Scuola Normale Superiore di Pisa
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Corso di Perfezionamento in Scienze Chimiche
Ph.D. Thesis

Synthesis and use of bile acid derived organocatalysts

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A mio padre, a mia madre e a mia sorella

“Se non temi Dio, temi i metalli.”
GABRIEL GARCÍA MÁRQUEZ

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Summary

The enantioselective formation of C-C bonds promoted by chiral organic molecules (asymmetric organocatalysts) represents an attractive approach for the synthesis of enantiomerically enriched products. Organocatalysts are usually endowed with inertness and robustness, so that demanding reaction conditions, such as inert atmosphere or absolute solvents, are not required. These features make advantageous the use of organocatalysts instead of organic ligands in transition metal complexes to promote asymmetric transformations. For these reasons, since List and Barbas have reported the use of L-proline as organocatalyst for the direct asymmetric aldol reaction, the last nine years have witnessed an explosive growth of asymmetric organocatalytic methods. (Chapter I)

First, a lot of effort was devoted towards the synthesis of new organocatalysts and, in this context, a great deal of interest was addressed to the development of proline containing systems to be used in the asymmetric formation of C-C bonds via enamine pathway, such as aldol and Michael additions (Chapter I and Chapter V). The interest in the development of this kind of system lies in the consideration that some molecular architectures, where proline is linked, form a chiral cleft that can mimic the active site of the class I Aldolase enzymes, which catalyse enantioselective C-C bond forming reactions by an enamine pathway (Chapter I).

Secondarily, the organocatalytic version of a chemical transformation offers the advantage to be environmental friendly, because no metal

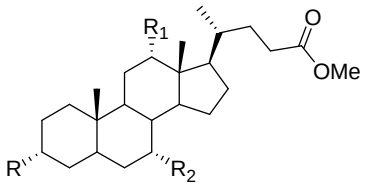
species are involved, and to perform the reaction without the use of inert atmosphere or absolute solvents (Chapter I).

Recently a new type of organocatalyst was proposed, that joins the features of proline and those of a chiral natural product. Among natural products, bile acids look very interesting for this purpose because not only they have been employed successfully by our research group as chiral auxiliaries in various chiral recognition processes, but also because the cholestanic backbone and the appended substituents form a chiral hole which could help the enantioselection (Chapter II).

These considerations have prompted us to propose, in this PhD Thesis, new organocatalytic systems based on proline moiety linked to bile acid scaffold. In fact the longstanding experience in bile acid chemistry of the research group where this Thesis was performed allowed us to develop and use a new concept related to the molecular recognizing properties of the cholestanic backbone (Chapter II): its simple and readily accessible semi-cavity can mimic the enzyme chiral cavity responsible for enantioselection in class I Aldolase; in this particular micro environment the prolinamide moiety can show catalytic activity in a really well defined concentrated organic phase. (Chapter III)

By transforming the hydroxyl groups of the cholic and deoxycholic acids into amino groups and derivatizing them with proline, new asymmetric organocatalysts have been obtained, by simple, selective and low cost synthetic methods. Since the enantiodiscrimination capability of bile acid derivatives depends strongly on the position

where the substituent is located, the three hydroxy-containing positions of cholic and deoxycholic acids were functionalized obtaining the 3,7 and 12 proline derivatives, shown in Figure 1; either L and D proline were introduced to evaluate the best match between the stereochemistry of the bile acid and the absolute configuration of proline for the asymmetric organocatalysis; moreover derivatives with free hydroxyl groups were synthesized to control the position of the substrate in the cavity of organocatalyst (Chapter III).



B1a R=OAc R1=*L*-Pro R2=H
B1b R=OAc R1=*D*-Pro R2=H
B1c R=OH R1=*D*-Pro R2=H
B2a R=*L*-Pro R1=OH R2=H
B2b R=*D*-Pro R1=OH R2=H
B3a R=R2=OAc R1=*D*-Pro
B3b R=R2=OH R1=*D*-Pro
B3c R=R2=OH R1=*L*-Pro
B4a R=R1=OAc R2=*L*-Pro
B4b R=R1=OAc R2=*D*-Pro
B4c R=R1=OH R2=*L*-Pro
B4d R=OH R1=OAc R2=L-Pro

Figure 1: structure of bile acid derived organocatalysts

In order to investigate also the possible cooperative effect of two proline moieties linked to the cholestanic backbone, we propose for the first time 12,7 bisprolinamide cholic derivatives, as reported in Figure 2 (Chapter III).

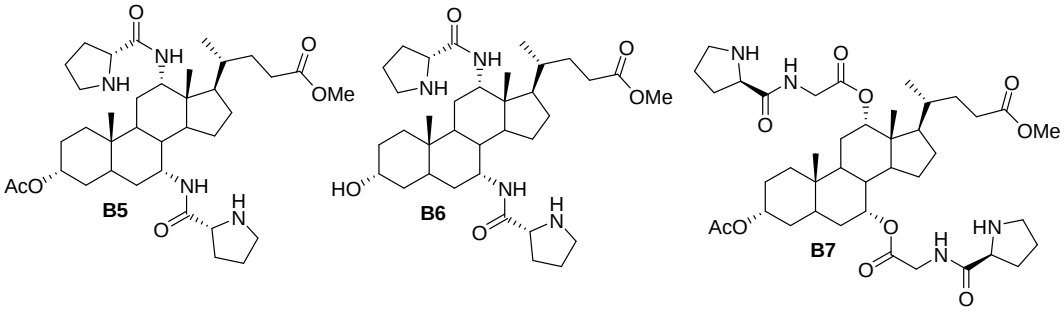


Figure 2: bisprolinamide bile acid derivatives

This new family of organocatalysts was first tested in organocatalytic aldol reaction between acetone and aromatic aldehydes and gave good results in terms of enantioselectivity and yield. In particular the bile

acid derivatives bearing the D-proline moiety at the position 12 of the steroid skeleton (**B1b, B3a, B3b**) were the most active and enantioselective. In addition, the presence of free OH groups at 3- and 7-positions of cholic acid afforded the most efficient organocatalyst, able to promote the asymmetric aldol reaction between acetone and 4-nitrobenzaldehyde even with a very low catalyst loading (2%) with e.e. up to 80% (Chapter IV).

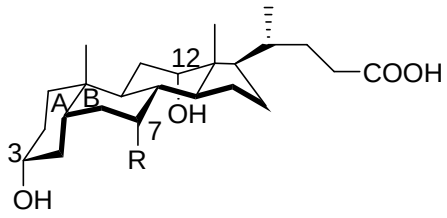
Further studies realized that organocatalyst **B3b** is able to catalyze without the presence of any additive also the aldol reaction between cyclic ketones and electron-poor aromatic aldehydes, affording aldol products in good yield and e.e.s up to 98%, in water as a solvent. Interestingly water made easy the recovery of the product, produced a great increase of reaction rate and, in the case of cyclopentanone, led to the achievement of the opposite diastereoisomer with respect to the use of organic solvent (Chapter IV).

With the idea of extending the scope of this new family of organocatalysts we used our new systems in the organocatalyzed Michael reaction between trans-β-nitrostyrene and cyclohexanone, evidencing a dependence of the behaviour of these systems on the solvent, which lead to the change of the asymmetric induction sense in passing from protic to aprotic solvents. In particular the derivative **B4b** bearing a D-proline in position 7 was very interesting for enantioselectivity in the Michael reaction between ketones and nitroolefins with good conversion in few days and e.e. up to 95% (Chapter V).

The formation of an enamine transition state between **B3b** organocatalyst and cyclopentanone was investigated by computational studies: we chose polarizable continuum model in the case of DCM as solvent and we performed calculations in the presence of explicit molecules in the case of water in order to describe the presence of H-bond. Structural information coming from NMR studies was used to build up the starting geometries for the calculations, which revealed energy differences for the enamine intermediates depending on the solvent. (Chapter VI)

Scope of this Thesis

The central idea of this work was design, synthesis and study of catalytic activity and enantioselectivity of new organocatalysts containing a chiral cavity mimicking the enzymatic pocket of Aldolase I. Following the longstanding interest of our research group in the use of bile acid derivatives in enantioselective processes, attention was addressed to the development of organocatalysts having bile acid structure, where, because of its concave structure, due to the *cis* junction of the A and B cyclohexanic rings (Figure 3), the cholestanic backbone and the appended substituents should form a chiral cleft that can help the enantioselection. In addition, the presence of free hydroxyl groups can constitute a further advantage by controlling, via hydrogen bonds, the position of the substrate in the cavity of organocatalyst.



R= OH cholic acid
R=H deoxycholic acid

Figure 3: cholic and deoxycholic acid structure

In particular in this project were synthesized:

- A wide class of monoprolinamide derivatives of bile acids, in order to find the right match between proline and cholestanic backbone. In particular synthesis of proline derivatives in different position of bile acid could throw light on the influence

of position of proline moiety in the cholestanic emicavity on selectivity of organocatalyst.

- A class of bisprolinamide derivatives of bile acids that could take advantage from the cooperative effect of two proline moieties.

In designing synthesis of these derivatives attention has been paid to the use of low cost, easy and fast procedures in order to improve availability of the new organocatalysts.

Activity and enantioselectivity of this new organocatalysts have been studied with particular attention to:

- Aldol reaction
- Michael reaction

During the activity studies evaluation of parameters that can improve rate of different reaction (temperature, solvents, catalyst loading, reagents…) was considered. The possibility to carry out reactions in water and with very low catalyst loading was checked, in order to evaluate ecosostenibility of the chemical process.

Experimental results were collected and analyzed with the help of conformational and computational studies.

Part of this work is collected in the following articles:

- Puleo, Gian Luigi; Masi, Matteo; Iuliano, Anna. *Synthesis of proline derivatives of bile acids and their evaluation as organocatalysts in the asymmetric direct aldol reaction*. Tetrahedron: Asymmetry **2007**, *18(11)*, 1364-1375

- Puleo, Gian Luigi; Iuliano, Anna. *Methyl 12-[D-prolinoylamino]cholate as a versatile organocatalyst for the asymmetric aldol reaction of cyclic ketones.* Tetrahedron: Asymmetry **2007**, *18(24)*, 2894-2900
- Puleo, Gian Luigi; Iuliano, Anna. *Substrate control by means of the chiral cavity of prolinamide derivatives of cholic acid in the organocatalyzed Michael addition of cyclohexanone to nitroolefins.* Tetrahedron: Asymmetry **2008**, *19(17)*, 2045-2050.

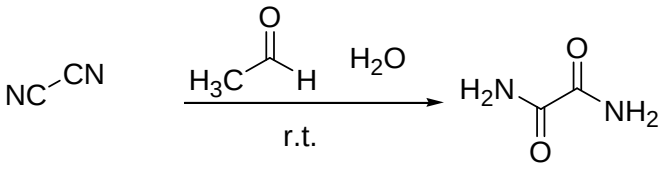
Chapter I

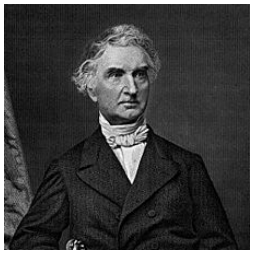
Organocatalysis by proline derivatives

1.1 Introduction

Organocatalysis is the acceleration of chemical reactions with a substoichiometric amount of an organic compound, which does not contain a metal atom.[i]

The word organocatalysis, proposed by David MacMillan[1], derives from "organic catalysis", a concept developed in 1929 by Wolfgang Langenbeck and originally indicating the use and study of a reaction catalyzed by an organic compound.[2a-b] Historically the first example of a completely organocatalyzed reaction was proposed in 1860 by Justus von Liebig,[2c] in his synthesis of oxamide from dicyan and water, using acetaldehyde as catalyst.





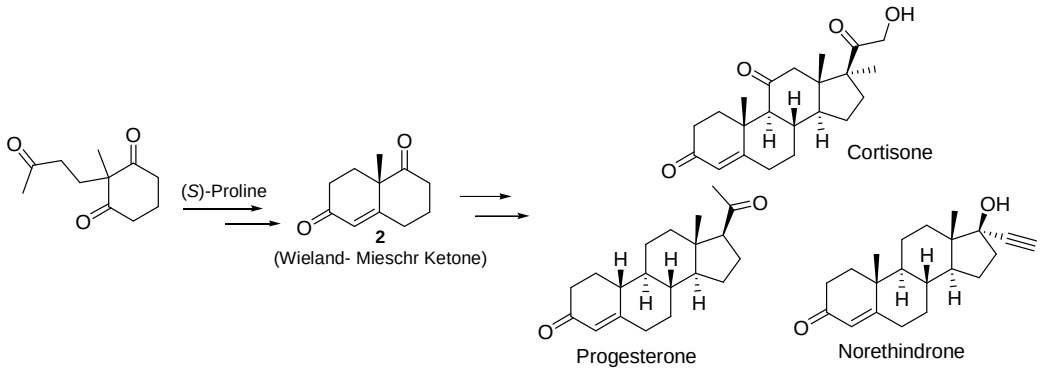
Scheme 1 First organocatalytic reaction and Jutus von Liebig, its father

In 1960s Yasnikov studied the catalytic activity of amino acid in aldol-like condensation and reported kinetic and mechanistic studies on activation of carbonyl group.[3] In early 1970s Hajos and Parrish at Hofmann La Roche proposed the first proline-catalyzed Robinson anulation,[4] and in the same year, Eder, Sauer and Wiechert,[5] with the

[i] In many cases it is difficult to define the boundary between organometallic and purely organic asymmetric catalysis. Organometallic reactions, in which a catalytic amount of an organic ligand participates, are not considered as organocatalytic reactions. The elements that can be contained in "organic" compounds can also be decided arbitrarily, in particular for metalloid elements. For example, according to general consensus, silicon is not considered to be a metal, but boron is. On the other hand, the absence of a metal is not an absolute criterion: Thus, in phase-transfer reactions a metal ion (e.g. Na^+, K^+, Cs^+) may play an indirect role through association with the base. For this reason, in organocatalytic reactions the "absence of metals" is more correctly considered within the context of the postulated "primary" catalytic cycle. For more information about organocatalyst see also P. I. Dalko, L. Moisan, *Angew. Chem.. Int. Ed.* **2004**, *43*, 5138-5175.

mechanistic interpretation offered by Agamina,[6] contributed to develop the Hajos-Parrish-Eder-Sauer-Wiechert protocol for this reaction that was used to synthesize starting materials for industrial synthesis of steroidal derivatives and other polycyclic interesting organic compounds.[5-7]

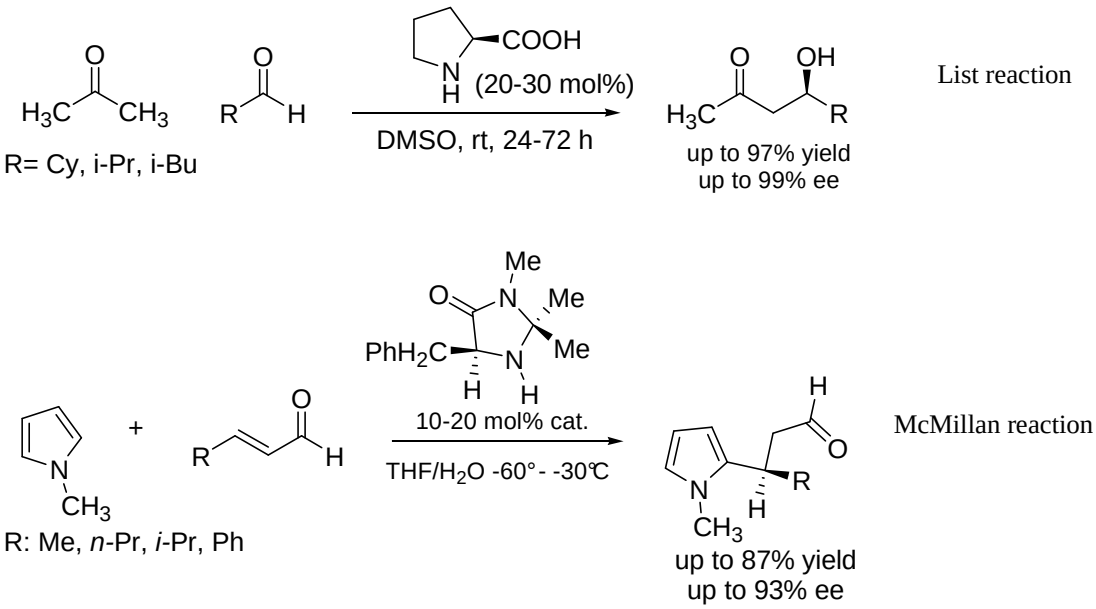
Between 1968 and 1997, there were only a few reports concerning the use of small organic molecules as catalysts for organic reactions, but in these early publications, there was no emphasis on the potential benefits of using organocatalysts or on the demonstration of new organocatalytic concepts.



Scheme 2 Hajos-Parrish-Eder-Sauer-Wiechert reactions

In the late 1990s, however, things began to change when Yian Shi,[8] Scott Denmark[9] and Dan Yang,[10] and their co-workers, demonstrated that enantiomerically pure ketones could be used to catalyse the enantioselective epoxidation of simple alkenes. Shortly afterwards, Eric Jacobsen[11] and Elias J. Corey,[12] and their co-workers, described the first examples of hydrogen-bonding catalysis, in a Strecker reaction, and Scott Miller and his co-workers[13] introduced the use of short peptides for the enantioselective kinetic resolution of alcohols. Although, collectively, these works did not conceptualize organocatalysis as a field of research, they demonstrated for the first

time that small organocatalysts could be used to solve important problems in chemical synthesis. It was not until 2000, however, that the field of organocatalysis was effectively launched, by two papers that appeared almost simultaneously: one from Carlos Barbas, Richard Lerner and Benjamin List,[14] and the other from MacMillan research group.[15]



Scheme 3 List's organocatalytic reaction and McMillan Michael reaction

The explosion of this field makes organocatalysis a good candidate to develop some new methodologies in asymmetric organic synthesis. The broad utility of synthetic chiral molecules as single-enantiomer pharmaceuticals, as electronic and optical devices, as components in polymers with novel properties and probes of biological functions, has made asymmetric catalysis a prominent area of investigation. Until a few years ago, it was generally accepted that transition-metal complexes and enzymes were the two main classes of very efficient asymmetric catalysts.

In comparison with metal asymmetric catalysts, organocatalysts are generally insensitive to oxygen and moisture, so there is no need for special experimental techniques or for ultra-dry reagents and solvents; a wide variety of organic reagents — such as amino acids, carbohydrates and hydroxy acids — are naturally available from biological sources as pure enantiomers. Preparation of organocatalysts is usually cheap and they are accessible in a range of quantities, suitable for small-scale reactions to industrial-scale reactions. Some critics suggest that low turnover numbers might limit the potential uses of organocatalysis for industrial applications, but, in general organocatalysts are cheaper than metal based catalysts and they can be used in larger amounts than metal-based ones at the same price. Moreover, organocatalysts are typically less toxic than metal catalysts, can be tolerated to a large extent in waste streams and are more easily removed, again mitigating the cost of high catalyst loadings.

Organocatalytic methodologies are also good alternatives to enzymatic reactions, working well both in organic and aqueous solutions without denaturation nor product inhibition. Extreme specificity of enzymes lead to the production of a little range of substrates and in a well defined configuration: for such a reason, enzymatic synthetic pathways to obtain two different enantiomers of the same molecules are in general longer than organocatalytic processes; in fact organocatalysts are more versatile in recognizing different substrates and, in a lot of reactions, changing of only one stereocenter in organocatalyst structure leads to complete inversion in

stereochemical outcome of reactions, broadening the scope of this methodology.

1.2 Enamine generalized catalytic cycle[18]

As proposed by List,[19] all organocatalyst mechanisms can be broadly classified as presented in Figure 4.

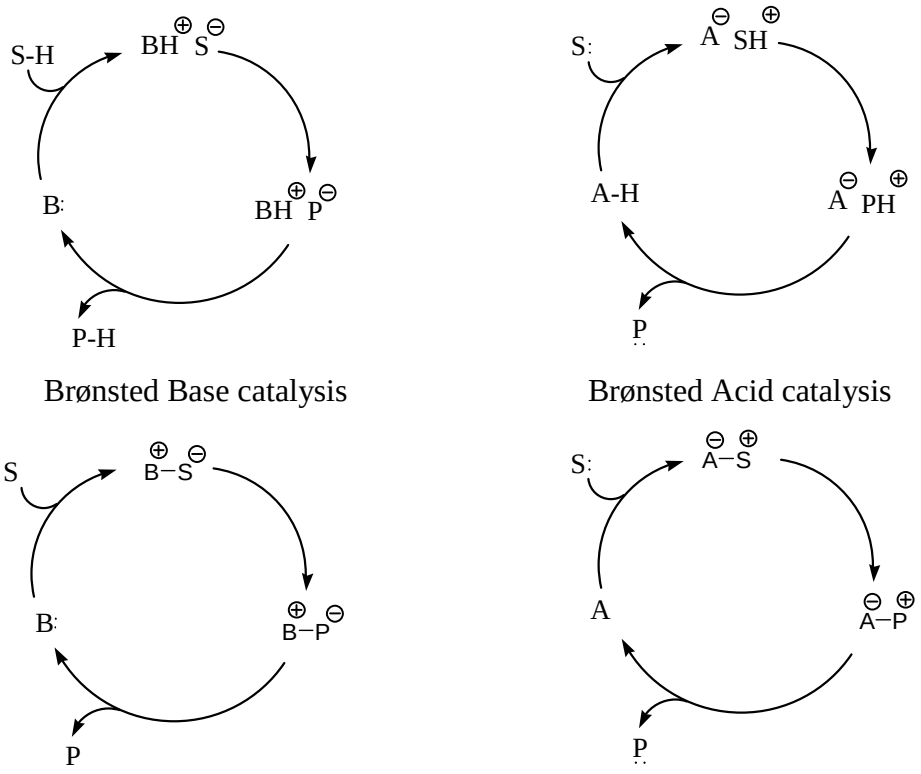


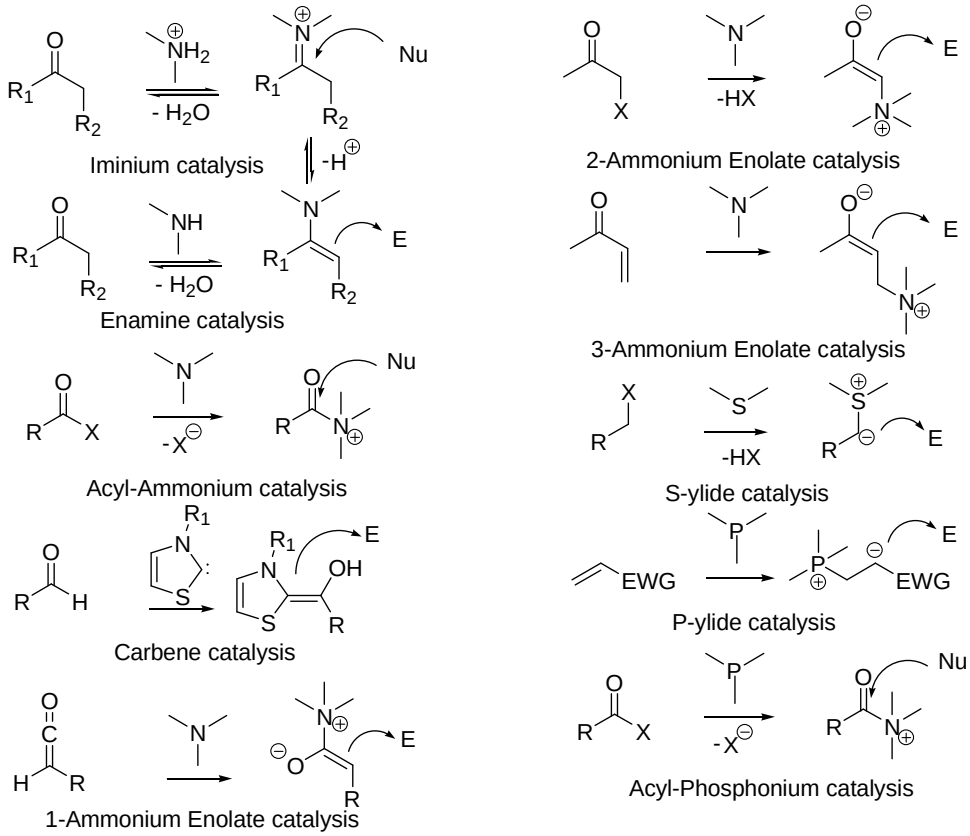
Figure 4: General mechanism of organocatalysis

Accordingly, Brønsted base and acid catalytic cycles are initiated *via* a partial or total deprotonation or protonation of substrate S; we can include in this group both general Brønsted bases, as guanidine and chinchona alkaloids derivatives, and general Brønsted acids, as phosphoric acids, thioureas and diols derivatives.

