(COD)Cl]_2$. The catalyst were applied for the asymmetric hydrogenation of acyclic imines with moderate enantioselectivity.[34]

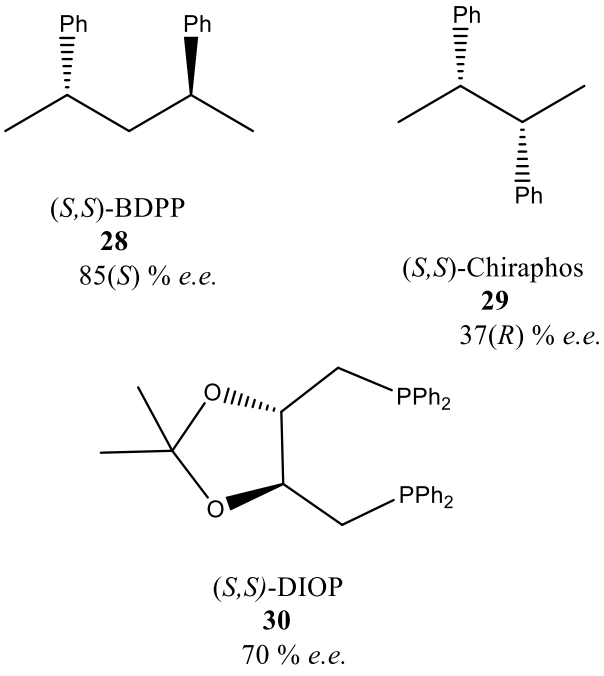
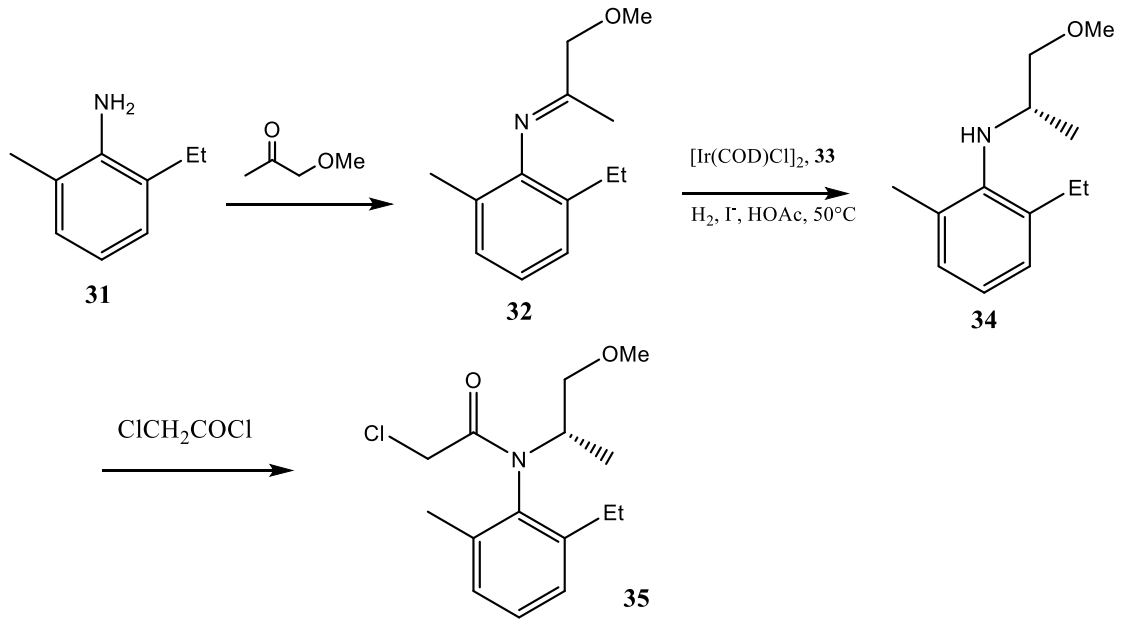


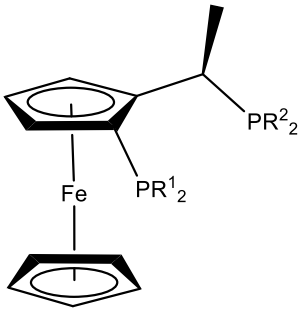
Fig 13 First chiral P-P ligands used to asymmetrical reduce imines.

The introduction of chiral ferrocenyl phosphine ligands by Zhang *et al.* in 2001 gave an high enantioselective system for the hydrogenation of N-arylimines achieving *e.e.* up to 99% in presence of iodine.[35] The same author described in 2009 the first example of efficient enantioselective hydrogenation of primary imines.[36] A relevant example in the use of chiral ferrocenyl diphosphines ligand,[37] is the production of (*S*)-metolachlor. (Scheme 4).[38]

The synthesis of this pesticide is the largest scale application of enantioselective hydrogenation, producing over 10,000 tons/year of the desired product with 79% *e.e*. The reduction of immine is performed with the catalyst prepared in situ from $[Ir(COD)Cl]_2$ and the JosiPhos ligand **33** developed by Togni and Blaser (Fig.14) In this case the presence of additive as iodide and acetic acid, is crucial for the activity of catalytic system.[39,40] Although the enantiselectivity is only 80%, it's sufficient to provide an economics advantage over the use of racemic mixture. Moreover, the catalyst cost could be offset by high catalytic activity (TOF >600.000 h^{-1}) and low catalyst usage (S/C ratio >2.000.000).



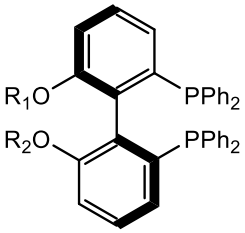
Scheme 4 Synthesis of (*S*)-Metolachlor.



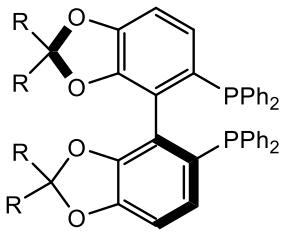
33 R^1 = Ph, R^2 = 3,5-$Me_2C_6H_5$

Fig 14 Josiphos ligand.

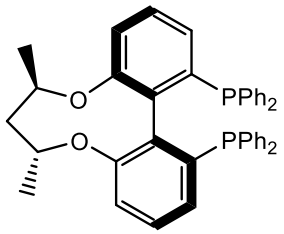
Also atropoisomeric diphosphines (Fig.15) were used in combination with Iridium giving highly efficient catalyst in the hydrogenation of cyclic imines.[41-49]



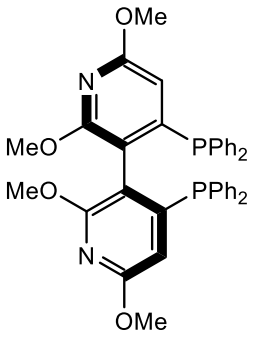
36 a R_1=R_2=Me
36 b R_1=*n*-$C_{12}H_{25}$, R_2=MeO-PEG



37 a R=H, (*S*)-segPhos
37 b R=F, (*S*)-difluorPhos



38 (*S*)-PQ-Phos

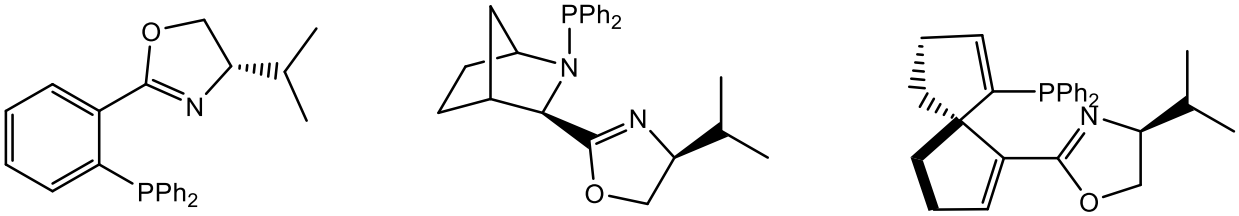


39 (*S*)-P-Phos

Fig.15 Selection of atropoisomeric diphosphines studied in the asymmetric hydrogenation of cyclic amines.

1.8.2 PHOSPHOROUS – NITROGEN LIGANDS

Chelating aminophosphines have a combination of hard (N) and soft (P) Lewis base centers which make these ligands particularly useful in a variety of catalytic reactions.[50-53] The catalytic system $RuCl_2$(diphosphine)-(diamine) developed by Noyori in 1990's for the reduction of ketones[54,55] was also shown to be useful for the reduction of imines.[56-58] In 2004, Morris developed $RuCl_2$(aminophosphine)$_2$ catalyst for the reduction of ketones and imines as well.[59] In 1997, Pfaltz *et al.* reported for the first time that Ir-complexes containing chiral phosphine-oxazolines (**40**, Fig.16) were highly efficient in the hydrogenation of imines.[60] In 2006 Andersson reported a new class of aminophosphine-oxazoline ligands with 2-azanorbornane backbone **41** that gave high *e.e.* values in the Iridium catalysed hydrogenation of imines.[61] In the same year Zhang and co-worker reported a phosphine-oxazoline ligands containing a spiranic backbone **42** which provided the best enantioselectivities for this family of ligands.[62]



40 **41** **42**

Fig. 16 Phosphine- oxazolines ligands.

1.8.3 NITROGEN – NITROGEN LIGANDS

Noyori, Ikariya and co-workers discovered a conceptually new chiral Ruthenium catalyst bearing N-sulfonylated 1,2-diamines that is highly efficient in asymmetric hydrogen transfer of imines.[63] Based on that system, Ikariya *et al.* recently reported the Iridium chiral complex, (**43**, Fig.17) [Cp*IrCl{(*S*,*S*)-Tscydn}] efficient for the asymmetric hydrogenation of acyclic imines. The addition of silver salts ($AgSbF_6$) caused an improvement in conversion and enantioselectivity.

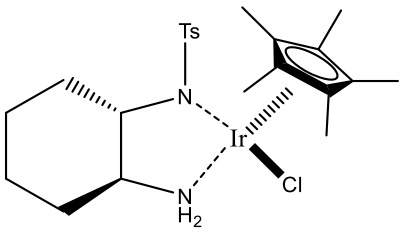


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Fig 17 Ir complex used as useful catalyst for the asymmetric reduction of acyclic imines.

Considering cyclic imines, is quite rare find an efficient hydrogenation system based on diamine-iridium complexes. The sole recent example is the use of (**44,** Fig.18) [Cp*Ir(OTf)(CF_3TsDPEN)}] as air stable and selective catalyst for the asymmetric reduction of quinolines.[64]

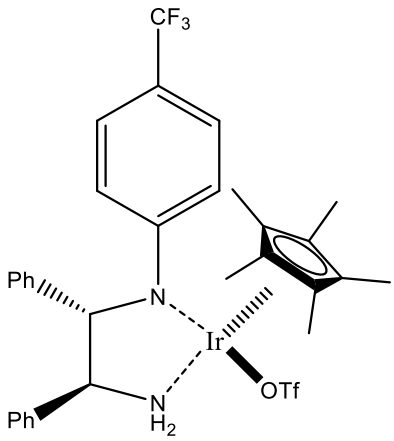

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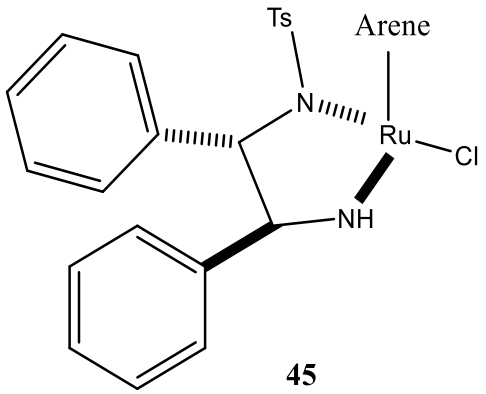
Fig.18 Rare example of Iridium complex applied in the reduction of cyclic imines.

Metals other than iridium were also tested in the asymmetric hydrogenation of cyclic imines and other substrates. Among them, ruthenium is the second most studied. It has provided modest results in hydrogenation using molecular hydrogen, which explains the fewer articles published in the recent years. However, it is commonly used and provides excellent results in asymmetric transfer hydrogenation.

1.9 ASYMMETRIC TRANSFER HYDROGENATION OF IMINES

Transfer hydrogenation has been used for many years with heterogeneous catalysts, but so far these are unsuitable for asymmetric synthesis. Starting from 1980s various groups showed that homogeneous catalysts, based on group VIII metals, were able to reduce C=O, C=C bonds with this mechanism. This catalysts, doesn't require the presence of Hydrogen gaseous under high pressure with an improvement of safety and an easier flow scheme. In 1981, Grigg *et al.* proposed C=N transfer hydrogenation, using Wilkinson's catalyst, $[RhCl[P(C_6H_5)_3]_3]$, in isopropanol to reduce aldimines into secondary amines.[65] Six years later, in 1987, a ruthenium catalyst, $Ru_3(CO)_{12}$, for transfer hydrogenation of imines was reported by Jones *et al.*[66] who successfully used it for the reduction of benzylideneaniline. In 1992 Maestroni[67] synthesized useful chiral bypiridine ligands but the efficiency (conversion and enantioselectivity) of such catalysts remained generally low. Despite many ligand modification the *e.e* was not sufficient until 1995 when Noyori, Ikariya and co-worker discovered a conceptually new chiral Ruthenium catalyst bearing N-sulfonylated 1,2 diamines.[68] (**45**, Fig.19).



45

Fig.19 (*S,S*)-Ru(II) catalyst

Since then, many efforts have been devoted toward the development for the asymmetric transfer hydrogenation of ketones and imines.[69] In this strategy different hydrogen source are used other than hydrogen gas. Typically the sources of hydrogen employed are isopropanol,

azeotrope mixture (formic acid and TEA) or formiate salts. Generally the catalysts consist of an arene or Cp-metal (Ru,Rh,Ir) complex with a chiral bidentate ligand (monotosylated 1,2 diamines or aminoalcohols) and an halide ligand. The backbone of the bidentate ligand is usually an ethylene bridge so that 1,2 relationship between the heteroatoms provides a stable five–member ring with the metal. Recently research is mainly focused on a more environmentally friendly used of TsDPEN. For this purpose, Deng *et al*. reported in 2006 the first asymmetric transfer hydrogenation of imines and iminiums salts in water using the soluble ligand reported in (**46**, Fig 20).

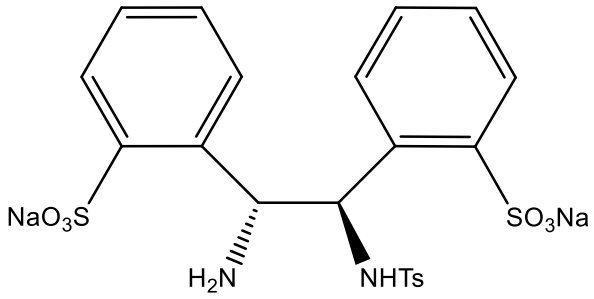


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Fig. 20 Soluble ligand for the transfer hydrogenation of imines.

Catalysis was performed with a water-soluble Ru(II)-catalyst and $NaHCO_2$ as hydrogen source. The enantioselectivity was improved by addition of CTAB, a surfactant which increased the solubility of the substrate, product, and catalyst.[70,71]

Also other routes to increase the water solubility of organometallic complexes have been reported:(see Fig.21) i) the use of polymer-supported catalyst (ligand **47**);[72] ii) incorporation of the catalyst in a biomolecular scaffold;[73] and iii) use of other types of ligand, e.g. based on a pyridine moiety (ligands **48**, **49** and **50**).[74–77]

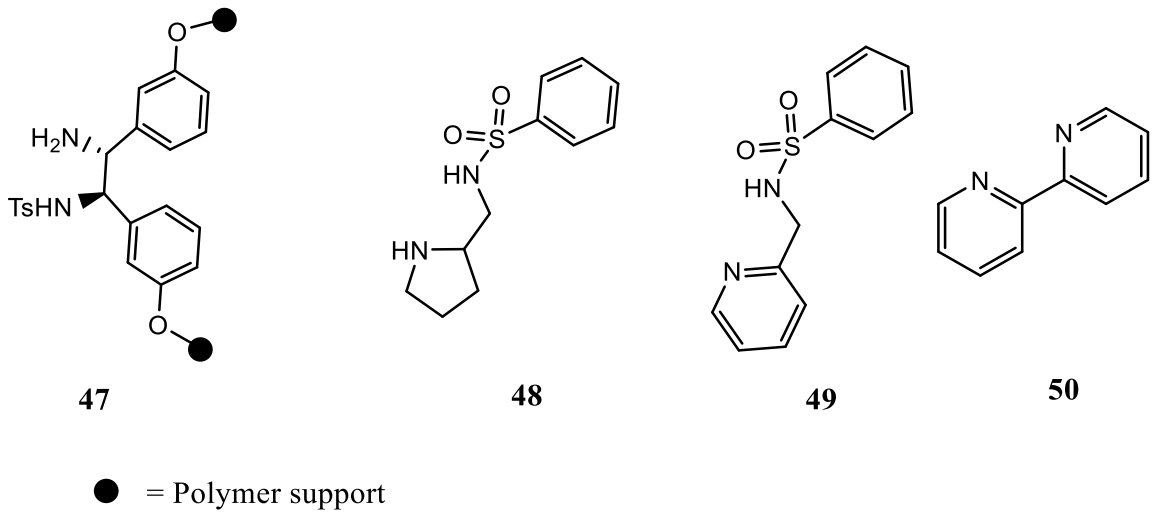


Fig 21 Ligands prepared with different strategies to improve the water solubility.

A key parameter for asymmetric transfer hydrogenation is the pH as highlighted by Xiao *et al*.in 2005. They reported that the pH of the reaction solution affects the catalyst performance in the aqueous-phase.[72] The observed variations of enantioselectivity according to the pH may be explained by the protonation of TsDPEN ligand under acidic conditions.[78] Depending on the nature of the aromatic sulfonamide moieties, the pKa of the corresponding N-H group varies between 4.5 (Ar = C_6F_5) and 7.6 (Ar = p-toluene).[79] The change in the sulfonamide protonation leads to an "on and- off" catalyst state observed by Xiao, as illustrated in Fig 22.

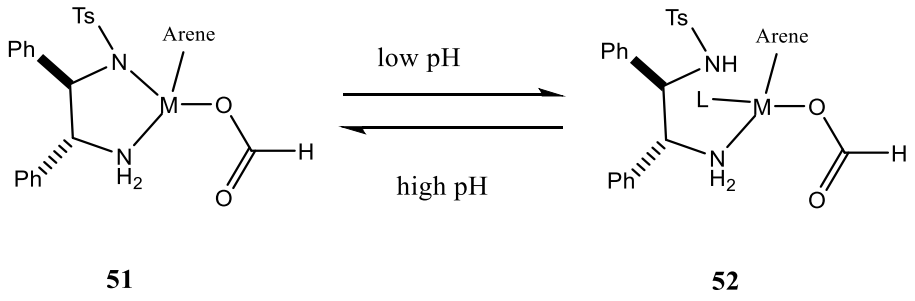


Fig 22 Different Metal –TsDPEN states correlated to basic (51) or acid (52)conditions. Ligand L may represented water molecule.

1.10 ENZYMES

Enzymes are macromolecular biological catalysts responsible for thousands[80] metabolic processes that sustain life.[81][82]Most of them are proteins, although some catalytic RNA molecules have been identified.[83][84] Folded polypeptides (Fig. 23) containing metallic ion cofactors in their active sites (Metalloenzymes) possess the ability to perform complex biological transformations (e.g. photosynthesis, respiration, water oxidation, molecular oxygen reduction and nitrogen fixation, etc.).

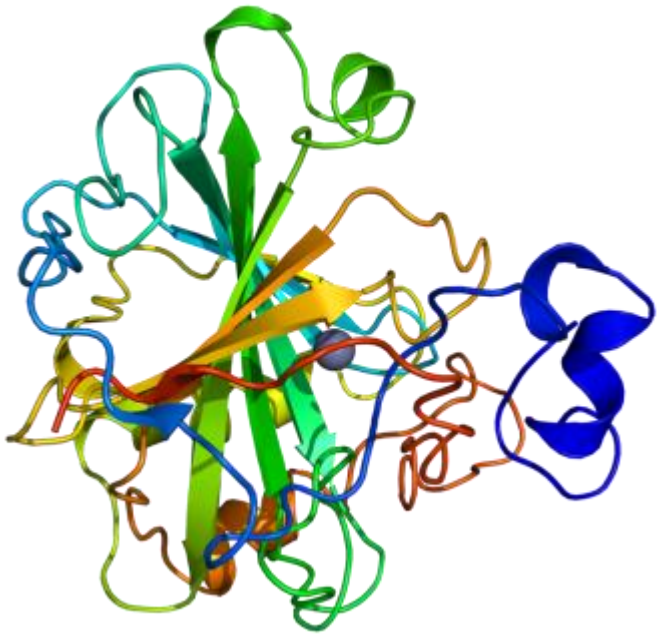


Fig 23 Picture of ribbon diagram of human carbonic anhydrase II. Zinc ion visible at center.

Humans have used enzymes for thousands of years for fermentation processes in order too produce and preserve foodstuff. Only in the 1858 Louis Pasteur placed a milestone of biocatalysis. He treated an aqueous solution of racemic tartaric acid ammonium salt with a culture of the mold *Pennicillium glaucum* leading to the consumption of (+) tartaric acid and enrichment of the (-) enantiomer.[85] This was the prototype of enzyme catalyzed racemic resolution widely used today in academia and industry.[86,87]

Enzymes as catalysts in synthetic organic chemistry gained importance in the latter half of the 20th century, when the advent of recombinant DNA[88] allowed to obtain proteins in large quantities for practical applications.

Moreover, with the development of directed evolution[89,90] beginning in the 1990s other problem such as narrow substrate scope, poor stereo- and/or regioselectivity, insufficient stability under operating conditions and product inhibition can be addressed. Nowadays, biocatalytic steps using fine-tuned biological scaffolds are implemented in industry in order to synthesize complex molecules, e.g. advanced pharmaceutical or insecticide intermediates under environmentally friendly conditions.[91–92]

1.11 ENZYMATIC CATALYSIS

Biocatalysis has many benefits to offer in the context of green chemistry.[93] Reactions are performed under mild conditions (physiological pH, room temperature and atmospheric pressure) with a biodegradable catalyst (an enzyme) that is derived from renewable resources and in an environmentally compatible solvent (water). Furthermore, reactions of multifunctional molecules proceed with chemo-, regio- and stereoselectivities and generally without the need of functional group activation, protection and deprotection steps required in traditional organic syntheses. These statement affords processes which are more economic, generate less waste and are, therefore, both environmentally and economically more attractive than conventional routes. As a direct consequence of the high regio and stereo- selectivities, coupled with milder reaction conditions, allowed to obtained products in higher quality than traditional chemical or chemo-catalytic processes.

An illustrative example is the green-by-design, two-step, three-enzyme process [94,95] for the synthesis of a key intermediate **57** in the manufacture of anticholesterolemic atorvastatin, (Lipitor®). (Fig. 24)

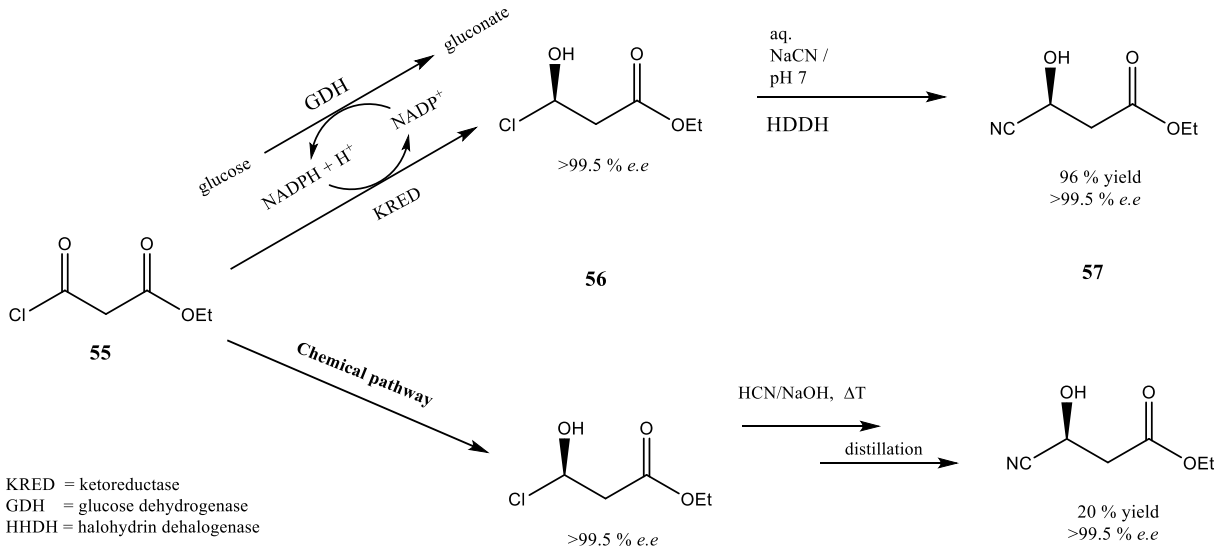


Fig 24 Synthesis of key intermediate for the manufacture of Atorvastatin.

All previous manufacturing routes to obtain the hydroxynitrile product **57** involved, as the final step, a standard SN_2 substitution of halide with cyanide ion in alkaline solution at high temperatures. However, both substrate and product are base-sensitive and extensive by-product formation is observed. To make things worse, the product is a high-boiling oil and a troublesome high-vacuum fractional distillation is required to recover product of acceptable quality, resulting in further yield losses and waste.

Using *in vitro* evolution strategies[96], Codexis led to the development of a very active and selective enzymes, and the process has been successfully commercialized on a multi-ton scale. The first step involves the biocatalytic reduction of ethyl-4-chloroacetoacetate **55** using a ketoreductase (KRED) in combination with glucose and a NADP-dependent glucose dehydrogenase (GDH) for cofactor regeneration. The (*S*)-ethyl-4-chloro-3-hydroxybutyrate product **56** was obtained in 96% isolated yield and 99.5% *e.e*. In the second step a halohydrin dehalogenase (HHDH) was employed to catalyze a nucleophilic substitution of chloride by cyanide using HCN at neutral pH and room temperature.

The improvement of the enzyme, increased the activity more than 2500-fold, compared to the wild-type, allowing the complete transformation of the substate in 8 h with an increased substrate loading to 160 g L^{-1}. Moreover, the problem connected to the formation of emulsion was overcome reducing the enzyme loading, and the downstream process became very easy. The E factor (Environmental Factor) of this process is 5.8 while Atom economy is 45%.

$$\text{E Factor} = \text{kg waste / kg product} \qquad \text{(Eq 1.2)}$$

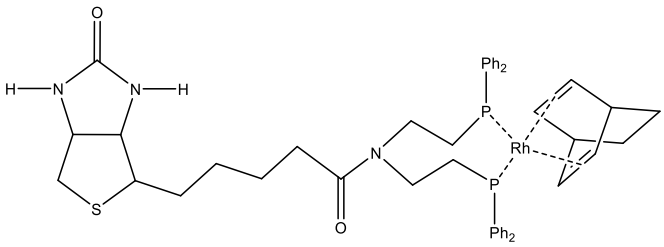
$$\text{Atom Economy} = \frac{\text{mass of atom product}}{\text{mass of atom reactants}} \times 100 \qquad \text{(Eq 1.3)}$$

1.12 ARTIFICIAL METALLO ENZYMES

The artificial metalloenzymes concept was created by Whitesides and Wilson in 1978 when they postulated:

> *"A globular protein modified by introduction of a catalytically active metal at an appropriate site could, in principle, provide an exceptionally well-defined steric environment around that metal, and should do so for considerably smaller effort than would be required to construct a synthetic substance of comparable stereochemical complexity."*[97]

In their pioneering work, Whitesides and Wilson incorporated one biotinylated phosphine-rhodium(I) complex (**58**, Fig.25) within avidin, for the hydrogenation of α-acetamidoacrylic acid, yielding a modest chiral induction (44% *e.e*).[97]



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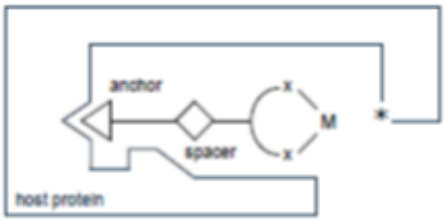


Fig.25 Biotinylated Rhodium complex **58** and graphical representation of artificial metalloenzymes based on supramolecular anchoring.

Considering the ability of macromolecules to selectively discriminate substrates and the number of chemical transformations catalysed by transition metals, which have not been observed to occur enzymatically, several research groups reasoned that a hybrid catalyst may combine some of the most attractive features of homogeneous and enzymatic catalysts (Tab.1).[98] Starting from the 70's, chemists have designed this catalyst, so called "artificial metalloenzymes", that exhibit high selectivity for the synthesis of enantiomerically enriched compounds in aqueous media.

	Homogeneous catalysis	**Enzymatic catalysis**	**Artificial metalloenzymes**
Substrate scope	Large	Limited	Large
Enantiomers	Both accessible	Single enantiomer	Both
Functional group tolerance	Small	Large	Small
Reaction repertoire	Large	Small	Large
Turnover number	Small	Large	Modest
Solvent tolerance	Organic>aqueous	Aqueous>Organic	Aqueous>Organic
Optimization	Chemical	Genetic	Chemogenetic
II coordination sphere	No defined	Well defined	Well defined
Reaction conditions	Harsh	Mild	Mild

Tab. 1 Comparison of homogeneous, enzymatic and Artificial metalloenzymes catalysis.

In more general terms, the development of a hybrid catalyst results from the combination of a biomolecular scaffold (e.g. proteins,[99,100] DNA[101] or peptides[102]) with an active catalytic moiety.

Such an endeavour is likely to meet with success thanks to the intimate knowledge of both the protein scaffold that hosts the metal entity and the interaction(s) between the metal and its chelating ligands (the first sphere of coordination) and the protein environment (the second sphere of coordination). (Fig 26)

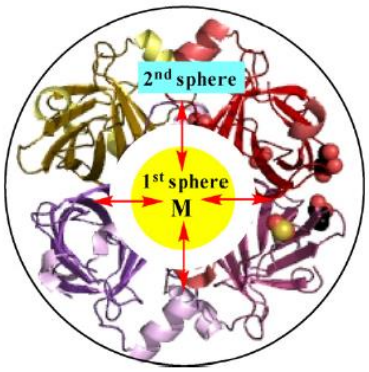


Fig. 26 Representation of first and second coordination sphere provided by ligand and biomolecules respectively.

The three key parameters in the design of an artificial metalloenzyme are a) the transition metal catalyst, b) the biomolecular scaffold, and c) the mode of attachment of the transition metal catalyst to the scaffold.

The choice of the transition metal catalyst is principally guided by the catalytic activity that is desired. Moreover the reactivity of the transition metal catalyst needs to be orthogonal to the biomolecular scaffold, (it should be inert to the chemical functionalities presented by the biomolecule) and the catalyst should be tolerant to water.

Considering the biological scaffold, two different strategies could be used:

1) *de novo* design
2) modification of an existing natural enzyme or protein.

Using the first strategy, the biomolecular scaffold is created from scratch. An interesting example is reported in 2014 by Pecoraro *et al* [103] in which protein design allow for the creation of artificial enzymes catalyzes the hydration of CO_2 with an efficiency within 1400-fold of the fastest carbonic anhydrase isoform. (Fig 27)

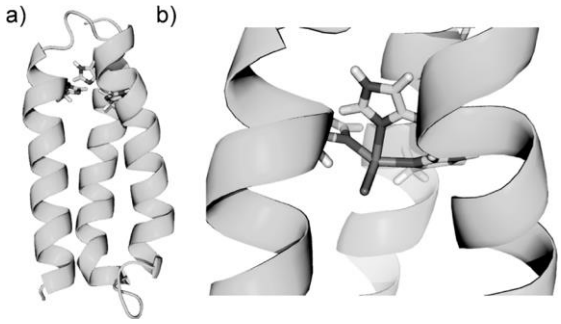


Fig. 27 a) the entire bundle (last four residues removed for simplicity) and b) the ZnII His3O site (PDB: 2A3D).[104]

Even if significant advances have been made in the computational methods that are required for this purpose, [105,106] our understanding of protein folding is still far from sufficient to allow the routine design of novel enzymes for any desired synthetic transformation. For this reason, the design of artificial metalloenzymes has focused on the creation of active sites in existing, native, biomolecular scaffolds, such as proteins and DNA. The design of active sites into native scaffolds offers more choices and it is, in principle, simpler than *de novo* design considering that most native scaffolds already have sufficient thermodynamic stability and tolerance for mutations. Furthermore, structural information for most of the scaffolds used to date is available, facilitating the design and optimization process considerably.