In the second case, Lewis base catalyst (B:), also known as donor, initiates the catalytic cycle *via* nucleophilic addition to the substrate

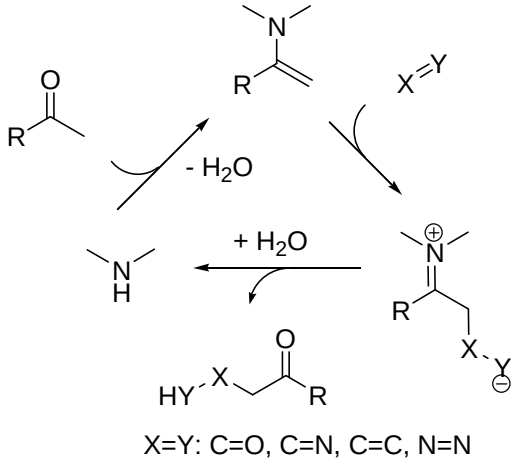
(S), that behaves as acceptor. The resulting complex undergoes reaction and then releases the product (P) and the catalyst for further turnover; in a similar way, Lewis acid catalyst (A), that displays acceptor role, activates nucleophilic substrates (S:), in this case donor, and then the reaction takes place, followed by the release of the product (P:).

While Lewis acid catalysis is used mainly to explain process acceleration in the presence of ammonium salts in biphasic systems, Lewis base catalysis is represented by the great majority of organocatalysts: amines, phospines, sulphides, carbenes, all of them possess an electron doublet to form a new bond with substrate and to active it to the following reaction. The most known Lewis base catalysis patterns are listed in Scheme 4.



Scheme 4: Lewis base catalysis patterns

The vast majority of organocatalytic reactions is represented by amine based reactions,[20] so we will focus on one of the most used concept in organocatalysis, in order to offer a good background for the comprehension of this research work: the enamine generalized catalytic cycle (Scheme 5).



Scheme 5 The enamine catalysis cycle

The donor molecule can be activated through the formation of an enamine, which leads to an increase in the electron density at the reactive center or centers. Chiral secondary amine catalysts can form imonium ions with ketones or aldehydes. These intermediates react by imine–enamine tautomerism or a related mechanism to form a nucleophilic enamine species, which can be trapped conveniently by an activated electrophile, for example, an aldehyde, ketone, or azodicarboxylate.

Normally organocatalysts possess more than two reactive centres that can react in different manner, either by Brønsted acid-base mechanism and by Lewis acid-base mechanism, as reported below. Most organocatalysts used currently are bifunctional, commonly with a Brønsted acid and a Lewis base center.[21] Bifunctional organocatalysts

activate both the donor and the acceptor, thus resulting in a considerable acceleration of the reaction rate.

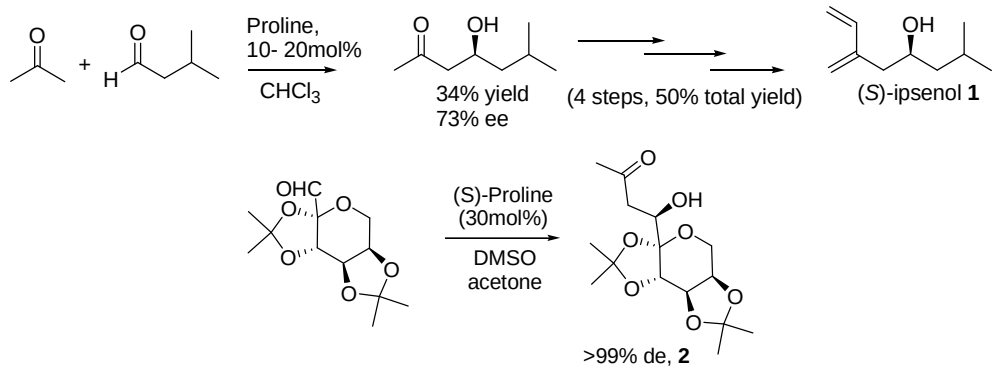
1.3 Structural features of enamine based organocatalysts

Organocatalysts that display a typical enamine generalized catalytic cycle present a huge variety of structures, but the simplest structure proposed for an organocatalyst, derived by the simplification of peptide structure, is aminoacidic. The most used aminoacid as organocatalyst is proline, an abundant chiral molecule that is inexpensive and available in both enantiomeric forms. In addition, there are various chemical reasons that contribute to the proline success in catalysis. Proline is bifunctional, with a carboxylic acid and an amine portion. These two functional groups can both act as acid or base and can also facilitate chemical transformations in concert, similar to enzymatic catalysis. While enzymes typically use several different functional groups in their catalytic machinery, bifunctional asymmetric catalysis has become a very successful strategy in the laboratory.[22]

While all of these criteria apply for all amino acids, proline is a secondary, cyclic, pyrrolidine-based amino acid. A unique consequence of this property is the increased pK_a value of its amino group compared to primary amino groups of other aminoacids. The most important difference with respect to other aminoacids is the effectiveness of proline aminocatalysis – a Lewis-base-type catalysis that facilitates iminium- and enamine-based transformations.[23] The unique nucleophilic reactivity of proline is primarily due to the

pyrrolidine portion, which forms iminium ions and enamines more readily than other amines, including cyclic ones such as piperidine.[24] The carboxylic function further contributes to proline aminocatalysis by acting as general Brønsted acid co-catalyst.

Thanks to List's seminal work, proline was used as organocatalyst in the synthesis of different compounds;[25] very interesting was the use of proline-catalyzed aldol reaction of acetone with α-unbranched aldehydes, starting step in the synthesis of the natural pheromone (S)-ipsenol **1**[26a] or the use of proline-catalyzed intermolecular aldol reaction with acetone in the synthesis of complex sugar derivatives **2**[26b] (Scheme 6).



Scheme 6 Synthetic application of proline organocatalysis

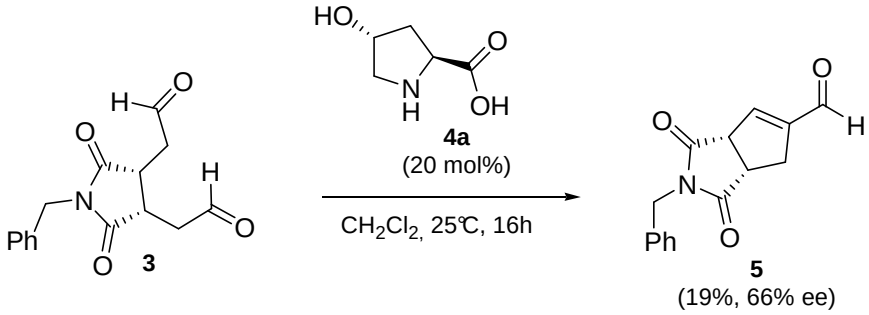
Proline is not the only organic molecule able to promote enamine based reactions, and not all enamine based reactions can be mediated by L-proline. Furthermore, synthetic shortcomings persist; for example, in the dimerization or oligomerization of α-unbranched aldehydes it is difficult to avoid competing reactions. Moreover proline is soluble only in water and aqueous-organic mixtures of solvents (like DMSO/water, DMF/water, THF/water) and this fact can limit the scope of this organocatalyst. Although proline continues to play a central role in aminocatalysis, its supremacy is being

challenged by new synthetic analogues able to overcome some typical proline drawbacks, such as the high catalyst loading (usually more than 20%) or failure of the proline organocatalyzed reactions with some substrates (low yield and selectivity with acetophenone or acetaldehyde).

1.4 Proline derived organocatalysts for aldol reaction[27]

The design of new organocatalysts is devoted to discovery of new functional groups that can enhance the performances of proline, in terms of catalytic activity and selectivity increasing, as well as broadening of application.

A solution to increase the stereoselectivity is to modify the substitution on proline ring, for example by using 4-hydroxyproline. For example, among more than forty organocatalysts tested in the enantioselective desymmetrization of meso-3,4-disubstituted-1,6-dialdehydes **3**, 4-hydroxyproline **4a** emerged as the best catalyst as far as enantioselectivity is concerned. The intramolecular aldol reaction gave the corresponding chiral bicyclic compound **5** in 66% e.e., albeit in low yield.[28] (Scheme 7)

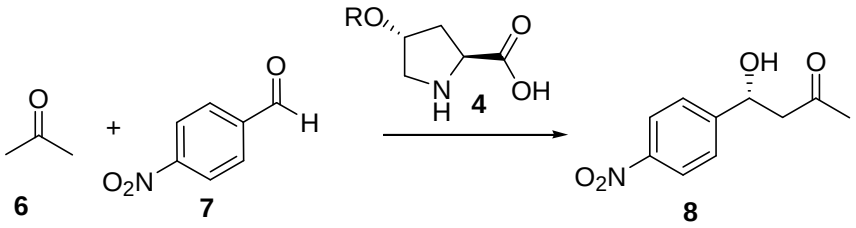


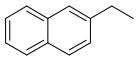
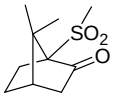
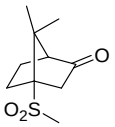
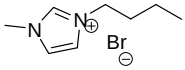
Scheme 7 Intramolecular aldol reaction catalyzed by 4-hydroxyproline **4**

The hydroxylic group of catalyst **4a** was derivatized with different groups, in order to obtain organocatalysts having better efficiency or

displaying recyclable properties. The first example was catalyst **4b**, which has a polyfluorous tail anchored to the hydroxyl group in order to improve solubility of this organocatalyst in different fluorous solvents (entry 1 in Table 1). As a result, the reaction could be performed in a biphasic trifluoromethylbenzene/acetone system affording the expected product **8** with similar results to those obtained using proline in DMSO. Decreasing the amount of catalyst **4b** to 7 mol % led to an important detrimental effect not only on the yield, but also on the enantioselectivity.[29]

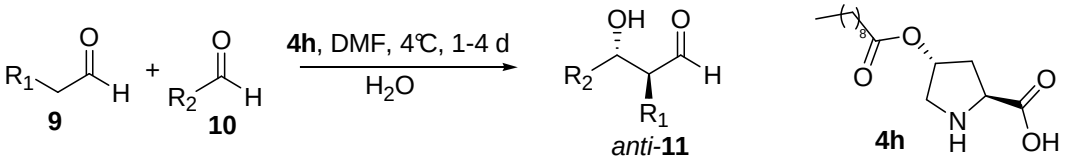
Table 1: Aldol reaction catalyzed by derivatives of 4-hydroxyproline



Entry	R	Catalyst	Reaction Conditions	Yield (%)	Ee (%)
1	$C_8F_{17}(CH_2)_2$	**4b**	Ref 29	72	73
2	Ph	**4c**	Ref 30	75	76
3	Ph	**4c**	Ref 30	81	75
4		**4d**	Ref 31	41	86
5		**4e**	Ref 32	60	81
6		**4f**	Ref 32	71	90
7		**4g**	Ref 33	94	82

Catalyst **4c** allowed the reaction to be carried out either in classical organic solvents or in ionic liquid.[30] The reaction using acetone as both source of nucleophile and organic solvent gave the expected

product **8** with similar results to those obtained using an ionic liquid (entries 2 and 3 in Table 1). However, the catalyst can be reused at least four-times with decrease of yield, under the latter reaction conditions. Similar yields and enantioselectivities were found when other highly electrophilic aromatic aldehydes were used, while results were accountably lower when benzaldehyde or p-methylbenzaldehyde were used as the electrophilic partners of the aldol reaction. In order to increase the solubility of the catalyst in common solvents, compound **4d** was prepared. However, the reaction had to be carried out at low temperature in order to improve the previous enantioselectivities, with the normal cost of decreasing the chemical yield (Table 1, entry 4).[31] The preparation of very hindered catalysts **4e** and **4f** allowed to reach very good levels of enantioselectivity (entries 5 and 6 in Table 1), with the camphorsulfonyl derivative **4f** giving better results even when using half amount of catalyst.[32] The introduction of an ionic liquid motif at the hydroxyl group in catalyst **4g** allowed to perform the reaction under ionic liquid phase conditions with very good results (entry 7 in Table 1). Although the enantioselectivity was constant after a six-fold recycling process, the chemical yield suffered a little decrease.[33] In addition to acetone **6**, other aliphatic ketones have been used as a source of nucleophile in the intermolecular aldol reaction.



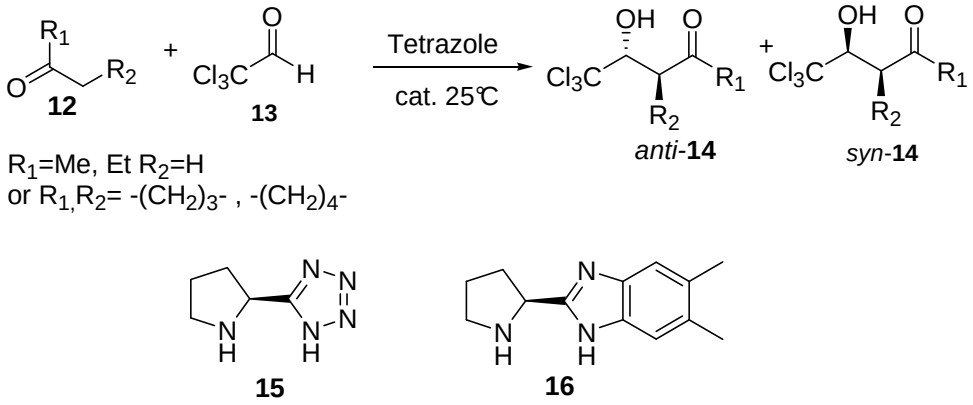
R_1=Me, nBu, iPr, CH_2Ph
R_2=Et, iBu, Ph, iPr

Scheme 8 Cross aldol reaction catalyzed by **4h**

Catalyst **4h** (10 mol %) emerged from another related ester derivative set as the best catalyst for the intermolecular aldol reaction using enolizable aldehydes **9** (5 equiv) as source of the nucleophile and water (18 equiv) as the additive (Scheme 8). The main product was the isomer *anti*-**11**, which was isolated after *in situ* reduction to the corresponding 1,3-diol (29–97% yield, 60–90% d.e., 77–99% e.e.). The length of the alkyl chain of the catalyst seemed to play an important role, with longer or shorter chains giving lower results; in fact this organocatalyst generates emulsion between water and DMF, allowing the reaction to take place in an emulsion environment that simulates slow addition conditions.[34]

In some proline derivatives, carboxylic group was substituted by another functional group with similar pK_a in the reaction medium. There are a lot of approaches in the design of these compounds and we want to discuss the best known in order to introduce our synthetic design choice.

Tetrazoles and carboxylic acids have similar aqueous pK_a values. However, tetrazole has a lower pK_a value in DMSO (8.2) than in acetic acid (12.3).[35] Moreover, tetrazoles show higher solubility, lipophilicity, and metabolic stability than the analogous carboxylic acids, being frequently used as their bioisosteres.



Scheme 9 : Aldol reaction catalyzed by tetrazole derivative

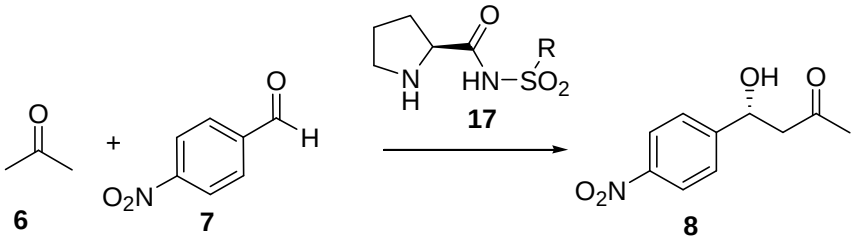
The reaction between different ketones **12** (2 equiv) and chloral monohydrate **13** (Scheme 9) has been catalyzed by tetrazole **15** (5 mol %) in acetonitrile, affording the expected products **14** with high yields (35–88%) and enantioselectivities (36– 97%).[36]

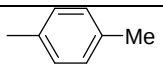
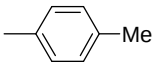
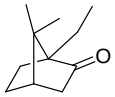
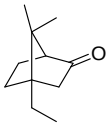
As usual, when the reaction was performed using cyclopentanone as the source of the nucleophile, isomer *syn*-**14** was the main product (80% d.e.), whereas using cyclohexanone, the main product was *anti*-**14** (92% d.e.). Other alkyl methyl ketones, as well as aldehydes (trifluoroacetaldehyde monohydrate or aqueous formaldehyde) could be used successfully, with the reaction always taking place at the methylene position of the ketone. Finally, it should be pointed out that the reaction can be also performed with aryl methyl ketones, this being the only example presented in the literature where aryl ketones have been used. Catalyst **15** (20 mol %) has shown its activity in the reaction between acetone **6** (34 equiv) and several aromatic or aliphatic aldehydes in DMSO/acetone mixture (4:1, v:v), achieving products **8** with good yields (65–82%) and enantioselectivities (63–99%) in very short reaction times (10 min–13 h). The high solubility of catalyst **15** permitted its use in other solvents, as well as in the

presence of 10 mol % of water without affecting the aforementioned results.[37] The related heterocyclic compound **16** (20 mol %), in conjunction with trifluoroacetic acid (20 mol %), has been used as a catalyst in the classical intermolecular aldol reaction giving good results.[38] For example, and only for comparison with other catalysts, the reaction between stoichiometric amounts of acetone **6** and p-nitrobenzaldehyde **7** in THF at -5°C gave the expected product **8** with 67% yield and 82% e.e..

The conversion of the carboxylic moiety of (*S*)-proline into the corresponding sulfonamide derivative would provide a catalytic system having, in general, similar acidity, but where the acidic, steric, and electronic properties could be finely tuned, just by a simple change of the sulfonyl moiety.

Table 2: Aldol reaction catalyzed by derivatives of sulfonimide



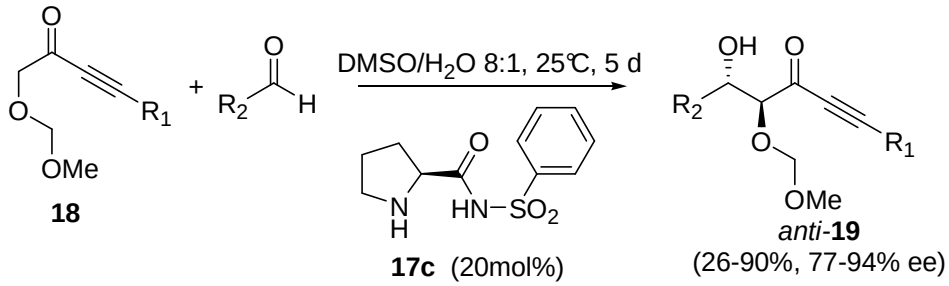
Entry	R	Catalyst	Reaction Conditions	Yield (%)	ee (%)
1		**17 a**	Ref 39	92	98
2		**17a**	Ref 40	96	84
3	Me	**17b**	Ref 41	78	79
4	Ph	**17c**	Ref 41	49	84
5		**17d**	Ref 42	47	63
6		**17e**	Ref 42	78	60

The synthesis of catalysts **17** was easily accomplished by coupling the corresponding aryl- or alkylsulfonamide with proline. The first example was imide **17a**, which gave excellent results in the intermolecular aldol reaction of acetone, performed in polar aprotic solvents such as DMSO or THF.[39] The use of protic solvents, such as methanol, led to a diminution of the yields and enantioselectivities.

Catalyst **17a** gave better results in the preparation of compound **8** (Table 2, entry 1), than simple (*S*)-proline, with these results being attributed to a better shielding of one of the two possible enantiotopic faces of the aldehyde by the aryl ring. Similar yields but slightly decreased enantioselectivities were obtained when the reaction was carried out using an ionic liquid as solvent (entry 2 in Table 2).[40]

Attempts to recycle catalyst **17a** under the aforementioned conditions failed, as a decrease in the yield and enantioselectivity was observed in the successive reaction cycles. These results were explained by a possible leaching of the catalyst during product extraction. Worse results were obtained when catalysts **17b** and **17c** were used (entries 3 and 4 in Table 2), performing the reaction in methylenechloride. Under these conditions different acyclic, as well as cyclic, ketones could be used as the source of the nucleophile, giving the expected products **8** with, in general, modest results (42–88% yield, 28–38% d.e., and 23–94% e.e.).[41] Attempts to improve the aforementioned results by using diastereomeric camphorsulfonamide derivatives **17d** and **17e** also failed (entries 5 and 6 in Table 2), both catalysts giving lower enantioselectivities independent of the diastereoisomer used.[42]

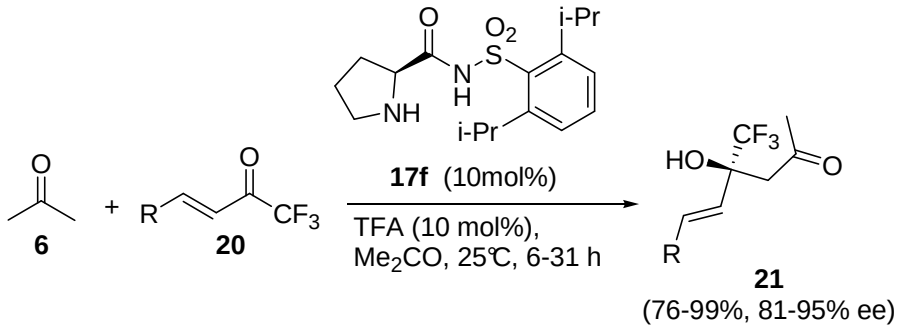
Catalyst **17c** has been surprisingly and successfully used in the aldol reaction using ynones **18** as the source of the nucleophile giving *anti*-**19** as the main product with good diastereoselectivities (50–90%) and enantioselectivities (Scheme 10), with the best results being obtained for the less bulky ynone (R_1=Me in **18**).[43]



Scheme 10: Aldol reaction between ynones and aldheydes catalyzed by sulfonimide

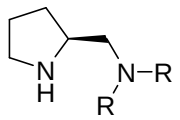
Compounds **19** are very unstable and are transformed into the corresponding 3-oxotetrahydrofuranone derivatives by the addition of an alkoxy moiety at the α-position of the triple carbon–carbon bond, catalyzed by phosphine compounds.