To rationally design an artificial metalloenzyme, two strategies for the attachment are available: the no-covalent strategy including dative and supramolecular interactions and the covalent anchoring strategy.[107] (Fig.28)

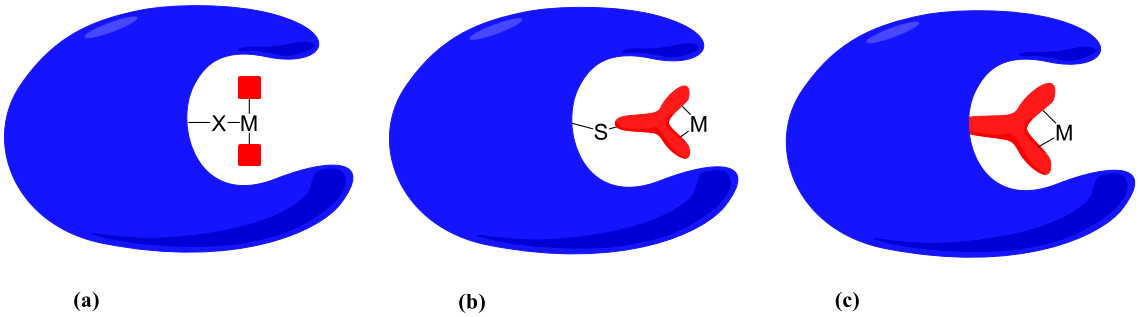


Fig. 28 Different anchoring strategies for the localization of precatalyst within macromolecule (blue). a) dative, b) covalent, and c) supramolecular. The first coordination sphere chemically synthesizd is rapresented in orange while M is the transition metal.

The covalent approach,[108–111] proposed for the first time by Kaiser in 1984,[112] commonly uses a cysteine as an anchoring residue. This method allows for precise localization of a metal complex within or on the surface of a biological scaffold, but a considerable drawback is that the optimization and redesign is not straightforward and is time-consuming, involving chemical modification and non-trivial purification steps.

The dative anchoring strategy relies on the coordination of a catalytic metal by chemical functionalities present directly on the surface of the macromolecular scaffold. An example are the sulfonamide ligands that bind hCA II zinc.[113–116]

In supramolecular approach [117,118] a strong and highly specific non-covalent interaction between bio and a small molecule derived from specific ligand/inhibitor structures to generate artificial metalloenzymes. This technique allows for easy chemical optimization of metal ligand features (first coordination sphere) and avoids uncertainties concerning the localization of the metal within the macromolecular scaffold.[119]

The above mentioned examples of artificial metalloenzymes based on the biotin/(strept)avidin interaction involve supramolecular anchoring. The success of this approach is related to the ease of self-assembly of these artificial metalloenzymes, which allows for rapid optimization. A potential complication is that there may be some ambiguity about where the catalyst might bind, depending on the strength and selectivity of the supramolecular interactions.

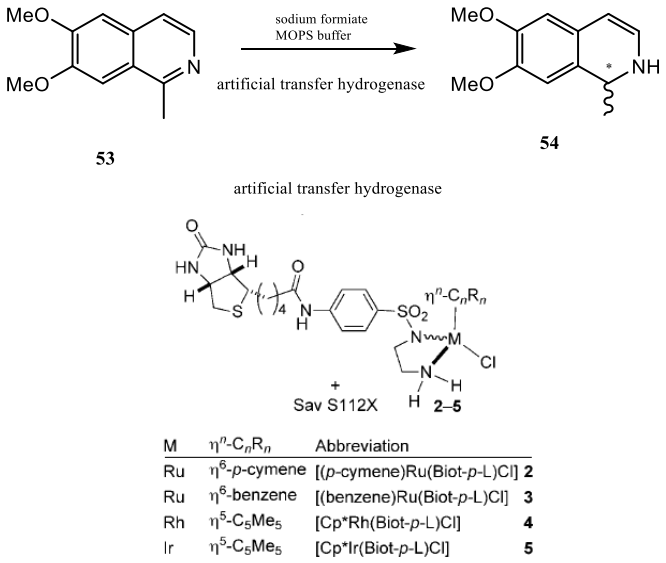
1.13 BIOTIN-STREPTAVIDIN TECHNOLOGY

As previously mentioned, the first example of a hybrid catalyst based on biotin-avidin technology was developed by Whitesides and Wilson in 1978.[97] A biotinylated Wilkinson's catalyst was embedded into avidin to obtain 44% *e.e.* for the reduction of α-acetamidoacrylic acid.

Several years after this system was used and optimized in the Ward group relied on the high affinity of (+)-biotin (also known as vitamin H) towards two proteins, streptavidin and avidin. To introduce the catalytic metal within the host protein, the biotin, used as anchor, was derivatized through *orto*, *meta* and *para* position with solfonammide moiety. Despite the modifications, the affinity towards the host protein remains high[120] and the metalloenzyme remains remarkably stable even under harsh catalytic conditions, e.g. 55 ◦C and mixture of water and organic solvents.[121]

Subsequently, rational chemo-genetic modifications (rational design, Section 1.8) of the hybrid catalyst, improved the enantioselectivity of the reaction up to 91% *e.e.* (*R*) as well as reversed of the enantioselectivity 75% *e.e.* (*S*) for the reduction of 4'-bromoacetophenone.[122,123]

Using the same technology this group realized, a novel artificial transfer hydrogenase for the enantioselective reduction of cyclic imines in 2011.[124] Imines were reduced under mild conditions using formiate as hydrogen source. Chemo-genetic optimization of the hybrid catalyst resulted in a system that yielded both enantiomers (*S*, 78% and *R*, 96 % *e.e.*) of salsolidine **54** (Fig. 29).



M	η^n-C_nR_n	Abbreviation	
Ru	η^6-*p*-cymene	[(*p*-cymene)Ru(Biot-*p*-L)Cl]	**2**
Ru	η^6-benzene	[(benzene)Ru(Biot-*p*-L)Cl]	**3**
Rh	η^5-C_5Me_5	[Cp*Rh(Biot-*p*-L)Cl]	**4**
Ir	η^5-C_5Me_5	[Cp*Ir(Biot-*p*-L)Cl]	**5**

Fig. 29 Reaction catalyzed by artificial transfer hydrogenase based on Biot-streptavidin technology.[124]

In a very recent paper they implemented the chemical diversity using three-leggend piano stool complexes tethering the biotin anchor on the Cp^* moiety.[125]
An alternative approach focused on directed evolution of hybrid catalysts was implemented by Reetz and co-workers.[126] They were able to enhance or invert the selectivity for the transfer hydrogenation of α-acetamidoacrylate obtaining 65% *e.e.* (*R*) or 7% *e.e.* (*S*) under optimized conditions.

Having demonstrated the potential of Artificial metalloenzymes for enantioselective transformation of prochiral substrates,[122,123] the biotin- streptavidin technology was also used for other reactions for which one of the substrate does not bind to the catalytic metal center in the transition state like asymmetric transfer hydrogenation,[124] dihydroxylation[127] and sulfoxidation.[128] (Fig. 30)

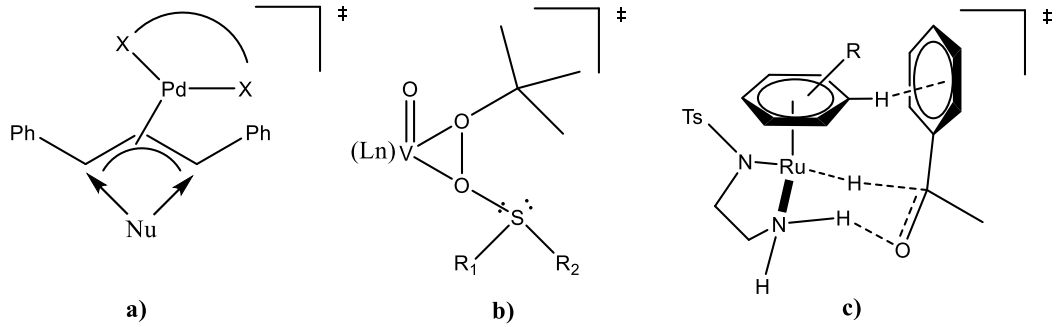


Fig. 30 Postulated transition states for catalysts where one of the substrates does not bind to the metal: a) asymmetric allylic alkylation, b) sulfoxidation, c) asymmetric transfer hydrogenation.

1.14 HUMAN CARBONIC ANHYDRASE II TECHNOLOGY

Wild-type human Carbonic Anhydrase II (hCA II, EC 4.2.1.1, M [g·mol−1] = 29227 pI 7.4) is a monomeric globular protein containing 259 amino acids[116]. This Protein is a metalloenzyme that catalyze the reversible hydration of carbon dioxide into bicarbonate with high efficiency ($k_{cat}/K_M = 1.5 \times 10^8\ M^{-1}\ s^{-1}$.)

$$CO_2 + H_2O \xrightleftharpoons{\text{hCA II}} HCO_3^- + H^+ \qquad \text{(Eq 1.4)}$$

Mutations or expression deficiency of the isozymes are involved in diseases such glaucoma, ureagenesis and lipogenesis.[129] Moreover, overexpression of hCA IX and hCA XII are found in certain forms of cancers[130,131] and inhibitors have been designed for diagnosis and therapy.[132,133]

In the active site, hCA II contains catalytic Zn II ion, tetrahedrally coordinated to three histidines (His94, His96 and His119) and a solvent molecule. This cofactor is located at the base of a funnel shaped cavity measuring roughly 15 Å in diameter at its mouth and 15 Å deep.[134]

Human carbonic anhydrase II has been extensively studied, and the parameters of its structural stability are well known and here reported in table below.

Temperature[135,136]	up to 55 ◦C
pH[116]	5.7—8.4
Metal [137]	low exchange rate
Organic solvent [138]	less than 20 % (DMSO)

Several characteristics of Human Carbonic Anhydrase II are attractive for the development of a new artificial metalloenzyme:

1) it's a monomeric protein is easy to overexpress in *E.coli* and to purify
2) hCA has a large binding pocket able to accommodate metal complexes
3) it is possible to apply computational design for rational tailoring of the active site to accommodate inhibitors compatible with soft transition metals[139,140]
4) the X-ray determination of hCA II structure is well established.[116]

Considering lock-and-key model described by Fischer in 1894, sulfonamide derivatives were proposed in 1940 by Keilin *et al.* as specific inhibitors of human Carbonic Anhydrase isozymes[141] because these molecules are analogs to the transition state of CO_2 hydration.
The sulfonamide nitrogen anion coordinates to the Zn II cofactor, and two hydrogen bonds are established with the protein scaffold as reported in (Fig.31). In addition the second oxygen of sulfonamide coordinates weakly to Zn II.

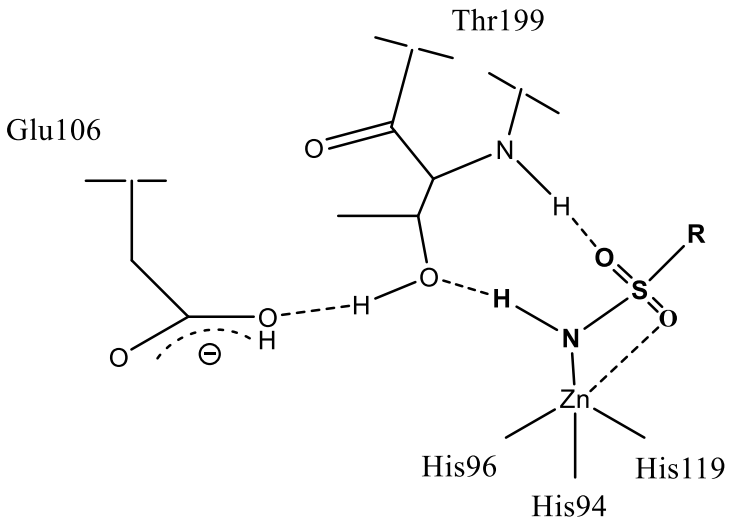


Fig. 31 Sulfonamide bound to the active site of hCA II.

Also the aromatic moieties of sulfonamide were found to interact significantly with the primary hydrophobic faces inside the funnel-shaped cavity,[142] and to further increase the binding affinity between ligands and protein, secondary recognition elements may be exploited.

Whitesides and Jain reported in 1994, that *para*-substituted benzenesulfonamides bearing benzyl moieties can interact with the hydrophobic upper rim of the funnel-shaped cavity of hCA II increasing the binding between protein and small molecule (K_d =2 nM).[143]
In 2002 the same group computationally designed *para*-benzenesulfonamide derivatives and reported an inhibitor with the highest known affinity for hCA II (K_d =30 pM).[144]
Another approach to further increase the affinity of inhibitors, was made by Fierke groups. They introduced fluorine substituents on the molecular scaffold (K_d =0.29 nM). [145] The presence of fluorine moieties also increase the metabolic stabilization of inhibitors when used as drugs.

The application of hCA II in hybrid catalyst was fruitfully explored by Ward group, and in 2013 they reported the used of this scaffold as host protein for the creation of artificial metalloenzymes able to asymmetrically reduce imines by transfer hydrogenation.[146] (Fig.32)

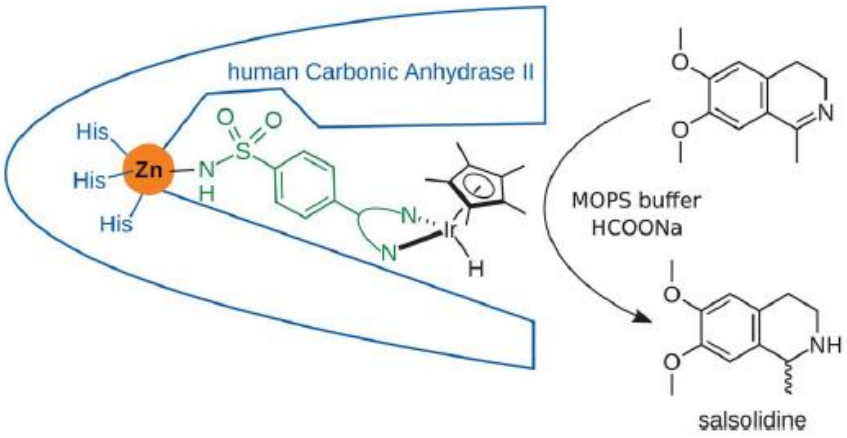


Fig. 32 Hybrid catalyst for imine reduction obtained combining hCA II (blue) and an aryl- sulfonamide bearing bidentate ligand (green) able to anchor an IrCp* moiety within the protein. Picture from publication.[146]

1.15 CATALYST OPTIMIZATION: BIOLOGICAL AND CHEMICAL DIVERSITY.

Directed evolution is one of the most powerful tools to engineer enzymes enhancing the activity and the selectivity of enzymes. [147,148]
This evolutionary approach involves the introduction of random mutations into the genes, thus creating a library of mutant proteins. These enzymes variants are screened for catalytic activity and selectivity, and the best candidates taken forward to another round of random mutagenesis.
In the case of hybrid catalysts, this process turns out to be complicated as for the need to use purified (or semi-purified) protein for screening.

To optimize hybrid catalysts, the chemo-genetic approach, previously described, was found to be the most suitable solution.[149,150] It concerned two distinct optimizations:

1) genetic modification of the protein scaffold, based on computational calculations and X-ray structures. Particular attention is given to the active site of enzyme (<10 Å around the catalytic metal center);[151,152]

2) chemical fine-tuning of the catalytic moiety, to adjust the localization of the catalytic moiety inside the funnel-shaped cavity of the protein.

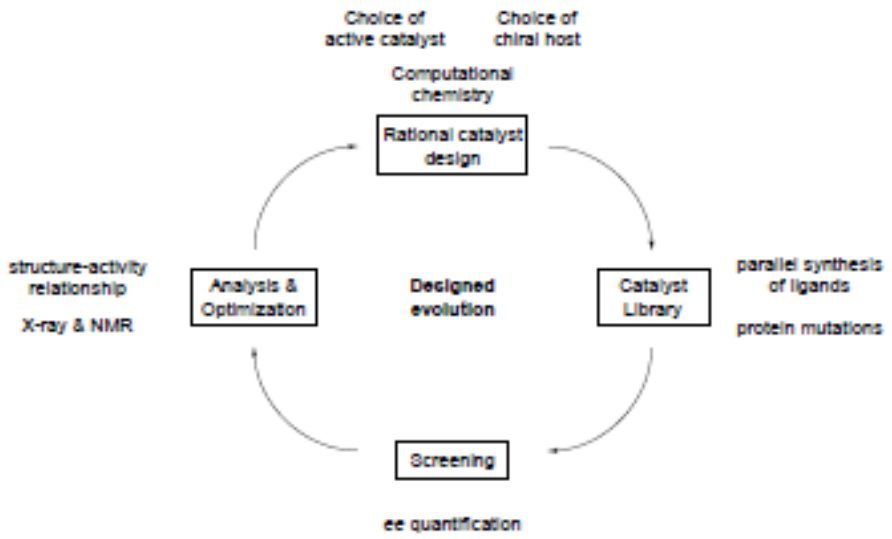


Fig. 34 Design evolution cycle.

The parallel use of this techniques allow the design of a small collection of hybrid catalysts with improved activity and selectivity screenable on a relatively short time frame.
This operation can also be performed iteratively as shown in Figure 34 resulting in a process which has been called “designed evolution”.[153]

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2

RESULTS AND DISCUSSION

2.1 Synthesis of chiral synthons

Several yeasts were tested for the asymmetric reduction of substrates **1**, with the aim to discover an efficient biocatalytic tool for the preparation of enantiopure chiral alcohols **2**.

Among 20 different microorganisms, *Rhodotorula rubra* MIM 147 and *Sporidiobolus pararoseus* SD2 showed their wide capabilities in the asymmetric reduction of carbonylic group in α-position to aromatic ring as reported in Fig.1

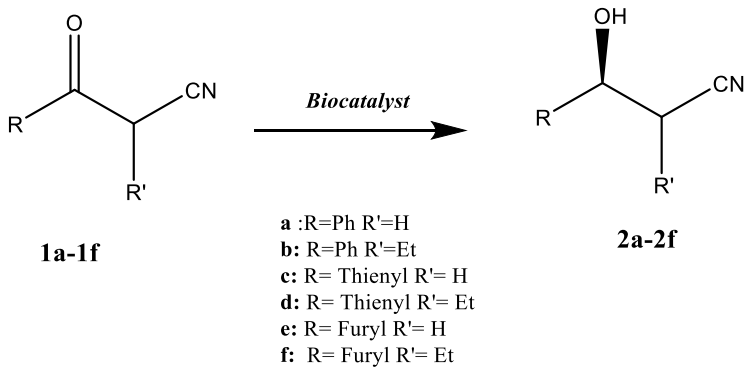


Fig.1 Scheme of synthesis for the preparation of chiral alcohol employing biotrasformation.

Compounds **1a,1c** and **1e** are commercially available, while compounds **1b,1d** and **1f** were synthesised in our laboratory. As shown in Fig.2, to introduce the ethyl group in compounds **1b, 1d** and **1e**, a condensation reaction was performed using *Saccharomyces cerevisiae* as biocatalyst, obtaining full conversion in 48h.

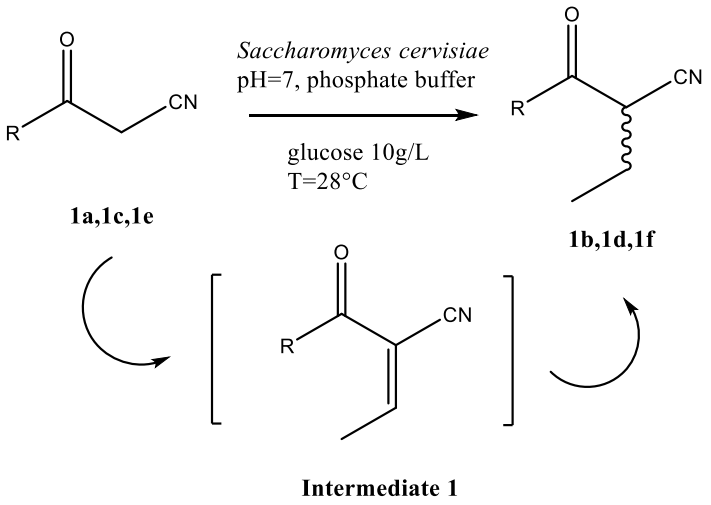
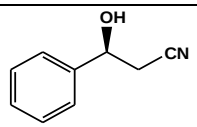
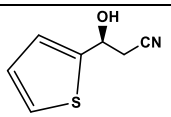
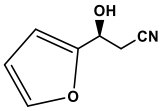
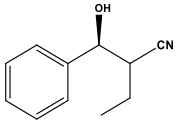
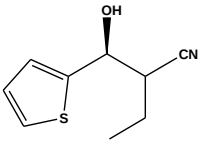
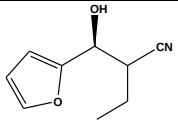


Fig.2 Biotrasformation reaction using Baker's yeast allows to introduce the ethyl group in β position.

Acetaldehyde, produced in presence of glucose by Baker's yeast as intermediate by alcoholic fermentation, performs an electrophilic attack at the C2 in α position to the nitrile and keto groups. Indeed the presence of these two electron withdrawing moieties allow the chemical condensation between the active methylene and the aldehyde leading the formation of unsatured carbonyl compound (intermediate 1). Finally the double bond reduction performed by yeast gives the saturated product as racemate.[1]

In particular, using *R.rubra* MIM 147, we were able to prepare in quantitative yields six enantiopure alcohols in *S* configuration, as reported in Table 1.

	%C	*e.e.*	*e.d.*
	95	>99[a]	-
	90	>99[a]	-
	92	>99[b]	-
	94	>99[a]	92[a]
	89	>99[a]	70[a]
	92	>99[b]	73[b]

Tab 1. Results obtained using *R.rubra* MIM 147 after 48 h. a) HPLC equipped with OD-H coloumn, eluent 95/5 hexane/*i*PrOH, flow= 0.8mL/min. b) GC using a chiral stationary phase column (MEGA DMT β, 25 m, internal diameter 0.25 mm), 180°C.

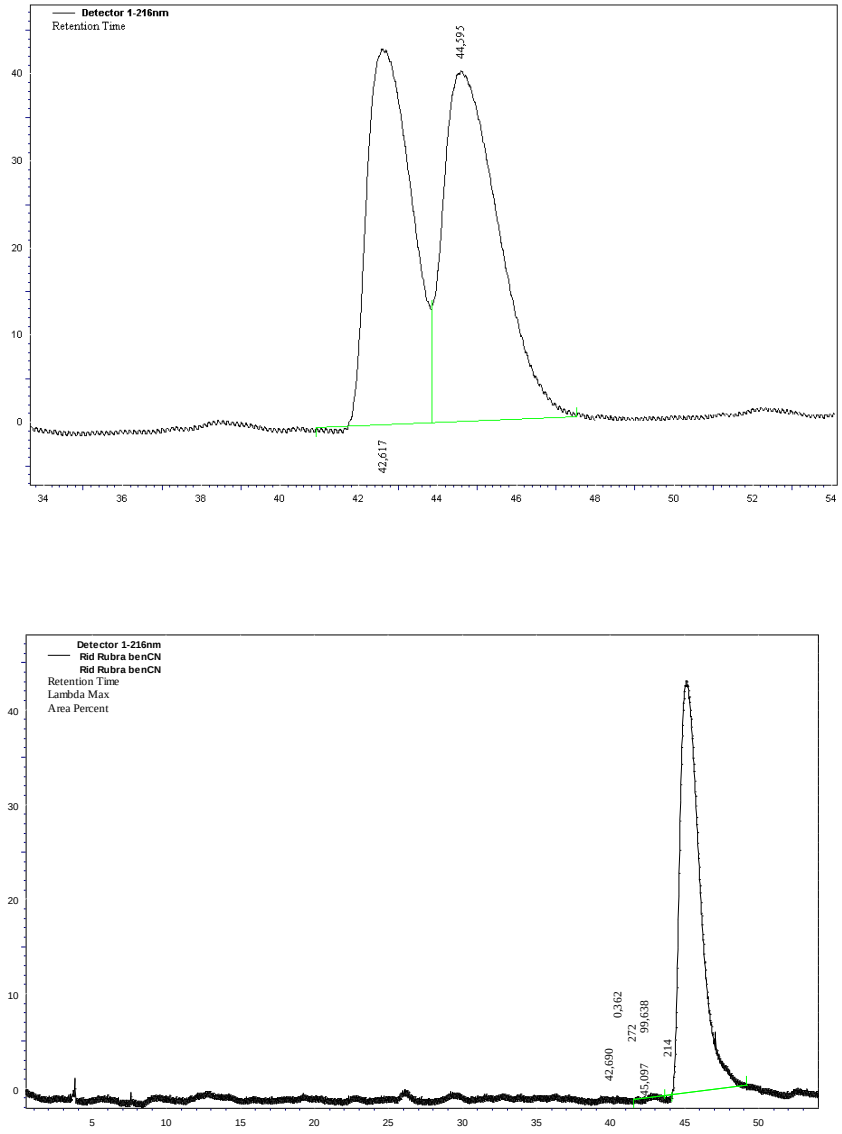


Fig.3 HPLC chromatograms of compound ***S*-2a** obtained using *R.rubra* MIM 147. OD-H Chiralcel, eluent: hexane/*i*PrOH = 95:5, flow = 0.8 mL/min, λ = 216 nm; rt: (*R*) = 42.6 min, (*S*) = 44.6 min.

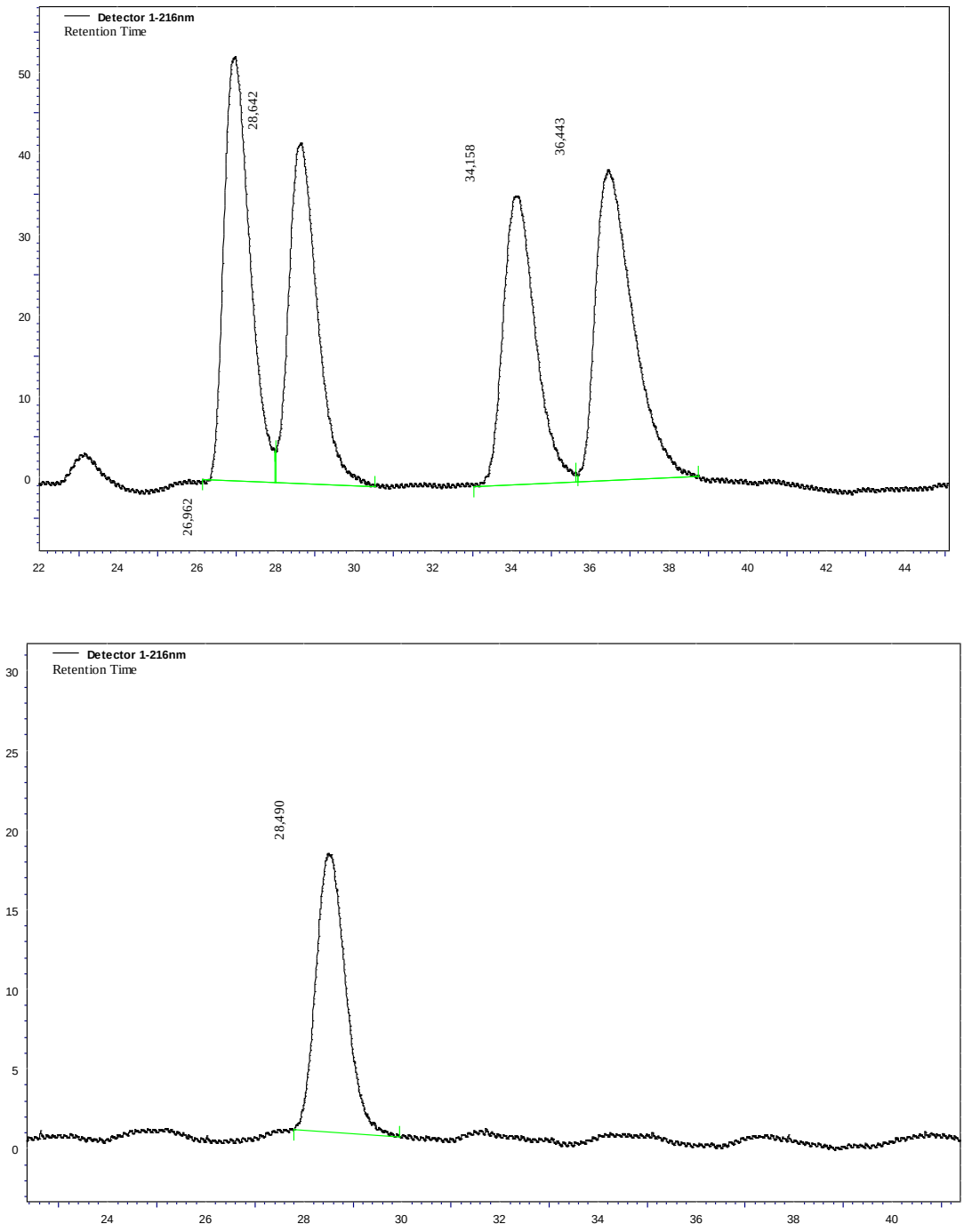
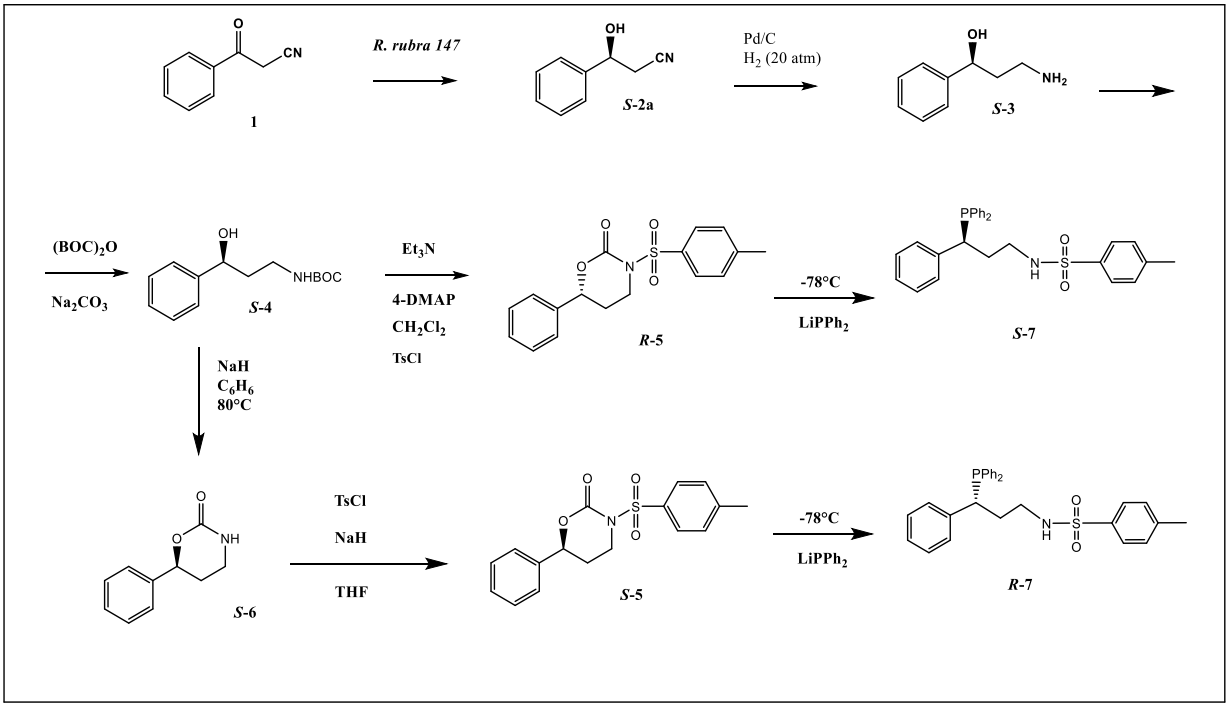


Fig.4 HPLC chromatograms of compound **2b:** OD-H Chiralcel, eluent: hexane/*i*PrOH= 95:5, flow = 0.8 mL/min, λ = 216 nm; rt: (*R*,*S*) = 26.9 min, (*S*,*S*) = 28.6 min, (*S*,*R*) = 34.2 min, (*R*,*R*) = 36.4 min.

2.2 First generation ligands: 1,3 Ts-aminophosphines

Chiral benzyl alcohol **2a** was used for the preparation of tosyl aminophosphines as useful ligands for the preparation of transition metal complexes. New ligands containing both phosphine and tosyl amino moieties are synthesised as shown in Scheme 1.



Scheme 1

Starting from (***S***)-alcohol (obtained in 95% yield in 48 h with *e.e.* up to 99% by biocatalysis), we were able to obtain both enantiomers (***R*-5**, ***S*-5**), using different synthetic approaches. The synthesis proceeded with the same pathway until the formation of compound ***S*-4 (**$[\alpha]_D$= -18,9), then different mechanisms of cyclization were exploited. Cyclic compound ***S*-6** was obtained by acid-base reaction without affecting chiral centre (retention of configuration), while the synthesis of compound ***R*-5** was led by SN_2 mechanism with an inversion of configuration. The enantiomeric relationship, due to the different mechanisms of cyclization, was confirmed by $[\alpha]_D$ data ($[\alpha]_D$ = +24,7 and –24,6 respectively for compound ***R*-5** and ***S*-5**) and these results are comparable to those reported in literature.[2,3] Final step of synthesis allow to introduce phosphine moiety at the chiral centre with an inversion of configuration respect to the previous cyclic compound as confirmed by $[\alpha]_D$ data ($[\alpha]_D$ = -124,7 and +124,9 respectively for compound ***S*-7** and ***R*-7**). In this way we were able to prepare both enantiomers of tosyl aminophosphine starting from the same chiral alcohol avoiding the need to identify another catalyst for the synthesis of ***R***-alcohol.

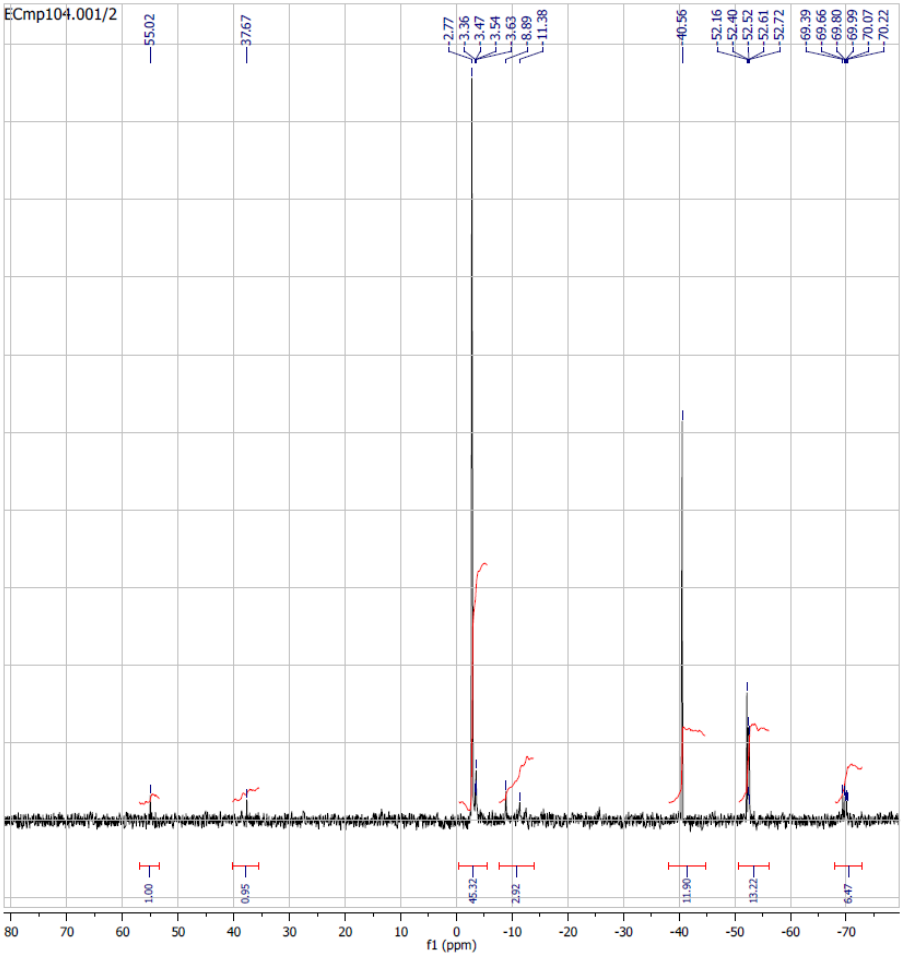


Fig.4 ^{31}P- NMR of ***S*-7** or ***R*-7**: -2.77 ppm

2.2.1 Tosylaminophosphine transition metal complexes

The Ts-aminophosphine ligands, shown above, were used for the synthesis of the corresponding transition metal complexes. Precatalysts in Fig.5 were synthesised using different metal complexes as starting material: $[Ir(COD)Cl]_2$, $[Ru(p\text{-Cymene})Cl_2]_2$, $[RuCl_2(PPh_3)_3]$, $[Rh(COD)_2ClO_4]$.

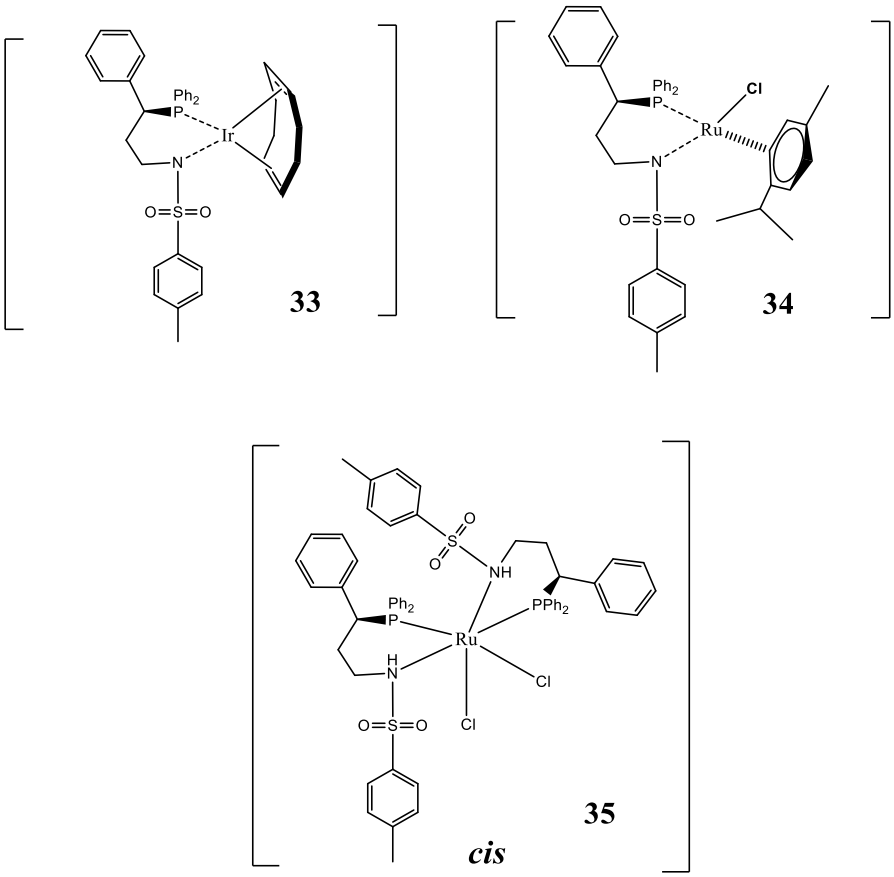


Fig.5

The synthesis of Rh(I) complex was not successful, while complexes containing Ru(II) and Ir(I) were fully characterized (^1H, ^{31}P,^{13}C, ESI^+). ^{31}P–NMR, reported below, confirm the phosphorous coordination of Ts-aminophosphine ligands and the formation of transition metal complexes. Ir(I) complex **33** has a single pick to +26.32 ppm, $[Ru(\boldsymbol{S}\text{-}\mathbf{7})(p\text{-cymene})]^+$ complex **34** shows a single pick to +28.70 ppm.(Fig.6-Fig.7)

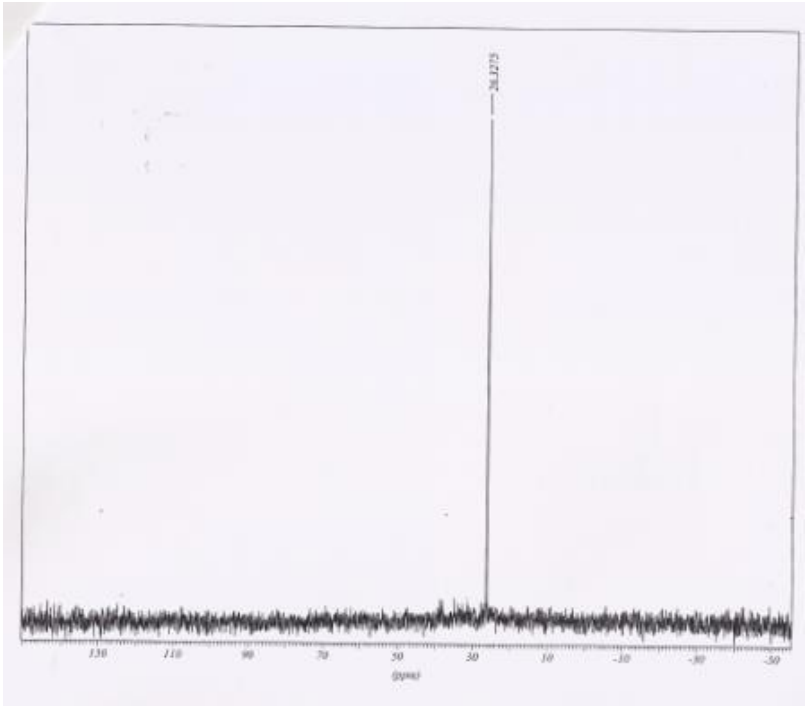


Fig.6 ^{31}**P-NMR** of **33**: +26.32 ppm

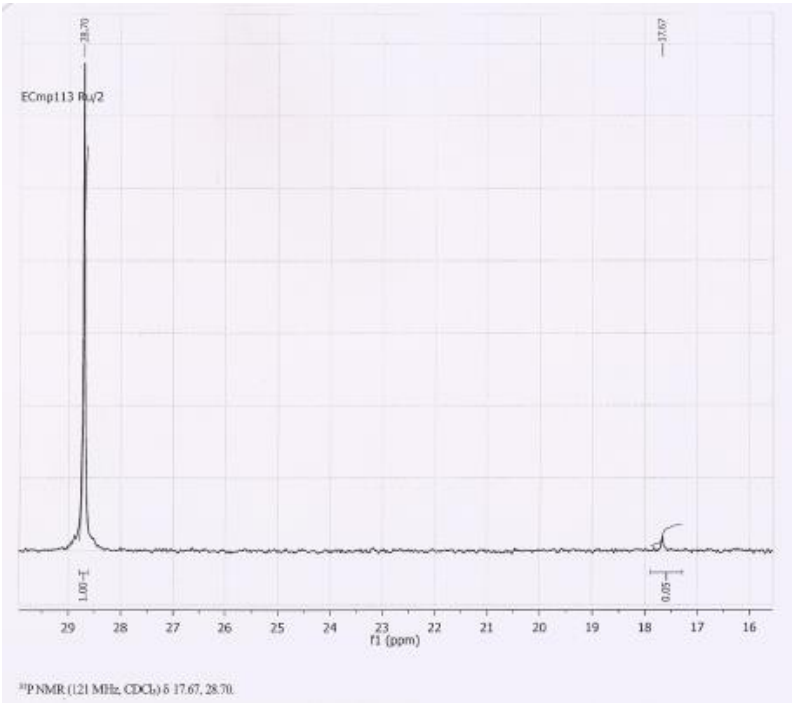


Fig.7 ^{31}**P-NMR** of **34**: +28.70 ppm

Complex obtained with $[RuCl_2(PPh_3)_3]$, **35**, shows four dublet between 52-62 ppm due to *cis* isomers Fig.8).

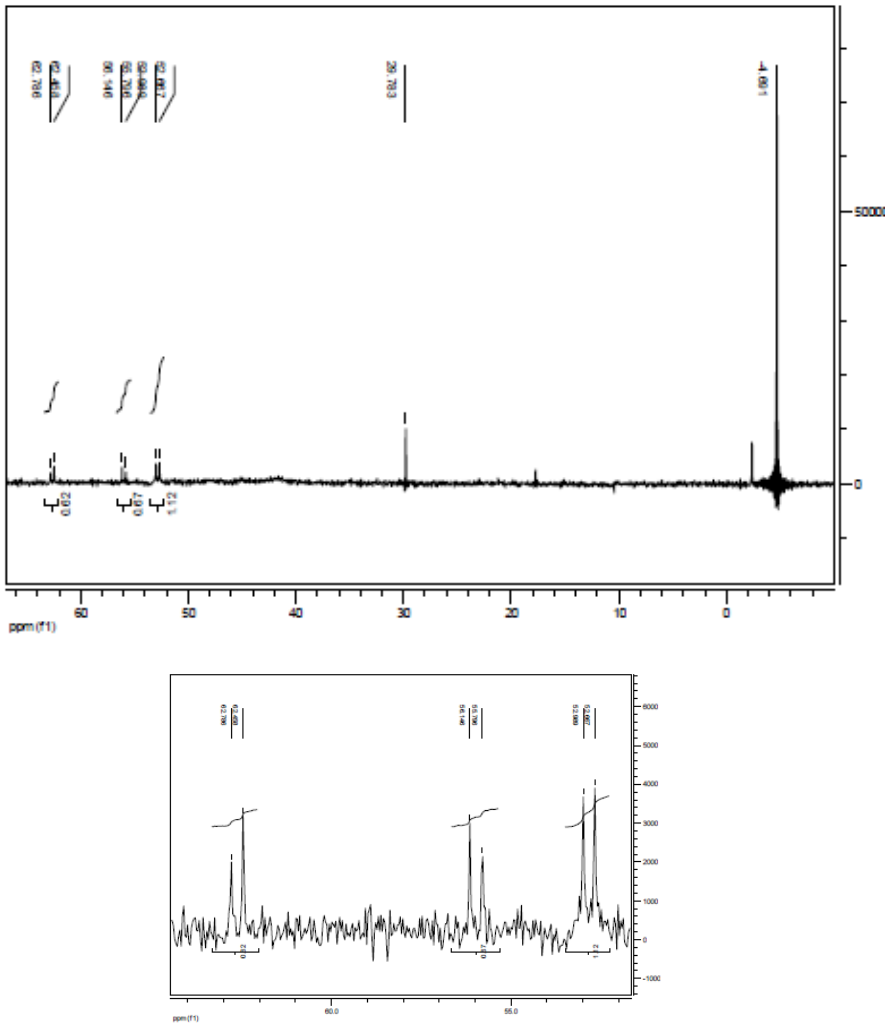


Fig.8 ^{31}P-NMR of **35**: +61.65, +56.35, +52.85 ppm

2.2.2 Catalytic results of tosyl aminophosphine precatalyst

Precatalysts were used in hydrogenation reactions for the asymmetric reduction of different prochiral substrates reported in Fig.9. For this reaction the solution containing substrate and catalyst was transferred with a cannula in stainless steel autoclave and pressurized to 20 atm.

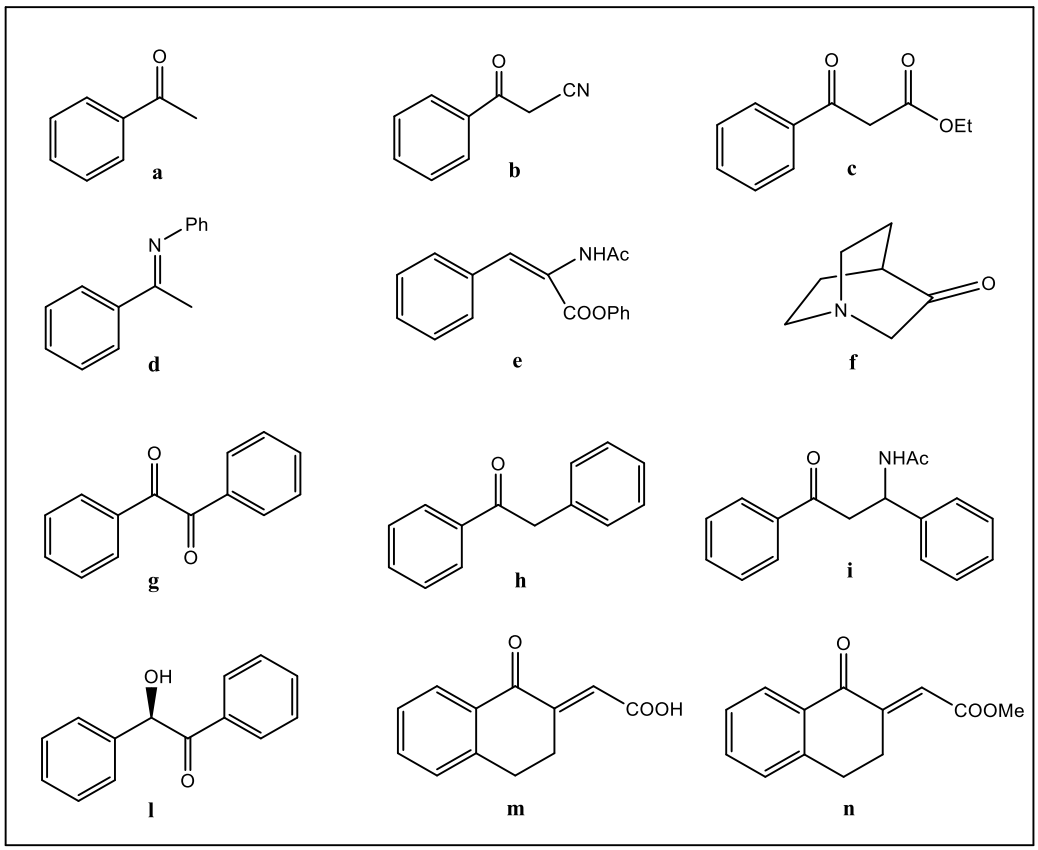
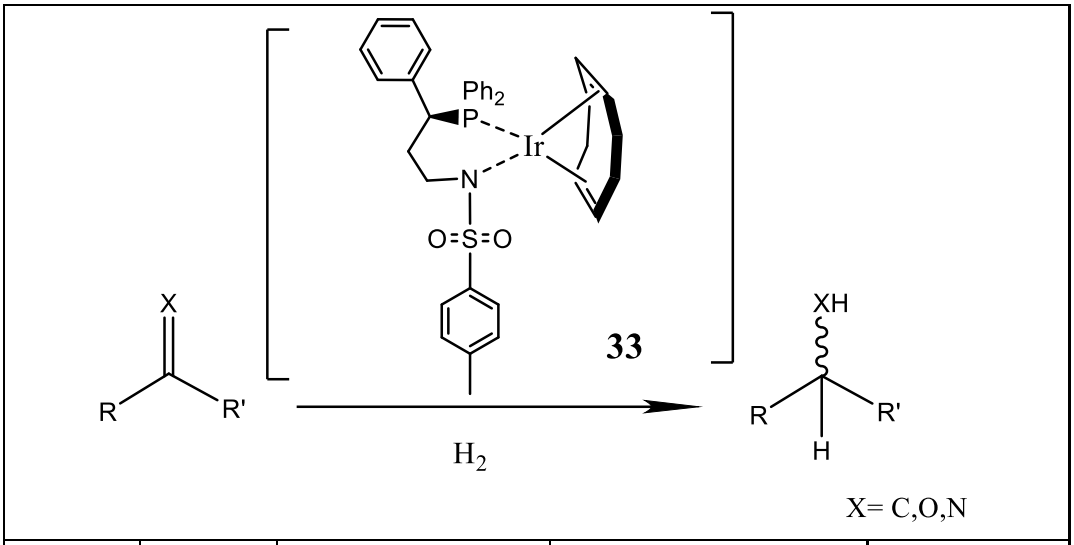


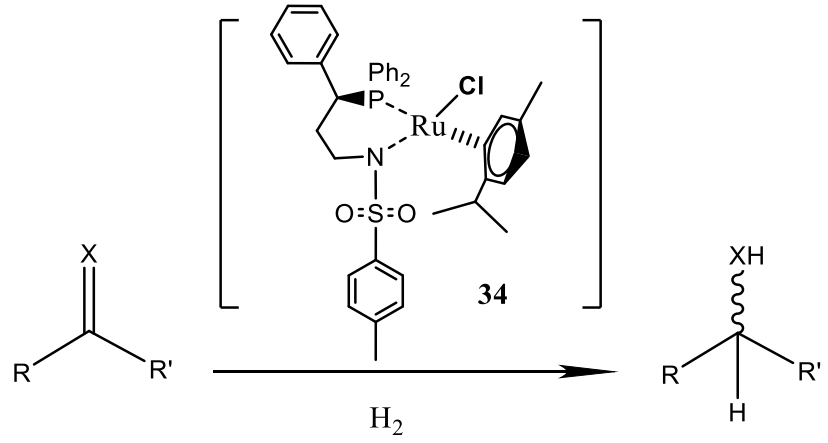
Fig.9 Aromatic ketons, imines and inactivated double bonds used as substrates for hydrogenation reaction.

All the samples were analyzed by ^{1}H-NMR, GC or HPLC equipped with chiral column after 24 h. Results in term of conversion, enantiomeric excess and TON are summarized in Table 2 and Table 3.



		%C	e.e	TON
1	a [1]	10	0	52.5
2	g [2]	98	0	295
3	l [2]	100	0	303
4	f [1]	100	0	639
5	d [3]	0 (**95**)	0	0 (**397**)
6	e [4]	10	0	36
7	m [5]	100 (**100**)	0	316 (**316**)
8	n [3]	100 (**100**)	0	291 (**291**)

Table 2. Results obtained with Ir(I) precatalyst **33**. In bold results obtained in absence of potassium *ter*-butanoate. 1) GC Iso 120°C, 2) OJ-H, 90.10(hexane/*i*-PrOH) flow=0.8mL/min, 3) OJ-H 90.10 (hexane/*i*-PrOH) flow=1mL/min, 4) OD-H 85.15 (hexane/*i*-PrOH) flow=0.8mL/min, 5) OJ-H 98.2 (hexane/*i*-PrOH, 0.1% TFA) flow=1mL/min.



X= C,O,N

		%C	e.e	TON
1	a [1]	60	0	315
2	b [1]	50	0	218
3	c [2]	50	0	165
4	g [3]	28	0	85
5	l [2]	100	0	322
6	h [2]	0	0	0
7	i [4]	0	0	0
8	f [1]	**90**	0	**575**
9	m [5]	60	0	190

Table 3. Results obtained with Ru(II) precatalyst **34**. In bold results obtained in absence of potassium *ter*-butanoate. 1) GC Iso 120°C, 2) OD-H, 90.10 (hexane/*i*-PrOH) flow=1mL/min; 3) OJ-H 90.10 (hexane/*i*-PrOH) flow=0.8mL/min; 4) OJ-H 90.10 (hexane/*i*-PrOH) flow=1mL/min; 5) OJ-H 98.2 (hexane/*i*-PrOH, 0.1% TFA) flow=1mL/min.

From the obtained results, general considerations have been summarized:

1) tosyl aminophosphine catalysts showed a high versatility to reduce different prochiral functional groups as aryl ketones, imine and inactivated double bonds.
2) tosyl aminophosphine catalysts showed a high activity, reducing most substrates with good TON and excellent conversion.
3) chiral Ts-aminophosphine Ir(I) or Ru(II) catalysts showed no stereoselectivity, in all reductions.

Iridium complex seems to have greater activity than Ruthenium one. For imine reduction, the absence of *t*-BuOK, improves the performance of the catalytic system (Table 2, entry 5), while for the reduction of inactivated double bonds, the presence of this salt doesn't affect the outcome of the reactions (Table 2, entry 7and 8).

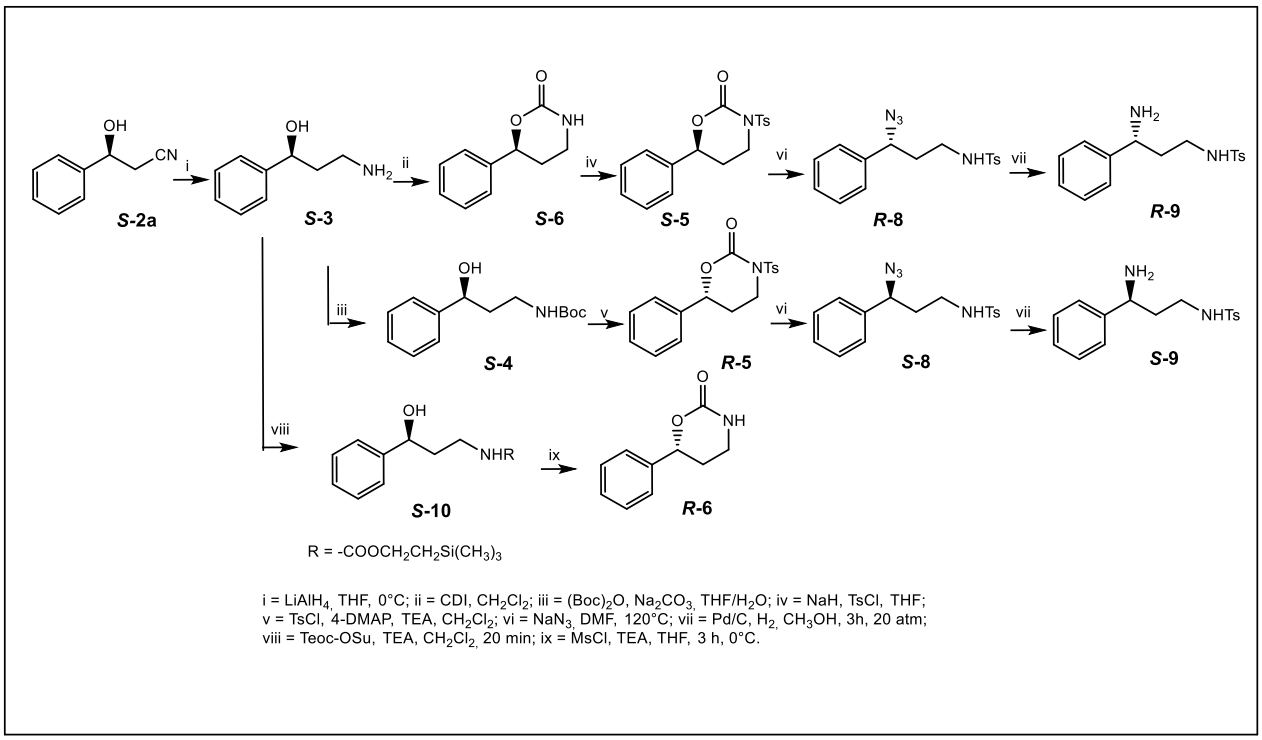
The fact that all the reactions proceed without stereoselectivity means that the first coordination sphere between substrates and chiral six member ring catalyst isn't sufficient to address the outcome of the reactions in term of enantioselectivity. An explanation could be found thanks to stereochemical considerations on chelating six member ring complexes. In presence of one substituent, a chair conformation is favored, and the substituent occupies an equatorial site. This cause a σ-symmetry and suggest a little discrimination in binding, giving a pre-catalyst which acts as achiral complex.[4]Another explanation for lack selectivity could be due to the instability of the catalyst in hydrogenation reaction.

2.3 Second generation ligand: 1,3 tosyl diamine

The chelating six member ring tosyl aminophosphine metal catalysts shown before, demonstrated promising activity but an implementation of stereoselectivity should be addressed. To reach this goal we decided to synthesised linear and branched 1,3 tosyl diamines containing tosyl moiety present in different position. These compounds have better features in respect to tosyl aminophosphine ones. Indeed they are more easy to synthesize, to functionalize and are more easy to handle because are not air sensitive. Moreover these compounds are water soluble and act as suitable ligands for hydrogen transfer reaction reductions.

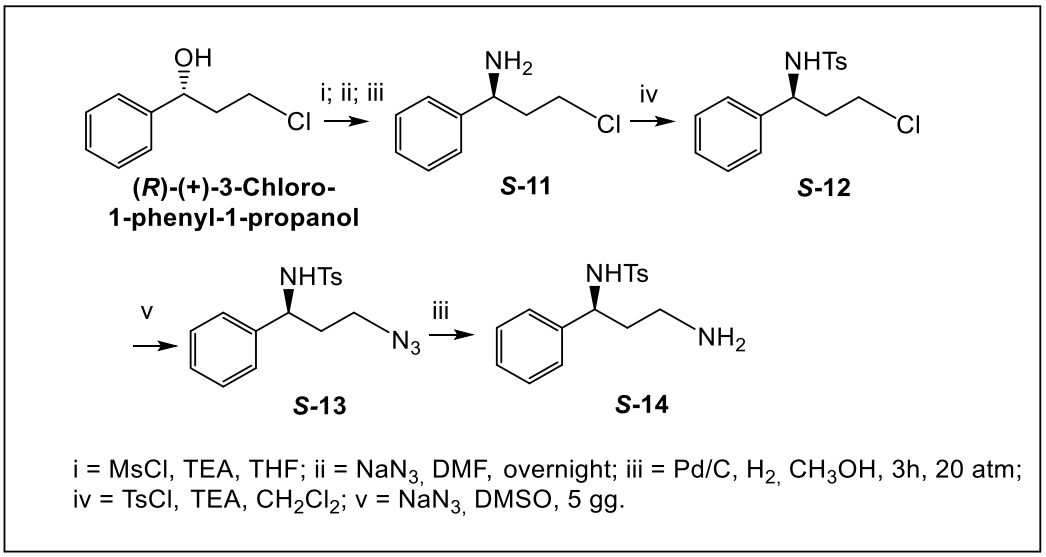
2.3.1 Linear 1,3 tosyl diamines

In Schemes 2 and 3 are reported the synthetic pathways used for the preparation of linear 1,3 tosyl diamines in which tosyl and amino moieties are introduced in different positions respect to chiral centre. This change was made with the purpose to understand if and how these different feature affecting the catalytic properties of the ligands.



Scheme.2

Considering Scheme 2, the enantiopure alcohol ***S*-2**, obtained by biotrasformation (see Section 3.1), was used as asymmetric starting compound, while the amino alcohol ***S*-3** represents the branching point for the synthesis of tosyl diamines ***R*-9** and ***S*-9** in which is possible to produce both enantiomers containing the amino moiety at the chiral centre**.** In this case we used CDI[3] to made cycling compound ***S*-6** which maintains the configuration at the chiral centre, while cyclic compound ***R*-5** was synthesised as shown before (see Section 3.2). The attempt to prepare ligand ***R*-9** by mesylation of ***S*-10** failed because in presence of mesylchloride this intermediated gave the cyclic compound ***R*-6** instead of the corresponding mesylated compound.



Scheme.3

To introduce tosyl moiety close to the chiral centre, pathway reported in Scheme 3 was applied. In this case we used as starting material the commercially available (***R***)-3-chloro-1-phenyl-1-propanol. Product ***S*-14** was obtained with an inversion of configuration.