The very bulky sulfinimide **17f** is able to catalyse the intermolecular aldol reaction using highly electrophilic ketones, such as compounds **20** (Scheme 11). The addition of trifluoroacetic acid (10 mol %) was crucial in order to obtain products **21** with good results. The absolute configuration of the final aldol was determined on the basis of crystallographic data. Finally, it should be pointed out that other different methyl alkyl ketones could be used with similar results, the reaction always taking place at the methyl group.[44]



Scheme 11 Aldol reaction between ketones **20** and acetone catalyzed by very sterically hindered sulfonimide

Shortly after (*S*)-proline was reported as a suitable catalyst for the intermolecular aldol reaction, prolindiamine derivatives **22** were tested in the same type of transformation; pK_a of ammonium species present in reaction medium can be quite similar to that of carboxylic acid and, moreover, amine itself can activate the acceptor by Brønsted acid catalysis. Several diamines derived from proline in combination with protic acids were screened in the aldol reaction between acetone **6** and aldehydes **7**.[45]



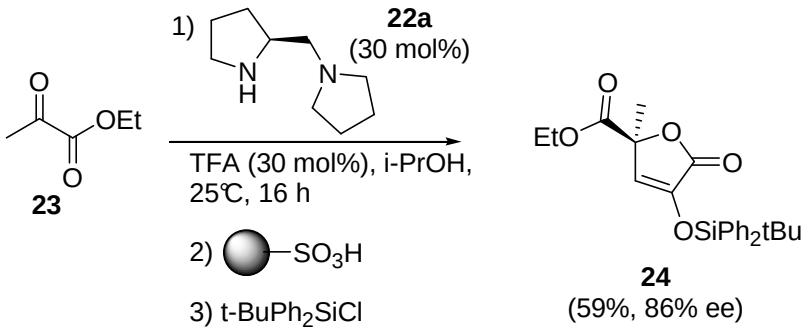
22a R-R=$(CH_2)_4$
22b R=$(CH_2)_9CH_3$

Among the catalysts tested, prolinamines bearing a tertiary amine group gave better results, with the reaction rate decreasing as the above moiety become bulkier. The catalyst **22a** (3 mol %) showed an excellent catalytic efficiency in the reaction of acetone (source of nucleophile and solvent) with aldehydes in the presence of carboxylic acids: for instance, using trifluoroacetic acid (3 mol %), the aldol compound **8** could be obtained after 2 h at 30°C in 51% yield and 82% e.e., but together with the corresponding α,β-unsaturated compound. In order to minimize the formation of this byproduct, the amount of carboxylic acid was reduced, although the decrease of the by-reaction

was marginal. The reaction has been also expanded to other ketones, such as cyclic ketones and 3-pentanone, which gives the main diastereoisomer *anti*-**8** with very low enantioselectivities (81–97% yield, 84–96% d.e., and 8–48% e.e.). The hydrophobic catalyst **22b** (10 mol %) in combination with trifluoroacetic acid (10 mol %) has been used in the intermolecular aldol reaction between ketones (2 equiv) and aromatic aldehydes in water as solvent.

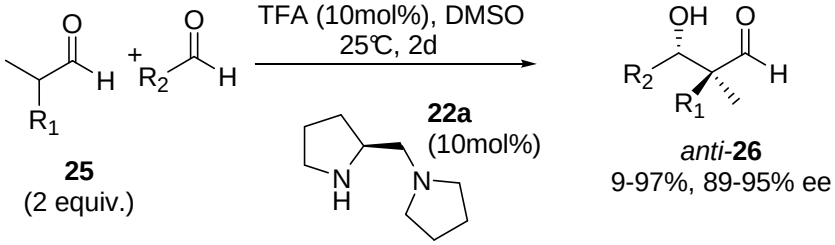
The expected products **8** were obtained with good yields (46–99%), when highly electrophilic aldehydes were used, and very low to good diastereoselectivities (8–82%) and enantioselectivities (22–99%). The presence of the carboxylic acid was of vital importance, since the reaction in the absence of trifluoroacetic acid gave the product as a racemic mixture.[46]

Ethyl pyruvate **23** has been used as both the source of the nucleophile and electrophile in the aldol reaction promoted by catalyst **22a** (Scheme 12). Initially, the reaction gave a complicated mixture of different products; however, the use of polymer-supported sulfonic acid Amberlist 15, in order to eliminate the catalyst, and final treatment of reaction mixture with a silylating agent, permitted the isolation of isotetronic acid derivative **24**.[47]



Scheme 12 Condensation of ethyl-pyruvate catalyzed by **22a**

Catalyst **22a** in combination with trifluoroacetic acid has permitted the intermolecular aldol reaction between α-methylaldehydes **25** (source of the nucleophile) and aromatic aldehydes in DMSO at 25°C (Scheme 13), affording *anti*-**26** as the main diastereoisomer, although with moderate diastereoselectivity (24–70% d.e.).[48]



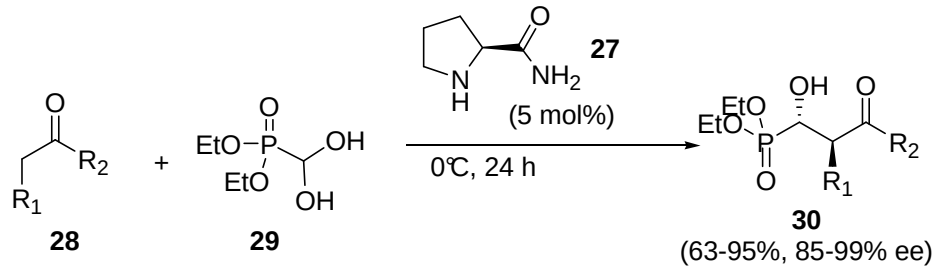
Scheme 13: Cross aldol reaction between α-methylaldehydes and aromatic aldeydes catalyzed by **22a**

On the contrary, the enantioselectivities were homogeneous, and independent of the size of the R_1 group and on the electronic nature of the substituent on the aromatic ring of the electrophilic aldehyde, the chemical yields depended strongly on the last factor. As a matter of fact, compound **26** was obtained in low chemical yields with aldehydes bearing electron-donating groups.

1.5 Prolinamide derived organocatalysts in aldol reaction[27]

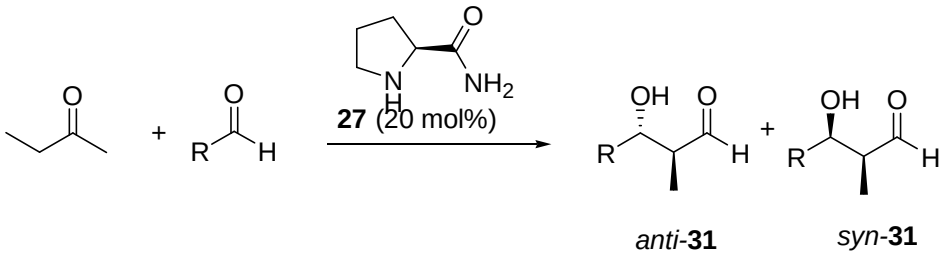
The most commonly used family of proline derivatives are prolinamides. Some general facts make these derivatives useful. First, their easy preparation starting from (*S*)-proline. Second, the robust amide linkage provides very stable compounds, which in some cases can be recovered and reused without detrimental effects. Finally, the hydrogen of NH moiety is acidic enough to activate electrophiles by hydrogen bonding, as in a general Brønsted acid catalysis. Although the simple prolinamide **27** failed in catalyzing the intermolecular aldol

reaction between two carbonyl compounds,[49] this organocatalyst has shown its efficiency in the intermolecular aldol reaction between ketones **28** and diethyl formylphosphonate **29**, affording the expected secondary α-hydroxyphosphonates **30** (Scheme 14).[50] The best results were found when the ketone was used as both the source of the nucleophile and solvent, with moderate to good diastereoselectivities (30–90%).



Schema 14: Aldol reaction between α-hydroxyphosphonates and ketones catalyzed by prolinamide **27**

The auto-aldol dimerization reaction between aldehydes (Scheme 15) has been reported very recently. The reaction of neat propionaldehyde catalyzed by (*S*)-prolinamide **27** (20 mol %) in the presence of 20 equiv of water gave the expected product *syn*-/*anti*-**31** as a 1.3:1 diastereoisomeric mixture. The enantiomeric excess of both diols obtained after reduction with $NaBH_4$ was practically identical (78% and 74% e.e. for *anti*- and *syn*-**31**, respectively).[51]

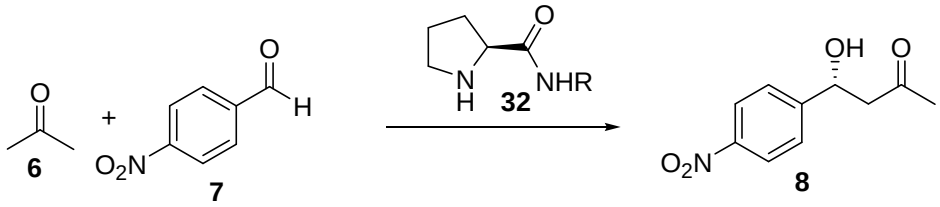


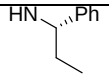
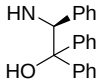
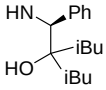
Scheme 15: auto-aldol dimerization reaction between aldehydes

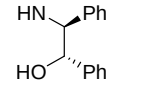
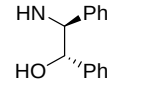
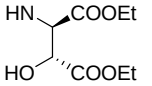
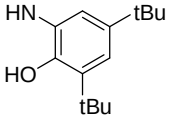
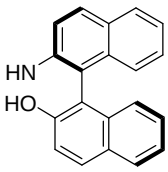
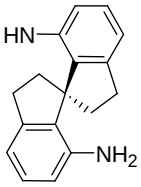
The use of amides derived from proline and chiral amines has allowed the intermolecular aldol reaction to be performed successfully. The

comparison of different systems can be made on the basis of the data reported in Table 3. The reaction between p-nitrobenzaldehyde **7** and acetone **6** to give the corresponding aldol product **8** can be carried out in the presence of simple N-alkyl prolinamide derivatives. However, the best results were obtained using the corresponding hydrobromide **32a** in the presence of water (Table 3, entry 1). In addition, the use of the diastereomeric amide derived from (*R*)-1-phenyl-1-propylamine showed higher reactivity but lower enantioselectivity. When the reaction was carried out using different aromatic aldehydes, the best results were found for those possessing electron-withdrawing groups, with the ortho-substituted aromatic aldehydes giving higher enantioselectivities than the related para-substituted ones.[52] The use of prolinamides derived from 1,2-aminoalcohols has been more successful for this purpose. Thus, prolinamides **32b** and **32c** (entries 2 and 3 in Table 3, respectively) showed good performances in this reaction, although the reaction must be performed at -40°

Table 3: Intermolecular aldol reaction catalyzed by prolinamide **32**



Entry	NHR	Reaction conditions	Yield(%)	Ee(%)
1		H_2O/Me_2CO (1:1, v:v), **32a** (20 mol %)[a], **6** (10 equiv), 25 °C, 8 h	83	46
2		Me_2CO, **32b** (5 mol %), **6** (13.1 equiv), -40°C, 1 d	70	99
3		Me_2CO, **32c** (10 mol %), **6** (13.1 equiv), -40°C, 2 d	78	85

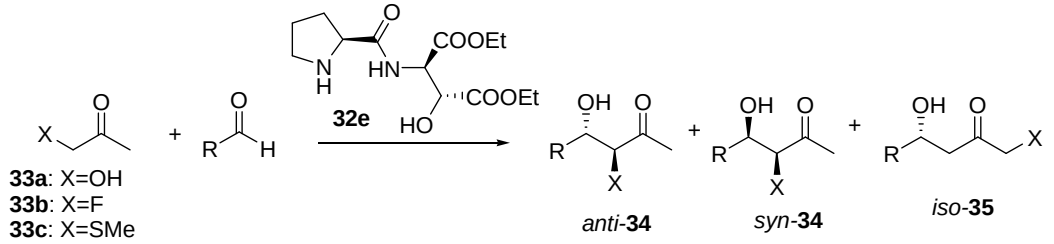
4		Me_2CO, **32d** (20 mol %), **6** (27.2 equiv), -25°C, 2 d	66	93
5		[bmim]BF_4, **32d** (20 mol %), **6** (27.2 equiv), 0°C, 1 d	82	94
6		Me_2CO, **32e** (2 mol %), **6** (27.2 equiv), -25°C, 1 d	62	99
7		Me_2CO, **32f** (20 mol %), **6** (27.2 equiv), 25°C, 3 d	16	68
8		hexane, **32g** (5 mol %), **6** (3 equiv), 25°C, 10 h	99	70
9		Me_2CO, **32h** (1 mol %), **6** (27.2 equiv), -25°C, 5 h	82	55

[a] Catalyst used as hydrobromide derivative.

The replacement of diphenyl or di-isobutyl moieties by other less hindered alkyl groups or by hydrogen led to a dramatic decrease of the enantioselectivities: this effect can be attributed to higher conformational homogeneity, higher hydrogen bonding ability and higher solubility of compounds **32b** and **32c**. In addition, the use of the corresponding diastereomeric amides gave worse results.[53a] Both catalysts **32b** and **32c** have shown their efficiency in the reaction between ketones and aromatic aldehydes when the reaction is performed in brine at -5°C, affording the corresponding aldol products with even better results.[53b] Other 1,2-aminoalcohols bearing two stereogenic centers have been used in the preparation of the corresponding amides **32**. The use of the hindered amide derived from (*S*,*S*)-1,2-diphenyl-2-aminoethanol **32d** gave excellent

enantioselectivities (entry 4 in Table 3).[54] The use of other electrophilic aldehydes besides **7** afforded different results depending on the nature of the aldehyde: good yields and enantioselectivities were obtained with aromatic aldehydes (48–93% and 81–93% e.e.) and modest yields and excellent enantioselectivities using aliphatic aldehydes (12–77% and 86–99% e.e.).[54a] When alkyl methyl ketones, such as butanone, were used, the reaction took place mainly at the methyl group giving the corresponding *iso*-regioisomer derivative with moderate yields and high enantiomeric excesses.[54b] Better results were obtained when an ionic liquid ([bmin]BF_4) was used as the reaction medium (Table 3, entry 5), permitting the twice catalyst recycling, without losing of the initial activity and enantioselectivity.[54c] The replacement of the phenyl groups of the above organocatalyst with electron-withdrawing groups, such as ethoxycarbonyl, led to a new organocatalyst **32e** showing stronger acidity, and therefore forming stronger hydrogen bonds.[55] This catalyst gave slightly better results than the previous one (compare entries 4 and 6 in Table 3), with homogenous yields and enantioselectivities for the reaction between acetone and aromatic aldehydes, increasing the enantiomeric excess until to only one enantiomer in the case of α-branched aldehydes. For methyl alkyl ketones, such as butanone, the reaction using **32e** mainly gave the *iso*-regioisomer derivative (43– 62% yield) with excellent enantiomeric excess (98–99%), together with a minor amount of *anti* isomer (21–42%, 98% d.e., and 98–99% e.e.). The results were also excellent for cyclic ketones, although the diastereomeric excess depended on the

ring size (90% de for the *anti*-product with cyclohexanone and 0% de for cyclopentanone).[55a] The high activity of this catalyst has permitted the use of other less reactive ketones as a source of the nucleophile. In the reaction of α-hydroxyacetone **33a** (15 equiv) in THF/H_2O (2:1 v:v) at -15°C (Scheme 16), only the regioisomer *iso*-**35** was isolated with good yields, with similar results being found for the related α-fluoroacetone (X = F in **33b**). In contrast, when the reaction was performed only in THF, the main product for the reaction of α-fluoroacetone was the isomer *anti*-**34** (X = F) obtained in 89–96% yield, 33–60% d.e. and 94–98% e.e..[55b] The use of α-(methylsulfanyl)acetone (X = MeS in **33c**) drove the reaction to give only the regioisomer *iso*-**35** up to 99% e.e..[55c]

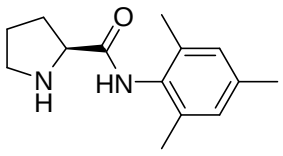


Scheme 16: Aldol reaction of α-branched ketones with different aldehydes catalyzed by prolinamide **32e.**

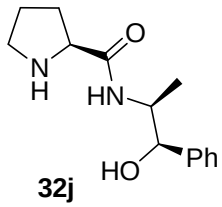
Although the change of the hydroxy group of the alcohol with a more acidic phenol derivative seems to be inefficient (entry 7 in Table 3), the results obtained in water, when a large excess of cyclohexanone (11.7 equiv) was used as the source of the nucleophile, were very good, affording aldol products with high yields and selectivities.[56] The NOBIN-prolinamide derivative **32g** has been shown to be an active catalyst for the aldol reaction under unusual conditions, such as the use of hexane as reaction medium or the use of only 3 equiv of ketone (entry 8 in Table 3).[57] The important role of phenolic OH was

demonstrated by the very scarce results obtained when the analogous methyl ether derivative was used as catalyst. Catalyst **32g** has been used in combination with trifluoroacetic acid in pure water in the aldol reaction between cyclic ketones (only 2 equiv used) and aromatic aldehydes, affording good results (53–99% yield, 40– 98% d.e., 62–97% e.e.).[58] The use of prolinamide derivatives bearing a stereogenic axis has been further explored with the spiro compound **32h**, which showed so high activity that a reduction of the amount of catalyst to only 1 mol % (entry 9 in Table 3) was possible, but afforded modest asymmetric inductions.[59]

Other prolinamides have been used as catalyst in the direct intermolecular aldol reaction. Thus, the simple amide **32i** was used in the reaction between α-chloroacetone and aromatic aldehydes, affording mainly *anti* product (18–57% yield, 66–94% d.e., 91–98% e.e.) with a minor amount of *iso*-product (X = Cl).[60]



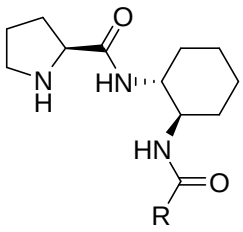
32i



32j

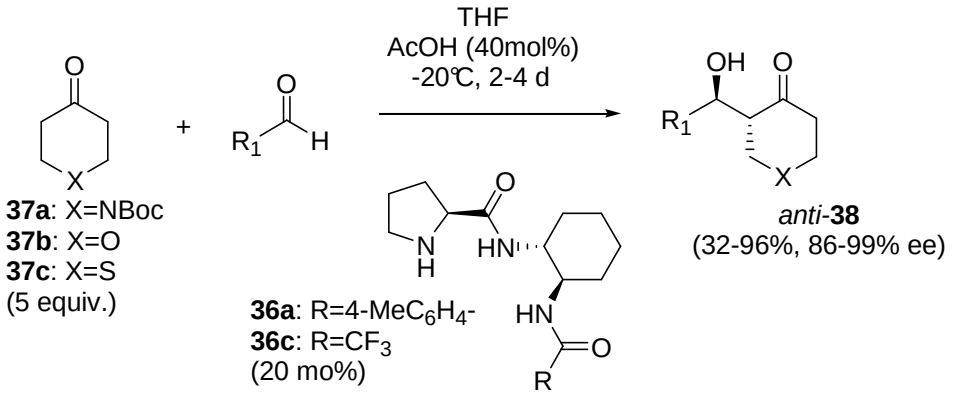
The norephedrine derivative **32j** has also been used as a catalyst. The reaction of different aldehydes in neat acetone at -40°C gave modest results (22–67% yield and 60– 80% e.e.).[61] Prolinamides derived from chiral diamines have been synthesized and employed as catalysts in the intermolecular aldol reaction. The results achieved with bisprolinamides are generally superior to those obtained with other diamides. The first example of these types of compounds was diamide **36a**, which bears only one unit of proline and is used in

substoichiometric amounts (20 mol %) in the aldol reaction between cyclohexanone (19.2 equiv) and different aromatic aldehydes. For this catalyst, the use of 20 mol % of acetic acid was beneficial in order to enhance its catalytic activity.[62] Better results were obtained with bisprolinamide catalyst **36b**, affording aldol *anti* products with high diastereo- (60–98%) and enantioselectivities (77–97%).[63]



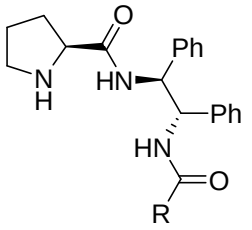
36a: RCO=4-MeC_6H_4CO
36b: RCO= (*S*)-proline

It has been hypothesized that the NH group of diamide plays an important role in the stabilization of the transition state, activating the electrophile. Therefore, the change of the R group in **36** could have an important effect on the selectivity of the reaction, since the acidity is altered. For instance, catalyst **36c**, which has a lower pK_a, has been used in the reaction of N-Boc-4-piperidone **37a** with different aromatic and heteroaromatic aldehydes giving mainly the isomer *anti*-**38** with diastereoselectivity higher than 90% (Scheme 17). However, catalyst **36a** gave better results in the reaction of tetrahydro-4H-pyran-4-one **37b**.[63] Catalyst **36a** has shown its superiority in the aldol reaction between tetrahydro-4H-thiopyran-4-one **37c**, affording the product *anti*-**38** in 37–99% yield, 78–99% d.e. and 90–99% e.e..[63]



Schema 17: Aldol reaction with cyclic ketones as nucleophiles, catalyzed by prolinamide **36a,c**

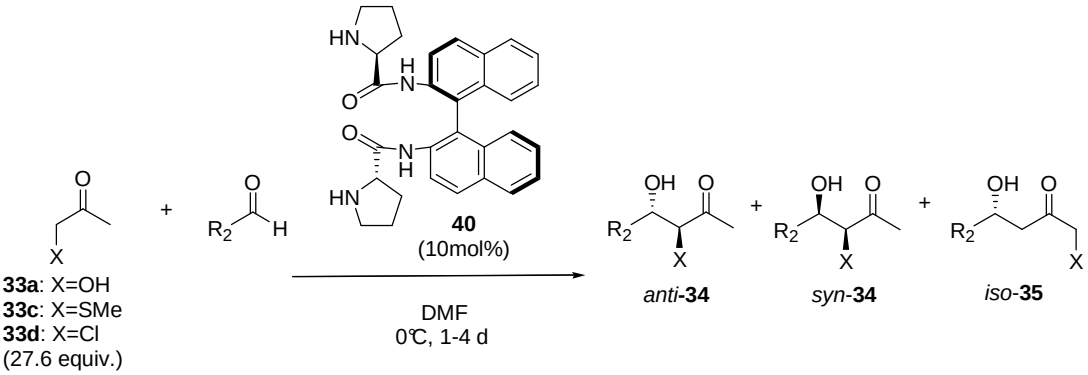
The presence of two units of proline in derivatives **39** increases the catalytic activity; In fact, the reaction between acetone **6** (27.2 equiv) and p-nitrobenzaldehyde **7** at -35°C catalyzed by bisprolinamide **39b** (10 mol %) gave the expected compound **8** with an excellent result (75% yield, 98% e.e.) in only 5 h, with this result being not quite dependent upon the nature of the electrophilic aldehyde (aromatic or aliphatic) used. Conversely, the same reaction catalyzed by amide **39c** gave worse results.[64]



39a: RCO=4-MeC$_6$H$_4$CO
39b: RCO= (*S*)-Proline
39c: RCO= MeCO

The bisprolinamide derived from 1,1'-binaphthyl-2,2'-dimine (BINAM) **40** has attracted a great deal of attention as a possible catalyst for the intermolecular aldol reaction. The matched combination was constituted by (*S*)-proline and (*Sa*)-BINAM units. Different reaction conditions were proposed for aldol reaction

catalyzed by this system: the mixture of 1,4-dioxane/ketone (4:1, v:v) at 4°C, affording the corresponding aldols with yields ranging from 9% to 79% and enantiomeric excess from 50% to 88%;[65] the mixture DMF/water (1:1, v:v) at 0°C or DMF at 25°C, giving the corresponding products with slightly better results (52–99% yield, 78–95% e.e.) and permitting the recovery of catalyst **40** just by an aqueous acidic extraction and its reuse three times without detrimental effect on yields and enantioselectivities;[66] the mixture $CHCl_3$:ketone (1:1, v:v) at -27°C, which provided worse results than the previously reported ones.[67] Catalyst **40** has been used in the reaction between α-substituted-acetones **33** and aldehydes (Scheme 18) to mainly give regioisomer **34**, with small amounts of corresponding *iso*-**35**. The diastereoselectivity depended upon the nature of the X group, giving always compound *anti*-**34** as the main product, with the enantioselectivity reaching values up to 99%.[68] It should be pointed out that simple α-hydroxyacetone **33a** can be also used as a source of nucleophile, but in this case the best reaction conditions were DMSO at 25°C, affording *anti*-**34** with 85% e.e..



33a: X=OH
33c: X=SMe
33d: X=Cl
(27.6 equiv.)

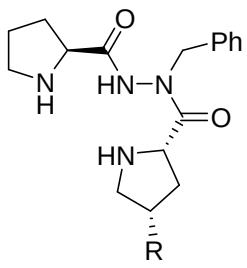
Schema 18: Aldol reaction between α-substituted ketones and aromatic aldehydes catalyzed by **40**

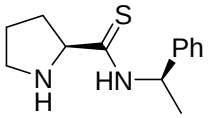
The addition of substoichiometric amounts of carboxylic acids[69-70] permitted the enhancement of the reaction rate, in particular benzoic acid (20 mol%) reduced the reaction time from 3 days to 1.5 h, while maintaining the enantioselectivity of **8**. This fact permitted a decrease in the reaction temperature from 25°C to -20°C, increasing the corresponding enantioselectivity (86–99%).[70] The amount of ketone could be reduced to 3 equiv, just by using water and a micellar agent, stearic acid (20 mol %) as a co-catalyst at 2°C.[71] Under these conditions, aldol adducts (61–99% yield, 58–93% e.e.) were obtained in 12 h. The combination of catalyst **40** (10 mol %) and benzoic acid (20 mol %) in either DMF or pure water permitted the use of less reactive ketones as the initial source of nucleophile. The reaction of α-(methylsulfanyl)acetone **33c** (X = MeS) with p-nitrobenzaldehyde **7** could be performed, giving *iso*-**35** as the main product in either DMF/water or pure water with 93% yield.[72]

The reaction of chloroacetone **33d** (X = Cl) with aromatic aldehydes catalyzed by amide **40** (10 mol %) and benzoic acid (20 mol %) gave the isomer *anti*-**34** as the main product (27–96% yield, 50–98% d.e., and 40–97% e.e.), which could be easily converted into the corresponding chiral (*3R,4S*)-*trans*-epoxide, by treatment with triethylamine, with excellent enantioselectivities.[73]

Other catalytic systems that can be included into this group are hydrazide derivatives and thioamides. The additional nitrogen atom at the hydrazide derivatives **41** provides a new hydrogen-bonding site, improving the activity of these catalysts compared to simple prolinamides **32**. The reaction between cyclohexanone (27.2 equiv)

with different aldehydes in toluene at 0°C using catalyst **41a** (20 mol %) and trifluoroacetic acid (20 mol %) gave the expected aldol product with good enantioselectivities (for comparison with Table 3, compound **8** was obtained in 7 h, 95% yield and 96% e.e.). Worse results were obtained with aromatic aldehydes bearing electron-donating groups.[74]



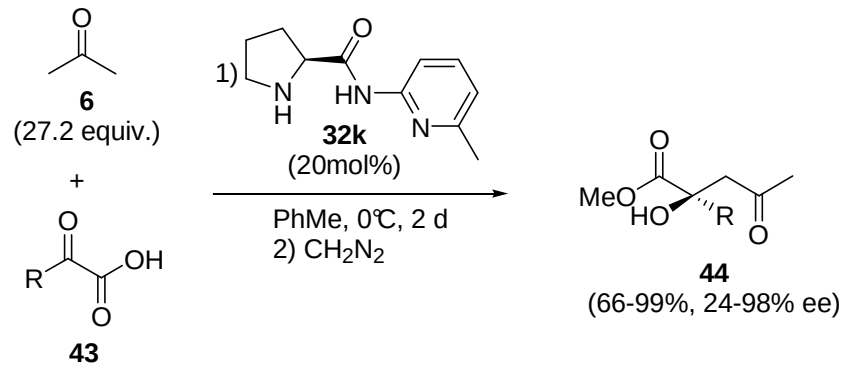


42

41a: R=H
41b: R=OH

The use of hydroxyl derivative **41b** did not change the aforementioned results.[75] The conversion of the amide into the corresponding thioamide derivative increases the acidity of the NH hydrogen and therefore would form a stronger hydrogen bond, improving its catalytic activity. With this idea in mind, catalyst **42** was prepared and tested in the intermolecular aldol reaction.[76] Thus, the standard reaction between acetone (27.2 equiv) and p-nitrobenzaldehyde **7** at 4°C catalyzed by thioamide **42** (20 mol %) in the presence of trifluoroacetic acid (20 mol %) gave the expected compound **8** in 81% yield and 94% e.e.. Although this result seems to confirm that thioamides could perform the reaction more selectively than the related amides (compare with entry 1 in Table 3) and other results using aromatic aldehydes bearing strong electron-withdrawing groups are in keeping with the previous one, the reaction with less reactive aldehydes gave lower enantioselectivities. Other thioamides assayed

gave lower or similar results, as well as other acidic catalysts. For instance, the use of stronger acids than trifluoroacetic acid led to the deactivation of catalyst **42**, whereas the use of acids with similar pK_a such as trifluoro-, difluoro-, or dichloroacetic acid gave similar results.[77] Thus, the aldol reaction between cyclic ketones and aromatic aldehydes catalyzed by thioamide **42** (10 mol %) and dichloroacetic acid (10 mol %) could be performed in brine as the reaction medium and using only 1.2–3 equiv of ketone as the source of the nucleophile. Moderate to good results (32–97% yield, 20–90% d.e., and 68–98% e.e.) were obtained in the formation of the corresponding *anti* products.[78] As presented above, the use of ketones as a electrophilic partners of the aldol reaction is a more challenging task.



Scheme 19: Aldol reaction between acetone and β-ketocarboxylic acid **43** catalyzed by **32k**

However, it has also been accomplished by using the amide catalyst **32k** (Scheme 19). In order to obtain good results, the ketone partner should have a carboxylic acid moiety and the catalyst, a pyridine ring. The use of either ester derivative of **44** or non-heteroaromatic ring catalyst had a strongly detrimental effect on the yields and enantioselectivities. This has been attributed to the presence of a strong interaction between the hydrogen from the carboxylic moiety and the basic nitrogen atom of pyridine ring, which help the approach

of reagents to each other in enantioselective way.[79] The reaction is not only restricted to acetone but other ketones, such as cyclopentanone, could be used with a slightly lower enantioselectivity. Finally, it should be pointed out that catalyst **32k** can be recovered and reused threefold just by aqueous acidic–basic extraction.

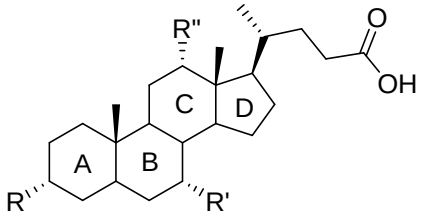
Chapter II

Bile acids as scaffold in organic chemistry

2.1 General features of bile acids.

In the field of chiral recognition, natural substances represent a class of very interesting compounds, because they possess different functional groups and multiple stereogenic centers. Among these, compounds with steroidal structures have attracted a great deal of attention. In particular bile acids and their derivatives have found applications in different cases: as a matter of fact, a lot of application of bile acids or their derivatives as molecular tweezers,[80,81] chiral inclusion complexes,[82-85] gelling agents,[86-88] HPLC stationary phases[89] and also as chiral auxiliars in asymmetric synthesis[90-91], are found in the literature.

Their success is due to their commercial availability, low cost and very easy functionalization without use of inert or dry atmosphere. The bile acid structure is formed by a (cyclopentane)perhydrophenanthrenic backbone with an aliphatic tail, containing 24 C atoms, named cholanic acid.



45 cholanic acid R=R'=R"=H
46 cholic acid R=R'=R"=OH
47 deoxycolic acid R=R"=OH, R'=OH

trans-5α-cholanic acids

cis-5β-cholanic acids

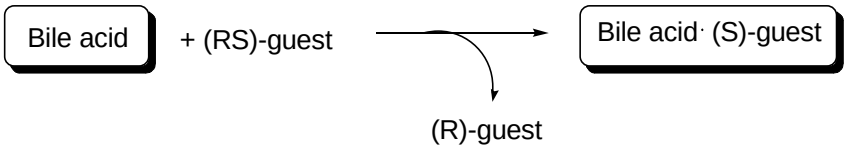
The family of cholestanic derivatives is obtained from **45**, and it is possible to evidence the presence of two isomeric structures, depending on the junction between A and B rings, *cis* for the acids 5β cholanic and *trans* for the 5α ones.[92]

Probably the best known compound of this natural product family is the 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid, also named cholic acid, a primary bile acid, directly derived by hydroxylation of cholanic acid. Hydroxy groups of this molecule, even if all secondary, could be derivatizated selectively,[93] because of high asymmetry of this structure that does not possess two equal positions on the stereoidal backbone. Hydroxy groups at positions 7 and 12 are axial, and the one at position 3 is equatorial, and then more reactive. This fact is true for a lot of reactions, such as acetylation, hydrogenation, following the reactivity order 3-OH>7-OH>12-OH,[93] while in the case of oxidation with NBS position 7 is the most reactive, following the order 7-OH>12-OH>3-OH.

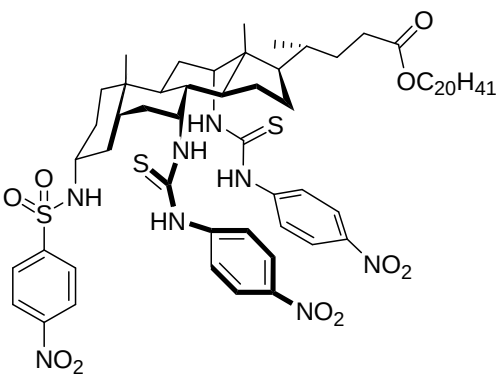
Also, the acid group in position 24 is particularly useful in enantiorecognition chromatographic processes, for the anchorage of this structure to a support (polymer[94a], silica gel[94b-d],…), in inclusion processes, for the host recovery by acid-base treatment[94e], in spectroscopic analysis, for the linkage of fluorophores[94f] sensitive to complexation of bile acid with different analytes, and in medicinal chemistry for the linkage of pharmacophores[94g].

2.2 Bile acid derivatives as scaffolds in recognition processes

The bile acids, with their polyfunctional and rigid structures, are one of the best sources of host molecules in host-guest recognition processes. The concavity of the bile acid backbone transforms these compounds into good systems for incorporation of different substrates by interactions with functional groups present in the steroid structure.



Their curved amphiphilic architecture is generally suitable for binding polar species, and especially so for binding anions.[95] With this idea in mind, Davis and co-workers[96a] proposed in 1999 cholapodal structure **48**, which binds chloride anion in chloroform with the exceptional K_a of 10^{11} M^{-1}, the best result among those obtained with synthetic chloride receptors; further studies developed analogous systems as anionophores through lipids membranes, which attracted interest in medicinal chemistry as a valid substitute of natural anion transporter;[96b-e] in particular, among the new steroids investigated, bis-4-nitrophenylthiourea **49** showed unprecedented activity, giving measurable transport through membrane with a transporter/lipid ratio of 1:250000 (an average of <2 transporter molecules per vesicle)[97].



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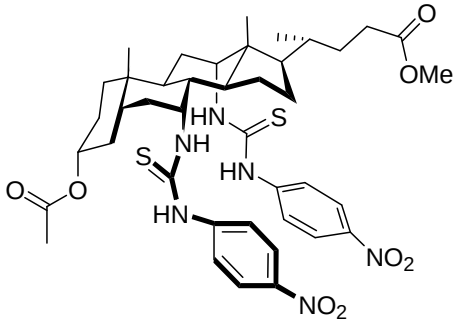

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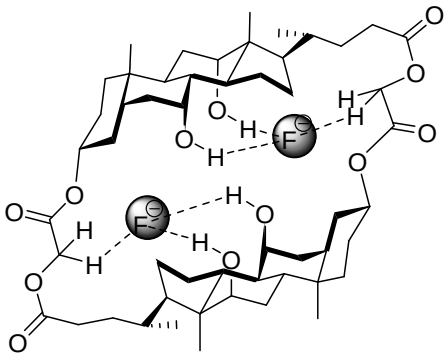
A different structural solution for binding anions was proposed by Maitra et al.[98] with the synthesis of cholaphane **50**, a head-to-tail dimer of cholic acid which can bind two fluoride anions in chloroform solution with a K_1 of 10^3 M^{-1} and a K_2 of 10^{-1} M^{-1}, estimated by NMR titration; NMR studies of chemical shift in glycolate bridge in

difluoride complex showed indeed that in this complex an interaction is present between CH_2 and F^- by hydrogen bonding.

In 2008 Davis et al.[99] transformed cholic acid into cyclotrimeric toroidal amphiphile **51** with inward-directed ammonium substituents that display an influence on the transport of chloride through bilayer membrane.



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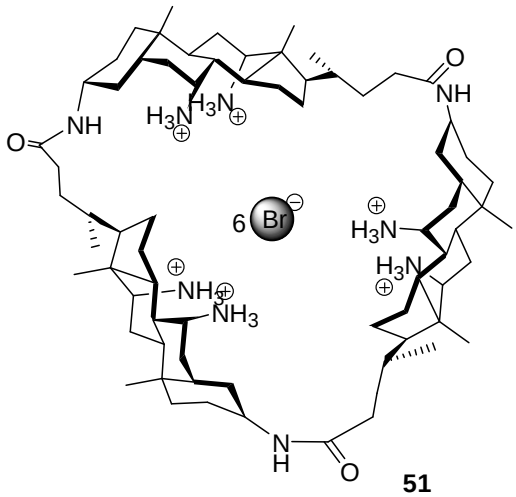
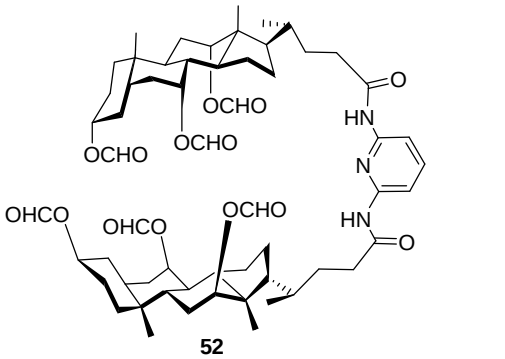


51

Particular structural features of bile acids are used also in recognition of organic molecules, like nucleotides, flavines or adenosine nucleotides. For example Pandey[100] proposed derivatives **52** and **53** which can recognize uracil[100a] and flavines,[100b] with a particular hydrogen bond network, and, more recently, Chan and co-workers[101] proposed cyclic derivatives **54** that can be used as chemosensors for ATP, displaying a selectivity for this molecule of 33-124 times higher than for other nucleotides. As evidenced by fluorescence binding studies, **55** is a highly sensitive probe; as little as 30 nM ATP can cause 15% fluorescence quenching of the sensor.



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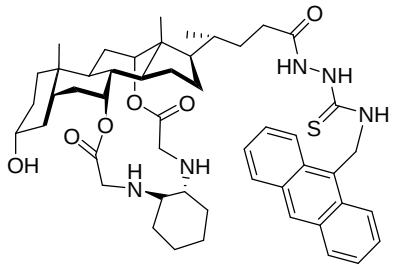

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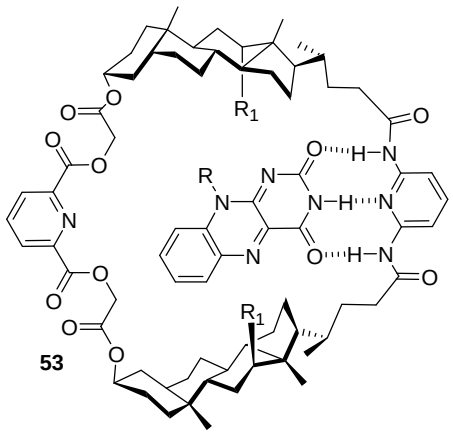


53

54a R_1=H
54b R_1=OH

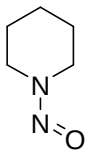
R=Hexyl, Octyl

2.3 Bile acids as scaffolds in chiral discrimination processes

Moreover the bile acid cavity is a chiral cavity and can be used successfully in host-guest chiral recognition processes.[102-103]

It is possible to resolve racemic mixtures by this technique, with great advantage on quantitative recovery both of the host molecule and the guest molecule under very mild conditions.[104,105] There are numerous procedures that we could classify into absorbing methods,[106] i.e. host and guest simply are left together for a determinate period, and crystallization method,[107] i.e. host is dissolved in the guest and recrystallized from this. The very high enantiodiscriminating ability is well revealed in different applications of these systems, among the other the resolution of lactones,[108] cyclic carbonates,[109] alcohols,[107] sulphoxides, amines,[110] epoxides, cyclic amides.[111]

Polonski and co-workers[112] illustrated the efficacy of cholic acids in chiral discriminations of low molecular weight N-nitrosamines.



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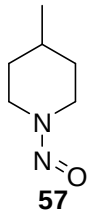

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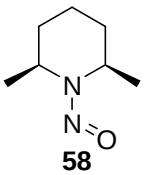


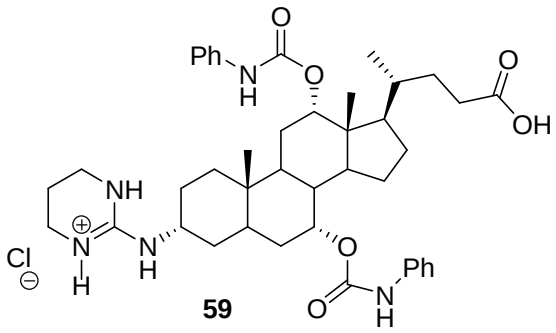
58

The chirality of N-nitroso pyperidines is only due to the hindered rotation around the N-N bond, because of its partially double bond character. Co-crystallization of cholic acid with these molecules leaded to crystals containing an inclusion complex between the two molecules. Crystal analysis by solid state CD and X-ray studies suggested that these complexes are made by selective incorporations of one of the two enantiomers: in the case of **56** and **57** there is the formation of a host guest crystal with only one enantiomer, while, in the case of **58**, authors report a preferential complexation of *E* with respect to *Z* (geometric enantiomerism) form of N-nitroso pyperidines.

The papers of Fantin and Pedrini[113] reported some examples concerning the resolution of cyclic and bicyclic ketones. In particular the authors obtained by chiral resolution the enantiomerically pure form of bicyclo[3.2.0]-ept-2-en-6-one, a very important starting material for the synthesis of prostaglandins,[114,115] very difficult to obtain in high enantiomeric excess by normal resolution procedures.

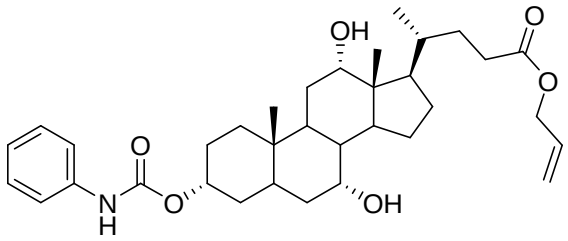
The inclusion in the cholic acid afforded the enantiomer (-)-(*1S,5R*) with an e.e. of 65%; in order to obtain an increase of this excess the procedures was repeated twice and 95% e.e. was reached. By using deoxycholic acid, the opposite enantiomer was obtained: this fact suggested that the discrimination is completely dependent on the host structure and little structural variation could be of fundamental importance.

For solution-phase separation a series of molecules derived from cholic acid was synthesized and studied: in particular **59**, obtained by introducing a guanidinic function at position 3 and two phenylcarbamate groups at positions 7 and 12, was revealed to be very efficient in the enantioselective extraction in organic solvent of different amino acids.[116]



59

A suitable application of chiral recognition by bile acid derivatives is offered by chiral HPLC: in fact, the possibility of opportune derivatizations on cholestanic backbone and the presence of a carboxylic function, useful to connect these molecules to silica gel, transforms these natural substances into very good candidates for the development of new chiral stationary phases (CSP) very useful in the resolution of racemic mixtures.

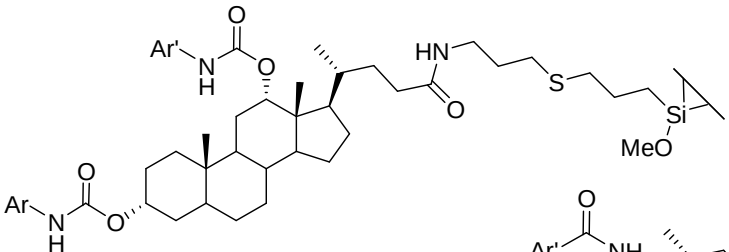


60

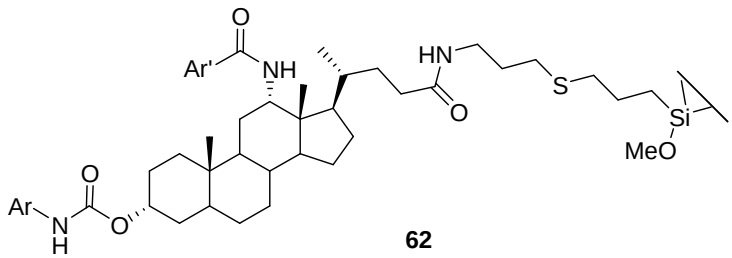
The first use of cholic acid in this field concerned the synthesis of derivative **60**, which was anchored on silica gel by hydrosilylation of the double bond, followed by transesterification between the silane obtained and hydroxyl groups on silica surface. The use of this

compound as CSP allowed the resolution of 3,5-dinitrobenzoyl amine and amino acid derivatives, of the 2,2'-dihydro-1,1'-binaphtyl and heterocyclic prescursors of aminoacids.