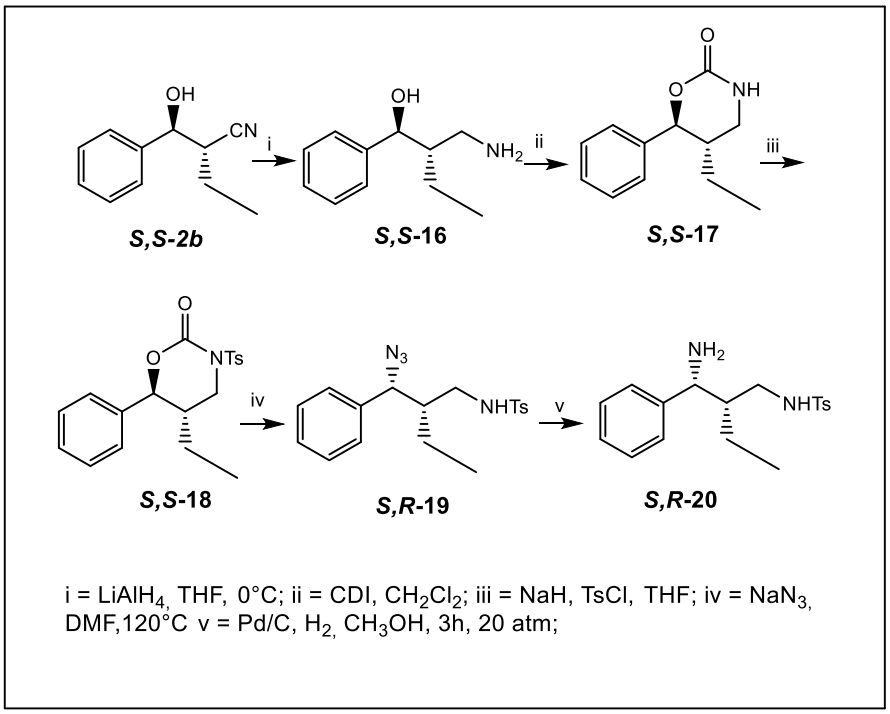
2.3.2 Branched 1,3 tosyl diamines

Scheme 4 and 5 show the pathways used to prepare branched 1,3 tosyl diamines containing amino and tosyl moieties in different positions. This approach was used to achieve two different aims:

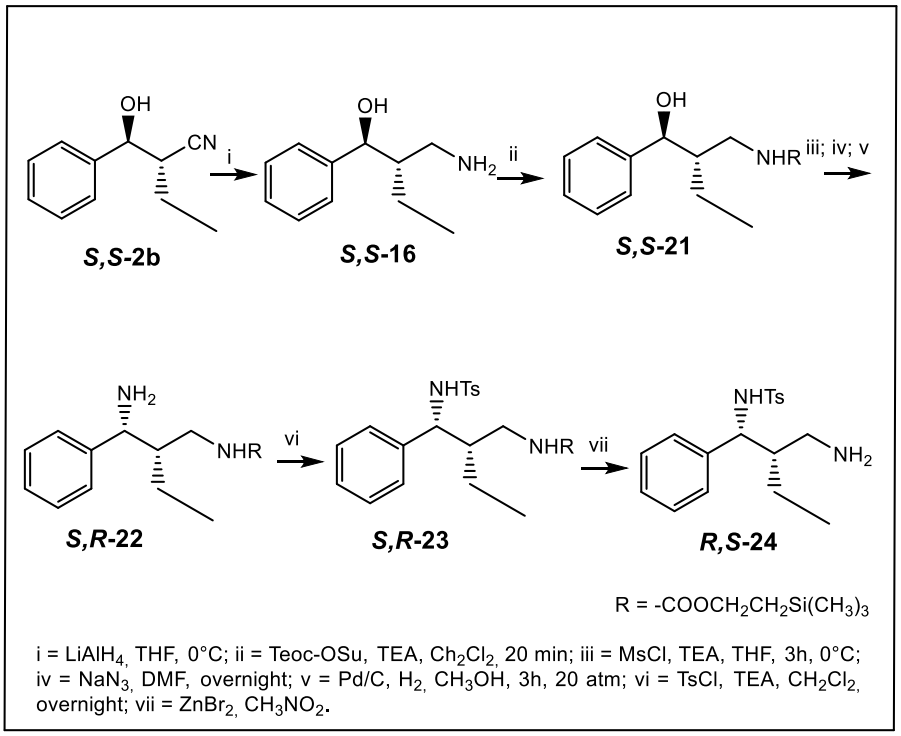
i) to identify if the introduction of a second chiral centre in α position to the preexisnting stereocentre allowed to introduce the conformational stability and rigidity to the six member ring during coordination as evidenced in the case of Chairphos and Skewphos[4].

ii) to understand if the different position of amino and tosyl moieties affects the catalytic properties of the ligands.

To introduce the ethyl group, a condensation reaction was performed using *Saccharomyces cerevisiae* and this intermediate was used to obtained the chiral alcohol ***S,S*-2b** by asymmetric biocatalysis with *Rhodotorula rubra* MIM 147 in 24 h with excellent *e.e.* and *d.e.* values (Section 3.1).



Scheme 4



Scheme 5

These schemes of synthesis are strictly related the previously reported (see Section 3.3.1). Interesting to be note that for the preparation of ***R,S*-24** the use of Teoc-OSu (N-[2-(Trimethylsilyl)ethoxycarbonyloxy]succinimide) as protecting amino group was exploitable. In this case cyclization reaction doesn't occur and the last step of the reaction scheme allows to obtain the terminal amino moiety in quantitative yield using zinc bromide in nitromethane.

2.3.3 Tosyl diamines transition metal complexes

Linear and branched 1,3 tosyl diamines ligands shown above, were used for the synthesis of the corresponding transition metal complexes. As first attempt $[Ru(p\text{-Cymene})Cl_2]_2$ was used for the preparation of precatalyst. Fig.10

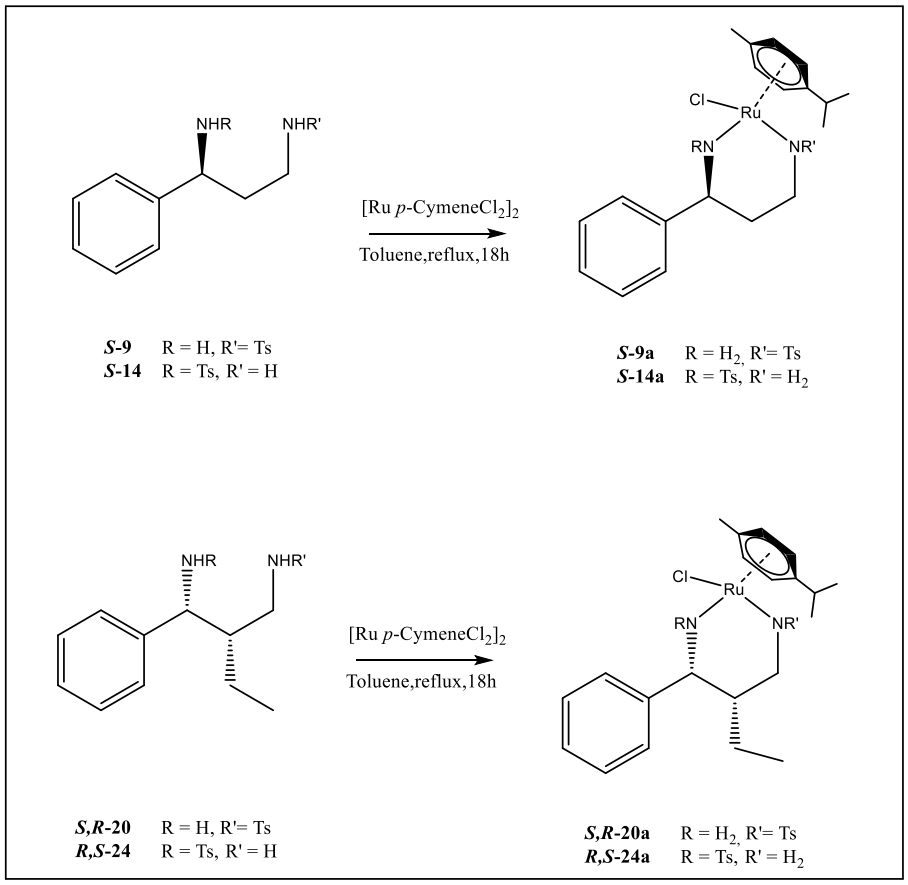


Fig.10

These four complexes were characterized and used for asymmetric transfer hydrogenation (ATH) revealing as a valid alternative to the use molecular hydrogen in obtaining enantiomerically pure compounds.[5] To improve the catalytic performances of this system, different parameters were considered such as solvent, temperature and hydrogen donor.

With regards to solvents, water was revealed the best choice, as when *i*PrOH or MeOH were employed the reaction conversion resulted significantly decreased. In the same way the selection of hydrogen donors[6] (HCOOH, HCOONa, azeotropic mixture HCOOH:TEA 5:2 and *i*PrOH) proved HCOONa among others, in ratio 10:1 with the substrate, as the best in

terms of enantioselectivity (pH=8.10). In fact using a different hydrogen donors, a racemic mixture of the product was obtained in all cases. An explanation of these behavior could be found considering the pH of the reaction as highlighted by Xiao *et al*. in 2005. They reported that the pH of the reaction solution affects the catalyst performance in the aqueous-phase.[7] (see Fig.11)

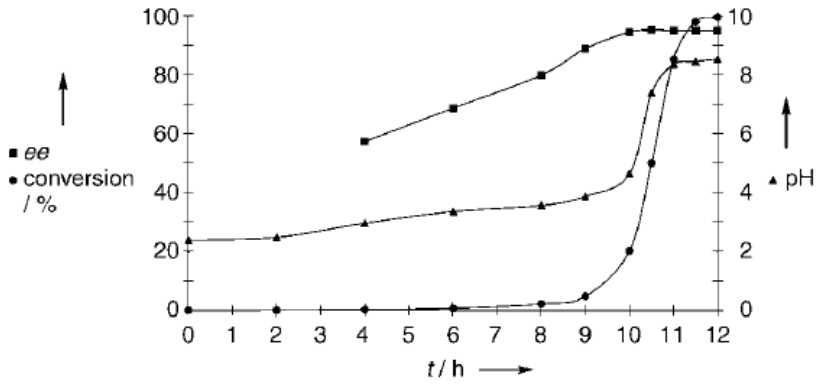
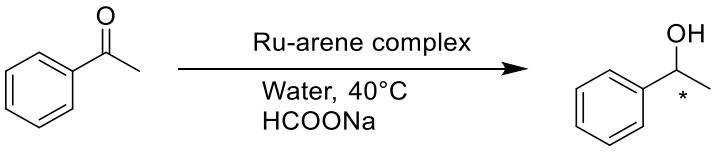


Fig 11. Graph of conversion (●), enantioselectivity (■) and pH (▲) versus time for the reduction of acetophenone in water with Ru(II) (*R*,*R*) Ts.DPEN at 40°C.[7]

In that case the observed variations of enantioselectivity according to the pH may be explained by the protonation of TsDPEN ligand under acidic conditions.[6] Depending on the nature of the aromatic sulfonamide moieties, the pKa of the corresponding N-H group varies between 4.5 (Ar = C_6F_5) and 7.6 (Ar = *p*-toluene).[8] The change in the sulfonamide protonation leads to an "on and- off"catalyst state observed by Xiao.

Another evaluated parameter in our case was the variation of temperature (20°C, 40°C or 60°C) which didn't show significant effect in conversion and enantioselectivity. In Table 3 were reported the results obtained for the four complexes under the set reaction conditions: water as solvent and HCOONa as hydrogen donor at 40°C.



Entry	Complex	Conversion (%)	*e.e.*%
1	***S*-9a**	97	56 (*R*)
2	**S-14a**	68	33 (*R*)
3	***S,R*-20a**	44	22 (*R*)
4	***R,S*-24a**	18	4 (*R*)

Table 3. ATH of acetophenone using Ru(II) complexes. Reactions were carried out at 40°C using 0.5 mmol of substrate with 0.5 mol % of ruthenium complex in 3 mL of water in presence of 10 equiv. HCOONa as hydrogen donor. Conversion and *e.e.* were determined by GC after 48 h.

Unexpectedly the presence of an additional chiral centre in 2 position in ligands ***S,R*-20a** and ***R,S*-24a** decrease the enantioselectivity and the activity of the catalysts (Entries 3 and 4 *vs* 1 and 2). This behaviour might be correlated to the instability of the catalysts,[9] an aspect already observed during the synthesis of the complexes that proceeded with lower yield than in the case of complexes **S-9a** and **S-14a**.

The results obtained by changing the position of tosyl moiety confirmed the importance of stereogenic centre to be in proximity of the amine involved in the catalytic cycle contributing to increase both the reaction conversion and enantioselectivity through a steric and/or an electronic effect (Entries 1 *vs* 2 and 3 *vs* 4).

Ligand ***S*-9** was then used for the preparation of Iridium Cp*complex as shown in Fig.12.

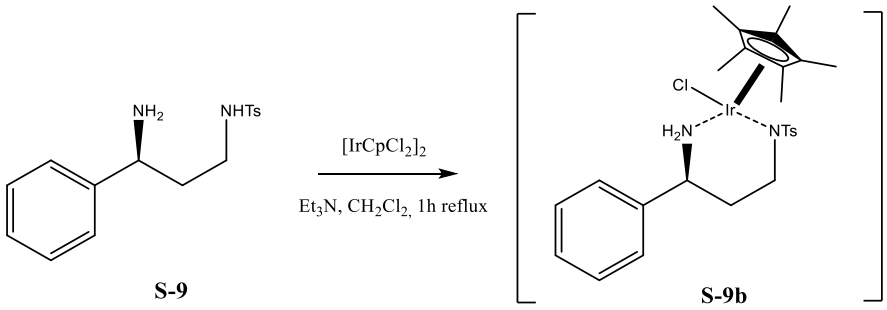
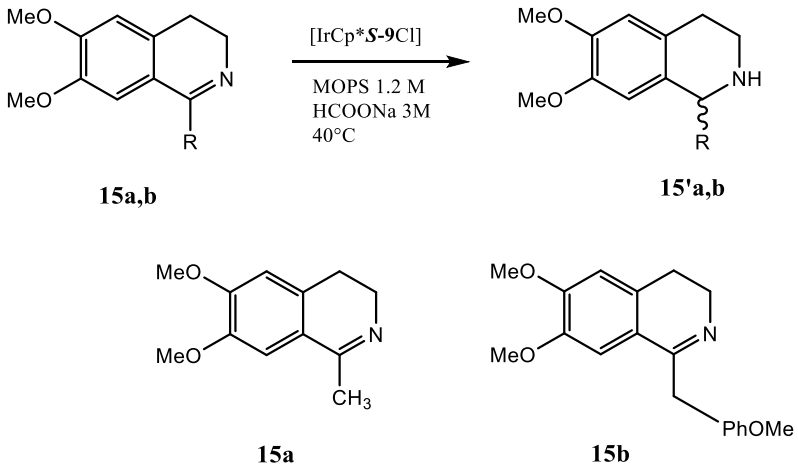


Fig.12 [IrCp* ***S*-9**Cl] complex. For the synthesis see Section 3.4.2.

This precatalyst was applied in ATH reactions for the reduction of imines in aqueous media (Scheme 6). Given the prevalence of the chiral 1,2,3,4-tetrahydroisoquinoline motif in natural alkaloids and pharmaceutical molecules,[10] the development of methods for their production is highly desirable. As model and challenging substrate were used dihydroisoquinoline analogues **15a,b** and results are reported in Table 4.



Scheme 6.

Entry	Substrate	Conversion (%)	*e.e.%*
1	***15a***	95[d]	2 (*R*)[a]
2	***15b***	95[d]	5 (*R*)[b]

Table 4. ATH of imines using Ir(III) complex ***S*-9b**. Reactions were carried out at 40°C using 5 mmol of substrate with 0.5 mol % of iridium complex in 1 mL of MOPS buffer pH=7 in presence of HCOONa 3M as hydrogen donor. Conversion was determined after 96 h by ^{1}H-NMR. *e.e.* were determined by HPLC using OD-H column hexane/EtOH /DEA 95/5/0.1, flow=0.8. a) r.t sub= 13.25, r.t (*S*)= 19.2, r.t (*R*)= 25.3. b) r.t sub= 12.39, r.t (*S*)= 9.13, r.t (*R*)= 11.68.

In our system, imines were reduced with excellent yield, in water and in absence of molecular hydrogen even if low enantioselection was observed. Again the conformation of chelating six member ring seems to play a primary role for the enantio discrimination of the reaction and we speculated that in this conditions a chair conformation was adopted.

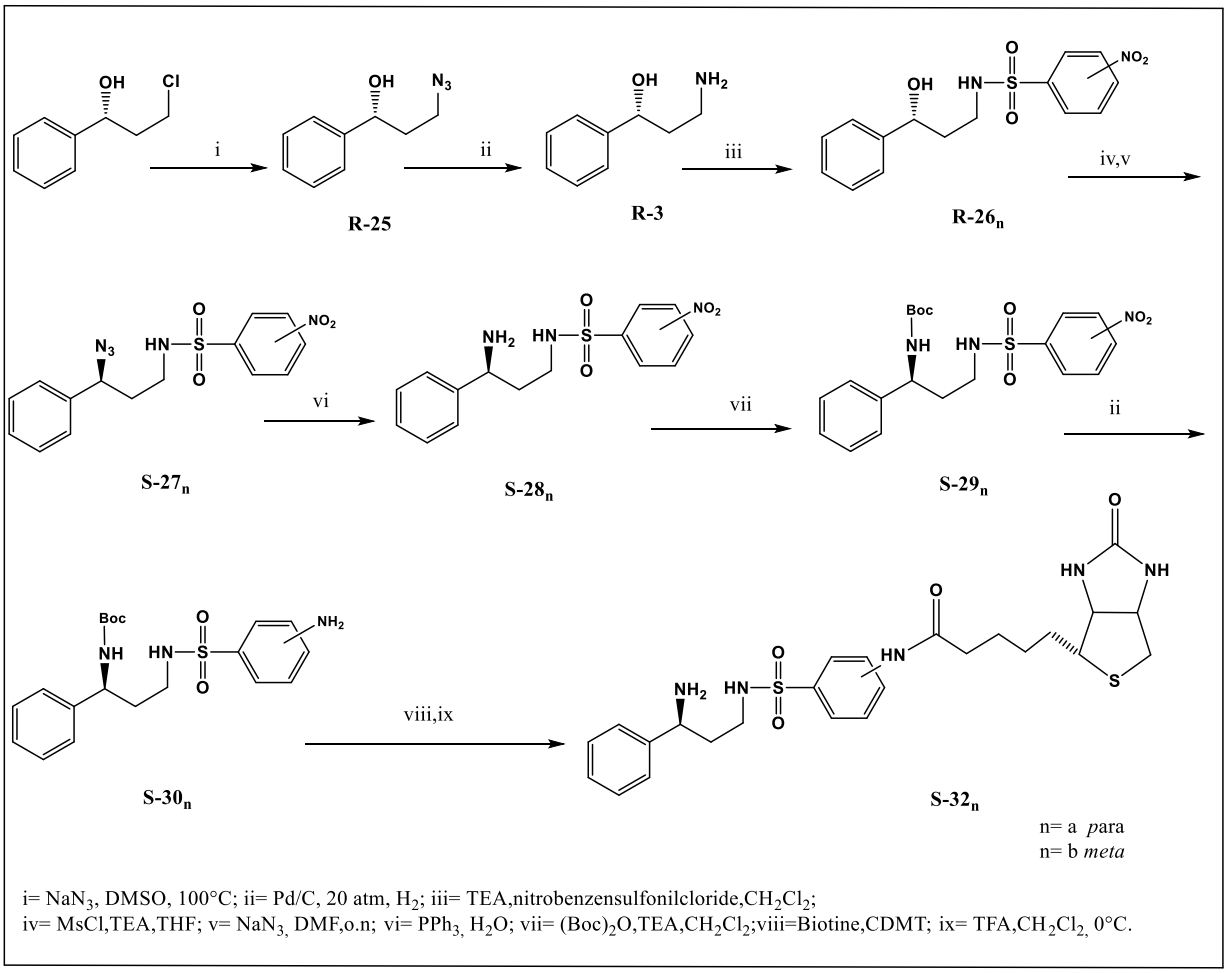
Starting from the assumption that the reduction of these substrates is not easy to obtain, the results using this catalyst, encouraged our research to improve the stereoselectivity of the system, and for this reason, transition metal catalysts was applied in the development of artificial metalloenzymes.[11]

2.4 Artificial metallo enzymes

2.4.1 Synthesis of biotynilated ligands

1,3 tosyl diamine ligands were used for the development of artificial imine reductases. For this scope we decided to use the well know biotin-streptavidin system.[12]

To obtain a suitable ligand for strepatavidine (Sav), a new scheme of synthesis was projected and used (Scheme 7). Considering the catalytic results shown before, we decided to prepare ligand which contains the amino group on the stereocentre, while the tosyl moiety was replaced by the corresponding nitro derivatives to allow the introduction of biotin in *para* or *meta* position.



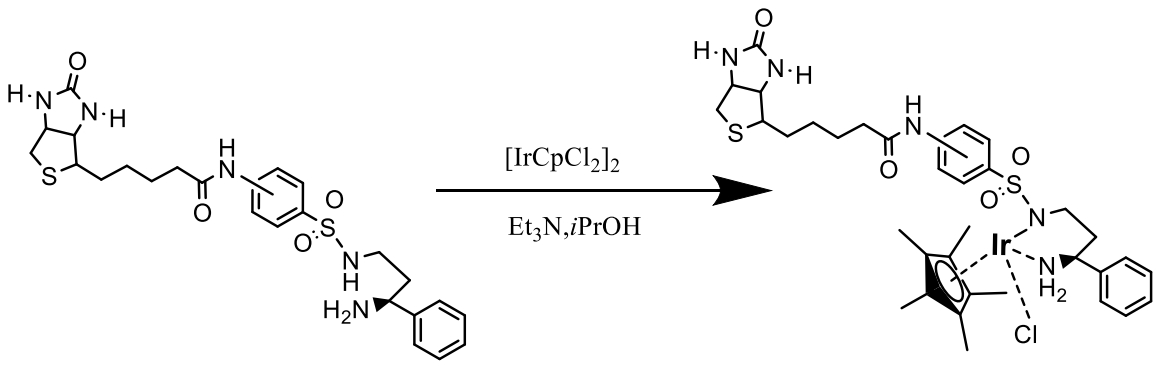
Scheme 7

The alcoholic function of nitro compounds ***R*-26$_n$** was activated involving the use of mesylchloride and then substituted with azido group. The selective reduction of this moiety respect to nitro moiety was achieved using Staudinger reduction [13]to get compound ***S*-28$_n$**.

The amino function was protected employing di-*tert*-butyl dicarbonate ***S*-29$_n$** and finally nitro group was reduced using hydrogen in presence of Pd/C. Then a condensation reaction was performed in presence of biotin and the amino function at the chiral centre was restored using trifluoroacetic acid.

2.4.2 Synthesis of [Ir(Cp*) (diamine) Cl] biotynilated complexes

Biotinylated ligands were used for the preparation of the corresponding Ir(III) transition metal precatalyst. The synthesis is reported in Scheme 8 (see also Section 3.4.3) and allows to obtain both complexes in quantitative yield as orange-brown powder, analyzed by EA and ESI.



***S*- 32a**
***S*- 32b**

***S*- 33a**
***S*- 33b**

n= a *p*ara
n= b *meta*

Scheme 8

2.4.3 Application of Artificial Metallo Enzyme using Biot-[Ir(Cp*) (diamine) Cl] and Streptavidin.

Streptavidin was used as biological scaffold for the preparation of immine reductases exploiting its high affinity constant for biotin. This binding event is one of the strongest no-covalent interactions known in nature (K_d ranging from 1.9 10^{-13} M [14] to 10^{-15} M [15]. Protein consists of four homomeric subunits folded into eight-stranded β-barrels, which can each bind one molecule of biotin, also known as vitamin H, and results very stable under extreme conditions such as high temperatures, extreme pH values and high concentrations of guanidium chloride.

Streptavidin wt and mutants S112X used for this study were kindly gift from the group of Prof. T. Ward from the university of Basel. The molecular mass of non-glycosilated wt protein is 65.7 kDa (16.4kDa for the monomer) and each monomer consists of 159 amino acids (mature streptavidin) including a tag for secretion of 24 amino acids. The first 15 amino acids of the mature streptavidin, however, are replaced by a T7 tag (aa 1-11, introduced by Cantor *et al.*, who thought that it might increase the solubility of the protein) and three amino acids (aa 12-14), which are a result of cloning strategy. Sav wt and mutants were expressed using *E.coli* and subsequently purified by affinity chromatography using imino biotin coloumn. The free binding sites per tetramer were determined using an assay with biotin-4-fluorescein,[16]and used for the calculation of final concentration of free binding sites.Tab.5

Mutant	Free binding sites /tetramer
Savwt	3.2
S112H	3.4
S112Y	3.6
S112T	3.6
S112M	3.6
S112Q	4
S112A	3.8
S112C	3.9
S112R	3.8
S112E	3.3
S112K	3.2

Tab.5 Free binding sites per tetramer of Sav wt and mutants used in this work.

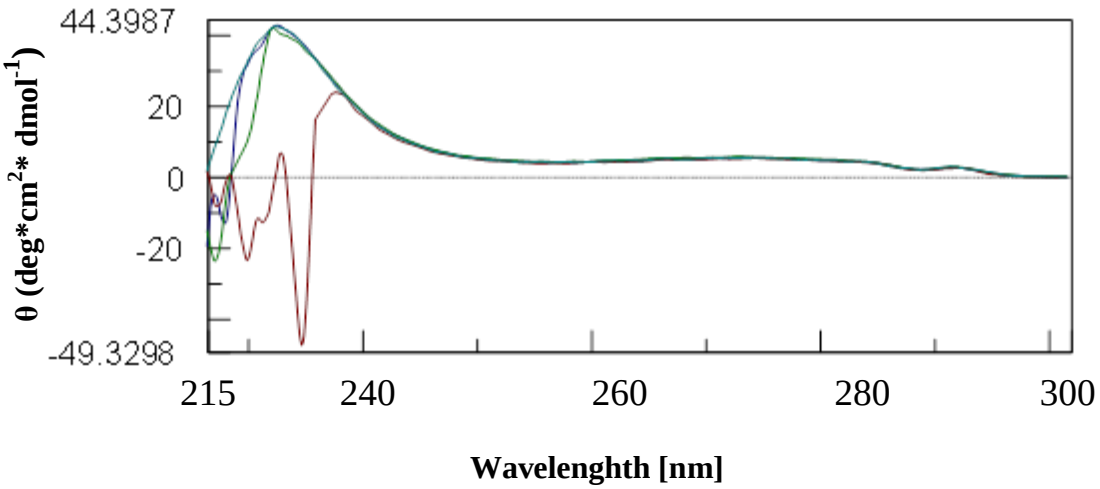
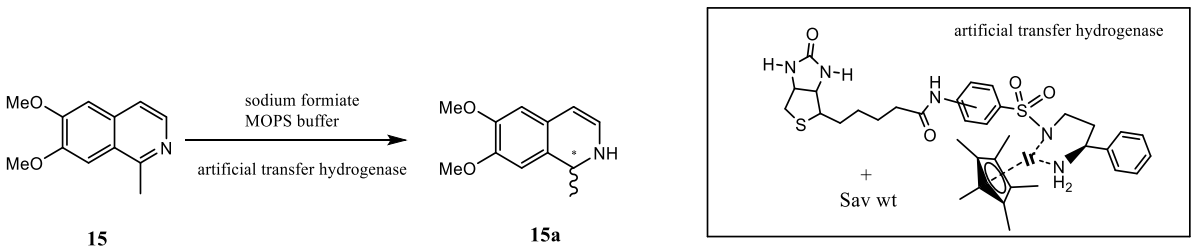


Fig.13.-Stability of SAV wt with DMF:(•) buffer, (•) 2%DMF, (•) 6% DMF, (•) 15% DMF.

Stability of the protein, under catalytic conditions, was evaluated as display in Fig.13. The CD spectra shows the loss of positive Cotton effect when DMF concentration increases to 15%, revealing the denaturation of the protein. The concentration of 1.6% was fixed for the stability of the protein during the catalytic reaction.

As a starting screening, we investigated the catalytic activity of the d^5 Ir –pianostool bearing the biotinylated aminosulfonamide ligand (***S*-33a** and ***S*-33b**) as bare catalysts and their combination with wild-type streptavidin (Sav) for the production of salsolidine **15a** (Scheme 9), taking account of different reaction parameters such as pH, temperature, substrate concentration and catalyst loading.



Scheme 9.

Protein	Entry	Complex	%C	*e.e.*
None	1[a]	***S***-33a	>99	rac
Sav wt	2[a]	***S***-33a	83	10(***R***)
None	3[b]	***S***-33a	99	rac
Sav wt	4[b]	***S***-33a	82	18(***R***)
None	5[c]	***S***-33a	81	rac
Sav wt	6[c]	***S***-33a	10	16(***R***)
None	7[d]	***S***-33a	99	rac
Sav wt	8[d]	***S***-33a	65	9(***R***)
None	9[e]	***S***-33b	83	rac
Sav wt	10[e]	***S***-33b	76	8(***R***)

Table 6. ***a)*** $[sub]_f$ =150mM, $[cat]_f$ =0.68 mM, 30°C, pH=6.5; ***b)*** $[sub]_f$ =50mM, $[cat]_f$ = 0.34mM, 30°C,pH=6.5; ***c)*** $[sub]_f$ =150mM, $[cat]_f$= 0.68mM, 10°C,pH=6.5; ***d)*** $[sub]_f$ =50mM, $[cat]_f$ =0.34 mM, 30°C, pH=7.5, ***e)*** $[sub]_f$ =35mM, $[cat]_f$ =0.34 mM, 30°C, pH=6.5. e.e was evaluated using HPLC OD-H column hexane/EtOH 95/5 0.1% DEA flow=0.8mL/min. Conversion was obtained using correction factor of 1.45 at 230nm.

Results, displayed in Table 6, showed very high activity of the iridium pianostool *para* biotinylated aminosulfonamide catalyst except when temperature decreases to 10°C (entries 1,3,7 *vs* entry 5) but in all cases absence of stereoselectivity occur and similar behavior can be observed also in the case in which *meta* biotinylated aminosulfonamide iridium complex was used. Differently, when transition metal catalyst is embedded to Sav wt, a little decreasing of activity and an improvement of strereoselectivity was observed (entry 2,4,6,8,10). For both types of *meta* and *para* iridium catalysts, the ones containing biotine moiety in *para* position revealed best performances. Finally, although the mechanism of imine reduction is not already well understood and continuously debated, different authors demonstrated that only protonated imines are reduced in ATH catalysis.[17]

Even if mechanistic study are far to our goal, data in Table 6, agree in principle with an ionic mechanism proposed. Best results were obtained when catalysis was carried out at pH 6.5 and 30°C.

With the aim to investigate the possibility to improve the selectivity of our catalyst (**S-33a**, **S-33b**), genetic optimization of the biological scaffold was performed. For this reason and thanks to the collaboration with Prof. Ward from the University of Basel, ten mutants in position 112 were tested.

This choice for the genetic optimization site is suggested by literature docking studies[18] and X-ray structure [19] which reveal that this position lies close to the biotinylated metal upon incorporation into streptavidin.

Catalytic results are reported in Table 7 and 8.

Protein	Entry	AA	Complex	%C	*e.e.*
None	1[b]		***S***-33a	>99	rac
Sav wt	2[b]	***Serine***	***S***-33a	83	18(***R***)
112K	3[a]	***Lysine***	***S***-33a	12	7(***R***)
112M	4[a]	***Methionine***	***S***-33a	15	4(***R***)
112R	5[a]	***Arginine***	***S***-33a	30	rac
112C	6[b]	***Cysteine***	***S***-33a	40	58(***R***)
112H	7[b]	***Histidine***	***S***-33a	65	13(***R***)
112Y	8[b]	***Tyrosine***	***S***-33a	76	17(***R***)
112T	9[b]	***Threonine***	***S***-33a	69	10(***R***)
112Q	10[b]	***Glutamine***	***S***-33a	76	16(***R***)
112A	11[b]	***Alanine***	***S***-33a	79	13(***R***)
112E	12[b]	***Glutamic acid***	***S***-33a	80	19(***R***)

Table 7 ***a)*** $[sub]_f$ =150mM, $[cat]_f$ =0.68 mM, 30°C, pH=6.5; ***b)*** $[sub]_f$ =50mM, $[cat]_f$= 0.34mM, 30°C,pH=6.5.

Changing the amino acid residue respect to the serine, present into SAV wt , an important improvement in stereoselectivity was observed when cysteine residue was substituted. (Tab 6, Entry 6).

The presence of other amino acids tested, gave results comparable to the wt assuming that the hindrance and the nature of them didn't affect the second coordination sphere among catalyst and substrate. The decrease of activity observed when cysteine was present suggest a partial interaction among transition metal catalyst and thiol group present in mutant 112C.[20,21,22]

This two consideration led to hypotized that when our type of catalyst was used, the position 112 is not involved as recognition element for the substrate while it is important for the transmission of chirality at the metal.

Protein	Entry	A.A	Complex	%C	e.e
None	1		***S*-33b**	83	rac
Sav wt	2	***Serine***	***S*-33b**	76	8(***R***)
112K	3	***Lysine***	***S*-33b**	70	7(***R***)
112M	4	***Methionine***	***S*-33b**	82	2(***R***)
112R	5	***Arginine***	***S*-33b**	83	4(***R***)
112C	6	***Cysteine***	***S*-33b**	10	40(***R***)
112H	7	***Histidine***	***S*-33b**	58	8(***R***)
112Y	8	***Tyrosine***	***S*-33b**	67	4(***R***)
112T	9	***Threonine***	***S*-33b**	68	2(***R***)
112Q	10	***Glutamine***	***S*-33b**	72	2(***R***)
112A	11	***Alanine***	***S*-33b**	77	6(***R***)
112E	12	***Glutamic acid***	***S*-33b**	79	12(***R***)

Table 8. $[sub]_f$ =35 mM, $[cat]_f$ =0.34 mM, 30°C, pH=6.5.