Starting from bile acids and using the different reactivity of the hydroxyl groups, new biselective CSP were developed that, by the simultaneous presence of π-acid and π-basic functionalities, were able to resolve a huge variety of racemic mixtures.[117] Particularly interesting were CSP **61** obtained by introducing selectively arylcarbamate groups with different electronic characteristics, by reacting deoxycholic acid with 3,5-dimethylphenylisocyanate, the electron rich moiety, and 3,5-dichlorophenylisocyanate, the electron poor moiety. The spatial disposition of the two hydroxy groups on cholestanic system indeed proved very favourable to build up these new FSC: in fact the two hydroxyl groups are 6.5 Å apart and they are almost parallel, so interacting independently with the substrates. Some examples of these new CSPs are reported below and present homo and hetero derivatizations.[118]



61

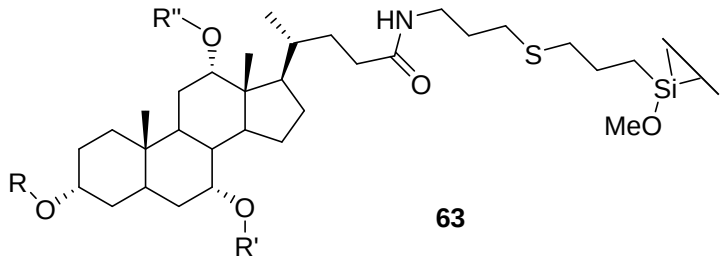


62

Substitution of arylcarbamate group with arylamide group improved enantiodiscrimination in some cases but generated less versatile

systems, as reported for CSP **62** in the chiral resolution of benzodiazepines.[119]

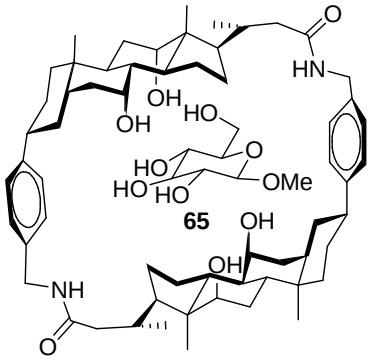
Recently, in order to improve the enantiodiscrimination by increasing the number of interactions, the attention was captured by cholic acid, that presents one other hydroxyl group at position 7. The stationary phases **63,** which possesses all the hydroxyl groups functionalizated[120] or only one free hydroxyl group,[121] showed good selectivity in the resolution of binaphtols or alkylarylcarbynols.[120]



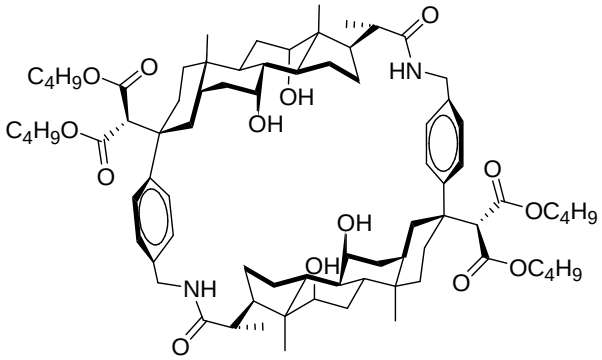
63

R, R', R'' = OH, arylcarbamate

The chiral cavity of a cholaphane was used by Davis and co-workers[122] as chiral recognizer for sugars, as reported for derivatives **64a-b**; in fact, as previously demonstrated in early works by the same authors,[123] the three dimensional arrangement of polar functionalities in cholaphane is particularly suitable for the recognition of carbohydrate nuclei in non polar media, and ^{1}H NMR studies confirmed the capability of the bile acid derivative **64b** to transfer methyl β-D-glucoside **65** from aqueous to organic medium while no transfer was observed in the absence of this receptor.



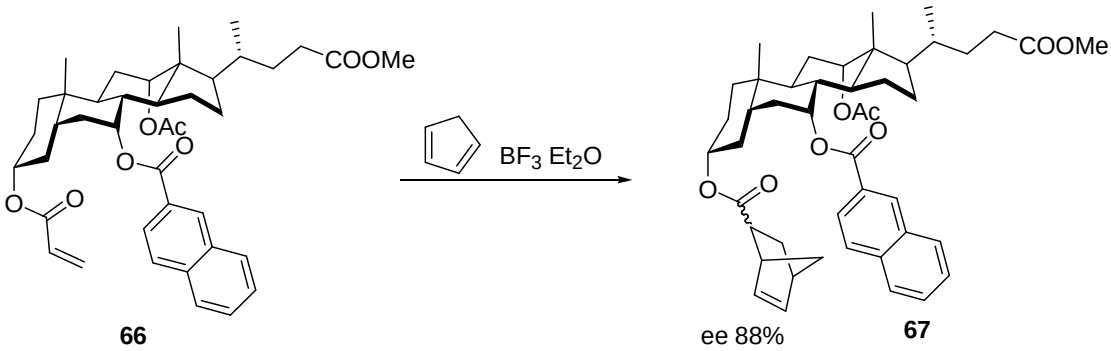
64a



64b

2.4 Cholestanic backbone as chiral structure for enantioselective reactions

The stereodefined structure of the cholestanic backbone suggested its use as chiral auxiliary in organic reactions. Maitra and co-workers,[124] by using the reactivity of the hydroxyl groups in cholic acid, synthesized compound **66** that was used as chiral auxiliary in asymmetric Diels-Alder reaction (Scheme 20).

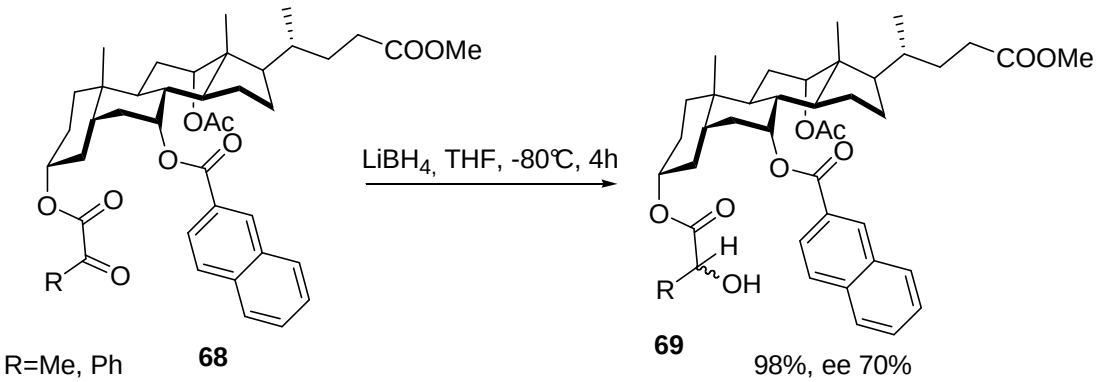


66 ee 88% 67

Scheme 20: Asymmetric Diels-Alder stereocontrolled by auxiliary **66**

The dienophile was bound at position C3, while at position C7 was bound the naphthoate group, in order to cover one of the two faces for the attack of the substrate, and then to increase the enantioselectivity of the reaction (e.e. up to 88%). This steric effect is possible thanks to the *cis* junction between A and B rings that allows the two substituents to be faced. The same chiral auxiliary was used in the asymmetric

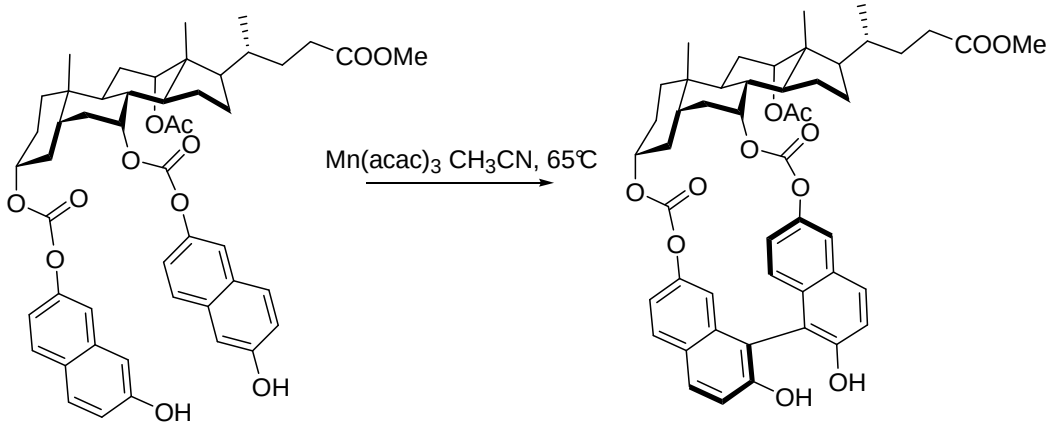
reduction of α-ketoesters to the corresponding α-hydroxyesters.[125] Also in this case the naphthoate group, by shielding one of two faces of α-ketoesters, allowed the attack of $LiBH_4$ only on one side of carbonylic group (Scheme 21).



R=Me, Ph **68** **69** 98%, ee 70%

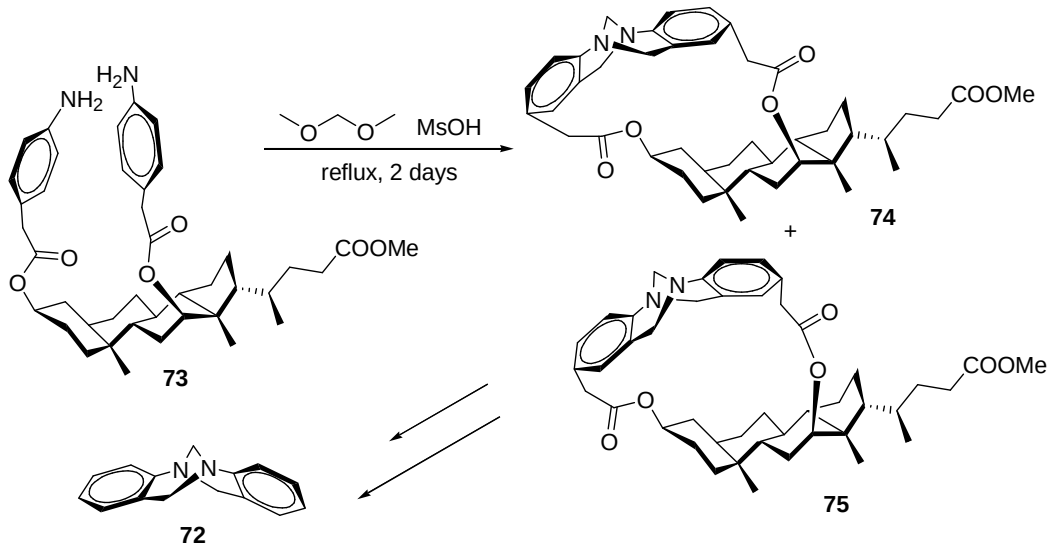
Scheme 21: Asymmetric reduction of α-ketoester to α-hydroxyester in presence of auxiliary **68**

Bandyopadhyaya and co-workers[126] exploited the geometric characteristics of deoxycholic acid for the asymmetric synthesis of 2,2'-dihydroxy-1,1'-naphthyl. Using 2,7-dihydroxynaphthalene, the authors could easily anchor the substrates by simple esterification; a spacer was firstly introduced in order to have C atoms in 1 and 1' positions at the best distance for the formation of the new bond: using as spacer a carbonate unit it was possible to obtain 99% of e.e. in the case of binaphthyl derivatives **70** (Scheme 22).



Scheme 22: Asymmetric synthesis of 2,2'-dihydroxy-1,1'-binaphthyl stereocontrolled by derivatives **70**

Deoxycholic acid has been revealed as a good chiral auxiliary for the synthesis of some analogues of Tröger base **72**:[127] in fact, the spatial disposition of hydroxyl groups 3 and 12 made possible the asymmetric coupling of two aniline moieties linked at these positions by means of a spacer, affording a diastereoisomeric mixture of **74** and **75** (ratio 1:2.5), that can afford pure **75** after crystallization from ethanol (Scheme 23).

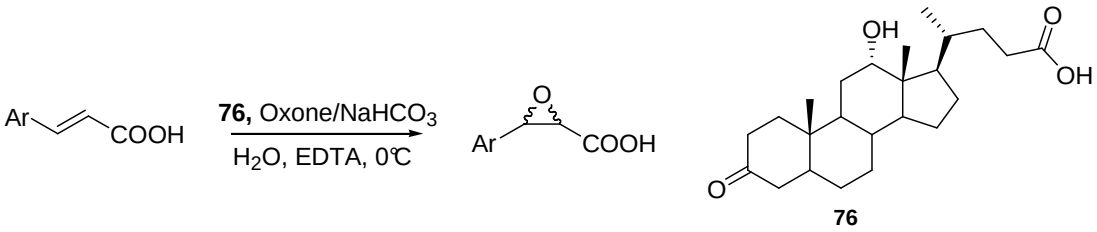


Scheme 23: Asymmetric synthesis of Troger base controlled by derivative **73**

Covalent functionalization of substrates is not the only way to use bile acid derivatives in promoting chiral processes. In particular, good enantiomeric excesses are obtained in epoxidation reaction of different cinnamic acid derivatives using Oxone® as oxidizing agent and dehydrocholic acid as chiral inducer in stoichiometric amount. The presence of a carboxylic group allowed the reaction to be performed in different solvents, also in aqueous solvents.

On this basis Bortolini and co-workers[128] performed a systematic study where a family of dehydrocholic derivatives was tested, in order to check the influence that the position of carbonyl group in the steroid backbone, the oxidation state of the other substituents in position 3,7 and 12 (H, OH, OAc), the steric hindrance and the absolute configuration of the position where these are linked have on enantiomeric excess and configuration of prevailing product.

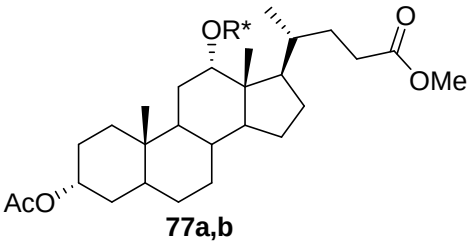
In this manner it is possible to understand that the presence of carbonyl group in position 3 is fundamental for the complete conversion of substrate in short time, while the substituents at positions 7 and 12 influence enantioselectivity, affording 95% e.e. with **76** (Scheme 24).



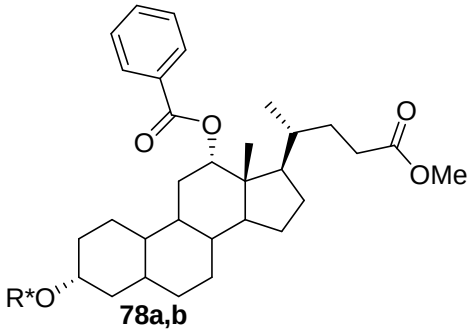
Scheme 24: Asymmetric epoxydation of cinnamic acid promoted by **76**

A new approach for the use of bile acid derivatives in asymmetric synthesis is the preparation of phosphorous ligands. Deoxycholic acid was used to synthesize phosphites **77** and **78**, as ligands in the Cu(I)

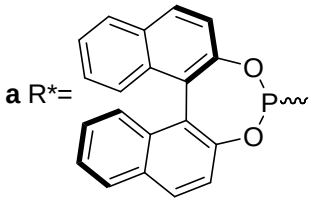
catalyzed asymmetric conjugate addition of diethylzinc to cyclic[129] and acyclic enones[130]. In particular derivative **77a**, which presents a *R*-binaphtyl phosphite unit in position 12, gave good asymmetric induction (e.e. up to 78%) in this reaction.[130]

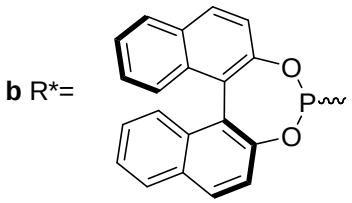


77a,b



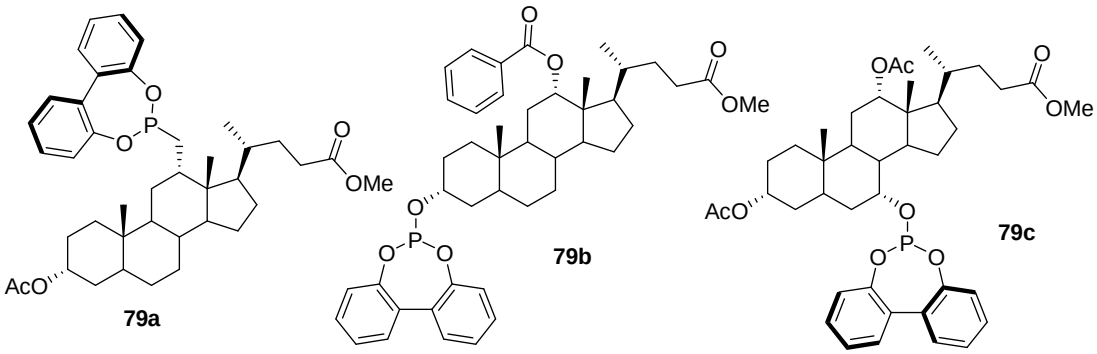
78a,b



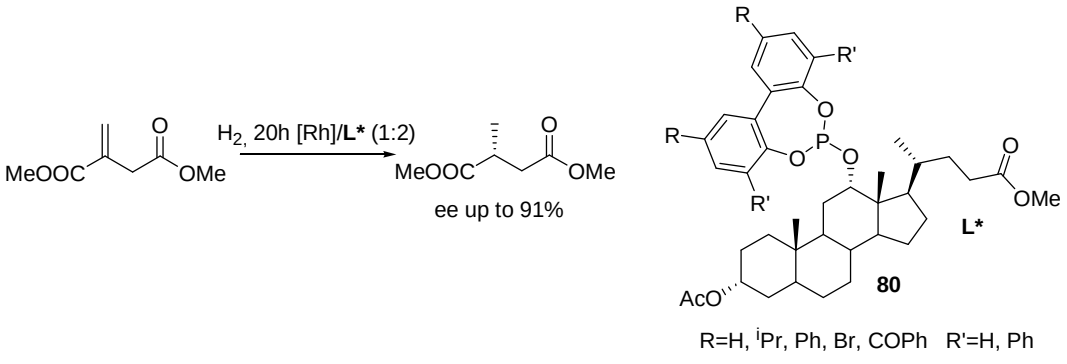


A second generation of these derivatives[131] was designed with the idea that the bile acid backbone can display a diastereoisomeric control on a *tropos* moiety. According to such a concept, phosphite ligands **79a-c** based on a bile acid unit and a *tropos* biphenol moiety have been studied: their main characteristic lies in the capability of the configurationally stable cholestanic unit to induce a prevalent screw sense in the flexible biphenol moiety, as evidenced by NMR and CD studies. In particular, phosphite **79a** showed an unusual dependence of the sense of twist of the biphenyl moiety on the solvent that definitively pointed out its *tropos* nature: the equilibrium between the two M and P forms of the biphenyl unit is shifted toward the M form in CH_3CN and toward the P form in THF. The achievement of a bile acid-based biphenyl phosphite having opposite screw sense depending

on the solvent represented an important result, as far as its use as a chiral ligand in the copper-catalyzed conjugate addition of diethylzinc to enones. In fact, the *tropos* nature of phosphite **79a**, joined to a low interconversion M-P barrier of its biphenyl moiety, which determines the dependence of its sense of twist on the solvent, allowed the different enantiomers of the same product to be obtained using the same chiral inducer, simply by changing the reaction solvent.



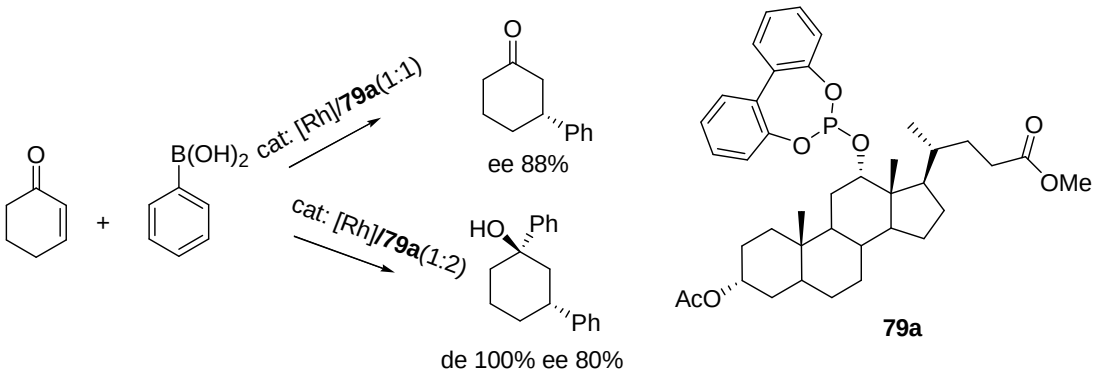
Recently different deoxycholic acid derived biphenylphosphites **80**, whose *tropos* nature was ascertained by NMR and CD measurements, were used in the rhodium-catalyzed asymmetric hydrogenation of dimethylitaconate achieving enantiomeric excesses up to 91% (Scheme 25).[132]



Scheme 25: Asymmetric hydrogenation of dimethylitaconate catalyzed by [Rh(I)]**L***$_2$ complex

The comparison of these results to those obtained using the corresponding atropoisomeric binaphthyl analogues, together with NMR and CD measurements on the rhodium complexes of some phosphites, shed light on the nature of the active catalytic species and on the asymmetric induction process and hence allowed to recognize the most appropriate stereochemical features to reach good levels of enantioselectivity.

Moreover the use of the phosphites **79a** as ligands for Rh(I) in conjugate addition of boronic acids to cyclic enones (Scheme 26) has shown an original switching in reactivity based on metal-ligand ratio: if an equimolar amount of ligand is used a monosubstituted metal complex is obtained which gives the conjugated addition product (e.e. up to 88%) while when two equivalents of phosphite are used a disubstituted complex is formed and double addition product is obtained with complete diastereoselectivity and a good e.e. (80%).[133]



Scheme 26: Asymmetric conjugate addition of boronic acids to cyclic enones catalyzed by [Rh]/**79a** complex

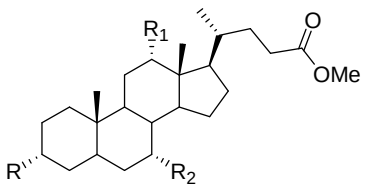
Variable temperature ^{31}P NMR of Rh monocoordinate complex has shown decoalescence of the signals of the M and P diastereoisomers at -80°C, in agreement with the *tropos* nature of this complex.

Chapter III

Synthesis of organocatalysts

3.1 Prolinamide derivatives

As reported in Chapter I, in the transition state of reactions proceeding via enamine pathway a hydrogen bond between an acidic proton of the catalyst and an acceptor group on the substrate is present.[135] As a result, we judged that the proline moiety had to be linked to the cholestanic skeleton by means of an amide bond. This required that one of the hydroxyl groups of bile acids was selectively transformed into an amino group. In addition, since it is known that the enantioselectivity of bile acid derivatives depends not only on the nature of the appended moieties, but also on their position on the cholestanic backbone,[134d,g] derivatives containing L or D proline at 3, 7 and 12 positions were synthesized, in order to find the best appendage position and the best match between the stereochemistry of the bile acid and the absolute configuration of proline for the asymmetric organocatalysis, which guarantee the achievement of high asymmetric inductions (Figure 5). The synthesis of analogous systems having free hydroxyl groups was also designed in order to evaluate the effect of the presence of these groups on the asymmetric organocatalysis.



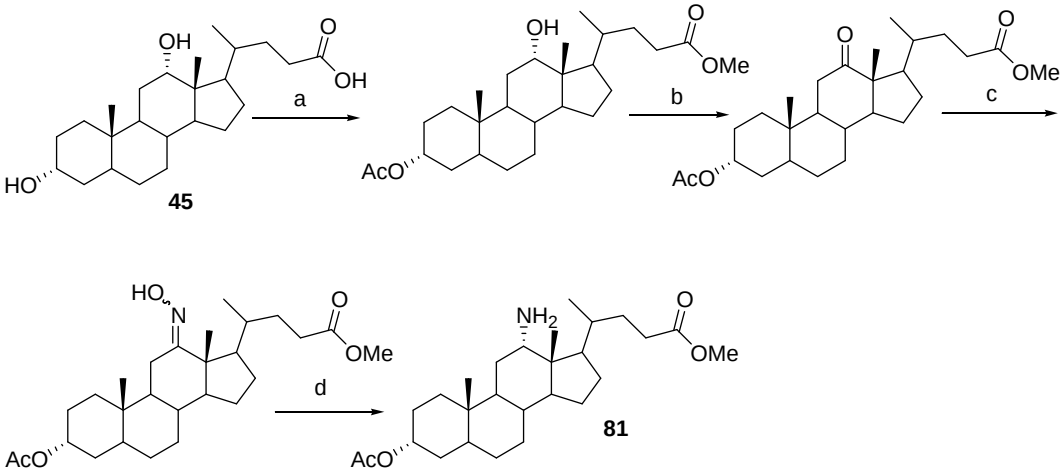
B1a R=OAc R_1=*L*-Pro R_2=H
B1b R=OAc R_1=*D*-Pro R_2=H
B1c R=OH R_1=*D*-Pro R_2=H
B2a R=*L*-Pro R_1=OH R_2=H
B2b R=*D*-Pro R_1=OH R_2=H
B3a R=R_2=OAc R_1=*D*-Pro
B3b R=R_2=OH R_1=*D*-Pro
B3c R=R_2=OH R_1=*L*-Pro
B4a R=R_1=OAc R_2=*L*-Pro
B4b R=R_1=OAc R_2=*D*-Pro
B4c R=R_1=OH R_2=*L*-Pro
B4d R=OH R_1=OAc R_2=L-Pro

Figure 5: structure of bile acid derived organocatalysts

The amino derivatives of deoxycholic acid, from which organocatalysts **B1a-c** and **B2a-b** can be prepared, were synthesized as previously described.[136]

Briefly, as reported in scheme 27, 3-hydroxyl and carboxylic groups of deoxycholic acid **45** were protected with one pot double esterification; then oxidation of 12-OH group, followed by transformation of the carbonyl function in oxime and reduction with H_2 and PtO_2 gave amine **81**

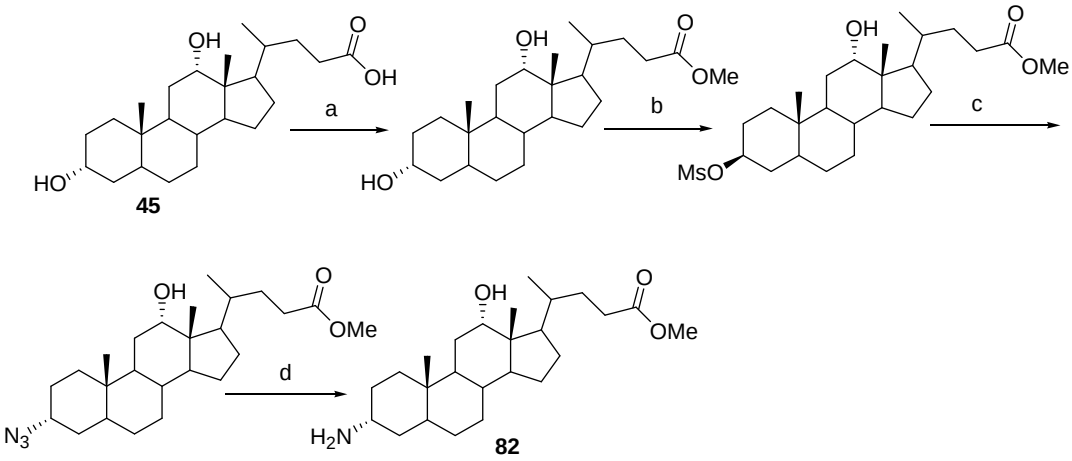
Scheme 27: synthesis of 12 amine derivatives of deoxycholic acid



Reagents and conditions: (a) TsOH H_2O, AcOMe, H_2O, reflux, 24 h, 67%; (b) $K_2Cr_2O_7$, H_2O, AcOH, r.t. 20 h, 90%; (c) NaOAc, NH_2OH HCl, MeOH, reflux, 4.5 h, 88%; (d) PtO_2 xH_2O, AcOH, H_2 (2 bar), r.t. 6 d, then Zn, r.t., 12 h, 70 %.

A different path was followed to obtain amine **82** from deoxycholic acid **45** according to scheme 28. After esterification of the carboxylic group with DBU and MeI, we performed a Mitsunobu reaction[136h] in presence of MsOH, DEAD and PPh_3 to obtain mesyl derivative that was treated with sodium azide to afford the corresponding azide. Reduction of this product with H_2 and Pd/C led to desired amine **82**.

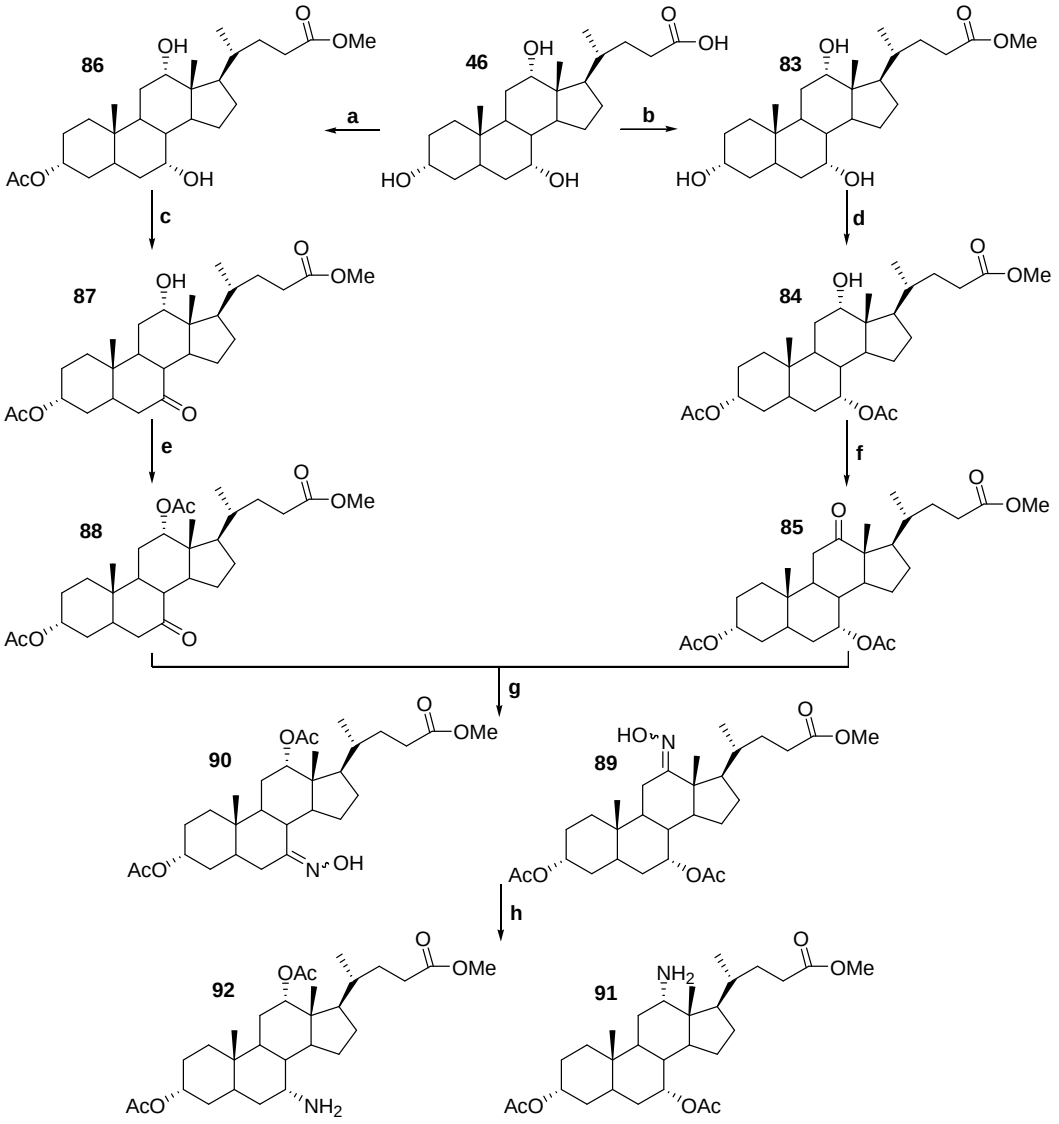
Scheme 28: synthesis of 3 amine derivatives of deoxycholic acid



***Reagents and conditions**:* (a) MeI, DBU, CH_2Cl_2, r.t., 12 h, 84%; (b) PPh_3, DEAD, MsOH, THF, 40℃, 24h, 43%; (c) NaN_3, DMF, 40℃, 48 h, quant.; (d) H_2, Pd/C, AcOEt/MeOH, r.t., 24 h, 91%.

The synthetic route to 12- and 7-amino derivatives of cholic acid was performed in this work for the first time and is reported in Scheme 29. It is quite similar for the two compounds, requiring the oxidation of 7- or 12-hydroxyl groups to ketones that can be transformed into oximes, easily reduced to amino groups.[136] In fact, due to the axial position of 7 and 12 hydroxyl groups, Mitsunobu transformation does not work; in addition, because of the asymmetric structure of the cholestanic backbone, the catalytic hydrogenation of an oxime at these positions proceeds with complete stereoselectivity.[136a]

Scheme 29: synthesis of amine derivatives of cholic acid



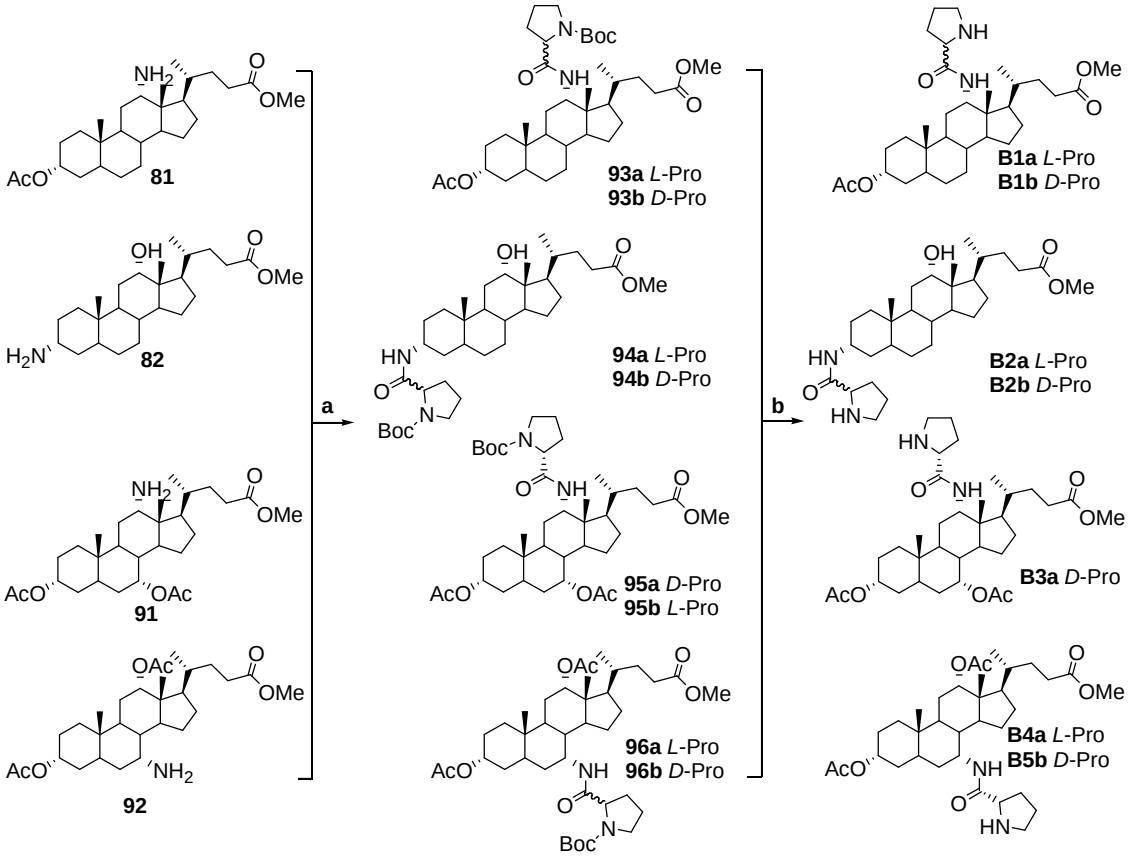
Reagents and conditions: a) AcOMe, TsOH, H_2O, reflux, 24 h; b)MeI, DBU, CH_2Cl_2, r.t. 12 h; c)NBS, acetone/H_2O, r.t., 30 h; d)Ac_2O, Py, toluene, r.t. 24 h; e) Ac_2O, DMAP, Et_3N, THF, r.t., 24 h; f)$K_2Cr_2O_7$, H_2O, AcOH, r.t. 20 h; g)NH_2OH HCl, NaOAc, MeOH, reflux, 3 h; h)PtO_2 xH_2O, AcOH, H_2 (2 bar), r.t., 6 d, then Zn powder, r.t., 12 h.

To obtain ketone **85**, the carboxylic function of cholic acid was transformed into a methyl ester, then the 3- and 7-hydroxyl groups were acetylated by reacting **83** with acetic anhydride and pyridine:[136g] under these experimental conditions the 12-OH group does not react and **7** was obtained in 72% yield, after chromatographic purification. The oxidation of the 12-OH group, performed with potassium

bicromate,[136a] gave **85** in quantitative yield. Ketone **88** was obtained in four steps from cholic acid, which was firstly reacted with methyl acetate[136] to give **86** in 75% yield, after chromatographic purification. The selective oxidation of the 7-hydroxy group with NBS in acetone solution[136a] afforded **87** in quantitative yield, which was then reacted with acetic anhydride and triethylamine in presence of DMAP giving **88** in 88% yield. Both 12- and 7-keto derivatives **85** and **88** were transformed, under standard reaction conditions,[136] into the corresponding oximes, which were eventually reduced, with complete stereoselectivity, to the amino derivatives by means of catalytic hydrogenation followed by reaction with Zn in acetic acid.[136]

Synthesis of monoamides at 12α- and 7α-position is quite delicate because of the steric demand of these positions on aminocholic skeleton; in particular amides of 7α,12α-diaminocholic acid methyl ester are known in the literature in some cases, as precursors in the synthesis of scaffolds for medicinal chemistry, as reported by Davis and Savage. Before this work, no article reports the synthesis of a 12α- or 7α-monoamide of correspondent aminocholic acid methyl esters, even if there is a lot of synthesis of amides in position 3α, where steric request is less important.

Scheme 30: Reactions of condensation of bile acid amino derivatives and proline



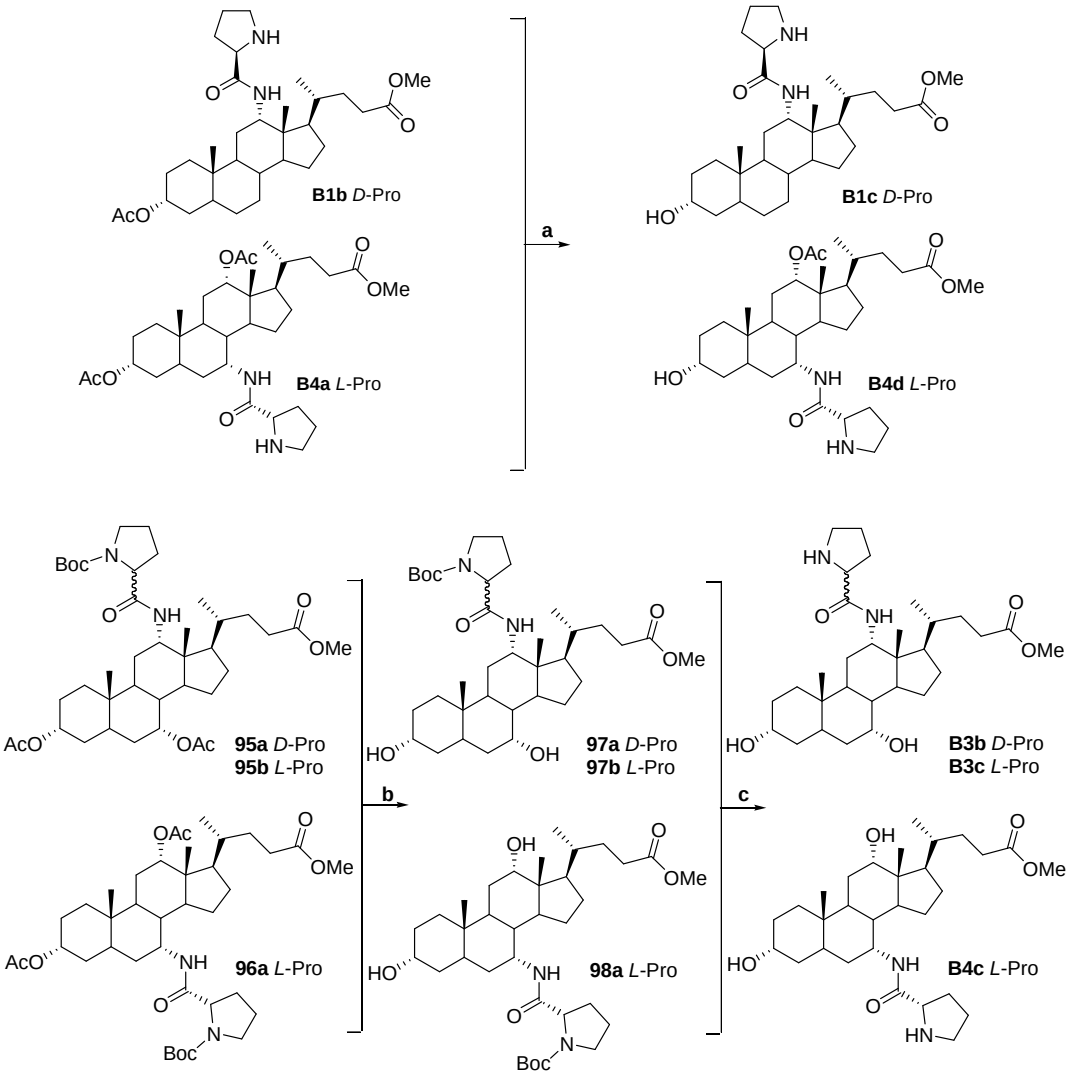
Reaction conditions: a) (L)- or (D)-Boc- Pro, isobutylchloroformate, NMM, CH_2Cl_2, 0℃, 26h; b) CH_2Cl_2, TFA, r.t. 15'

We tried to synthesize the amides **93a-b**, **94a-b**, **95a-b**, **96a-b,** via the mixed-anhydride method, by coupling the corresponding amino derivatives of cholic acid with L- or D-BOC-proline, using a classical and readily available coupling reagent, EEDQ, but in this case the mixed-anhydride was too sterically demanding to react efficiently with the amino groups at the positions 12α or 7α (yield ≤20%). Also the use of ethyl chloroformate lead to a low yield (30%) with the presence of carbamate derivatives of cholic acid as byproducts.

Finally, the desired derivatives **93a-b**, **94a-b**, **95a-b**, **96a-b** were obtained by reacting the corresponding amino derivatives **81, 82, 91, 92** with L- or D-BOC-proline (Scheme 30) in the presence of isobutyl

chloroformate:[137] by working at room temperature, the corresponding prolinamides of bile acids were obtained in 50% yield, after chromatographic purification. The final products **B1a-b**, **B2a-c**, **B3a**, **B4a-b** were obtained in quantitative yield by removing the BOC protecting group by means of trifluoroacetic acid in dichloromethane solution.[138]

Scheme 31: Hydrolysis of 3-acetyl group and 7,12-acetyl group on organocatalysts



Reaction conditions: a) HCl conc., MeOH, r.t., 24 h; b) MeONa/ MeOH 10%, r.t., 24 h; c) TFA, CH_2Cl_2, r.t., 15'.

To obtain analogous systems having free hydroxyl groups, the acetyl moieties of compounds **B1b**, **B4a**, **95a-b** and **96a** were removed

(Scheme 31). The hydrolysis of the 3-acetyl group of **B1b** was performed by reacting **B1b** with HCl in methanol at room temperature,[138a] reaction conditions that do well because the 3-acetyl group is equatorial and hence very reactive and afforded **B1c** in quantitative yield. This experimental procedure cannot be used for removing the acetyl groups at 7- and 12-positions, since they are axial and thus less reactive. The same methodology was used to obtain **B4d**, a cholic derived 7-prolinamide with only one free hydroxy group in position 3. Therefore, to obtain **B3b-B3c** and **B4c** the hydrolysis was carried out with sodium methoxide in methanol at room temperature on **95a-b** and **96a**:[139] under these reaction conditions the methylester group is stable and, after BOC deprotection, **B3b-B3c** and **B4c** were obtained in 46% and 50% yield, respectively, after chromatographic purification.

3.2 Bis-prolinamide derivatives

Bisprolinamide derivatives were designed to resemble the peptides involved in the active pocket of enzyme.[140] In that case the achieved stereoselectivity is reasonably due to the presence of several amide bonds with restricted rotation, as well as to the possible cooperation between proline residues, which create a non covalent bonding environment similar to that of enzyme.[140d] The goal of our approach was to find a new system with the broad substrate applicability of proline and the specificity of aldolase enzymes for the direct aldol reaction. Therefore, we synthesized three new bisprolinamides **B5-B7** derivatives of cholic acid (Figure 6).

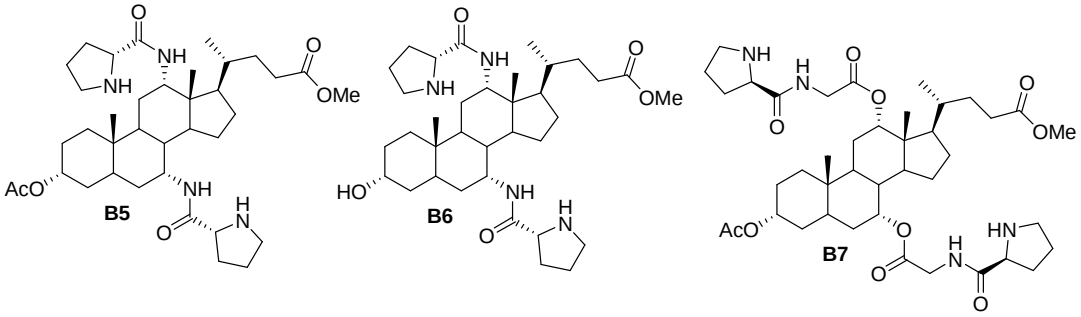
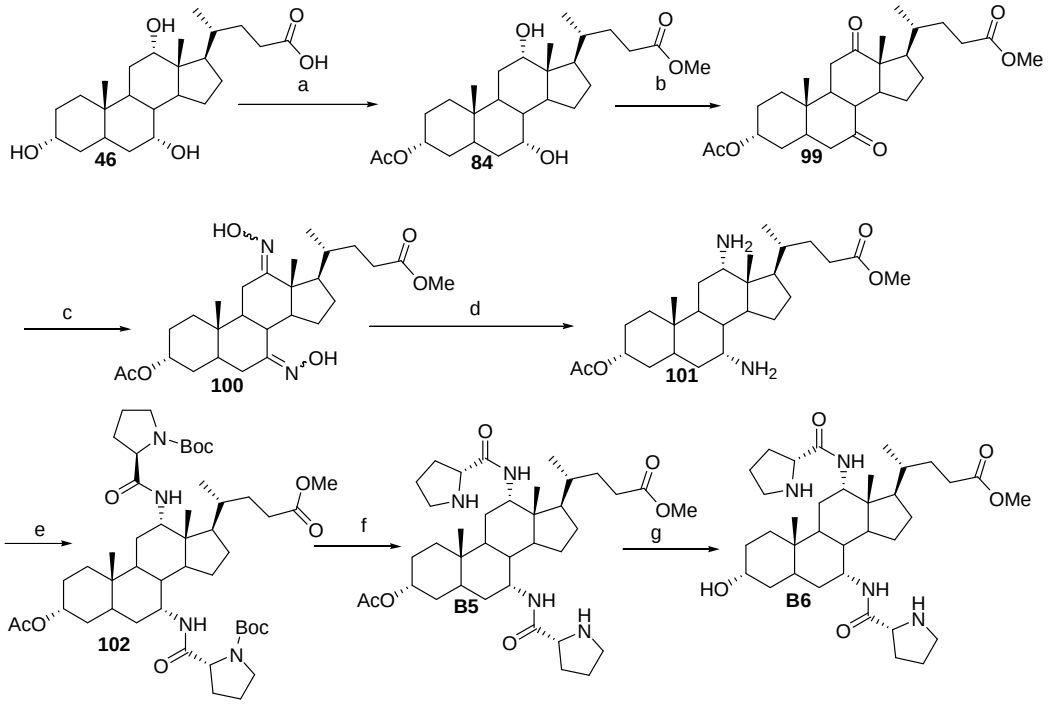


Figure 6: bisprolinamide bile acid derivatives

The design of these catalysts is based on the following facts: the cholestanic backbone would provide restricted rotation around the bile acid moiety due to steric hindrance of polycyclic structure.[140d] This structural motif would look like the β-turn pocket associated with the presence of proline residues in the active sites of several enzymes, and it could work as specifically as the proper enzyme.[140d] Moreover, the presence of two proline moieties in a very close position could lead to cooperative effect between the two residues.[140a]

Synthesis of organocatalysts **B5** and **B6** is quite similar to the synthesis of **B3-B4** derivatives and is briefly presented in Scheme 32.