A similar behaviuor was observed when *meta* biotynilated catalyst was used. In this case a depletion of activity and selectivity were observed comparing data present in Table 7 with 8. For clearly, bubble chart of all catalytic results was presented in Fig.13

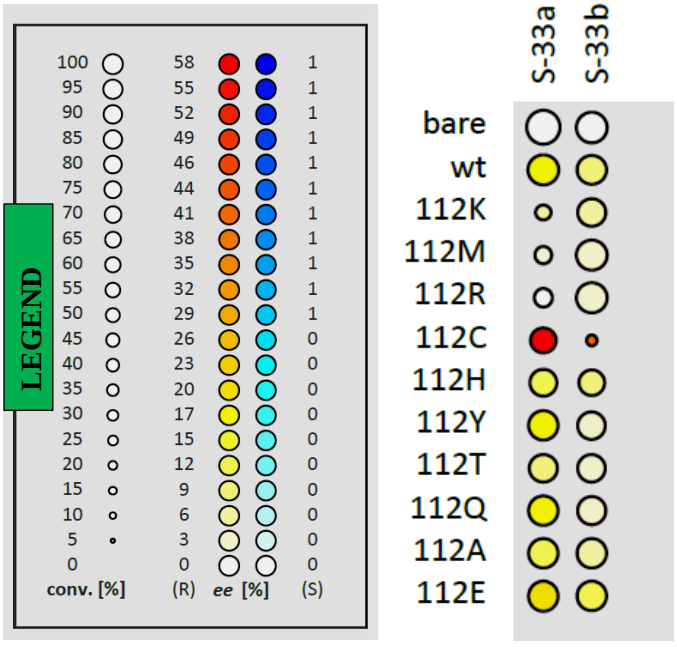


Fig.13. Bubble chart of data reported in table 6 and 7.

Finally, our best artificial metalloenzyme, resulting from chemo-genetic optimization (Sav112C+***S***-33a), was tested working at fixed substrate concentration and varying the catalyst loading. Data reported in Table 9 shown that high ratio of [sub]/[cat] dramatically decrease the activity of artificial imine reductase. (entry 1). This poisoning behaviour can be due to a strong coordination among imine and catalyst which affects the catalytic activity.

Protein	Entry	A.A	Complex	%C	e.e	TON	[cat]/[sub]
112C	1[a]	***Cysteine***	***S***-33a	4	63	12	1/294
112C	2[b]	‘’	***S***-33a	40	58	58	1/147
112C	3[c]	‘’	***S***-33a	90	66	66	1/74

Tab 9. Optimization of 112C with ***S***-33a**.** All reaction were carried out at in MOPS buffer 1.2M, NaCOOH 3M, 30°C, pH=6.5and $[sub]_f$ =50mM. ***a)*** $[cat]_f$ =0.17 mM. ***b)*** $[cat]_f$= 0.34mM; ***c)*** $[cat]_f$= 0.68mM.

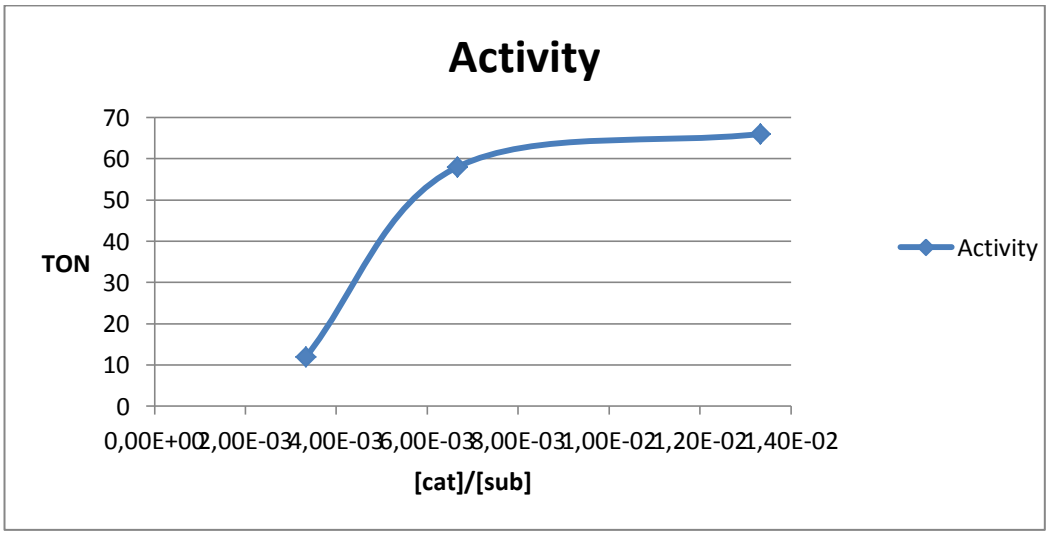


Fig.14

In Fig 14 is graphically described the correlation between TON and [cat]/[sub] ratio. In the first part of this curve, a double loading of catalyst (0.17mM to 0.34mM) improves 4.8 times the turn over number of the reaction, while doubling further the load of catalyst, TON increases only 1.14 times, even if this catalytic conditions allow to obtain the best enantiomeric excess.(entry 3)

In conclusion, the combination of chelating six member ring transition metal complexes containing *para* biotinylated aminosulfonamide and Sav S112C results in the formation of imino reductases able to reduce dihydroisoquinoline **15a** with 66% *e.e* and TON=66 in ATH conditions. While the chiral d^5 Ir pianostool *para* biotinylated aminosulfonamide acts as

achiral ligand in the reduction of our reference compound, the presence of biological scaffold allows to introduce a new source of chirality with good activity and enantioselectivity.

How the presence of the protein directs the enantio selectivity of the reaction is far to be clarified, it reasonable seems due to the interaction between the iridium and thiol residue in position 112 when cysteine is used instead of serine.

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3

EXPERIMENTAL SECTION

3.1 GENERAL EXPERIMENTAL CONDITIONS

3.1.1 Solvents and reagents

Materials and reagents were purchased at the highest commercially available grade and used without further purification.

Solvents used for reactions correspond to the quality "puriss". For analytical and high performance liquid chromatography (HPLC), HPLC-grade solvents were used. Commercially reagent grade solvents were dried according to standard procedures and freshly distilled under nitrogen before use. All manipulations involving air sensitive materials were carried out in an inert atmosphere using standard Schlenk techniques in oven-dried glassware.

3.1.2 Separation and purification methods

Reactions were monitored by thin layer chromatography (TLC) using Merck silica gel 60 F254 plates. Flash chromatography was performed using Merck silica gel 60, particle size 40-63 μm.

Compounds were visualized by gas chromatography analysis using a chiral stationary phase column (MEGA DMT β, 25 m, internal diameter 0.25 mm) or by HPLC analysis with Merck-Hitachi L-7100 equipped with Detector UV6000LP and chiral column (OD-H Chiralcel or AD Chiralpak).

3.1.3 Spectroscopic methods

^{1}H and ^{13}C and ^{31}P NMR spectra were recorded in $CDCl_3$, DMSO or CD_3OD on Bruker DRX Avance 300 MHz equipped with a non-reverse probe. Chemical shifts (in ppm) were referenced to residual solvent proton/carbon peak. and coupling constants (J) are reported in Hertz (Hz).

The multiplicity's are abbreviated as: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet and br = broad.

FTIR spectra were collected by using a Perkin Elmer (MA, USA) FTIR Spectrometer "Spectrum One" in a spectral region between 4000 and 450 cm^{-1} and analysed by transmittance technique with 32 scansions and 4 cm^{-1} resolution.

Circular dichroism (CD) spectroscopy analyses were performed on Jasco 810 spectrophotometer. Spectra were obtained from 210 nm to 320 nm with 0.1 nm step and 1 s collection time per step, taking three averages. The spectrum of the solvent was subtracted to

eliminate interference. The CD spectra were plotted as mean residue ellipticity θ (degree x cm^2 x $dmol^{-1}$) versus wave length λ (nm).

Polarimetry analyses were carried out on Perkin Elmer 343 Plus equipped with Na/Hal lamp.

3.1.4 Spectrometric methods

MS analyses were performed by using a Thermo Finnigan (MA, USA) LCQ Advantage system MS spectrometer with an electronspray ionisation source and an 'Ion Trap' mass analyser. The MS spectra were obtained by direct infusion of a sample solution in MeOH under ionisation, ESI positive or negative.

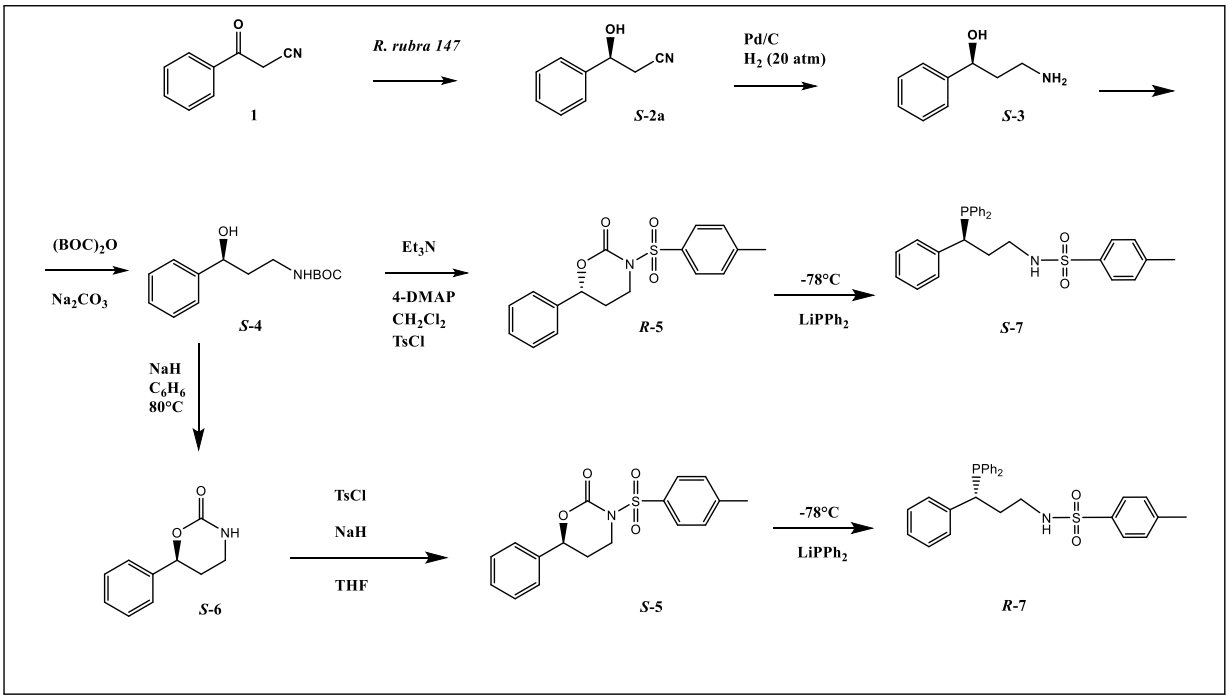
3.1.5 Other methods

The elemental analyses (EA) were measured using Perkin Elmer Series II/CHNS/O 2400 Analyzer.

3.2 SYNTHESIS OF LIGAND

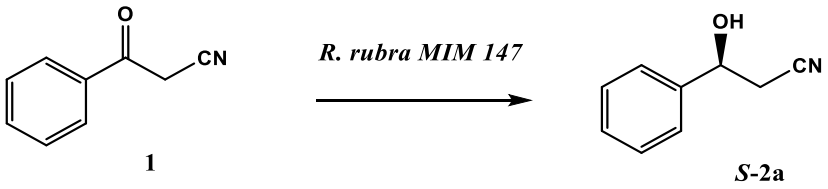
3.2.1 Synthesis of chiral tosyl aminophosphine ligands

General scheme of synthesis



Scheme 1

3.2.1.1 Synthesis of (*S*)-3-hydroxy-3-phenylpropanenitrile *(S*-2)



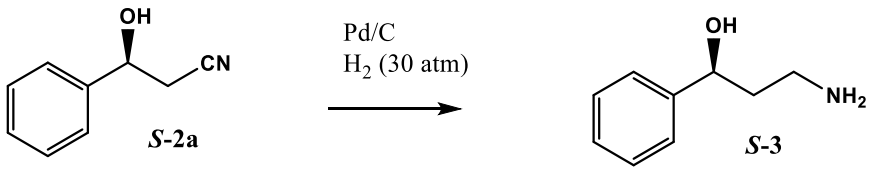
1 *S*-2a

Chiral starting compound for the synthesis of several ligands was synthetized by using biotransformation reaction employing *Rhodotorula rubra* MIM 147.

Procedure of biotransformation with *Rhodotorula rubra* MIM 147: *Rhodotorula rubra* MIM 147 was routinely maintained on malt extract slants (8 g L^{-1}, yeast extract 5 g L^{-1}, agar 15 g L^{-1}, pH 5.6). The strain, grown on malt extract slants for 72 h at 28 °C, was inoculated into 1000-mL Erlenmeyer flasks containing 150 mL of the same liquid medium and incubeted on a reciprocal shaker (100 spm) for 48 h at 28°C. Cells obtained by centrifugation (4000×*g* for 15 min at 4 ◦C) of the culture broth (1L) were washed with tap water (3x200 mL) and re-suspended in 500 mL of 0,1M phosphate buffer pH = 7 containing 50 g L^{-1} of glucose. The substrate dissolved in DMSO was added to the biotransformation system in 2 g L^{-1} (1). The biotransformation system was shaken with mechanic stirrer at 28°C for 24 h. The cells were separated by centrifugation and both were extracted with diethyl ether (3x150 mL), dried with Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was purified by flash chromatography (ethyl acetate/cyclohexane = 7:3) to give 786 mg of ***S*-2** (78% yield).

***S*-2:** All characterization data are in agreement with previously reported literature.[1, 2] $[\alpha]^{20}_{D}$= -63.8 (c=1, $CHCl_3$). HPLC data: OD-H Chiralcel, eluent: hexane: 2-propanol = 95:5, flow = 0.8 mL/min, λ = 216 nm; rt: (*R*) = 42.6 min, (*S*) = 44.6 min.

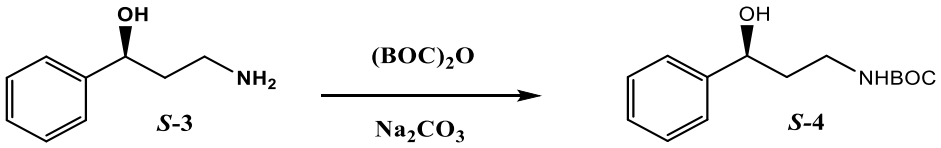
3.2.1.2 Synthesis of (*S*)-3-amino-1-phenylpropan-1-ol (*S*-3)



In a stainless steel autoclave (20 ml), equipped with temperature control and a magnetic stirrer, purged five times with hydrogen, a solution of ***S*-2** (550 mg, 3.74 mmol) in methanol with 1% of Pd/C was transferred. The autoclave was pressurised at 20 atm and kept under stirring at room temperature for four hours. The mixture was then filtered on Celite and the solvent was evaporated in *vacuo* to give the product as a yellow oil, without any further purification step (537 mg, 95% yield).

$[\alpha]^{20}_{D}$= -44.38 (c=0.3, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.80 (m, 2H), 2.97 (m, 4H), 4.92 (dd, 1H, *J*=4.03, 8.06 Hz), 7.21 -7.38 (m, 5H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 145.16, 128.43, 128.14, 127.20, 125.85, 125.54, 75.44, 40.57, 39.87 ppm; IR ν = 3360, 2917, 2874, 1601, 1492, 1453, 1337, 1062, cm^{-1};MS (ESI) of $C_9H_{13}NO$ *m/z* 152.0 $[M+H]^+$, 174.1 $[M+Na]^+$.

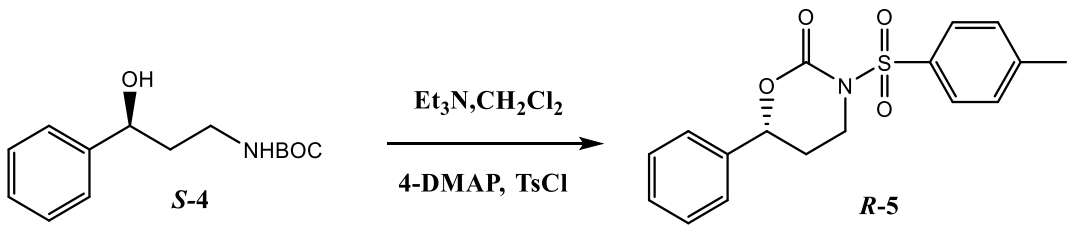
3.2.1.3 Synthesis of *tert*-butyl-(*S*)-(3-hydroxy-3 phenyl propyl) carbamate (*S*-4)



To a solution of ***S*-3** (445 mg, 2.94 mmol) in a mixture of THF/water 1:1, Na_2CO_3 was added (720 mg, 6.76 mmol). The solution was then cooled to 0°C and a solution of di-*tert*-butyl dicarbonate (770 mg, 3.53 mmol) in 5 mL THF was added dropwise. After 1 h stirring at 0°C, the solution was warmed to room temperature and stirred for further 3 h. The reaction was monitored by TLC using dichloromethane/diethyl ether 1:1 as eluent. After 4 h the reaction was complete and water was added to the mixture and extracted with diethyl ether (3x10mL) to give the product as a yellow oil (575 mg, 78% yield).

$[\alpha]^{20}_{D}$= -18.9 (c=0.4, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.41 (s, 9H), 1.78 (m, 2H), 3.18 (m, 2H), 4.15 (s, 1H), 4.64 (t, J = 8.79 Hz, 1H), 5.23 (s, 1H), 7.28 (m, 5H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 157.05, 144.54, 128.65, 127.59, 125.87, 79.76, 71.95, 39.77, 37.83, 28.63 ppm; IR ν = 3363, 3274, 2975, 1677, 1546, 1291, 1180, 1025, 981 cm^{-1};MS (ESI) of $C_{14}H_{21}NO_3$ *m/z* 274.10 $[M+Na]^+$.

3.2.1.4. Synthesis of (*R*)-6-phenyl-3-tosyl-1,3-oxazinan-2-one (*R*-5)

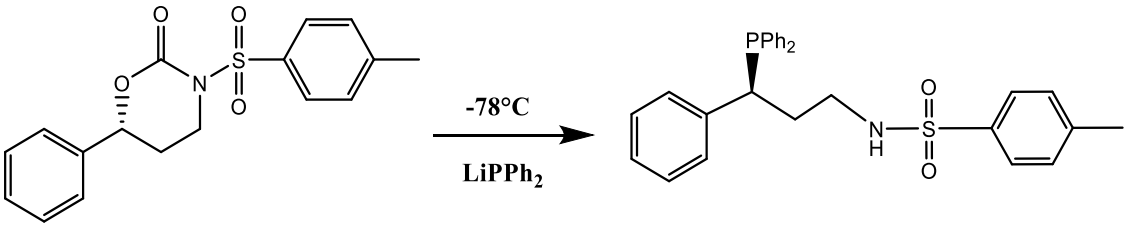


To a solution of ***S*-4** (270 mg, 1.08 mmol) in fresh-distilled dichloromethane, 4-dimethylaminopyridine (99 mg, 0.81 mmol) and triethylamine (2 mL, 14.04 mmol) were added. The reaction mixture was then cooled to -10°C and stirred for half an hour. A solution of tosyl chloride (267 mg, 1.4 mmol) in dichloromethane was then dropped into the former solution and stirred overnight allowing the reaction mixture to reach room temperature. The reaction was monitored by TLC using dichloromethane/diethyl ether 1:1 as eluent.

After 24 h the reaction is completed. The desired product was obtained as a white solid by slow diffusion of hexane into the acetone solution (152 mg, 43% yield).

$[\alpha]^{20}_{D}$= + 24.7 (c=0.25, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.27 (m, 2H), 2.45 (s, 3H), 4.04 (m, 2H), 5.34 (dd, 1H, *J* = 2.93, 9.53 Hz), 7.23-7.37 (m, 7H), 7.94 (d, 2H, *J* = 8.43Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 145.44, 137.61, 135.37, 129.68, 129.16, 129.06, 129.03, 125.75, 79.67, 44.22, 29.89, 21.93 ppm; IR ν = 3436, 2976, 2923, 1709, 1354, 1174, 1148, cm^{-1}; MS (ESI) of $C_{17}H_{17}NO_4S$ *m/z* 354.1 $[M+Na]^+$.

3.2.1.5 Synthesis of (*S*)-N-(3-(diphenylphosphanyl)-3-phenylpropyl)-4 methyl benzene sulphonamide (*S*-7)

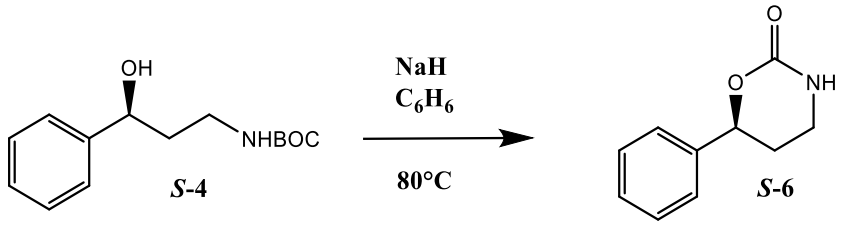


***R*-5** ***S*-7**

Compound ***R*-5** (24 mg, 0.07 mmol) was dissolved in anhydrous THF and the solution was cooled to -78°C. After 20 minutes a solution of $LiP(Ph)_2$ was added drop wise (700 µl, 0.075 mmol) and the mixture was stirred for an hour. Solvent was removed under vacum and compound ***S*-7** (15 mg, 45% yield) was purified using preparative TLC ($CHCl_3$/ EtOAc 9/1) Rf = 0.6

$[\alpha]^{20}_D$= -124.7 (c=0.25, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.97 (m, 2H), 2.42 (s, 3H), 2.8-3.01 (m, 2H), 4.56 (m, 1H), 5.9 (m, 1H), 7.01 (d, 2H, 8.4 Hz), 7.23-7.57 (m, 15H), 7.72 (d, 2H, 8.4 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 143.62, 139.41, 137,30, 134.99-134.2, 132.4-132.0, 129.96-126.3, 74.54, 39.73, 36.79, 21.74. ^{31P} NMR (75 MHz, $CDCl_3$): δ = -2.35 ppm; MS (ESI) of $C_{28}H_{28}NO_{2P}S$ *m/z* 496.1 $[M+Na]^+$.

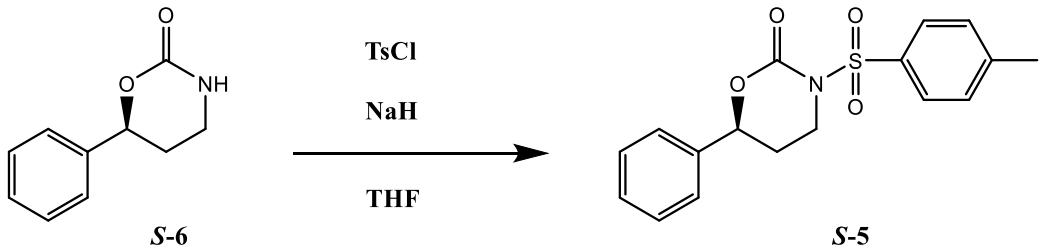
3.2.1.6 Synthesis of (*S*)-6-phenyl-1,3-oxazinan-2-one (*S*-6)



To a solution of ***S*-4** (526 mg, 2.1 mmol) in anhydrous benzene, sodium hydride (84 mg, 2.1 mmol) was added. The suspension was warmed to 80°C and stirred overnight. Reaction was allowed to reach room temperature, quenched with water and extracted with dichloromethane (3X10 ml). The collected organic layers were dried on Na_2SO_4, filtered and evaporated to give a pale oil. The desired product was obtained as a white solid by recrystallization in acetone/hexane (152 mg, 43% yield).

$[\alpha]^{20}_D$= -37.4 (c=0.7, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.17 (m, 2H), 3.42 (m, 2H), 5.34 (dd, 1H, *J* = 2.93, 9.53 Hz), 5.81 (s, 1H), 7.26-7.39 (m, 5H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 154.61, 138.67, 128.85, 128.59, 125.85, 78.83, 39.17, 28.91 ppm; IR ν = 3389, 2966, 2878, 1797, 1682, 1494, 1456, 800, 702 cm^{-1}; MS (ESI) of $C_{10}H_{11}NO_2$ *m/z* 200.1 $[M+Na]^+$.

3.2.1.7 Synthesis of (*S*)-6-phenyl-3-tosyl-1,3-oxazinan-2-one (*S*-5)

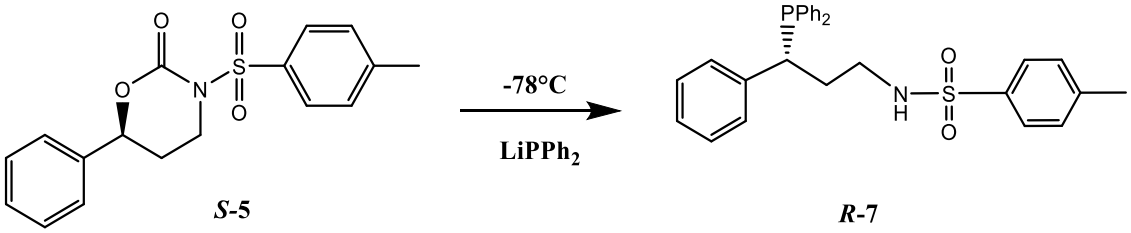


***S*-6** ***S*-5**

To a solution of ***S*-6** (300 mg, 1.69 mmol) in anhydrous THF at 0°C the stoichiometric amount of NaH was added (68 mg, 1.69 mmol). After thirty minutes the solution of tosyl chloride in THF (387 mg, 2.03 mmol) was dropped into the former solution and stirred at room temperature overnight. The resulting solution was quenched with water and extracted with trichloromethane (3x10mL). The collected organic layers were dried on Na_2SO_4, filtered and evaporated to give a yellow oil then purified by crystallization in dichloromethane/hexane to provide the product as a white solid (440 mg, 80% yield).

$[\alpha]^{20}_D$= -24.7 (c=0.5, $CHCl_3$);^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.27 (m, 2H), 2.45 (s, 3H), 4.04 (m, 2H), 5.34 (dd, 1H, *J* = 2.93, 9.53 Hz), 7.23-7.37 (m, 7H), 7.94 (d, 2H, *J* = 8.43Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 145.44, 137.61, 135.37, 129.68, 129.16, 129.06, 129.03, 125.75, 79.67, 44.22, 29.89, 21.93 ppm; IR ν = 3436, 2976, 2923, 1709, 1354, 1174, 1148, cm^{-1}; MS (ESI) of $C_{17}H_{17}NO_4S$ *m/z* 354.1 $[M+Na]^+$.

3.2.1.8 Synthesis of (*R*)-N-(3-(diphenylphosphanyl)-3-phenylpropyl)-4 methyl benzene sulphonamide (*R*-7)



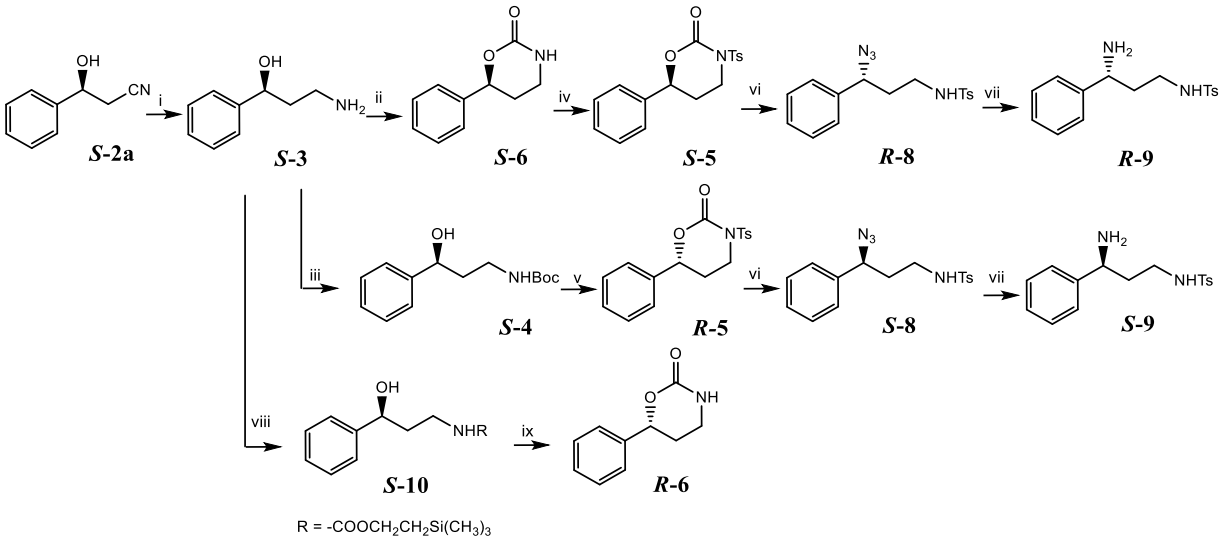
***S*-5** ***R*-7**

Compound ***R*-7** was synthesised according to the procedure reported for ***S*-7 (Section 3.2.1.5)** starting from ***S*-5** (100 mg, 0.3 mmol). ***R*-7** was obtained as a white solid (68 mg, 48 % yield). All characterization data are in agreement with previously reported for ***S*-7.**

$[\alpha]^{20}_{D}$= + 124.9 (c=0.25, $CHCl_3$).

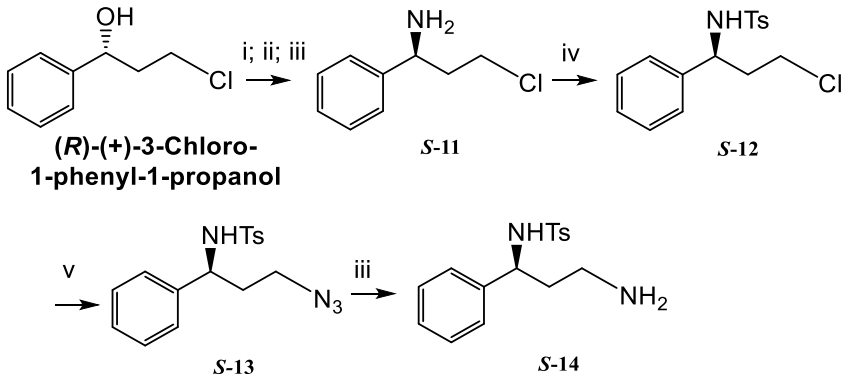
3.2.2 Synthesis of chiral 1,3 Tosyl Diamines ligands

General schemes of synthesis



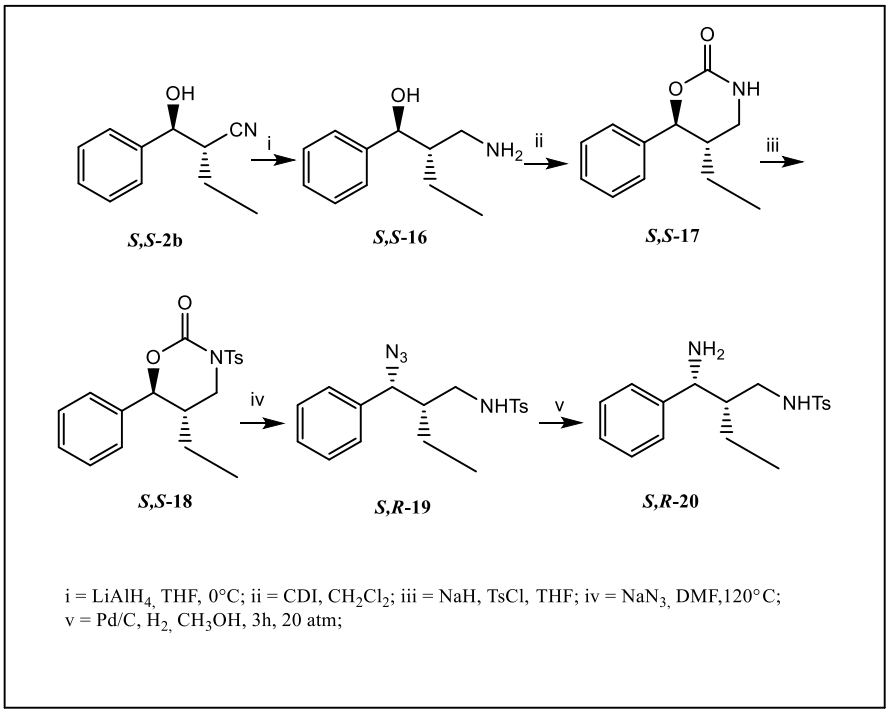
i = $LiAlH_4$, THF, 0°C; ii = CDI, CH_2Cl_2; iii = $(Boc)_2O$, Na_2CO_3, THF/H_2O; iv = NaH, TsCl, THF;
v = TsCl, 4-DMAP, TEA, CH_2Cl_2; vi = NaN_3, DMF, 120°C; vii = Pd/C, H_2, CH_3OH, 3h, 20 atm;
viii = Teoc-OSu, TEA, CH_2Cl_2, 20 min; ix = MsCl, TEA, THF, 3 h, 0°C.

Scheme 2

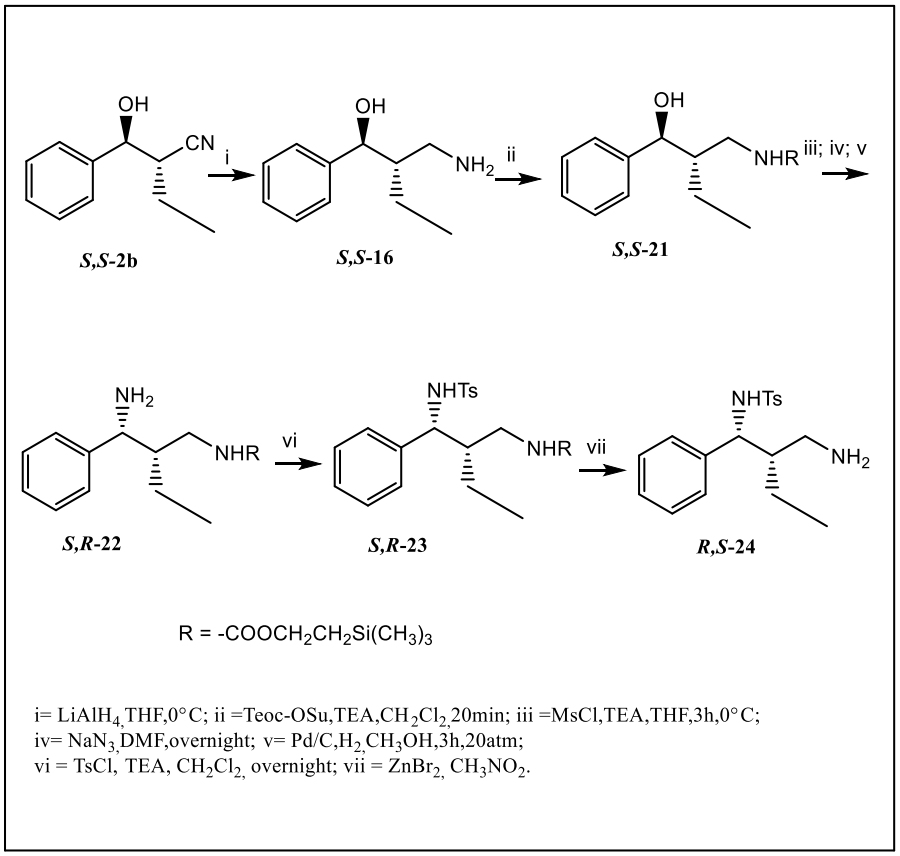


i = MsCl, TEA, THF; ii = NaN_3, DMF, overnight; iii = Pd/C, H_2, CH_3OH, 3h, 20 atm;
iv = TsCl, TEA, CH_2Cl_2; v = NaN_3, DMSO, 5 gg.

Scheme 3

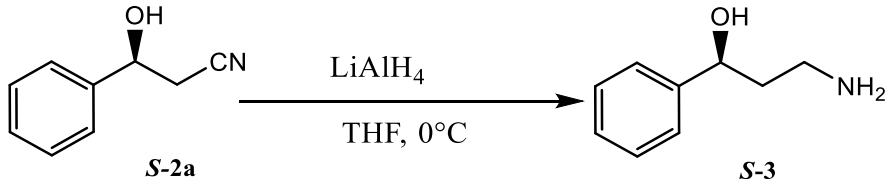


Scheme 4



Scheme 5

3.2.2.1 Synthesis of (S)-3-ammino-1-phenylpropan-1-ol (S-3)

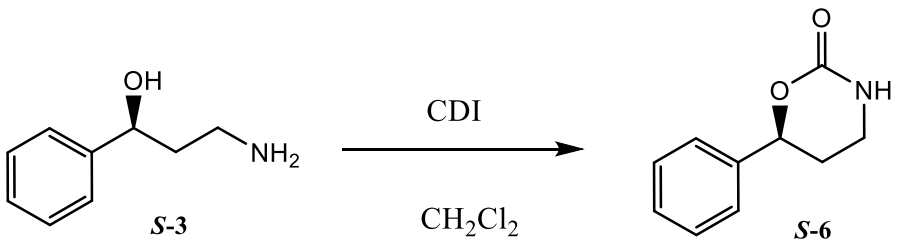


***S*-2a** ***S*-3**

To a solution of 3-hydroxy-3-phenylpropanenitrile ***S*-2** (250 mg, 1.72 mmol) in anhydrous TH F (10 mL), $LiAlH_4$ was added (100 mg, 2.6 mmol) and the resulting mixture was stirred under nitrogen atmosphere at 0°C. After 1 hour, some water was carefully added in order to quench the excess of $LiAlH_4$ and the solution was then reduced in volume and extracted with dichloro methane (3x15mL). The organic layers were dried on Na_2SO_4, filtered and evaporated to give the product as a pale yellow oil (200 mg, 77% yield).

All characterization data are in agreement with previously reported in section **3.2.1.2**

3.2.2.2 Synthesis of (*S*)-6-phenyl-1,3-oxazinan-2-one (*S*-6)

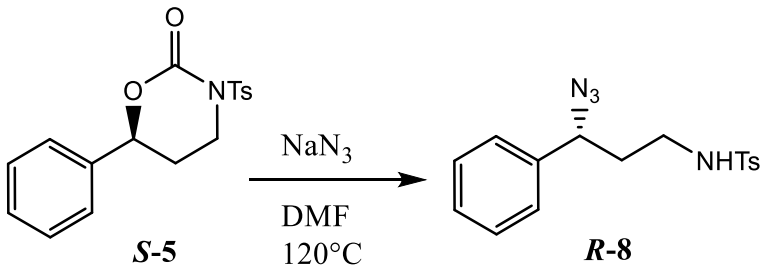


***S*-3** ***S*-6**

N,N'-Carbonyldiimidazole (218 mg, 1.35 mmol) was added to a solution of ***S*-6** (204 mg, 1.23 mmol) in CH_2Cl_2 at room temperature and the resulting mixture was stirred for 12 h. Then, the solvent was evaporated and the residue solved in ethylacetate and washed with aqueous HCl (0.1 M) and water. After drying and elimination of the solvent, crystallization by diffusion of hexane into the acetone solution afforded the product as a white solid (179 mg, 75% yield).

All characterization data are in agreement with previously reported in section **3.2.1.6**

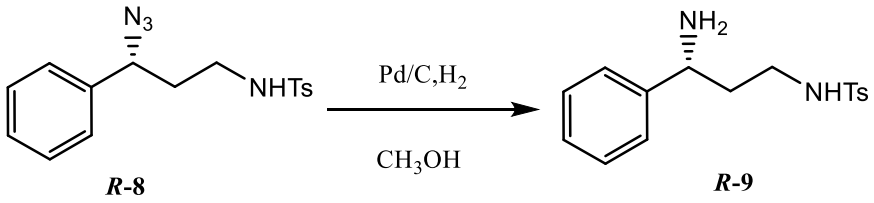
3.2.2.3 Synthesis of (R)-N-(3-azido-3-phenylpropyl)-4-methylbenzene sulfonamide (R-8)



To a solution of ***S*-5,** synthesised as reported in section **3.2.1.7,** in anhydrous DMF (50 mg, 0.30 mmol), NaN_3 was added (98.2 mg, 1.51 mmol). The solution was refluxed at 120°C for 3.5 h under N_2 atmosphere. After cooling to room temperature, water was added and the solution was extracted with diethyl ether (3x10mL). The collected organic layers were dried on Na_2SO_4, filtered and evaporated to give the product as an orange oil (50 mg, quantitative yield).

$[\alpha]^{20}_D$= +61.2 (c=0.5, $CHCl_3$), ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.88 (m, 2H), 2.42 (s, 3H), 3.03 (m, 2H), 4.52 (t, *J* = 7.33 Hz, 1H), 5.16 (t, 1H) 7.19-7.37 (m, 7H) 7.73 (d, *J* = 8.43Hz, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 143.79, 138.96, 137.02, 130.01, 129.15, 128.74, 127.34, 127.03, 63.78, 40.47, 36.24, 21.73, 1.26 ppm; IR ν = 3283, 3063, 3032, 2927, 2876, 2099, 1663, 1598, 1454, 1326, 1160, 1093, 909, 815 cm^{-1}; MS (ESI) of $C_{16}H_{18}N_4O_2S$ *m/z* 353.2 $[M+Na]^+$.

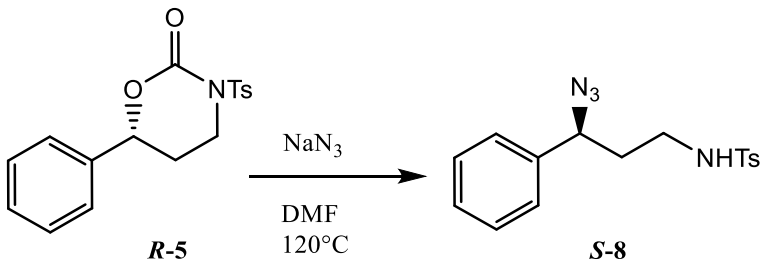
3.2.2.4 Synthesis of (*R*)-*N*-(3-amino-3-phenylpropyl)-4-methylbenzen sulfonamide (*R*-9)



In a stainless steel autoclave (20 ml), equipped with temperature control and a magnetic stirrer, purged five times with hydrogen, a solution of ***R*-8** (50 mg, 0.303 mmol) in methanol with 1% of Pd/C was transferred. The autoclave was pressurised at 20 atm and kept under stirring at room temperature for four hours. The mixture was then filtered on Celite and the solvent was evaporated in *vacuo* to give the product as a yellow oil, without any further purification step (44 mg, 95% yield).

$[\alpha]^{20}_{D}$= +8.0 (c=0.4, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.89 (dd, J = 5.87, 12.09 Hz, 2H), 2.42 (s, 3H), 2.94 (m, 2H), 3.76 (br, 2H), 4.04 (t, J = 5.87 Hz, 1H) 7.29-7.17 (m, 7H),7.72 (d, J = 8.07 Hz, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 143.10, 142.74, 136.82, 129.60, 128.73, 127.64, 127.05, 126.29, 54.34, 40.71, 36.43, 21.42 ppm; IR ν = 3350, 3293, 2917, 2099, 1650, 1598, 1454, 1323, 1156, 1094, 951, 815 cm^{-1}; MS (ESI) of $C_{16}H_{20}N_2O_2S$ *m/z* 305.2 $[M+H]^+$.

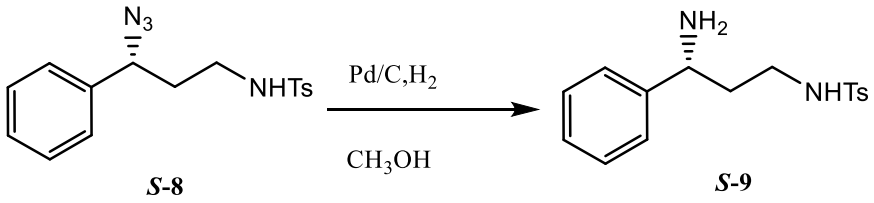
3.2.2.5 Synthesis of (*S*)-*N*-(3-azido-3-phenylpropyl)-4-methylbenzen sulfonamide (S-8)



This intermediate was synthesised according to the procedure reported for ***R*-8** (**section 3.2.2.3**) starting from ***R*-5** (48 mg, 0.145 mmol). ***S*-8** was obtained as an orange oil (24.6 mg, 51% yield). All characterization data are in agreement with previously reported for ***R*-8.**

$[\alpha]^{20}_{D}$= -75.0 (c=0.25, $CHCl_3$).

3.2.2.6 Synthesis of (*S*)-*N*-(3-amino-3-phenylpropyl)-4-methylbenzen sulfonamide (S-9)

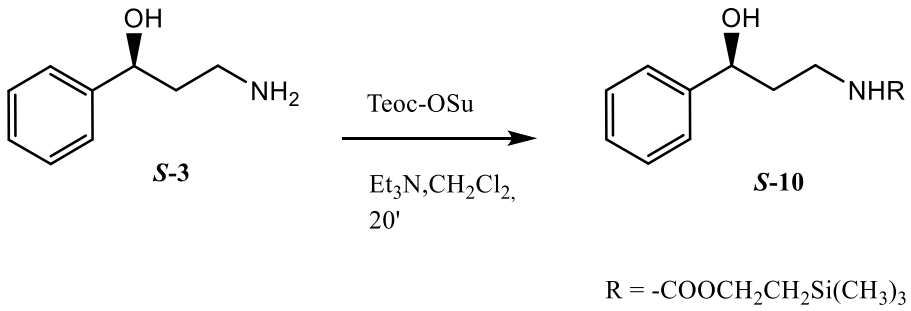


***S*-8** ***S*-9**

This product was synthesised according to the procedure reported for ***R*-9 (section 3.2.2.4)** starting from ***S*-8** (24 mg, 0.07 mmol). ***S*-9** was obtained as a pale yellow oil (24 mg, quantitative yield). All characterization data are in agreement with previously reported for ***R*-9.**

$[\alpha]^{20}_{D}$= -7.6 (c=0.24, $CHCl_3$).

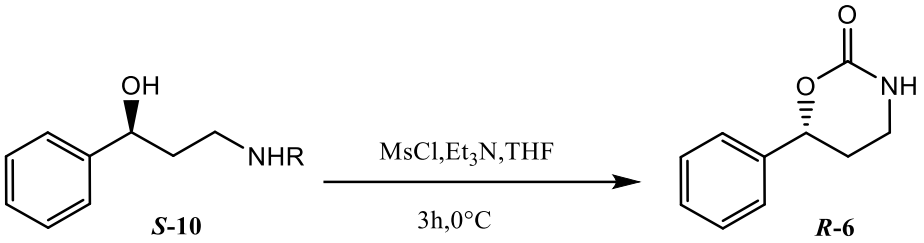
3.2.2.7 Synthesis of 2-(trimethylsilyl)ethyl(*S*)-(3-hydroxy-3 phenylpropyl) carbamate (*S*-10)



The synthesis proceeded according to methodology reported in literature.[3] ***S*-10** was obtained as a colourless oil (405 mg, 93% yield).

$[\alpha]^{20}_{D}$= -12.3 (c=1.5, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.02 (s, 9H), 0.89 (t, *J* = 8.43 Hz, 2H), 1.75 (q, *J* = 6.6 Hz, 2H), 3.11-3.27 (m, 2H), 4.05 (t, *J* = 8.43 Hz, 2H), 4.60-4.66 (dd, *J* = 5.50 Hz, 1H), 5.42 (br, 1H), 7.14-7.26 (m, 5H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 157.59, 144.79, 125.89-128.61, 71.98, 70.67, 63.15, 39.32, 25.60, 17.95, -1.27 ppm; IR ν = 3403, 2953, 1743, 1694, 1525, 1251, 1062, 860, 838 cm^{-1};MS (ESI) of $C_{15}H_{25}NO_3Si$ *m/z* 318.2 $[M+Na]^+$.

3.2.2.8 Synthesis of (*R*)- 6-phenyl-1,3-oxazinan-2-one (*R*-6)



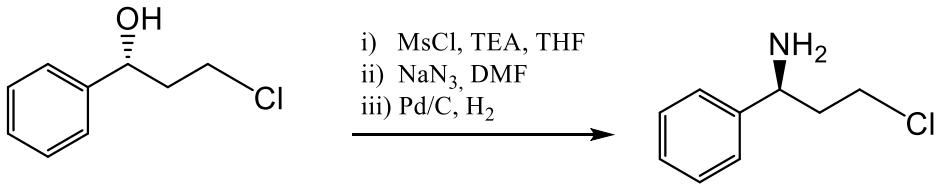
***S*-10** ***R*-6**

R = -$COOCH_2CH_2Si(CH_3)_3$

A solution of ***S*-10** (405 mg, 1.37 mmol) and triethylamine (380 µl, 2.74 mmol) in anhydrous THF (10 ml), was cooled to 0°C. Mesyl chloride (130 µl, 1.64 mmol) in THF (2 ml) was added dropwise. The reaction was stirred for 2 h, filtrated and the solvent evaporated in *vacuo*. ***R*-6** was obtained as a white solid (179 mg, 74% yield). $[\alpha]^{20}_D$= +40.0 (c=0.5, $CHCl_3$).

All characterization data are in agreement with previously reported in section **3.2.1.6**

3.2.2.9 Synthesis of (*S*)-3-chloro-1-phenylpropan-1-amine (*S*-11)



(*R*)-(+)-3-Chloro-1-phenyl-1-propanol **S-11**

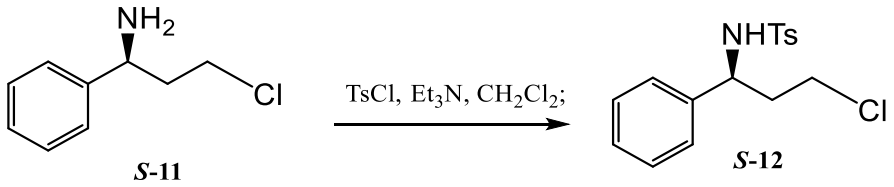
A solution of (*R*)-(+)-3-chloro-1-phenyl-1-propanol (115 mg, 0.68 mmol) and triethylamine (190 µl, 1.36 mmol) in anhydrous THF (5 ml), was cooled to 0°C. Mesyl chloride (65 µl, 0.81 mmol) in THF (1 ml) was added dropwise. The reaction was stirred for 2h, filtrated and the solvent evaporated in *vacuo*. The mesylated intermediate was used without any other purification step. The compound was dissolved in dry DMF (5 ml) and NaN_3 (65 mg, 1 mmol) was added. After stirring for 12 h at room temperature, water (2 ml) was added and the solution was extracted with diethyl ether (3x10 ml). The collected organic layers were washed with an aqueous solution of $NaHCO_3$, dried on Na_2SO_4, filtered and evaporated to give the azido compound (130 mg, 97% yield).

$[\alpha]^{20}_D$= -123.8 (c=0.5, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.81 – 6.99 (m, 5H), 4.95 – 4.59 (dd, *J* = 8.4, 6.0 Hz,1H), 3.82 – 3.35 (m, 2H), 2.45 – 1.99 (m, 2H).^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.80, 129.24, 128.86, 127.15, 63.33, 41.57, 39.16. ppm; IR ν = 3032, 2964, 2919, 2098, 1678, 1454, 1244, 760, 700 cm^{-1}; MS (ESI) of $C_9H_{10}ClN_3$ *m/z* 196.7 [M+H].

In a stainless steel autoclave (20 ml), equipped with temperature control and a magnetic stirrer, purged five times with hydrogen, a solution azido intermediate (130 mg, 0.67 mmol) in methanol with 1% of Pd/C was transferred. The autoclave was pressurised at 20 atm and kept under stirring at room temperature for four hours. The mixture was then filtered on Celite and the solvent was evaporated in *vacuo* to give the product ***S*-11** as a yellow pale oil. (100 mg, 89% yield).

$[\alpha]^{20}_D$= +5.4 (c=1.0, CH_3OH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.12 (dd, *J* = 13.5, 7.0, 3.1 Hz, 2H), 3.01 (br, 2H), 3.35-3.65 (m, 2H), δ 4.15 (t, *J* = 7.0 Hz, 1H), 7.21-7.39 (m, 5H) ppm; ^{13}C NMR (75 MHz, CD_3OD): δ = 143.80, 128.64, 127.82, 127.49, 126.78, 126.48, 126.24, 57.51, 53.38, 41.56, 41.15, 30.01, 9.77. ppm; IR ν = 3352, 3270, 2933, 1602, 1453, 1348, 1072, cm^{-1}; MS (ESI) of $C_9H_{10}ClN$ *m/z* 170 $[M+H]^+$.

3.2.2.10 Synthesis of (*S*)-N-(3-chloro-1-phenylpropyl)-4-methylbenzene sulfonamide (*S*-12)

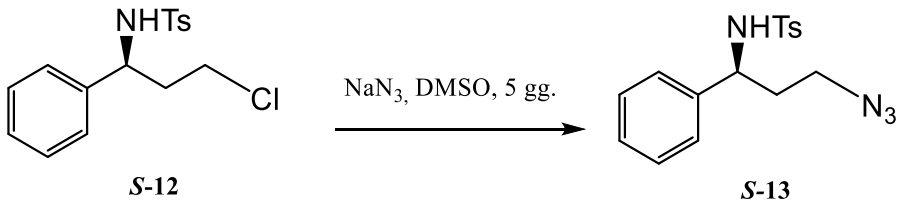


***S*-11** ***S*-12**

To a solution of **S-11** (90 mg, 0.53 mmol) in fresh-distilled dichloromethane, triethylamine (112 µL, 0.79 mmol) was added. The reaction mixture was then cooled to 4°C and stirred for half an hour. A solution of tosyl chloride (126 mg, 0.66 mmol) in dichloromethane was then dropped into the former solution and stirred overnight allowing the reaction mixture to reach room temperature. The reaction was monitored by TLC using EtOAc/hexane 1:1 as eluent. The desired product was obtained as a white solid by slow diffusion of hexane into the chloroform solution (80 mg, 50%yield).

$[\alpha]^{20}_{D}$= -6.5 (c=0.25, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.57 – 1.92 (m, 2H), 2.35 (s, 3H), 3.62 – 3.19 (m, 2H), 4.52 (q, *J* = 7.4 Hz, 1H), 5.55 (d, *J* = 7.6 Hz, 1H), 7.00-7.16 (m,7H), 7.57 (d, *J* = 8.2 Hz, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 143.39, 139.81, 137.62, 129.95, 129.47, 128.88, 128.56, 127.96, 127.30, 126.79, 126.68, 126.36, 55.98, 41.31, 40.16, 21.64 ppm; IR ν = 3436, 3265, 2965, 1600, 1458, 1325, 1161, cm^{-1};MS (ESI) of $C_{16}H_{18}ClNO_2S$ *m/z* 346.3 $[M+Na]^+$.

3.2.2.11 Synthesis of (*S*)-N-(3-azido-1-phenylpropyl)-4-methylbenzene sulfonamide (*S*-13)

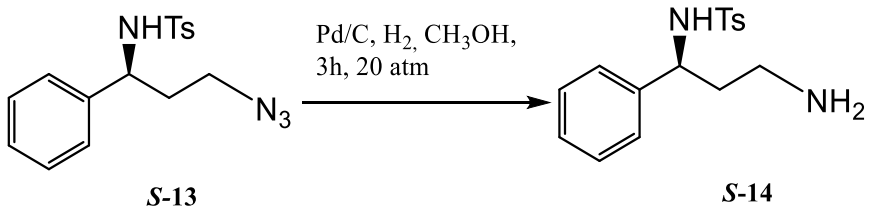


***S*-12** ***S*-13**

Compound ***S*-12** (40 mg, 0.124 mmol) was dissolved in dry DMSO (5 ml) and NaN_3 (80 mg, 1.24 mmol) was added. After stirring for five days at room temperature, water (2 ml) was added and the solution was extracted with diethyl ether (3x5 ml). The collected organic layers were washed with an aqueous solution of $NaHCO_3$, dried on Na_2SO_4, filtered and evaporated to give the product ***S*-13** as white solid (40 mg, 97% yield).

$[\alpha]^{20}_D$= -14.3 (c=0.4, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ =1.8-2.10 (m,2H), 2.57 (s, 3H), 3.01-3.33 (m, 2H), 4.31 (dd, *J* = 14.8,8.1Hz,1H), 6.65 (d, *J* = 8.2, 1H), 6.98-7.16 (m, 7H), 7.46 (d, *J* = 7.8, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 143.43, 140.02, 137.72, 129.60, 129.45, 128.90, 128.57, 127.95, 127.28, 126.77, 126.56, 126.29, 56.12, 48.28, 36.59, 21.59 ppm; IR ν = 3232, 2963, 2091, 1599, 1455, 1323, 1156, 1088 cm^{-1};MS (ESI) of $C_{16}H_{18}N_4O_2S$ *m/z* 353.3 $[M+Na]^+$.

3.2.2.12 Synthesis of (*R*)-N-(3-amino-1-phenylpropyl)-4-methylbenzene sulfonamide (*S*-14)

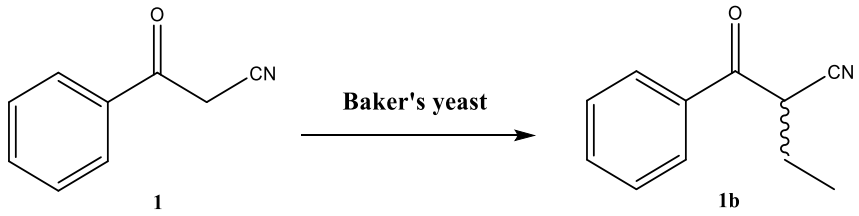


***S*-13** ***S*-14**

In a stainless steel autoclave (20 ml), equipped with temperature control and a magnetic stirrer, purged five times with hydrogen, a solution of ***S*-13** (40 mg, 0.121 mmol) in methanol with 1% of Pd/C was transferred. The autoclave was pressurised at 20 atm and kept under stirring at room temperature for four hours. The mixture was then filtered on Celite and the solvent was evaporated in *vacuo* to give the product ***S*-14** as a yellow pale oil. (35 mg, 95% yield).

$[\alpha]^{20}_{D}$= +5.4 (c=1.5, CH_3OH). ^{1}H NMR (300 MHz, $CDCl_3$): δ =1.85 – 2.05 (m,2H), 2.30 (s, 3H), 2.77-2.9. (m, 2H), 4.25-4.6 (br, 4H), 6.97-7.16 (m, 7H), 7.46 (d, J = 7.8) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 142.82, 141.22, 138.12, 129.89, 129.36, 128.45, 127.21, 126.77, 126.56, 57.69, 38.89, 38.47, 21.57 ppm; IR ν = 3352, 3270, 2933, 2103, 1652, 1453, 1328, 1152, 1091, 953, 817 cm^{-1};MS (ESI) of $C_{16}H_{20}N_2O_2S$ *m/z* 305.4 $[M+H]^+$.

3.2.2.13 Enzymatic synthesis of rac-2-benzoylbutanenitrile (1b)



1 1b

Commercial Baker's yeast (50 g L^{-1}) was suspended in a phosphate buffer (200 mL, 0.1 M, pH 7) containing 50 g L^{-1} of glucose and 5 g L^{-1} of the substrate **1**. The biotransformation system was shaken with mechanic stirrer at 28°C. When the total conversion was achieved, the cells were separated by centrifugation. Both the aqueous phases and the cells mixture were extracted with diethyl ether (3x50 mL), dried with Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was purified by flash chromatography (CH_2Cl_2/hexane/ethyl acetate = 4:1:1) to give 860 mg of **1b** (86% yield).

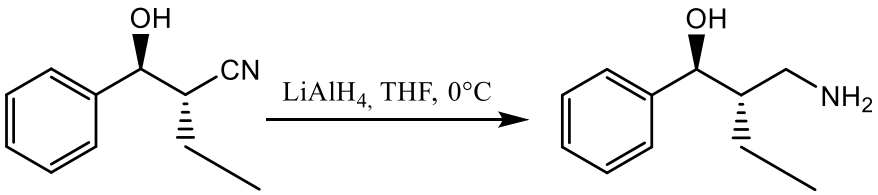
^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.16 (t, J = 7.7 Hz, 3H), 2.02-2.15 (m, 2H), 4.30 (dd, J = 6.2, 4.3 Hz, 1H), 7.49-7.56 (m, 2H), 7.65 (d, J = 7.6 Hz, 1H) 7.95 (d, J = 6.7 Hz, 2H) ppm;^{13}C NMR (75 MHz, $CDCl_3$): δ = 190.97, 170.91, 128.92-134.63, 117.41, 41.69, 23.77, 11.71, ppm. IR ν = 3467, 2975, 2936, 2249, 1694, 1597, 1449, 1265, 1233, 1208, 1000, 696 cm^{-1}. MS (ESI) of $C_{11}H_{11}NO$ (*m/z*) : 196.1 [$M+Na^+$].

3.2.2.14 (*S*)-2-((*S*)-hydroxy(phenyl)methyl)butanenitrile (*S,S*-2b)

Rhodotorula rubra MIM 147 was routinely maintained on malt extract slants (8 g L^{-1}, yeast extract 5 g L^{-1}, agar 15 g L^{-1}, pH 5.6). The strain, grown on malt extract slants for 72 h at 28 °C, was inoculated into 1000-mL Erlenmeyer flasks containing 150 mL of the same liquid medium and incubated on a reciprocal shaker (100 spm) for 48 h at 28°C. Cells obtained by centrifugation (4000×*g* for 15 min at 4 ◦C) of the culture broth (1L) were washed with tap water (3x200 mL) and re-suspended in 500 mL of 0,1M phosphate buffer pH = 7 containing 50 g L^{-1} of glucose. The substrate (**1b)** dissolved in DMSO was added to the biotransformation system in 1 g L^{-1} of substrate concentration and 1% of solvent. The biotransformation system was shaken with mechanic stirrer at 28°C for 24 h. The cells were separated by centrifugation and both were extracted with diethyl ether (3x150 mL), dried with Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was purified by flash chromatography (ethyl acetate/cyclohexane = 7:3) to give 287 mg of ***S,S*-2b** (57% yield).

^{1}H NMR ($CDCl_3$, 300 MHz, 25°C): δ = 1.09 (t, *J* = 7.7 Hz, 3H), 1.51-1.69 (m, 2H), 2.76-2.83 (m, 2H), 4.79 (d, *J* = 6.2 Hz, 1H), 7.33-7.56 (m, 5H) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, 25°C): δ = 145.05, 128.58, 128.32, 128.07, 127.14, 126.73, 126.55, 79.45, 47.11, 43.42, 22.36, 11.79 ppm. IR ν = 3390, 2964, 1494, 1453, 160, 1103, 1038, 702 cm^{-1}. MS (ESI) of $C_{11}H_{11}NO$ (*m/z*): 198.3 [M+Na^+]. $[\alpha]^{20}_D$= -46.4 (c=0.5, $CHCl_3$). HPLC data: OD-H Chiralcel, eluent: hexane: 2-propanol = 95:5, flow = 0.8 mL/min, λ = 216 nm; rt: (*R,S*) = 26.9 min, (*S,S*) = 28.6 min, (*S,R*) = 34.2 min, (*R,R*) = 36.4 min.

3.2.2.15 Synthesis of (1*S*,2*S*)-2-(aminomethyl)-1-phenylbutan-1-ol (*S*,*S*-16)

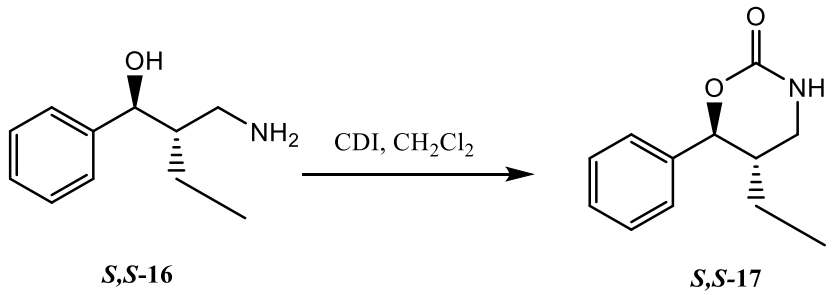


S,S-2b **S,S-16**

To a solution of ***S,S*-2b** (250 mg, 1.43 mmol) in anhydrous THF (10 mL), $LiAlH_4$ was added (550 mg, 14.3 mmol) and the resulting mixture was stirred under nitrogen atmosphere at 0°C. After 1 hour, some water was carefully added in order to quench the excess of $LiAlH_4$ and the solution was then reduced in volume and extracted with dichloromethane (3x15mL). The organic layers were dried on Na_2SO_4, filtered and evaporated to give the product as a pale yellow oil (215 mg, 84% yield).

$[\alpha]^{20}_D$= -18.3 (c=2.0, $CHCl_3$); ^{1}H NMR (300MHz, $CDCl_3$): δ = 0.88 (m, 3H); 1.28 (m, 2H); 2.88 (m, 2H); 2.96 (m, 1H); 3.09 (s, 2H); 4.71(d, J = 6.59 Hz, 1H); 7.38-7.23 (m, 5H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 145.05, 128.58, 128.31, 128.07, 127.14, 126.73, 126.55, 79.45, 47.11, 43.42, 22.36, 11.79 ppm; IR ν=3367, 3305, 2960, 2929, 2874, 1601, 1493, 1453, 1043, 1026, 701cm^{-1}. MS (ESI) of $C_{11}H_{17}NO$ *m/z* 180.1 $[M+H]^+$.