Scheme 32: synthesis of organocatalyst **B5-B6**



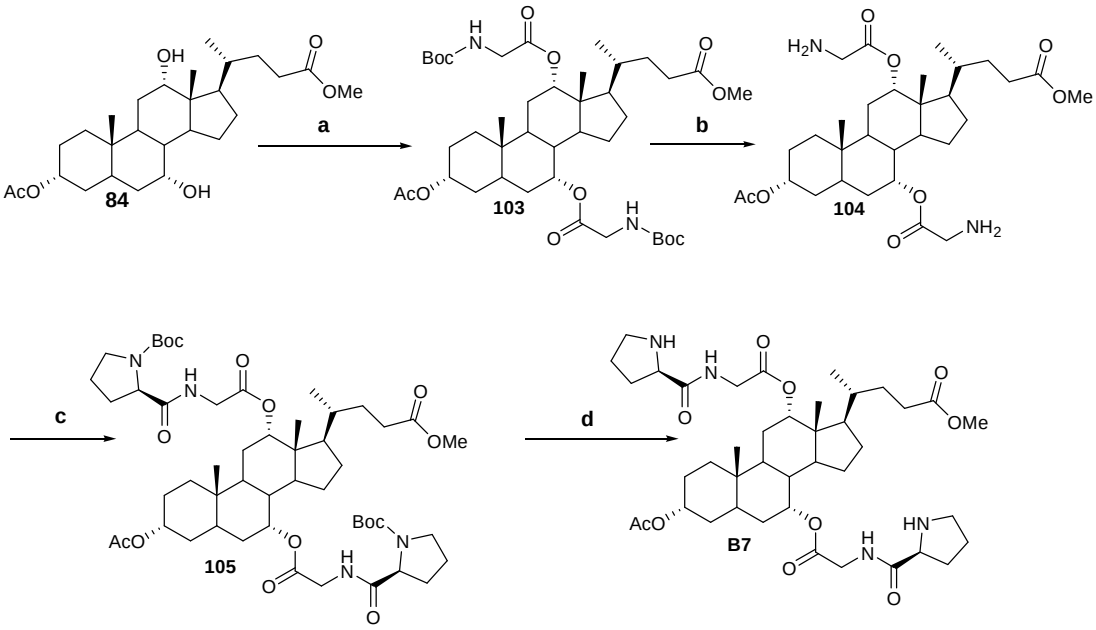
Reagents and conditions: a) AcOMe, TsOH, H_2O, reflux, 24 h; b)$K_2Cr_2O_7$, H_2O, AcOH, r.t. 20 h; c)NH_2OH HCl, NaOAc, MeOH, reflux, 3 h; d)PtO_2 xH_2O, AcOH, H_2 (2 bar), r.t., 6 d, then Zn powder, r.t., 12 h,e) (D)-Boc- Pro, isobutylchloroformate, NMM, CH_2Cl_2, 0°C, 26h; f) CH_2Cl_2, TFA, r.t. 15'; g) HCl conc., MeOH, r.t. 24 h.

Diketone **99** was obtained in two steps from cholic acid, which was reacted with methylacetate[136] to give **86** in 70% yield, after chromatographic purification. The oxidation of the 12 and 7-hydroxy groups with bichromate in AcOH solution[15a] afforded **99** in quantitative yield, which was transformed to the corresponding dioxime **100**, under standard reaction conditions;[136] this last was eventually reduced, with complete stereoselectivity, to the diamino derivative **101** by means of catalytic hydrogenation, followed by reaction with Zn in acetic acid.[136] The bis-prolinamide **B5** was obtained starting from the corresponding diamino derivative **101** in two steps. By reacting **101** with L- or D-BOC-proline in the presence of isobutyl chloroformate[137] at room temperature the corresponding bis-prolinamide of bile acid **102** was obtained in 50% yield, after

chromatographic purification. The final product **B5** was obtained in quantitative yield by removing the BOC protecting group by means of trifluoroacetic acid in dichloromethane solution.[138]

Deprotection of the 3-hydroxy group by reacting with HCl in MeOH, as reported for organocatalyst **B1c**, afforded derivative **B6**. The synthesis of organocatalyst **B7** is shown in Scheme 33**.**

Scheme 33: synthesis of organocatalyst **B7**



Reagents and conditions: a)N-Boc-Glycine, DMAP, DCC, CH_2Cl_2, r.t., 12 h; b)TFA, CH_2Cl_2, r.t. 15'; c)(L)-Boc-Pro, isobutylchloroformate, NMM, CH_2Cl_2, 0℃, 26h; d) CH_2Cl_2, TFA, r.t. 15'.

Diamine **104** was obtained from derivative **86** in two steps: esterification with N-BOC-Glycine promoted by DMAP and DCC,[141] giving quantitative yield of **103**, and successive removing of BOC groups with TFA in CH_2Cl_2[138]. By reacting **104** with L-BOC-proline in the presence of isobutyl chloroformate[137] at room temperature the corresponding prolinamide of bile acid **105** was obtained in 40% yield, after chromatographic purification. The final product **B7** was

obtained in quantitative yield by removing the BOC protecting groups by means of trifluoroacetic acid in dichloromethane solution.[138]

Chapter IV

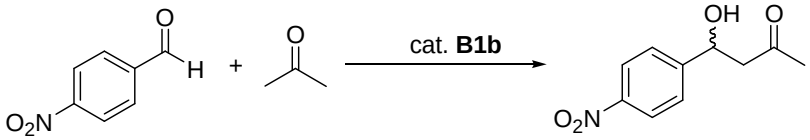
Organocatalytic aldol reaction

4.1 Direct asymmetric aldol reactions

The aldol reaction[142a-c] is one of the most successful reactions in organic chemistry because it readily allows the formation of a C–C bond by reaction of an enolizable carbonyl compound acting as a source of nucleophile with itself or another carbonyl compound acting as an electrophile to give a β-hydroxy carbonyl compound, known as aldol. In particular this transformation is a convenient methodology to obtain by an atom economic way one or more stereogenic centers starting from achiral molecular sources. For this reason, this transformation has been chosen historically as a chemical test to prove the efficiency of new methodologies, especially asymmetric ones.[142c-m] Therefore organocatalysts **B1-B4** were assayed in the direct asymmetric aldol reaction between acetone and 4-nitrobenzaldehyde. The effect of different reaction parameters, such as the solvent, temperature, catalyst loading, presence of additives, was investigated using **B1b** as organocatalytic system and the results obtained are listed in Table 4. All the reactions were stopped when conversion of the substrate was complete or did not proceed further, as judged by TLC analysis. The first run, performed by mixing p-nitrobenzaldehyde (0.25 mmol) and **B1b** (0.025 mmol) in acetone, used as reaction solvent,[143a-b] at room temperature gave complete conversion of the substrate in 48 hours and the condensation product was obtained in 27% e.e. (entry 1). However, together with the aldol product, appreciable amounts of other condensation products together with the product coming from the dehydration of the aldol were obtained, and their formation was observed even performing the reaction at 0°C.

This problem was avoided using a different reaction procedure, which involves the preactivation of the organocatalyst obtained by reacting **B1b** and acetone for 1 hour, then adding the aldehyde.[143c]

Table 4:Reaction of p-nitrobenzaldehyde with acetone in presence of organocatalyst **B1b** in different conditions.



Entry[a]	Solvent	T (°C)	E.e. (%)[b]	Conv.(%)[c]
1	neat	r.t.	27	>99
2	neat	0	46	>99
3	neat	-20	34	>99
4[d]	neat	0	37	>99
5[e]	neat	0	44	>99
6[f]	$CHCl_3$	0	32	>99
7[f]	CH_3CN	0	38	50
8[f]	THF	0	17	73
9[f, g]	toluene	0	29	>99
10[f]	DMSO	10	23	26
11[f]	H_2O/DMF 2:1	0	49	72
12[f]	H_2O/DMF 2:1	r.t.	36	>99
13[f]	DMF	0	37	36
14[f, h]	H_2O/DMF 1:1	0	n.d.	0
15[e, h, i]	H_2O/acetone 4:1	r.t.	14	26

[a] Reagents and conditions: p-nitrobenzaldehyde (0.5 mmol), acetone (0.5 mL), catalyst (generally 10 mol%); reactions are monitored by TLC;

[b] Determined by HPLC on chiral stationary phases (Chiralpack AS); the absolute configuration of the prevailing enantiomer was *S*;

[c] Determined by ^{1}H NMR

[d] With 20% of catalyst

[e] With 5% of catalyst

[f] Solvent/ acetone ratio 4:1, in a mL of volume

[g] Reaction stopped after 96 h of conversion

[h] With 10% of TFA as additive

[i] Reaction stopped after 120 h of conversion

Under these experimental conditions, at 0°C complete conversion of the aldehyde was reached in 48 h, and neither other condensation products nor the dehydration product were observed; in addition the aldol was obtained in 46% e.e. (entry 2). Further lowering of the reaction temperature, as well as increasing the catalyst loading, caused worsening of enantioselectivity (entries 3 and 4). The use of a lower amount of catalyst did not improve the enantioselectivity, even if the activity of the catalyst remained unchanged, complete conversion being reached in 48 hours (entry 5). Changing of the solvent gave, in general, inferior results both in terms of substrate conversion and enantioselectivity (entries 6-13). In fact, using $CHCl_3$ or toluene complete conversion of the substrate was observed, but 96 hours were required in toluene solution and in both cases the e.e.s were lower (entries 6 and 9). In a polar solvent as THF we obtained low conversion and very low e.e. (entrie 8). According to what was proposed by List and Barbas in early works,[143] we try to use DMSO with worse results (entry 10) and also the mixture DMF-water, affording a slightly higher e.e., unfortunately associated with lower substrate conversion (entry 11). Raising of the temperature in the presence of this solvent mixture gave complete conversion of the substrate but lower enantioselectivity (entry 12). In some cases the literature proposed the use of acid additives[63, 73, 76] in order to favour the formation of the enamine intermediate but, in our case, the use of trifluoroacetic acid as additive did not afford positive results, neither in DMF-H_2O solution (entry 14) where no reaction took place, nor in

acetone (entry 15), where a very low conversion of the substrate and 36% e.e. of the product were obtained.

On the basis of these results, for comparative purposes, the other organocatalysts were assayed, under the experimental conditions which afforded the best results in terms of conversion and enantioselectivity, i.e. the use of acetone as solvent at 0°C with the pre-activation of the catalyst. The results are listed in Table 5.

Table 5: reaction of p-nitrobenzaldehyde with acetone in presence of organocatalyst **B1a-c**, **B2a-b**, **B3a-b**, **B4a-c** at 0°C.

Entry[a]	Catalyst	e.e. (%)[b]	Absolute configuration[c]
1	**B1b**	46	*S*
2	**B1a**	12	*S*
3	**B2a**	32	*R*
4	**B2b**	38	*S*
5	**B4a**	21	*R*
6	**B4b**	21	*S*
7	**B4c**	20	*R*
8	**B1c**	41	*S*
9	**B3a**	42	*S*
10	**B3b**	64	*S*

[a] Reaction conditions: p-nitrobenzaldehyde (0.5 mmol), acetone (solvent), organocatalyst (10 mol%), 0°C. All reaction are stopped after 48 h at complete conversion.

[b] Determined by HPLC on chiral stationary phase (Chiralpack AS).

[c] Determined by comparison with literature data[4e]

The activity of the different organocatalysts was comparable, complete conversion of the substrate being reached in 48 hours in all cases. Compound **B1a**, which possesses L-proline moiety linked at the 12-position of the deoxycholic acid, gave a remarkably lower enantioselectivity (entry 2), suggesting that a mismatched couple is obtained when L-proline is linked at the 12-position. However the

sense of asymmetric induction remained unchanged, the *S* enantiomer being obtained with both the diastereoisomers (entries 1 and 2): this result suggests that the sense of asymmetric induction mainly depends on the stereochemistry of the cholestanic backbone. Lower enantioselectivity is obtained when the proline moiety is linked at the 3-position of deoxycholic acid, as in **B2a** and **B2b** (entries 3 and 4), suggesting that, as observed with other kind of bile acid derivatives,[134g] derivatization of position 3 affords less enantioselective chiral auxiliaries. Again, the matched couple is constituted by the diastereoisomer bearing the D-proline, although, in this case, the difference in the extent of asymmetric induction is smaller. The sense of asymmetric induction changes in passing from one to another diastereoisomer, suggesting that now it depends on the aminoacid moiety. Very unusual results were obtained using, **B4a** and **B4b** as organocatalysts, where L- or D-proline moiety is linked at the 7-position of the cholic acid. Both the diastereoisomers gave products having the same e.e. (entries 5 and 6) but opposite absolute configuration, suggesting a scarce influence of the cholestanic moiety on the asymmetric induction, which seems dependent only on the aminoacid moiety. The use of **B1c**, the analogue of **B1b** possessing a free OH group at the 3-position of the deoxycholic moiety, did not give better results than **B1b** (entry 8), suggesting that a free OH group at this position does not help the enantioselectivity of the reaction, by controlling the position of the substrate at the inner of the cavity of the catalyst. This is likely due to the high distance between moieties linked at the positions 3 and 12 of the cholestanic backbone, which

prevents interaction of the 3-OH group with a substrate that lies near the 12-position, where the active moiety of the catalyst is located. Even the use of **B3a**, the analogue of **B1b**, bearing D-proline moiety linked to the 12-position of 3,7-diacetyl cholic acid, did not work better than **B1b**, affording the aldol product in 42% e.e. On the contrary, the use of **B3b**, the analogue of **B3a** bearing two free OH groups at 3 and 7 positions, gave the best enantioselectivity (entry 10). The higher e.e. can be explained taking into account that groups at 7 and 12 positions are closer than groups at 3 and 12 positions,[136] therefore the 7-OH group can interact with a substrate reacting with the group linked at 12-position, by controlling its position inside the chiral cavity and then affording higher asymmetric induction. This result suggested that the behaviour of **B3b** could be different from that one exhibited by the other organocatalysts and prompted us to investigate the effect of various reaction parameters on activity and enantioselectivity of this system. The results are reported in table 6.

Table 6: Effect of reaction parameters on activity and enantioselectivity of **B3b** organocatalyst

Entry[a]	Catalyst (mol%)	T(°C)	Time (h)	Solvent	Conversion (%)[b]	E.e. (%)[c]
1	10	0	48	Acetone	>99	64
2	10	0	48	THF	8	45
3	10	0	48	DMF	53	65
4	10	0	48	THF/H_2O	>99	67
5	10	0	24	DMF/H_2O	>99	64
6	5	0	24	DMF/H_2O	>99	67
7	2	0	48	DMF/H_2O	>99	65
8	5	-20	24	DMF/H_2O	>99	75
9	5	-40	48	DMF/H_2O	>99	78

[a] Reagents and conditions: p-nitrobenzaldehyde (0.5 mmol), acetone (0.5 mL), catalyst (generally 10 mol%); reactions are monitored by TLC;

[b] Determined by ^{1}H NMR;

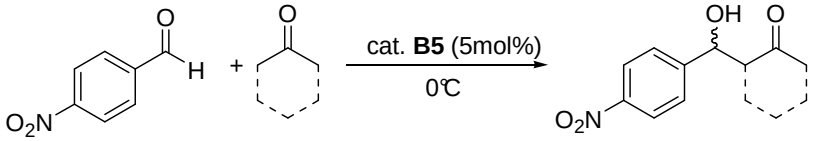
[c] Determined by HPLC on chiral stationary phases (Chiralpack AS); the absolute configuration of the prevailing enantiomer was *S*.

Changing of the solvent from acetone to THF or DMF did not improve the previous result. In fact using THF as solvent the activity remained unchanged but the extent of asymmetric induction was lower (entry 2). On the contrary, the use of DMF did not change the enantioselectivity to a significant extent, but worsened the activity of the catalyst (entry 3). The use of THF/H_2O or DMF/H_2O mixtures afforded better results in terms of catalyst activity, giving complete substrate conversion in 24 hours instead of 48 (entries 4 and 5). In addition, the presence of water avoided the formation of by-products coming from the acetone auto-condensation giving a cleaner reaction. Using the DMF/H_2O mixture as reaction solvent allowed us to lower the catalyst loading to 5% without loss of activity and with a slight improvement of asymmetric induction (entry 6). Further lowering of the catalyst loading to 2% did not give rise to loss of asymmetric

induction and complete conversion of the substrate was still obtained, even if a longer reaction time was required (entry 7). Improvement of asymmetric induction was obtained by lowering the temperature to -20°C, without loss of catalytic activity (entry 8). The best result in terms of asymmetric induction was reached by further lowering the reaction temperature to -40°C (entry 9), but at this temperature the reaction was slower, affording complete substrate conversion in 48 hours.

Indeed organocatalysts **B5** and **B7** were subjected to a preliminary screening in aldol reaction between acetone and p-nitrobenzaldehyde in order to evaluate the catalytic activity and selectivity. Organocatalyst **B5** gave a behaviour similar to free proline in organic solvent (Table 7). DMF gave low yield (20% after 12 h) because of formation of polymer of acetone (entry 2); water had a dramatic effect on reaction rate but gave only 9% e.e. (entry 3). Interestingly use of an acid additive[140g-j] did not affect e.e. but lowered reaction rate (entries 4-5). Reaction with cyclohexanone in DMF and in water gave good stereoselectivity, respectively of 41 and 60% of e.e. (entries 6-7). All these results are comparable with those obtained with proline.[143]

Table 7 Reaction of 4-nitrobenzaldehyde with ketones in presence of organocatalyst **B5**[a]



Entry	Ketone	Time (h)	Solvent	Conv. (%)[d]	e.e. (%)[c]
1[a]	Acetone	48	Acetone	>98	16
2[a]	Acetone	12	DMF	20	25
3[a]	Acetone	12	H_2O	>98	9
4[a]	Acetone	120	Tol[e]	<2	19
5[a]	Acetone	120	H_2O[f]	<2	22
6[b]	Cyclohexanone	120	DMF	41	64 (71)
7[b]	Cyclohexanone	120	H_2O	62	60 (80)

[a] Reaction conditions: p-nitrobenzaldehyde (0.5 mmol), acetone (27 equiv.), organocatalyst (5 mol%), 0°C. Main enantiomer for this reaction is always *S*.

[b] Reaction conditions: p-nitrobenzaldehyde (0.5 mmol), cyclohexanone (2 equiv.), organocatalyst (5mol%), 0°C. Main diastereisomers is *anti* with d.e. of 19% (entry 6) and 51% (entry 7). E.e.s into brackets is referred to *syn* diastereoisomer

[c] Determined by HPLC on chiral stationary phase (Chiralpack AS).

[d] Determined by ^{1}H NMR.

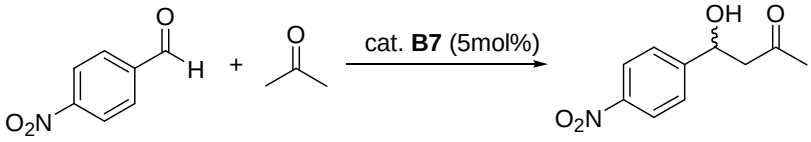
[e] 10mol% of TFA is added

[f] 10mol% PhCOOH is added

Similar results were obtained with organocatalyst **B7** (Table 8). Organic solvents DMF and $CHCl_3$ gave low conversion and low enantiomeric excess (entries 1-5): in particular aldol reaction in DMF produced a lot of polymerization products (entries 3-5) and acidity of solvent did not affect the selectivity and the conversion (entry 2). The presence of water increased the reaction rate with a detrimental effect on e.e. (entry 6). Eventually, TFA additive in combination with organic solvent[140g-j] gave good enantiomeric excess, up to 67 %, independently of amount of acid (entries 8-10). Probably in this case

cooperative effect between the two residues of proline could be postulated.

Table 8 Reaction of 4-nitrobenzaldehyde with ketones in presence of organocatalyst **B7**[a]



Entry	Time (h)	Solvent	Temperature (°C)	Conv. (%)[b]	e.e. (%)[c]
1	48	Acetone	25	32	25
2	48	$CHCl_3$	0	37	39
3	48	DMF	0	26	43
4	120	DMF	0	27	43
5	72	DMF[d]	0	26	45
6	96	DMF/H_2O[e]	0	>98	29
7	72	DMF	-20	12	50
8	120	DMF/TFA[f]	0	>98	57
9	120	DMF/TFA[g]	0	>98	58
10	120	DMF/TFA[h]	0	>98	67

[a] Reaction conditions: p-nitrobenzaldehyde (0.5 mmol), acetone (27 equiv.), organocatalyst (5 mol%), 0°C. Main enantiomer for this reaction is always *R*.

[b] Determined by ^1H NMR.

[c] Determined by HPLC on chiral stationary phase (Chiralpack AS).

[d] Withuot preactivation

[e] DMF/H_2O 2:1

[f] 5mol% TFA is added

[g] 20mol% TFA is added

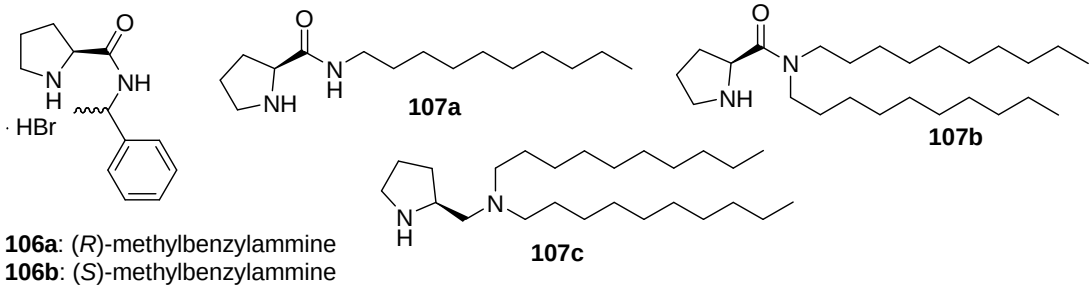
[h] 10mol% TFA is added

4.2 Water as solvent in organocatalytic aldol reactions

The use of pure water as solvent is considered, in general, to be of high interest because water is an inexpensive, safe, and environmentally benign solvent. Importance of water as solvent in organocatalytic processes was widely discussed in academia in 2005-2006, in particular by Janda and Hayashi, both of them involved in a

deep diatribe about the water-media concept.[144] Today we consider reasonably the organocatalytic reactions in water as reactions performed in an organic concentrated phase and a good term to define it is “reaction performed in the presence of water”, as proposed by Sharpless et al.[145]

Proline derived organocatalysts are reported to be effective for aldol reaction even in the presence of large amounts of water but some additives were added in order to facilitate the formation of enamine intermediate. In 2005 Chimni and coworkers[146] proposed firstly prolinamide **106** hydrobromide salts in the enantioselective direct aldol reaction between acetone and p-nitrobenzaldehyde in the presence of water, obtaining high yield and good enantioselectivity.