3.2.2.16 Synthesis of (5*S*,6*S*)-5-ethyl-6-phenyl-1,3-oxazinan-2-one (*S*,*S*-17)

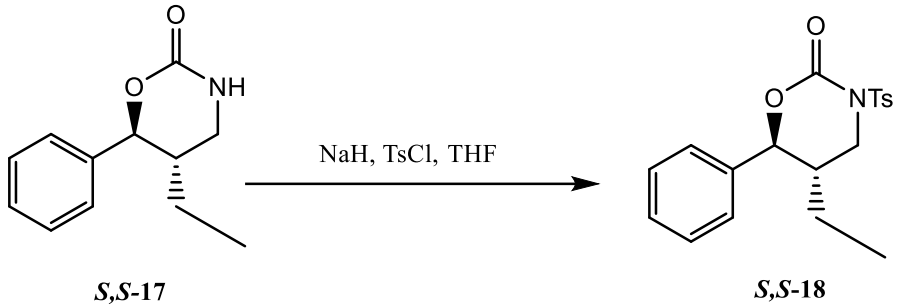


***S*,*S*-16** ***S*,*S*-17**

This intermediate was synthesised according to the procedure reported for *S*-**6 (Section 3.2.2.2)** starting from **S,S-16 (**215 mg, 1.20 mmol). **S,S-17** was obtained as a white solid (177 mg, 72% yield).

$[\alpha]^{20}_{D}$= -7.0 (c=0.7, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.84 (t, *J* = 7.33Hz, 3H); 1.24 (m, 2H); 2.04 (m, 1H); 3.12 (t, *J* = 9.89 Hz, 2H); 3.46 (m, 1H); 4.97(d, *J* = 8.79 Hz, 1H); 5.27 (s, 1H); 7.36-7.29 (m, 5H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.38, 138.14, 128.86, 128.75, 128.55, 127.15, 126.05, 84.04, 43.81, 38.79, 22.31, 11.09 ppm; IR ν= 3435, 2961, 2925, 2854, 1698, 1457, 1355, 802, 761 cm^{-1}. MS (ESI) of $C_{12}H_{15}NO_2$ *m/z* 206.1 $[M+H]^+$.

3.2.2.17 Synthesis of (5*S*,6*S*)-5-ethyl-6-phenyl-3-tosyl-1,3-oxazinan-2-one (S,S-18)



***S,S*-17** ***S,S*-18**

This intermediate was synthesised according to the procedure reported for ***S*-5 (Section 3.2.1.7)** starting from ***S,S-17*** **(**76 mg, 0.37 mmol). ***S,S*-18** was obtained as a white solid (71 mg, 58% yield).

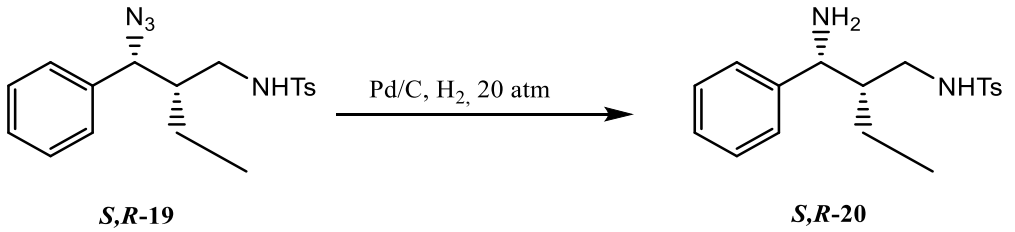
$[\alpha]^{20}_{D}$= -5.8 (c=0.6, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.87 (m, 3H), 1.32 (m, 2H), 2.1 (m, 1H), 2.46 (s, 3H), 3.65 (dd, *J* = 9.53, 2.19Hz, 1H), 4.11 (dd, 1H, *J* = 5.13, 6.59 Hz), 4.96 (d, *J* = 8.43 Hz, 1H), 7.43-7.19 (m, 7H) 7.97-7.91 (m, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 148.89, 145.33, 136.82, 135.52, 129.66, 129.28, 129.12, 128.97, 126.89, 84.63, 48.41, 40.31, 22.39, 21.85, 11.06 ppm. IR ν= 3426, 2964, 2882, 2101, 1719, 1353, 1175, 1158, 885, 700 cm^{-1}. MS (ESI) of $C_{19}H_{21}NO_4S$ *m/z* 360.2 $[M+H]^+$.

3.2.2.18 Synthesis of N-((*S*)-2-((*R*)-azido(phenyl)methyl)butyl)-4methyl benzene sulfonamide (S,R-19)

This intermediate was synthesised according to the procedure reported for ***R*-8 (Section 3.2.2.3)** starting from ***S,S*-18** (59 mg, 0.164 mmol). ***S,R*-19** was obtained as a pale yellow oil (40.1 mg, 68% yield).

$[\alpha]^{20}_{D}$= +103.5 (c=0.4, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.84 (m, 3H), 1.27 (m, 2H), 1.75 (m, 1H), 2.44 (s, 3H), 2.89 (t, *J* = 6.23 Hz, 2H), 4.61 (m, 2H), 7.36-7.13 (m, 7H), 7.69 (d, *J* = 6.59 Hz, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 146.76, 138.25, 136.95, 131.09, 130.71, 130.55, 130.40, 128.32, 86.06, 49.84, 41.75, 23.82, 21.43, 12.49 ppm; IR ν= 3282, 2964, 2933, 2101, 1711, 1666, 1328, 1160, 1093, 911 cm^{-1}. MS (ESI) of $C_{18}H_{22}N_4O_2S$ *m/z* 359.3 $[M+H]^+$.

3.2.2.19 Synthesis of N-((*S*)-2-((*R*)-amino(phenyl)methyl)butyl)-4-methyl benzene sulfonamide (S,R -20)



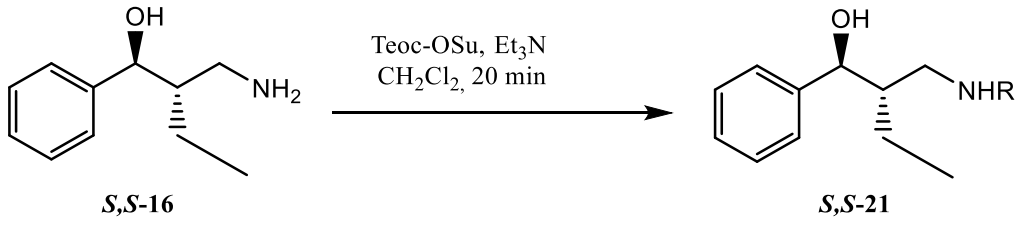
***S,R*-19** ***S,R*-20**

;

This product was synthesised according to the procedure reported for ***R*-9 (Section 3.2.2.4)** starting from ***S,R*-19** (40.1 mg, 0.11 mmol). ***S,R*-20** was obtained as a white solid (32.7 mg, 89% yield).

$[\alpha]^{20}_{D}$= +4 (c=0.3, CH_3OH). ^{1}H NMR (300 MHz, CD_3OD): δ = 0.86 (m, 3H), 1.21 (m, 2H), 1.74 (m, 1H), 2.45 (s, 3H), 2.89 (m, 2H), 4.15 (d, J = 3.29 Hz, 1H), 7.33-7.05 (m, 7H), 7.75 (d, J = 8.06 Hz, 2H) ppm; ^{13}C NMR (75 MHz, CD_3OD): δ = 143.51, 140.92, 136.64, 129.53, 128.54, 127.68, 126.96, 55.94, 45.51, 42.45, 20.27, 19.18, 9.75 ppm; IR ν= 3436, 3292, 2963, 2925, 1631, 1320, 1151, 1093, 803, 704 cm^{-1}. MS (ESI) of $C_{18}H_{24}N_2O_2S$ *m/z* 333.0 $[M+H]^+$.

3.2.2.20 Synthesis of 2-(trimethylsilyl)ethyl ((*S*)-2-((*S*)-hydroxyl (phenyl) methyl) butyl) carbamate (*S,S*-21)



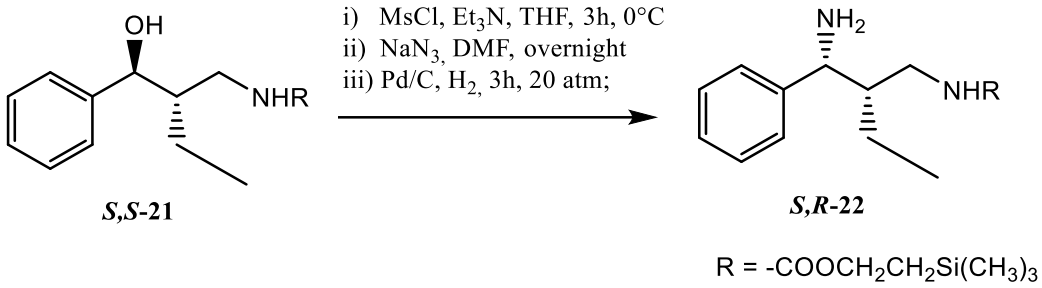
***S,S*-16** ***S,S*-21**

R = -$COOCH_2CH_2Si(CH_3)_3$

The synthesis proceeded as reported for ***S*-10 (section 3.2.2.7).** Product ***S,S*-21** was recovered as a white oil (374 mg, 1.16 mmol, 82% yield).

$[\alpha]^{20}_D$= -9.5 (c=0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ= 0.09 (s, 9H), 0.93 (t, *J* = 4.03 Hz, 3H), 0.97-1.02 (m, 2H), 1.17-1.28 (m, 2H), 1.69-1.75 (m, 1H), 3.18-3.25 (m, 2H), 4.15 (t, *J* = 9.16 Hz, 2H), 4.48 (d, *J* = 7.7 Hz, 1H), 4.85 (d, *J* = 3.67 Hz, 1H), 5.10 (br, 1H), 7.24-7.34 (m, 5H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 157.93, 143.54, 129.25, 128.60, 127.79, 126.77, 74.23, 63.44, 63.39, 47.73, 41.74, 21.61, 19.16, 17.99, 12.13, 1.24 ppm; IR ν = 3391, 2958, 1694, 1519, 1251, 1064, 1041, 860, 837 cm^{-1}; MS (ESI) of $C_{17}H_{29}NO_3Si$ *m/z* 346.3 $[M+Na]^+$.

3.2.2.21 Synthesis of 2-(trimethylsilyl)ethyl ((*S*)-2-((*R*)-amino(phenyl) methyl)butyl)carbamate (S,R-22)



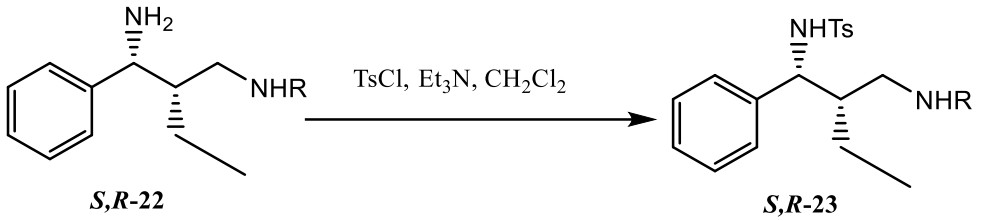
The synthesis proceeded as reported for ***S*-11 (section 3.2.2.9).** Product was recovered as colourless oil (130 mg, 35% total yield for three steps).

Intermediate 2-(trimethylsilyl)ethyl ((*S*)-2-((*R*)-azido(phenyl)methyl)butyl)carbamate

$[\alpha]^{20}_{D}$= +51.8 (c=1.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.07 (s, 9H), 0.92 (t, *J* = 7.70 Hz, 3H), 1.25-1.39 (m, 2H), 1.43-1.50 (m, 2H), 1.83-1.86 (m, 1H), 3.08-3.14 (t, *J* = 6.23 Hz, 2H), 4.13 (t, *J* = 9.89 Hz, 2H), 4.54 (d, *J* = 6.6 Hz, 1H), 7.28-7.40 (m, 5H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.98, 138.29, 129.03, 128.72, 128.48, 128.25, 127.44, 126.65, 77.89, 77.26, 76.62, 68.22, 63.25, 45.93, 41.33, 20.67, 17.98, 11.36, -1.25 ppm; IR ν = 3339, 2956, 2100, 1704, 1524, 1250, 1176, 860, 838 cm^{-1}; MS (ESI) of $C_{17}H_{28}N_4O_2Si$ *m/z* 374.3 $[M+Na]^+$.

Product ***S,R*-22**: $[\alpha]^{20}_{D}$= +6.46 (c=1.3, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.08 (s, 9H), 0.95 (t, *J* = 8.03 Hz, 3H), 1.15-1.42 (m, 4H), 1.63-1.74 (m, 1H), 2.85 (br, 2H), 3.88-3.21 (m, 2H), 4.12 (m, 3H), 5.85 (br, 1H), 7.21-7.38 (m, 5H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 157.19, 143.25, 128.60, 128.25, 127.41, 127.03, 126.65, 126.17, 62.99, 57.91, 45.98, 41.94, 20.10, 17.99, 11.90, -1.25 ppm; IR ν = 3339, 2596, 1704, 1519, 1250, 860, 837 cm^{-1}; MS (ESI) of $C_{17}H_{30}N_2O_2Si$ *m/z* 323.2 $[M+H]^+$.

3.2.2.22 Synthesis of 2-(trimethylsilyl)ethyl ((*S*)-2-((*R*)-((4-methyl phenyl) sulfonamido)(phenyl)methyl) butyl)carbamate (S,R-23)



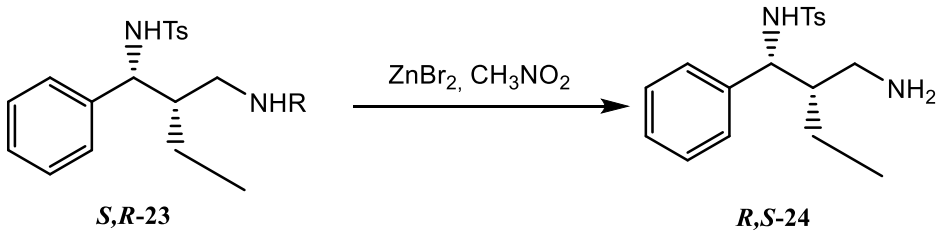
***S,R*-22** **S,R-23**

R = -$COOCH_2CH_2Si(CH_3)_3$

The synthesis proceeded as reported for ***S*-12 (section 3.2.2.10).** The product was recovered as colourless oil (50 mg, 40% yield).

$[\alpha]^{20}_D$ = + 20.4 (c = 0.9, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.05 (s, 9H), 0.88 (t, *J* = 8.06 Hz, 3H), 0.96 (t, *J* = 8.06 Hz, 2H), 1.44-1.62 (m, 2H), 2.30 (s, 3H), 3.18-3.24 (m, 2H), 4.18 (t, *J* = 6.78 Hz, 2H), 4.49-4.56 (m, 1H), 5.29 (br, 1H), 6.89-6.98 (m, 2H), 7.04-7.14 (m, 5H), 7.53 (d, *J* = 8.43, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 157.13, 143.34, 139.22, 137.52, 129.49, 128.44, 127.17, 126.56, 63.26, 58.20, 47.04, 41.22, 29.90, 21.63, 18.00, 11.82, -1.23 ppm; IR ν = 3382, 2597, 1694, 1532, 1251, 1160, 860, 838, 702 cm^{-1}; MS (ESI) of $C_{24}H_{36}N_2O_4SSi$ *m/z* 477.3 $[M+H]^+$.

3.2.2.23 Synthesis of N-((*1R,2S*)-2-(aminomethyl)-1-phenylbutyl)-4-methyl benzenesulfonamide (*R,S-24*)



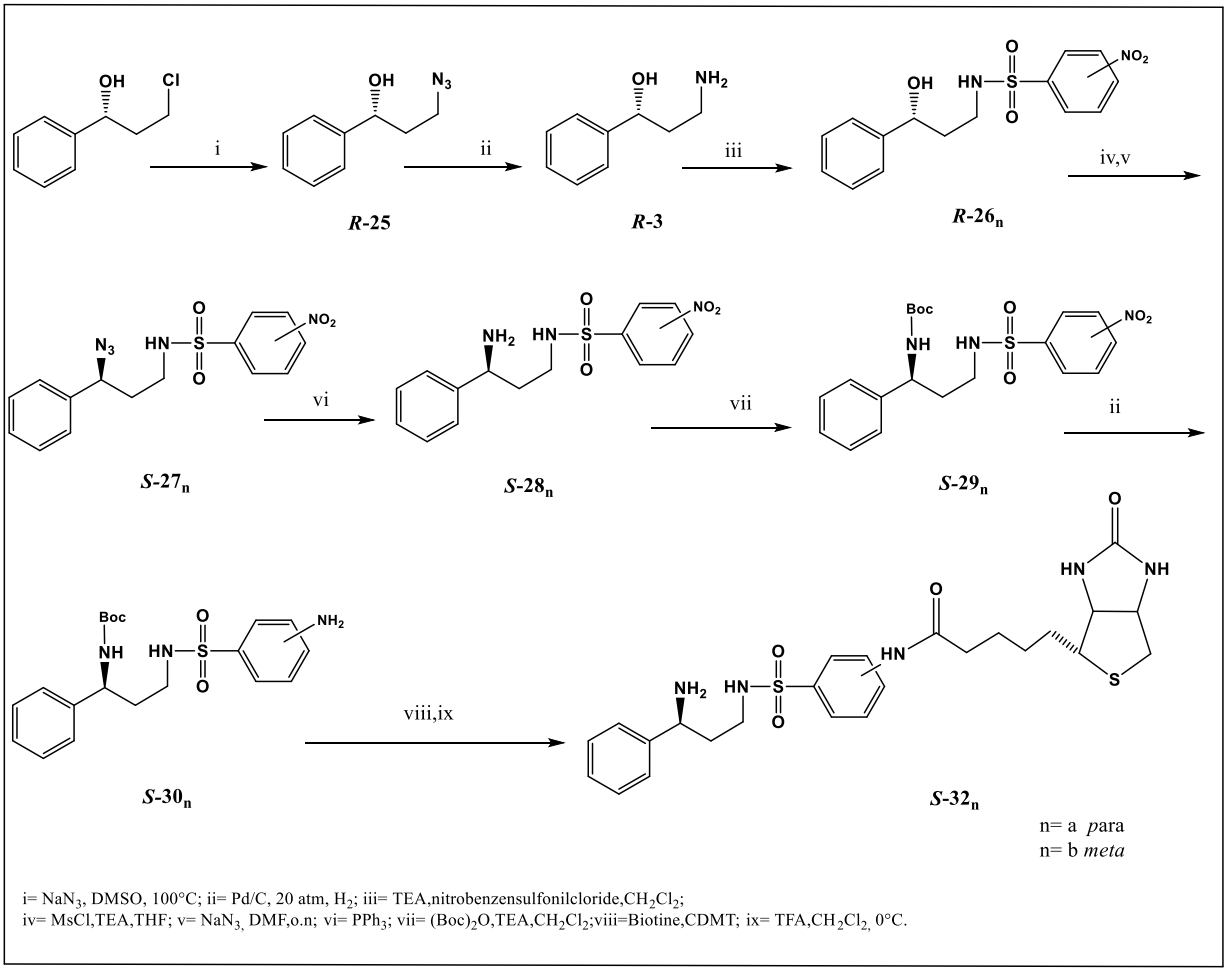
***S,R*-23** **R,S-24**

The synthesis proceeded as reported in literature[4]**.** The product was recovered as colourless oil (29 mg, 87% yield).

$[\alpha]^{20}_{D}$= +6.5 (c=1.3, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.86 (t, J = 7.33 Hz, 3H), 1.37-1.43 (m, 2H), 2.25 (s, 3H), 2.46 (d, J = 6.97 Hz, 1H), 3.73-3.82 (m, 2H), 4.50 (d, J = 5.50 Hz, 1H), 7.01-7.10 (m, 5H), 7.43-7.51 (m, 4H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 143.16, 138.47, 138.03, 129.02, 128.21, 127.12, 126.84, 126.76, 65.67, 58.19, 44.69, 20.10, 19.71, 10.32 ppm; IR ν = 3252, 3063, 2968, 2353, 1661, 1598, 1455, 1325, 1159, 1091, 969, 814 cm^{-1}; MS (ESI) of $C_{18}H_{24}N_2O_2S$ *m/z* 333.4 $[M+H]^+$.

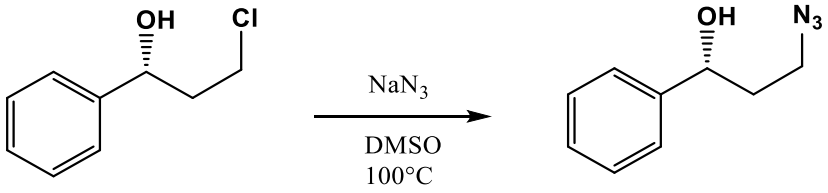
3.2.3 Synthesis of biotynilated ligands

General scheme of synthesis



Scheme 6

3.2.3.1 Synthesis of (*R*)-3-azido-1-phenylpropan-1-ol (R-25)



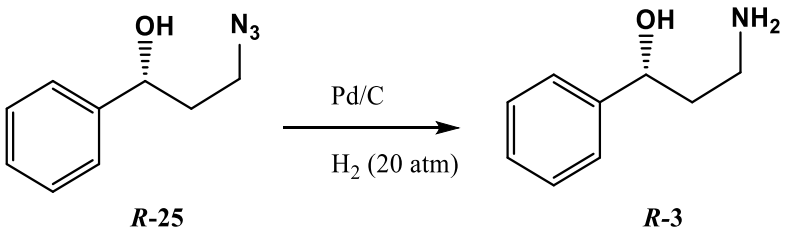
(*R*)-(+)-3-chloro-1-phenyl-1-propanol

***R*-25**

To a solution of (*R*)-(+)-3-chloro-1-phenyl-1-propanol (500 mg, 2.94 mmol), in anhydrous DMSO, NaN_3 was added (1912 mg, 29.4 mmol). The solution was refluxed at 100°C for 5 h under N_2 atmosphere. After cooling to room temperature, water was added and the solution was extracted with diethyl ether (3x10mL). The collected organic layers were washed with $NaHCO_3$ saturated solution, dried on Na_2SO_4, filtered and evaporated to give the product ***R*-25** as a pale yellow oil (456 mg, 88%).

$[\alpha]^{20}_D$= + 10.5 (c= 0.2 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.86-2.10 (m,3H), 3.37-3.61 (m, 2H), 4.82 (q, 1H), 7.31-7.40 (m, 5H), ^{13}C NMR (75 MHz, $CDCl_3$): δ = 144.07, 128.87, 128.09, 125.98, 71.89, 48.55, 38.02 ppm; IR ν = 3392, 2927, 2097, 1454, 1262, 1068 cm^{-1}; MS (ESI) of $C_9H_{11}N_3O$ *m/z* 178.8. $[M+H]^+$, 200 $[M+Na]^+$.

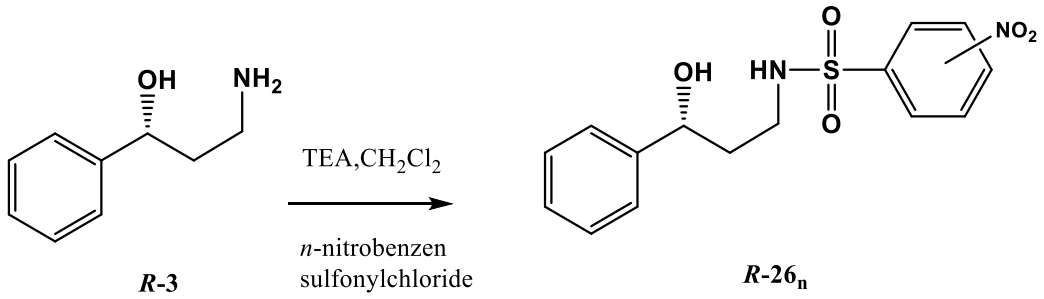
3.2.3.2 Synthesis of (*R*)-3-amino-1-phenylpropan-1-ol (*R*-3)



This intermediate was synthesised according to the procedure reported for *R*-**3 (Section 3.2.1.2)** starting from ***R*-25 (**456 mg, 2.58 mmol). ***R*-3** was obtained as a pale oil (390 mg, quantitative yield). $[\alpha]^{20}_{D}$= +44.5 (c=0.3, $CHCl_3$);

All characterization data are in agreement with previously reported in section **3.2.1.2**

3.2.3.3 Synthesis of (*R*)-N-(3-hydroxy-3-phenylpropyl)-(*n*)-nitro benzene sulfonamide (*R*-26$_n$)



***R*-3** ***R*-26$_n$**

n = a *para*
n = b *meta*

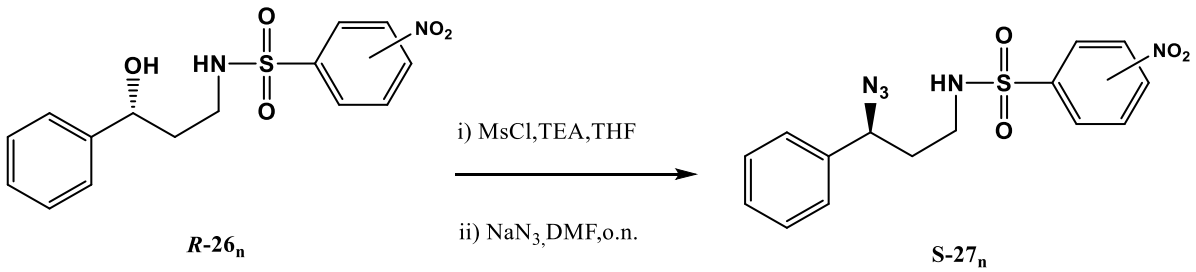
To a solution of ***R*-3** (390 mg, 2.58 mmol) in fresh-distilled dichloromethane, triethylamine (0.705 mL, 5.16 mmol) was added. The reaction mixture was then cooled to 5°C and stirred for half an hour. A solution of ***4*-**nitrobenzenesulfonyl chloride (679 mg, 3.09 mmol) in dichloromethane was then dropped into the former solution and stirred for 3h allowing the reaction mixture to reach room temperature. The reaction was monitored by TLC using EtOAc/hexane 1:1. Reaction solution was washed with water (3x 10 mL), dried, filtered and evaporated to give the product ***R*-26a**. (694 mg, 80% yield).

$[\alpha]^{20}_D$= + 22.22 (c=0.3, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ= 1.90 (m, 2H), 3.00 – 3.38 (m, 2H), 4.83 (t, J = 6.1 Hz, 1H), 5.67 (t, J = 5.3 Hz, 1H), 7.10–7.49 (m, 5H), 8.05 (d, J = 8.8 Hz, 2H), 8.36 (d, J = 8.8 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 150.22, 146.10, 143.62, 128.88, 128.55, 128.119, 125.66, 124.59,73.52, 41.33, 37.69 ppm; IR ν = 3513, 3305, 2923, 2874, 1703, 1530, 1350, 1163, 1093 cm^{-1}; MS (ESI) of $C_{15}H_{16}N_2O_5S$ *m/z* 359 $[M+Na]^+$.

Compound **R-26b** was obtained as yellow solid using the same procedure. (370mg, 60% yield).

$[\alpha]^{20}_D$= + 12.00 (c= 0.3 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.90 (dd, J = 11.6, 5.8 Hz, 2H), 3.19-3.35 (m, 3H), 4.83 (t, J = 5.8 Hz, 1H), 5.8 (br, 1H), 7.11–7.54 (m, 5H), 7.74 (t, J = 8.0 Hz, 1H), 8.20 (d, J = 8 Hz, 1H), 8.43 (d, J = 6.9, Hz, 1H), 8.71 (s,1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 148.61, 143.62, 124.70, 132.86-122.52, 73.73, 41.39, 37.65 ppm; IR ν = 3522, 3166, 2964, 1606, 1532, 1351, 1261, 1091, 1019, 798 cm^{-1}; MS (ESI) of $C_{15}H_{16}N_2O_5S$ *m/z* 335 [M-H].

3.2.3.4 Synthesis of(*S*)-N-(3-azido-3-phenylpropyl)-(*n*)-nitro benzene sulfonamide (*S*-27$_n$)



***R*-26$_n$** **S-27$_n$**

n = a para
n = b meta

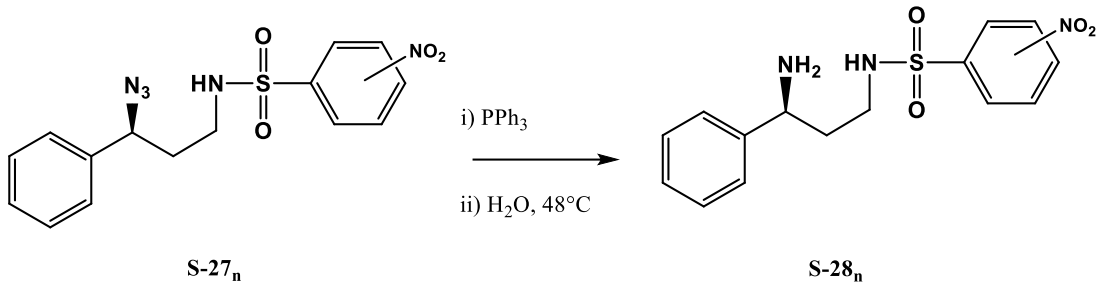
A solution of ***R*-26a** (200 mg, 0.60 mmol) and triethylamine (168 µl, 1.2 mmol) in anhydrous THF (5 ml), was cooled to 0°C. Mesyl chloride (56 µl, 0.72 mmol) in THF (1 ml) was added dropwise. The reaction was stirred for 2h, filtrated and the solvent evaporated in *vacuo*. The mesylated intermediate was used without any other purification step. The compound was dissolved in dry DMF (5 ml) and NaN_3 (58 mg, 0.9 mmol) was added. After stirring for 12 h at room temperature, water (2 ml) was added and the solution was extracted with diethyl ether (3x10 ml). The collected organic layers were washed with an aqueous solution of $NaHCO_3$, dried on Na_2SO_4, filtered and evaporated to give the azido compound ***S*-27a** as pale oil (193 mg, 78% yield).

$[\alpha]^{20}_D$= -63.4 (c=0.22, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.95 (dt, J = 13.2, 4.2 Hz, 2H), 2.99-3.22 (m, 2H), 4.56 (dd, J = 7.7, 6.4 Hz, 1H), 5.10 (t, J = 6.2 Hz, 1H), 7.11 – 7.49 (m, 5H), 7.93-8.12 (m, 2H), 8.29-8.42 (m, 2H), ^{13}C NMR (75 MHz, $CDCl_3$): δ = 150.36, 145.91, 138.48, 129.33, 129.04, 128.97, 128.55, 126.92, 124.69,124.61, 64.06, 40.83, 36.27 ppm; IR ν = 3542, 3306, 3106, 2935, 2874, 2461, 2100, 1660, 1531, 1164 cm^{-1}; MS (ESI) of $C_{15}H_{15}N_5O_4S$ *m/z* 360 [M-H]

Compound ***R*-27b** was obtained as yellow oil using the same procedure. (300 mg, 81% yield).

$[\alpha]^{20}_D$= -22.00 (c=0.1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.84-2.02 (m, 2H), 3.07 (m, 2H), 4.53 – 4.64 (m, 1H), 6.1 (br, 1H), 7.2-7.4 (5H), 7.72 (t, J = 8.0 Hz, 1H), 8.18 (d, J = 7.8 Hz, 1H), 8.41 (dd, J = 8.0, 2.0, Hz, 1H), 8.69 (t, J = 2.0 Hz, 1H),), ^{13}C NMR (75 MHz, $CDCl_3$): δ = 162.84, 142.75, 138.77, 132.80-128.50, 63.78, 40.58, 36.32 ppm; IR ν = 3296, 3089, 2932, 2875, 2100, 1661, 1532, 1352, 1168 cm^{-1};MS (ESI) of $C_{15}H_{15}N_5O_4S$ *m/z* 360 [M-H]

3.2.3.5 Synthesis of (*S*)-N-(3-amino-3-phenylpropyl)-(*n*)- nitro benzene sulfonamide (*S*-28$_n$)



S-27$_n$ **S-28$_n$**

n = a *para*
n = b *meta*

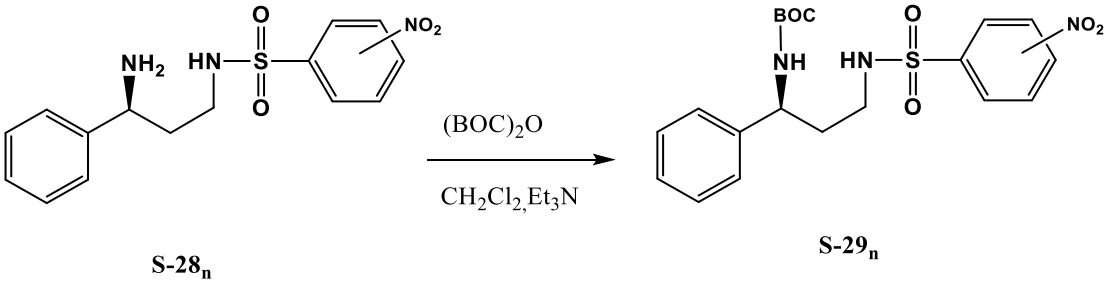
To a solution of ***S*-27a** (150 mg, 0.41 mmol, in anhydrous THF (4 ml), PPh_3 was added (120 mg,0.46 mmol). The reaction was stirred for 2h at 48°C, then water (300µl) was added and the mixture was stirred overnight at 48°C. The reaction was monitored by TLC using EtOAc/cyclohexane 7:3. The solution was evaporated under vacum and compound ***S*-28a** used for next step without purification.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.94 (m, 2H), 3.06 (m, 2H), 4.08 (t, J = 6.4 Hz, 1H), 7.11 – 7.37 (m, 5H), 8.02 (d, J = 8.9 Hz, 2H), 8.33 (d, J = 8.9 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 149.55, 143.38, 137.48, 129.33, 129.04, 128.97, 128.55, 126.92, 124.69,124.61, 52.50, 40.53, 38.27 ppm; MS (ESI) of $C_{15}H_{17}N_3O_4S$ *m/z* 334,38 [M-H]

Compound ***R*-28b** was obtained as yellow oil using the same procedure.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.96 – 1.87 (m, 2H), 3.05 (m, 2H), 4.05 (m, 1H), 7.40-7.72 (5H), 8.17 (d, J = 7.8 Hz, 1H), 8.40 (dd, J = 8.0, 2.0, Hz, 1H), 8.69 (t, J = 2.0 Hz, 1H), ^{13}C NMR (75 MHz, $CDCl_3$): δ = 148.55, 142.55, 141.57, 134.15-128.47, 55.15, 41.22, 35.92. ppm; IR ν 3366, 3058, 2871, 1668, 1531, 1351, 1171, 1120 cm^{-1} MS (ESI) of $C_{15}H_{17}N_3O_4S$ *m/z* 334,38 [M-H]

3.2.3.6 Synthesis of tert-butyl (*S*)-(3-(-(*n*)-nitrophenyl)sulfonamido)-1-phenylpropyl)carbamate (*S*-29n)



S-28$_n$ **S-29$_n$**

n = a *para*
n = b *meta*

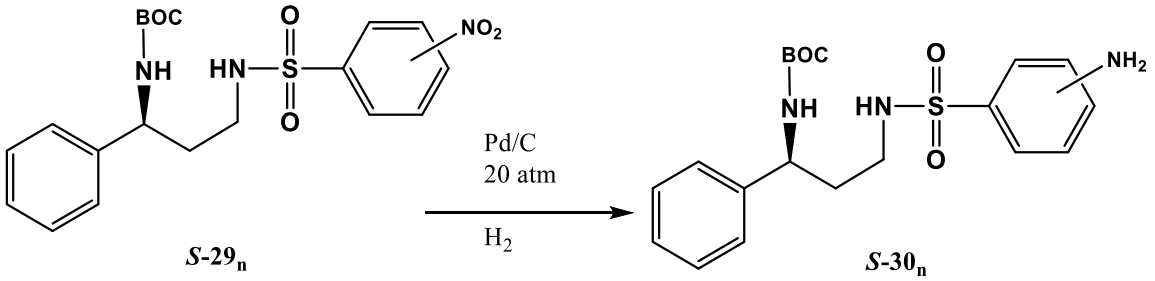
To a solution of compound ***S*-28a** (0.41mmol) and TEA (172µl, 1.23 mmol) in dry CH_2Cl_2 (5ml), a solution of $(BOC)_2O$ (134 mg, 0.62mmol) in the same solvent, was added slowly with magnetic stirring. The reaction was monitored by TLC using EtOAc/cyclohexane 7:3. Organic layer was washed with water (3x10 mL), dried, filtered and evaporated under vacum. Compound ***S*-29a** was obtained as yellow solid after crystallization using CHCl3 and hexane. (106 mg, 60 % yield for two step).

$[\alpha]^{20}_D$= -21.8 (c=0.23, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.29 (s,9H), 1.89 (m, 2H), 3.04 (m, 2H), 4.64 (m, 1H), 4.87 (m, 1H), 6.63 (m, 1H), 7.07 – 7.39 (m, 5H), 8.04 (d, J = 8.6 Hz, 2H), 8.28 (d, J = 8.6 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.41, 150.07, 146.65, 141.3, 132.38-124.48, 80.44, 52.08, 40.51, 37.03, 28.45 ppm IR ν = 3391, 2978, 2870, 1689, 1530, 1349, 1164, 1092 cm^{-1}; MS (ESI) of $C_{20}H_{25}N_3O_6S$ *m/z* 458,15 [M+Na]

Compound ***R*-29b** was obtained as yellow oil using the same procedure. (226 mg, 65 % yield for two step).

$[\alpha]^{20}_D$= -15.8 (c=0.18, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.34 (s, 9H). 1.87 – 1.96 (m, 2H), 2.8- 3.4 (m, 2H), 4.60-4.85 (m, 2H), 6.2 (br, 1H), 7.40-7.72 (m, 5H), 8.20 (d, J = 7.8 Hz, 1H), 8.40 (d, J = 8.0, 1H), 8.69 (s, 1H), ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.18, 148.45, 143.07, 142.20, 134.10-128.46, 79.96, 52.1, 40.68, 36.86, 28.46 ppm; IR ν = 3306, 3079, 2978, 1682, 1532, 1351, 1166 cm^{-1}; MS (ESI) of $C_{20}H_{25}N_3O_6S$ *m/z* 458,15 $[M+Na]^+$, 434 $[M-H]^-$

3.2.3.7 Synthesis of tert-butyl (*S*)-(3-(((n)-aminophenyl)sulfonamido)-1-phenylpropyl)carbamate (*S*-30n)



n = a *para*
n = b *meta*

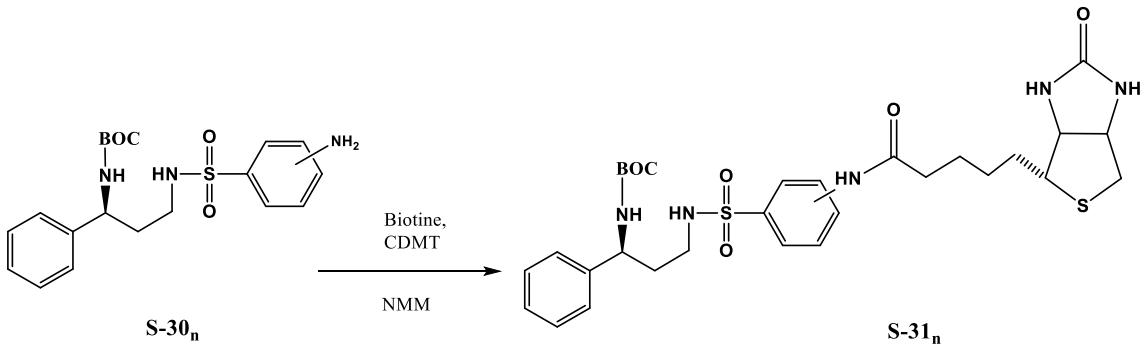
In a stainless steel autoclave (20 ml), equipped with temperature control and a magnetic stirrer, purged five times with hydrogen, a solution of **S-29a** (106 mg, 0.24 mmol) in methanol with 3% of Pd/C was transferred. The autoclave was pressurised at 20 atm and kept under stirring at room temperature for four hours. The mixture was then filtered on Celite and the solvent was evaporated in *vacuo* to give the product ***S*-30a** as a yellow pale oil. (96 mg, quantitative yield).

$[\alpha]^{20}_{D}$= -16.6 (c=0,2 CH_3OH). ^{1}H NMR (300 MHz, CD_3OD) δ = 1.35 (s, 9H), 1.65 – 1.97 (q, J = 7.3 6.9 Hz, 2H), 2.64 – 2.97 (t, J = 6.9 Hz, 2H), 4.56 (t, J = 7.3 Hz, 1H), 6.67 (d, 8.8 Hz, 2H), 7.15 – 7.31 (m, 5H), 7.52 (d, 8.8 Hz, 2H). ^{13}C NMR (75 MHz, CD_3OD) δ = 156.74, 155.76, 152.85, 143.45, 132.64-125.71, 113.43, 112.21, 79.8, 52.3, 40.12, 36.54, 27.64ppm; IR ν = 3371, 2977, 2930, 1688, 1597, 1504, 1310, 1151, cm^{-1}; MS (ESI) of *m/z* $C_{20}H_{27}N_3O_4S$ 428.3 [M+Na]

Compound ***R*-30b** was obtained as yellow oil using the same procedure. (210 mg, quantitative yield).

^{1}H NMR (300 MHz, CD_{3OD}): δ = 7.42 – 7.01 (m, 8H), 6.83 (dd, *J* = 8.4, 6.0 Hz,1H), 4.57 (q 1H), 2.86 (t, 2H), 1.81 (q, 2H).^{13}C NMR (75 MHz, CD_3OD): δ = 156.73, 152.85, 149.13, 140.98, 132.60-128.40, 118.43, 115.09, 112.34, 79.16, 52.47, 40.23, 36.60, 27.60 ppm; (ESI) of *m/z* $C_{20}H_{27}N_3O_4S$ 428,17 $[M+Na]^+$

3.2.3.8 Synthesis of tert-butyl ((1*S*)-3-((*n*-(5-((4*R*)-2-oxohexahydro -1H-thieno[3,4-d]imidazol-4-yl)pentanamido) phenyl)sulfonamido)-1-phenylpropyl)carbamate (*S*-31n)



n= a *p*ara
n= b *meta*

Compounds **S-31a** and **S-31b** were synthesised according to the procedure reported in literature.[5]

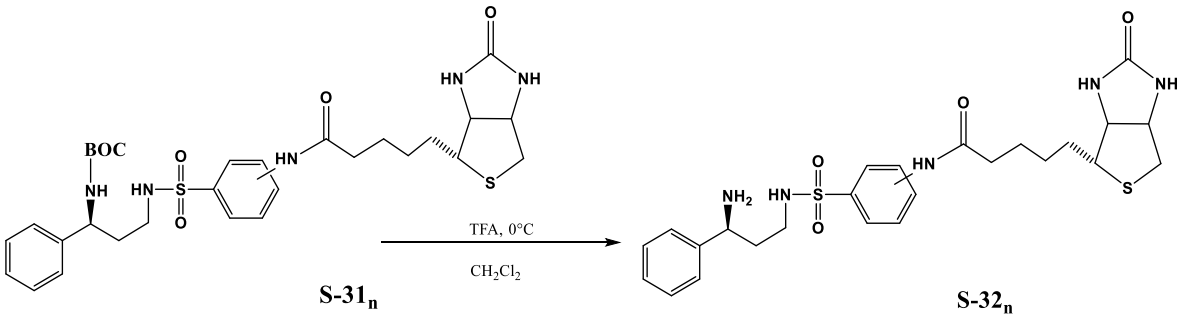
S-31a

^{1}H NMR (300 MHz, CD_3OD): δ = 1.33 (s, 9H), 1.45-1.94 (m. 6H), 2.38-2.42 (m, 2H), 2.70-2.97 (m, 5H), 3.20-3.35 (m,2H), 3.78 (s, 4H), 4.31 – 4.33 (m, 1H), 4.45 – 4.57 (m, 2H), 7.15-7.35 (m,5H), MS (ESI) of *m/z* $C_{30}H_{41}N_5O_6S_2$ 654.25[M+Na]; yield 40%.

S-31b

^{1}H NMR (300 MHz, CD_3OD): δ = 1.35 (s, 9H), 1.45-1.94 (m, 6H) 2.38-2.42 (m, 2H), 2.70-2.97 (m, 5H), 3.20(m,1H), 3.65-3.80 (m,2H), 4.31 – 4.33 (m, 1H), 4.20 – 4.65 (m, 2H), 7.10 (m, 5H), 7.40-7.80 (m,4H). ^{13}C NMR (75 MHz, CD_3OD): δ = 173.47, 169.52, 164.92, 156.70, 141.17, 139.74, 132.62-118.08, 79.17, 62.19, 60.48, 55.76, 55.01, 44.13, 40.27, 39.91, 36.50, 28.60, 28.35, 27.62, 25.40 ppm; MS (ESI) of *m/z* $C_{30}H_{41}N_5O_6S_2$ 632.0 [M+Na], 654.30[M+Na]; Yield 35%.

3.2.3.9 Synthesis of N-(*n*-(N-((*S*)-3-amino-3-phenylpropyl)sulfamoyl) phenyl)-5-((4*R*)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl) pentanamide (*S*-32n)



S-31$_n$ **S-32$_n$**

n= a *p*ara
n= b *meta*

Compound s **S-32a** and **S-32b** were synthesised according to the procedure reported in literature[5].

S-32a

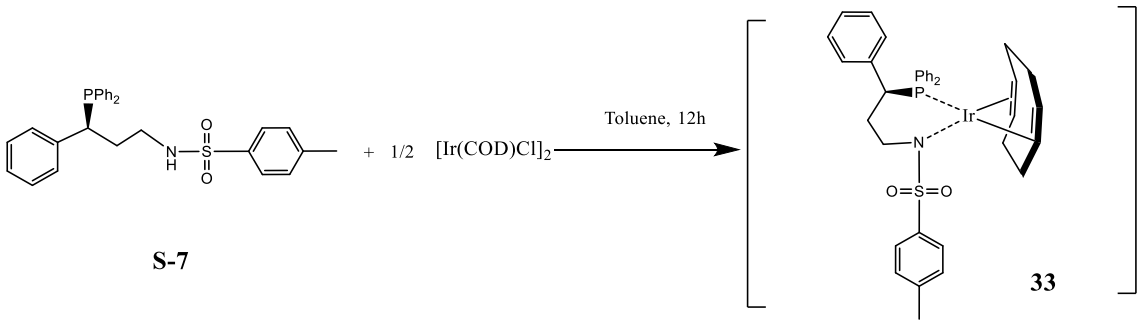
^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.81 – 6.99 (m, 5H), 4.95 – 4.59 (dd, *J* = 8.4, 6.0 Hz,1H), 3.82 – 3.35 (m, 2H), 2.45 – 1.99 (m, 2H). MS (ESI) of *m/z* $C_{25}H_{33}N_5O_4S_2$ 554.5 [M+Na]; quantitative yield.

S-32b

^{1}H NMR (300 MHz, CD_3OD): δ = 1.46-1.82 (m, 6H). 2.38-2.46 (t, J=6.8, 2H), 2.66-2.98 (m, 4H), 3.20 (m,1H), 3.66-3.75 (m,1H), 3.88-3.97 (m,1H), 4.27 – 4.52 (m, 3H), 7.39-7.76 (m, 9H), ^{13}C NMR (75 MHz, CD_3OD): δ = 173.62, 164.72, 140.67, 139.67, 136.07, 132.55-118.21, 62.23, 60.53, 55.73, 53.12, 44.88, 39.84, 39.15, 36.39, 28.53, 28.31, 25.35 ppm; MS (ESI) of *m/z* $C_{25}H_{33}N_5O_4S_2$ 532.2[M+H]; quantitative yield.

3.3 Synthesis of P-N complexes

3.3.1 Synthesis of [(*S*)-N-(3-(diphenylphosphanyl)-3-phenylpropyl)-4-methylbenzenesulfonamide Ir (COD)]Cl complex (33)

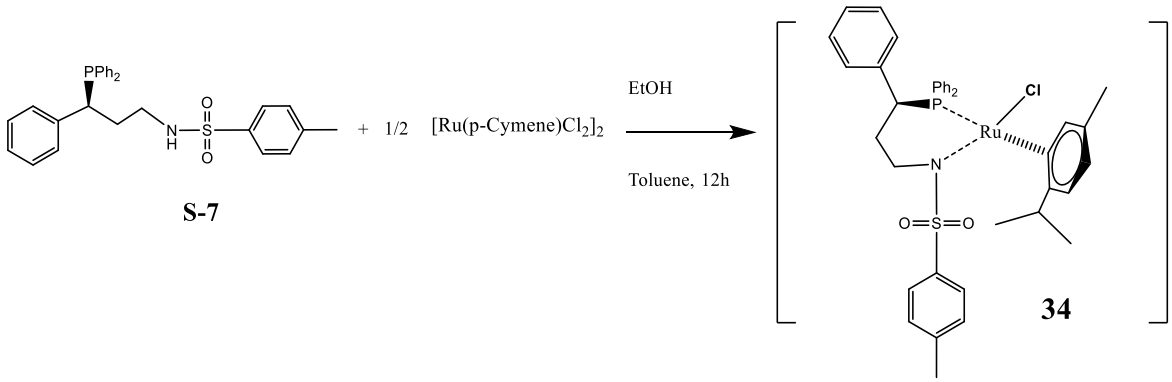


S-7 **33**

A 10 mL Schlenk tube was loaded with $[Ir(COD)Cl]_2$ (1 eq.), **S-7** ligand (2.2 eq.) and charged with distilled toluene(3 mL). The solution was stirred at RT for 12 h. Solvent was evaporated, and a yellow solid was obtained.. The complex was used in without other purification.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.8- 2.1 (m, 8H), 2.45 (s, 3H), 2.59-2.84 (m, 2H), 3.09-3.19(m, 2H), 5.40 (m, 1H),5.9 (m, 1H), 7.12-7.82 (m, 19H) ppm; ^{13}C NMR: *δ = 144.85, 139.60, 136.01, 135.25-125.69, 96.82, 76.93, 55.15, 46.66, 41.06, 39.56, 33.41-29.70, 21.88 ppm;* ^{31P} NMR (75 MHz, $CDCl_3$): δ = 26.32 ppm; MS (ESI) of *m/z* $C_{36}H_{40}ClIrNO_2PS$ 832,18 [M+Na]

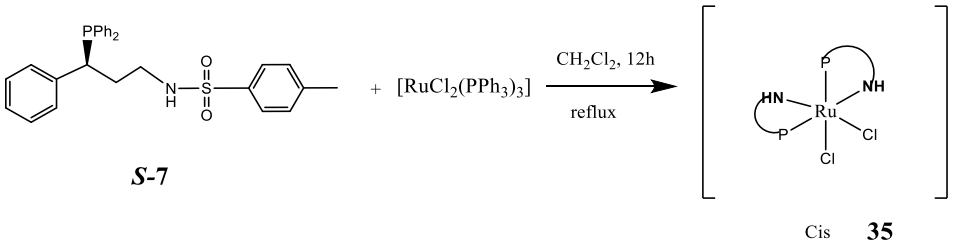
3.3.2 Synthesis of [(*S*)-N-(3-(diphenylphosphanyl)-3-phenylpropyl)-4-methylbenzenesulfonamide Ru(p-cymene)Cl]Cl complex (34)



A 10 mL Schlenk tube was loaded with [Ru(p-cymene)Cl_2]Cl_2 (1 eq.), **S-7** ligand (2.2 eq.) and charged with distilled EtOH (0.5 mL) and toluene (1.5 mL). The solution was stirred at RT for 12 h. Solvent was evaporated, and a brown solid was obtained.. The complex was used in without other purification.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.13-1.20 (m, 6H), 1.55 (m, 2H), 2.18 (s, 3H), 2.44 (s, 3H), 2.73-3.20 (m, 3H), 3.75 (m, 1H), 5.8 (m, 1H), 5.24-5.51 (m, 4H), 6.95-7.82 (m, 19H) ppm; ^{13}C NMR: *δ = 143.5, 140.33, 137.56, 136.13, 133.46-125.79, 111.21, 102.32, 98.15, 92.35, 88.96, 87.52, 81.32, 80.56, 76.21, 36.74, 38.21, 29.69, 22.50, 21.53, 17.40, 11.17 ppm;* ^{31}P NMR (75 MHz, $CDCl_3$): δ = 28.70 ppm; MS (ESI) of *m/z* $C_{38}H_{42}Cl_2NO_2PRuS$ 848,14 [M+3Na]

3.3.3 Synthesis of [(*S*)-N-(3-(diphenylphosphanyl)-3-phenylpropyl)-4-methylbenzenesulfonamide $Ru(PPh_3)Cl_2$] complex (35)



***S*-7**

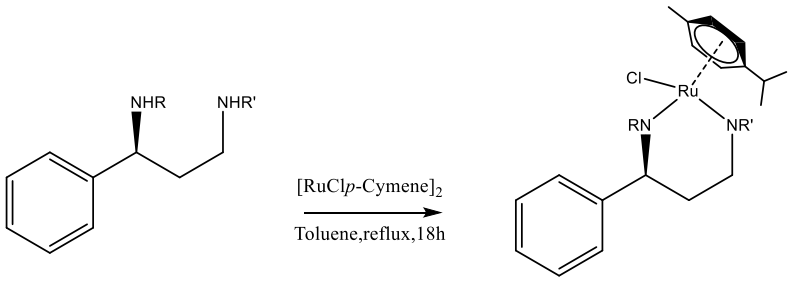
Cis **35**

A 10 mL Schlenk tube was loaded with $[RuCl_2P(Ph_3)_3]$ (1 eq.), **S-7** ligand (2.2 eq.) and charged with distilled diclorometane (3 mL). The solution was stirred at reflux for 12 h. Solvent was evaporated, and a brown solid was obtained.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.43 (m, 3H), 2.29-2.33 (m, 2H), 2.90-2.95 (m, 2H), 3.42-3.46 (m, 1H), 7.28-7.88 (m, 24H) ppm; ^{13}C NMR: δ = ^{31P} NMR (75 MHz, $CDCl_3$): δ = 61.65 (d), 56.35(d), 52.85(d) ppm;

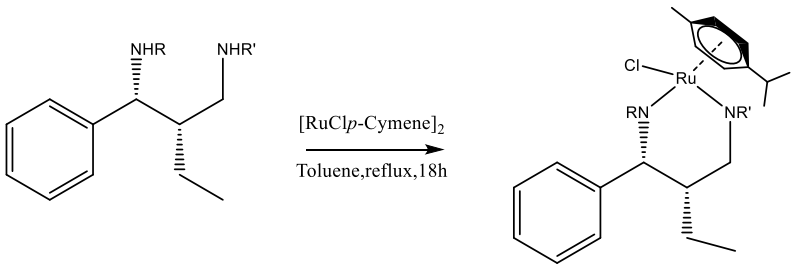
3.4 Synthesis of N-N complexes

3.4.1 General procedure for synthesis of [Ru(*p*-cymene)(diamine)Cl]Cl complexes:



S-9 R = H, R'= Ts
S-14 R = Ts, R' = H

S-9a R = H_2, R'= Ts
S-14a R = Ts, R' = H_2



S,R-20 R = H, R'= Ts
R,S-24 R = Ts, R' = H

S,R-20a R = H_2, R'= Ts
R,S-24a R = Ts, R' = H_2

A 10 mL Schlenk tube was loaded with $[RuCl_2(p\text{-cymene})]_2$ (1 eq.), diamine ligand (2.2 eq.) and charged with distilled toluene (3 mL). The solution was refluxed at 110°C for 18 h. After cooling an orange to red solid was obtained. The precipitate was completely separated from solution by filtration and washed with distilled toluene. The complexes were used in ATH without other purification.

***S*-9a**

^{1}H NMR (300 MHz, DMSO): δ= 1.00 – 1.30 (m, 6H). 1.98 – 2.18 (m, 2H), 2.27 (s, 3H), 2.72 (m,, 3H), 4.21 – 4.45 (m, 2H), 5.28 (m, 2H), 5.69 – 5.87 (m, 2H),), 7.15 (m, 7H), 7.39 – 7.54 (m, 2H),

***S*-14a**

^{1}H NMR (300 MHz, C_6D_6): δ = 0.91-1.22 (m, 6H). 2.06 (s, 3H), 2.22 (s, 3H), 2.80-3.21 (m, 3H), 4.22 (br, 1H), 4.79 -5.30 (m, 4H), 6.70 (d, J=7.2 Hz, 2H), 6.93 -7.38 (m, 5H), 7.93 (d, J=7.2 Hz, 2H),

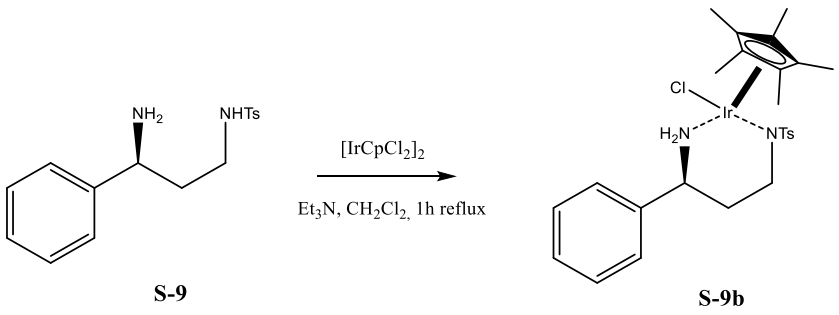
***S,R*-20a**

^{1}H NMR (300 MHz, DMSO): δ = 1.21 (m, 11H), 2.17 (s, 3H), 2.44 – 2.52 (m, 3H), 2.74 - 3.08 (m, 3H), 3.30 (br, 1H), 5.72 – 5.94 (m, 4H), 6.74 -7.51 (m, 9H),

***R,S*-24a**

^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.78 (dt, J = 14.5, 6.9 Hz, 3H). 1.23 – 1.35 (m, 6H), 2.07 – 2.24 (m, 5H), 2.42 (s, 3H), 2.72 -3.12 (m, 3H), 4.92 (dd, J = 15.6, 6.2 Hz, 2H), 5.40 (dd, J = 27.7, 6.0 Hz, 4H), 7.29 – 7.55 (m, 7H), 7.75 (d, J = 8.3 Hz, 2H),

3.4.2 Synthesis of [Ir(Cp)(S-9)Cl] complex (*S*-9b)

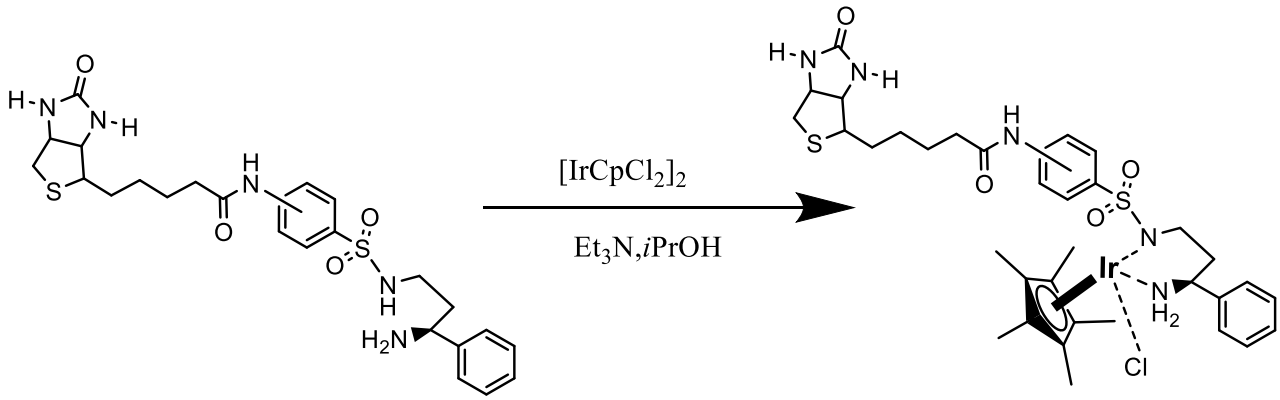


S-9 **S-9b**

In a 10 mL Schlenk tube **S-9** ligand (46mg, 0.16 mmol) and TEA (16.6 mg, 0.165 mmol) were dissolved in dry 5 mL of dichloromethane. Dimer $[\eta^5\text{-}(C_5Me_5)IrCl_2]_2$ (58 mg, 0.073 mmol), was added to the solution, and the mixture was refluxed for 1h. After cooling an orange solid was obtained. The precipitate was completely separated from solution by filtration and washed with cold CH_2Cl_2.

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50- 1.62 (s, 18H). 1.88 (m, 2H), 2.82 (m, 2H), 4.40 (m, 1H), 5.62 (br, 1H), 7.05 – 7.52 (m, 7H), 7.69 (d, J = 8.1 Hz, 1H), 7.99 (d, J = 7.9 Hz, 1H),

3.4.3 General procedure for synthesis of [Ir(Cp*) (diamine) Cl] biotynilated complexes:



***S*- 32a**
***S*- 32b**

***S*- 33a**
***S*- 33b**

n= a *p*ara
n= b *meta*

In a 10 mL Schlenk tube ***S*-32a** or ***S*-32b** ligands and Et_3N (1.1eq) were dissolved in 5 mL of dry and degased isopropanol. The dimer $[\eta^5\text{-}(C_5Me_5)IrCl_2]_2$ (0.5 eq), was added and stirred for 2 hours at room temperature. Then the red solution was refluxed for 1h and during this period the solution became orange. Solvent was evaporated under vacum and an orange/red solid was obtained. This precipitate was washed with water (3x1ml) and dry under vacum

33a

Chemical Formula: $C_{35}H_{46}ClIrN_5O_4S_2$; **MW:** 891.59**; ESI:** m/z 856.4[M-Cl]**; EA (C,H,N):** C, 46.99; H, 5.41; N, 7.83; found C, 43.29; H, 5.55; N, 5.8

33b

Chemical Formula: $C_{35}H_{48}ClIrN_5O_4S_2$; **MW:** 894.59**; ESI:** m/z 856.8[M-Cl]; **EA (C,H,N):** C, 46.99; H, 5.41; N, 7.83; found C, 45.51; H, 5.31; N, 5.38.

^{1}H NMR (300 MHz, DMSO): δ = 1.40-1.82 (m, 21H), 2.10 (s, 2H), 2.35 (m,2H), 2.80 (m, 4H), 3.02 (m, 2H), 4.12 (m, 1H), 4.25 (m,1H), 4.65(m, 1H), 6.40 (d, 2H), 7.21-7.62 (m,9H), 10.4 (d,1H),

3.5 General procedure for asymmetric reduction.

3.5.1 Asymmetric hydrogenation

In a Schlenk tube under nitrogen the substrate was added to the catalyst, followed by 10 ml of solvent (MetOH or iPrOH) and with or without t-BuOK. The solution was stirred for 15 minutes at room temperature and then it was transferred with a cannula in an autoclave. The stainless steel autoclave, equipped with temperature control and magnetic stirrer, was purged 5 times with hydrogen and then pressurized (pH_2 = 20 atm).

3.5.2 Asymmetric transfer hydrogenation using Ru diamine complexes

To a solution of acetophenone (0.5 mmol) in water (2 mL), [Ru(p-cymene) (diamine)Cl]Cl (0.0025 mmol) in 20 µL DMSO and HCOONa as hydrogen donor (5 mmol, 10 equiv) were added. The reaction mixture was stirred at 40°C for 48 h and extracted with ethyl acetate (2x1 mL). The combined organic layers were dried with Na_2SO_4 and analysed by GC.

3.5.3 Asymmetric transfer hydrogenation of imines using Ir diamine complexes:

To a 200 µl of buffer (NaCOOH 3M, MOPS 0.4M, pH=7), substrate and catalyst dissolved in DMSO were added. The reaction mixture was stirred at 40°C for 48 h and extracted with ethyl acetate (2x5 mL). The combined organic layers were dried with Na_2SO_4 and filtered. Solvent was removed under vacum and analysed by HPLC.

3.5.4 Asymmetric transfer hydrogenation using [biot-ligands-SAV]

~3 mg of SAV (final concentration of free binding sites 0.69 mM) was dissolved in a 200µl of buffer (NaCOOH 3M, MOPS 1.2 M) and catalyst (0.9 eq) previously dissolved in degassed DMF was added. The solution was stirred for 20 minutes at defined temperature and imines was added. For the work up 10 µl of NaOH 10M was added and aqueous media was extracted with Et_2O. The organic layers was dried with Na_2SO_4, filtered, removed under vacum and analysed by HPLC.

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