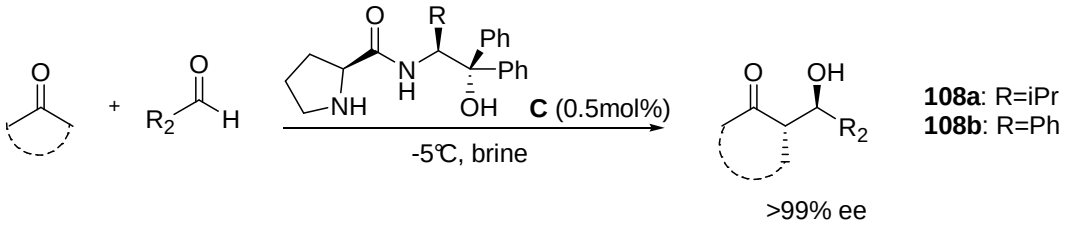


106a: (*R*)-methylbenzylammine
106b: (*S*)-methylbenzylammine

107a **107b** **107c**

In 2006 Barbas et al.[147] proposed to use emulsion obtained by proline derivatives **107** with long alkyl tails in order to form micelles in the reaction medium, sequestering reagents and increasing selectivity. In particular the diamine **107c**/TFA bifunctional catalyst system demonstrated excellent reactivity, diastereoselectivity, and enantioselectivity in the presence of water in reaction between aromatic aldehydes and cyclohexanone (yield up to 98%, d.e. up to 80% for *anti* product and e.e. up to 94%).

More recently Visnhu Maya and co-workers[148] exploited the electroconstriction effect obtained by very salted medium as a way to improve results in organocatalytic aldol reaction; prolinamide **108a** and **108b** catalyzed the direct aldol reaction of both acyclic and cyclic ketones with different aldehydes in an excess of water/brine (Scheme 34). Excellent enantioselectivities up to >99% and diastereoselectivities up to 99% with very good yields were obtained by using much lower catalyst loadings (0.5 mol %).



Scheme 34: Aldol reaction organocatalyzed by **108** in brine**.**

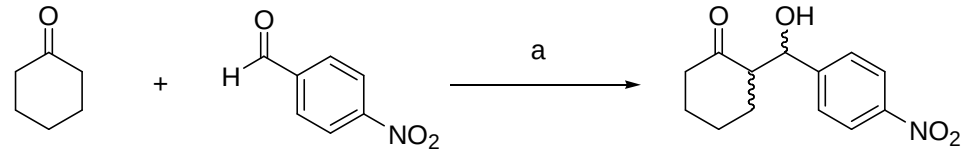
4.3 Direct aldol reactions in water and organic media

Taking into account these results, we tried to explore the possibility to use our best organocatalytic system **B3b** in reactions in aqueous solvent and moreover in the presence of a large amount of water, in order to study different reaction conditions and to compare results in water and in organic solvents. To pursue this objective, we chose as test system the aldol reaction between cyclohexanone and p-nitrobenzaldehyde.

As previously reported (see section 4.1), this reaction was performed using **B3b** as organocatalyst and according to the "pre-activation" method, which entails mixing of ketone and **B3b** at the reaction temperature for 1 hours, then adding the aldehyde.[149] Before studying the behaviour of this reaction in the presence of water we explored the

effect of different reaction parameters, such as solvent, catalyst loading, amount of ketone and temperature, as reported in Table 9.

Table 9 : Reaction of 4-nitrobenzaldehyde with cyclohexanone in presence of organocatalyst **B3b-B3c**[a]



Entry	Cat.(%)	Solvent	Temp. (°C)	Time (h)	Conv.(%)[c]	d.e.(%) (*anti*)[c]	e.e. (%)[b]
1	5	H_2O/DMF[d]	r.t.	24	>98	28	77
2	5	H_2O/DMF[d]	0	24	70	80	87
3	5	H_2O/DMF[d]	-20	24	50	82	90
4	5	MeOH	0	24	46	82	63
5	5	DMF	0	24	28	83	87
6	5	Toluene	0	24	97	83	85
7	5	Nitrobenzene	0	24	>98	84	86
8	5	DCM	0	24	>98	84	86
9	5	H_2O[e]	0	48	61	80	81
10	2	H_2O[e]	0	96	31	85	68
11	5	H_2O[e]	0	96	12[f]	95	68
12	2	DCM	0	48	>98	92	87
13	1	DCM	0	48	>98	94	87
14	5	H_2O/DMF[d]	0	48	>98	46	60[g]

[a] Reagents and conditions: p-nitrobenzaldehyde (0.25 mmol), cyclohexanone (0.5 mmoL), catalyst, solvent (usually 0.5 mL); reactions were monitored by TLC;

[b] Determined by HPLC on chiral stationary phase (Chiralpack AS, hexane: 2-propanol 85:15, 1 mL/min, 254 nm); main diastereoisomer was *anti*.

[c] Determined by ^{1}H NMR

[d] H_2O/DMF 1:2

[e] All this reactions are performed in 2 mL of H_2O

[f] 1 equivalent of ketone was used;

[g] Organocatalyst with L-*Pro* **B3c** was used; the opposite enantiomer was obtained in prevalence.

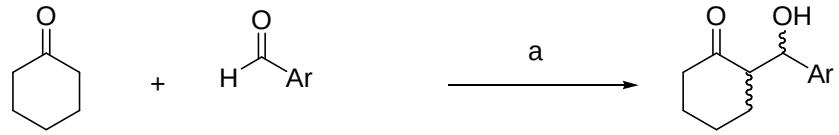
The use of the 1:2 H_2O-DMF mixture, the solvent giving the best result with acetone as donor substrate, as reported in section 4.1, with a 5% catalyst loading at room temperature and using only a twofold excess of ketone, afforded complete conversion of the aldehyde substrate in 24 hours (entry 1). The aldol product was obtained with low diastereoselectivity in favour of the *anti* isomer, which showed an e.e. of 77% (entry 1). Lowering of the temperature to 0 °C (entry 2) caused an improvement of both diastereoselectivity, which went to 80%, and enantioselectivity of the *anti* isomer, which was obtained in 87% e.e. This good result was flanked by a slowing down of the reaction rate, with a 70% of substrate conversion being obtained. Further lowering of the reaction temperature until -20 °C (entry 3) slightly improved both diastereoisomeric and enantiomeric excesses, but slowed down the reaction rate so that only a 50% conversion was obtained in 24 hours. Screening of various organic solvents (entries 4-8) showed that the best results in terms of conversion of the substrate as well as stereoselectivity were obtained when non-competing for hydrogen bond solvents, such as toluene, nitrobenzene or dichloromethane, were used. As a matter of fact, the use of methanol, a protic solvent, caused a worsening both in conversion and asymmetric induction (entry 4). It is notable that toluene a π-rich aromatic solvent and nitrobenzene a π-acceptor aromatic solvent gave the same results allowing us to conclude that π–π stacking between solvent molecules and aldehyde substrate was not involved in determining the outcome of the reaction. It seems likely that the organic solvent acts only as diffusion medium, when it cannot interact

with the substrate or the organocatalyst by means of hydrogen bonds. As a matter of fact, worsening of the reaction outcome occurs using a protic solvent (DMF, entry 5, caused only slowing down of the reaction rate), probably because hydrogen bonds between solvent and aldehyde substrate or organocatalytic species compete with hydrogen bonds between the reactive enamine species of the organocatalyst and the aldehyde, which are believed important for the stereoselectivity of the reaction.[9] Water behaved in a different way (entry 9): although the reaction was slower, when performed in pure water as a solvent, a good level of asymmetric induction was obtained. This is not surprising if we take into account that water does not solubilise either reagents or organocatalysts. The hydrophobic components are obliged by water to react in a "concentrated organic medium":[144b] in this sense water does not compete for hydrogen bonds behaving as, for example, dichloromethane and guarantees the achievement of good e.e.s. The lowering of catalyst loading or ketone amount had a detrimental effect both on reaction rate and asymmetric induction (entries 10 and 11). On the contrary, lowering of the catalyst loading in dichloromethane solution did not affect the extent of asymmetric induction, as well as the reaction rate (entries 12 and 13). The aldol reaction between 4-nitrobenzaldehyde and cyclohexanone can be carried out using a very low amount of **B3b** (1%), without loss of catalytic efficiency or asymmetric induction: in addition, the low catalyst loading had a positive effect on the diastereoselectivity of the reaction, with the aldol product being obtained in 94% d.e. (entry 13). The use of the diastereoisomeric analogue of **B3b**, which possesses an L-prolinamide

moiety linked at the 12-position of cholic acid **B3c** , gave the aldol product in high yield but in lower enantiomeric excess (entry 14), suggesting that the more effective diastereoisomer is obtained when D-proline moiety is linked at the 12-position of cholic acid. The opposite enantiomer of the *anti* product was obtained in prevalence: this result suggests that the sense of asymmetric induction is governed by the proline moiety linked to the 12-position of the cholic acid.

The conditions giving the best results in the asymmetric aldol reaction of cyclohexanone and 4-nitrobenzaldehyde, i.e. water with 5% of catalyst loading and dichloromethane with 1% of **B3b** at 0°C, were used to test the reaction with other substrates. The results concerning the aldol reaction of cyclohexanone and various aromatic aldehydes are reported in Table 10. The reactions carried out in water (entries 1-6) were faster than those performed in dichloromethane (entries 7-11) independently of the aldehyde substrate. This can be attributed mainly to the different catalyst loading that has a scarce influence in the case of the reaction of 4-nitrobenzaldehyde but affects to a greater extent the reaction of other kind of aldehyde substrates.

Table 10 : Reaction of aromatic aldehydes with cyclohexanone in presence of organocatalyst **B3b**[a]



Entry	Ar	Solvent	Catalyst (mol%)	Time(h)	Conv.(%)[c]	d.e. (%)[c]	e.e. (%)[b]
1	4-FC_6H_4	H_2O[d]	5	48	95	94	81[f]
2	4-ClC_6H_4	H_2O[d]	5	48	58	84	68[f]
3	4-$CF_3C_6H_4$	H_2O[d]	5	48	>98	97	63[g]
4	2-ClC_6H_4	H_2O[d]	5	48	93	95	62[h]
5	2-$NO_2C_6H_4$	H_2O[d]	5	48	46	99	62[g]
6	Ph	H_2O[d]	5	72	61	80	80[h]
7	4-FC_6H_4	DCM[e]	1	120	>98	80	80[f]
8	4-ClC_6H_4	DCM[e]	1	120	80	83	90[f]
9	4-$CF_3C_6H_4$	DCM[e]	1	48	74	85	84[g]
10	2-$NO_2C_6H_4$	DCM[e]	1	120	76	95	80[g]
11	Ph	DCM[e]	1	120	81	26	80[h]

[a] Reagents and conditions: aldehyde (0.25 mmol), cyclohexanone (0.5 mmoL), catalyst, solvent at 0°C

[b] Enantiomeric excess of the *anti* diastereoisomer.

[c] Determined by ^1H NMR; main diastereisomers was *anti*.

[d] All this reactions are performed in 2 mL of H_2O

[e] All this reactions are performed in 0.5 mL of DCM

[f] Determined by HPLC analyses on Chiralpack AD, 254 nm, hexane:2-propanol 90:10, 0.5 mL/min.

[g] Chiralcel OD-H, 254 nm, hexane:2-propanol 95:5, 1 mL/min.

[h] Chiralcel OD-H, 210 nm, hexane:2-propanol 99:1, 0.4 mL/min.

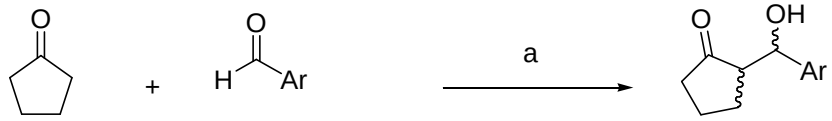
The extent of the substrate conversion depended on the electrophilic character of the aromatic aldehyde as well as the steric hindrance near the carbonyl group in both the solvents: as a matter of fact, benzaldehyde and 2-nitrobenzaldehyde gave lower conversion (entry 5) or required longer reaction times to afford good conversions (entries 6, 10 and 11). Only 4-chlorobenzaldehyde, an electrophilic

and unhindered substrate, exhibited in dichloromethane an unusual behaviour, giving a 58% conversion (entry 2). Both diastereomeric and enantiomeric ratios were affected by the solvent in opposite ways: water gave very high diastereoisomeric excess in favour of *anti* diastereoisomer (entries 1-6), whereas the highest enantiomeric excesses were obtained by performing the reaction in dichloromethane solution (entries 7-11). The extent of asymmetric induction depended on the nature of the aldehyde substrate more in water than in dichloromethane: aldehydes possessing a less polarisable aromatic ring gave the highest enantiomeric excess in water (entries 1 and 6), whereas the e.e.s in dichloromethane were around the 80% value (entries 7, 9-11) except for 4-chlorobenzaldehyde, which afforded the aldol product in 90% e.e. (entry 8).

The same conditions were used in the aldol reaction of cyclopentanone and various aromatic aldehydes: the results are reported in Table 11.

The conversions of the aldehyde substrates were similar in water and in dichloromethane, except for 2-chlorobenzaldehyde, 4-chlorobenzaldehyde and 4-nitrobenzaldehyde which gave remarkably higher conversions in water (entries 8, 10 and 14).

Table 11:Reaction of aromatic aldehydes with cyclopentanone in the presence of organocatalyst **B3b**[a]



Entry	Ar	Solvent	Catalyst(mol%)	Conv.[c](%)	d.r.[c] (%) (anti:syn)	e.r.[b] (%) (anti)	e.r.[b] (%) (syn)
1	4-$NO_2C_6H_4$	DCM[d]	1	36	69:31	97:3[g]	55:45[g]
2	4-FC_6H_4	DCM[d]	1	>98	53:47	93:7[h]	65:35[h]
3	4-ClC_6H_4	DCM[d]	1	83	54:46	90:10[g]	67:33[g]
4	4-$CF_3C_6H_4$	DCM[d]	1	>98	69:31	99:1[i]	71:29[i]
5	Ph	DCM[d,f]	1	93	72:28	95:5[j]	81:19[j]
6	2-$NO_2C_6H_4$	DCM[d]	1	53	43:57	97:3[k]	65:35[k]
7	2-ClC_6H_4	DCM[d]	1	51	44:56	95:5[l]	72:28[l]
8	4-$NO_2C_6H_4$	H_2O[e]	5	>98	41:59	98:2[g]	20:80[g]
9	4-FC_6H_4	H_2O[e]	5	89	39:61	80:20[h]	44:56[h]
10	4-ClC_6H_4	H_2O[e]	5	>98	41:59	73:27[g]	47:53[g]
11	4-$CF_3C_6H_4$	H_2O[e]	5	>98	36:63	95:5[i]	36:64[i]
12	Ph	H_2O[e,f]	5	87	54:46	5:95[j]	53:47[j]
13	2-$NO_2C_6H_4$	H_2O[e]	5	57	33:67	94:6[k]	72:28[k]
14	2-ClC_6H_4	H_2O[e]	5	91	28:72	41:59[l]	35:65[l]

[a] Reagents and conditions: aldehyde (0.25 mmol), cyclopentanone (0.5 mmoL), catalyst, solvent at 0°C for 60 h.

[b] Enantiomeric ratio: first eluted enantiomer:second eluted enantiomer.

[c] Determined by ^{1}H NMR.

[d] All this reactions are performed in 0.5 mL of DCM

[e] All this reactions are performed in 2 mL of H_2O

[f] Reaction time: 96 h.

[g] Determined by HPLC analyses on Chiralpack AS, 254 nm, hexane:2-propanol 85:15, 1 mL/min.

[h]Chiralpack AD, 210 nm, hexane:2-propanol 95:5, 0.5 mL/min.

[i]Chiralpack AD, 254 nm, hexane:2-propanol 90:10, 0.5 mL/min.

[j] Chiralcel OD-H, 210 nm, hexane:2-propanol 90:10, 0.5 mL/min.

[k] Chiralcel OD-H, 254 nm, hexane:2-propanol 95:5, 1 mL/min.

[l] Chiralpack AD, 220 nm, hexane:2-propanol 99.5:0.5, 1 mL/min.

Conversions depended, also with this ketone, on the electronic nature of the aldehyde as well as on the steric hindrance near the carbonyl

group (entries 5-7 and 12-14), with the sole exception of the reaction of 4-nitrobenzaldehyde in dichloromethane (entry 1).[150] As far as the diastereoisomeric ratios are concerned, low diastereoisomeric prevalence was observed in all the cases: this is not surprising, given that the use of cyclopentanone as donor substrate usually affords low diastereoisomeric excesses.[151] However, it is notable that in most cases the diastereoisomeric ratios are higher than those reported with other proline-derived organocatalysts.[151] The reactions performed in dichloromethane gave a prevalence of the *anti* diastereoisomer, except when 2-nitro and 2-chlorobenzaldehyde were used as substrates, suggesting that the ortho-substitution is a structural feature that helps the formation of the *syn* isomer. By contrast, when water was used as reaction solvent prevalence of the *syn* isomer was obtained, apart from the reaction of benzaldehyde (entry 12) that still gave prevalence of the *anti* isomer, but to a remarkably lower extent. These results suggest that water can stabilise the transition state leading to the formation of the *syn* product: such a kind of stabilisation gives rise to the inversion of the diastereisomeric ratio (entries 1-4 and 8-11), or to a lower prevalence of the *anti* product (entries 5 and 12) or to a higher prevalence of the *syn* isomer (entries 6, 7, 13, 14), in passing from dichloromethane to water. The *anti* products were obtained in good enantiomeric excesses in both solvents, apart from the reactions of 2- and 4-chlorobenzaldehyde in water (entries 10 and 14): as observed in the case of cyclohexanone, the best asymmetric inductions were achieved when reaction was performed in dichloromethane (entries 1-7). On the contrary, the enantiomeric excesses of the *syn* products

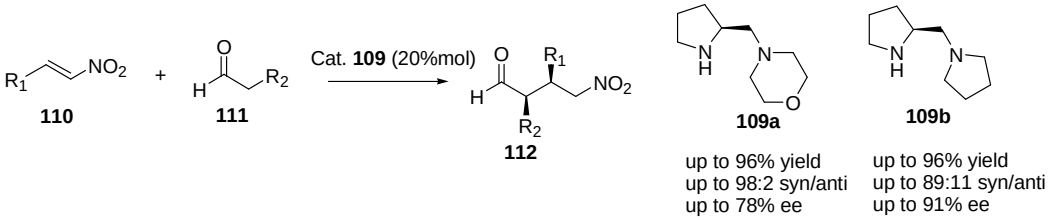
were lower and a general trend depending on the solvent cannot be found: the *syn* product coming from the reaction of benzaldehyde was obtained in 62% e.e. in dichloromethane (entry 5), whereas a 60% e.e. of the *syn* product coming from the reaction of 4-nitrobenzaldehyde was obtained in water (entry 8). The sense of asymmetric induction, as far as the *anti* product was concerned, changed in passing from dichloromethane to water in the case of benzaldehyde and 2-chlorobenzaldehyde (entries 5, 7, 12 and 14). As far as the *syn* product was concerned, the opposite enantiomer was obtained in prevalence in water in several cases (entries 8-11). These results point out a change of asymmetric induction mechanism depending on the solvent with some aldehyde substrates. This particular behaviour of organocatalyst **B3b** is very useful when benzaldehyde is reacted: actually, both enantiomers of the *anti* aldol product can be obtained in 90% e.e. simply by changing the reaction solvent (entries 5 and 12).

Chapter V

Organocatalytic Michael reaction

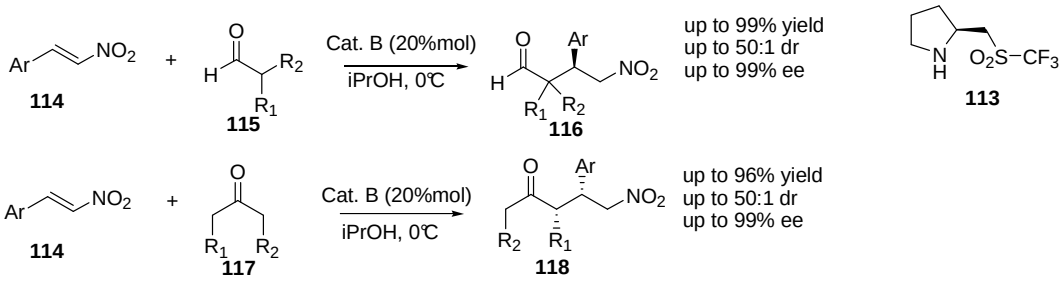
5.1 Organocatalytic Michael reactions[152]

The asymmetric Michael addition of carbon nucleophiles to nitro olefins has attracted a lot of attention because it allows functionalized products with multiple stereogenic centres to be obtained in a single step.[153] Moreover, Michael reactions of aldehydes and ketones with nitro olefins represent a convenient route to valuable building blocks in organic synthesis.[154] In fact, the nitro functionality can be easily transformed into a nitrile oxide, ketone, amine, or carboxylic acid, etc., providing a wide range of synthetically interesting compounds. Organocatalysis of the Michael reaction was pioneered using proline first by List[155a] and Barbas[155b] in 2001; afterward, excellent results have been obtained in the Michael addition of carbonyl compounds to nitroalkenes using chiral amines as organocatalysts,[156] which promote the reaction via enamine pathway.[157] Among these, proline derivatives bearing substrate orienting functional groups[158] succeeded in reaching good levels of asymmetric induction. Barbas described[159] for the first time highly diastereoselective direct catalytic Michael reactions involving the addition of unmodified aldehydes to nitro olefins using proline derivative organocatalysts (*S*)-2-(morpholinomethyl)pyrrolidine and (*S*)-1-(2-pyrrolidinylmethyl)pyrrolidine **109** as catalysts (Scheme 35). The reactions proceed in good to high yields (up to 96%) in a highly *syn*-selective manner (up to 98:2), with up to 91% e.e. values.



Scheme 35 Organocatalyzed Michael addition of aldehydes to nitrostyrene promoted by **109**

Chiral (*S*)-pyrrolidine trifluoromethanesulfonamide **113** has been shown by Wang's group[160] to serve as an effective catalyst for direct Michael additions of aldehydes and ketones to nitro olefins (Scheme 36). Studies with linear chain aldehydes revealed that these substrates reacted much more rapidly than the more hindered α,α-dialkyl aldehydes.

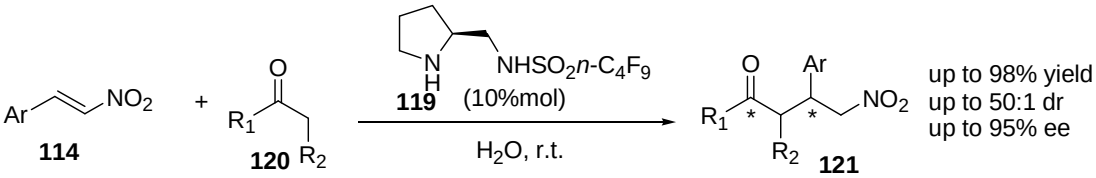


Scheme 36: Organocatalyzed Michael reaction promoted by **113**

More significantly, reactions with β-nitrostyrenes **114** bearing either electron-withdrawing or electron-donating substituents occurred with excellent levels of enantio- (94–99% e.e.) and diastereoselectivity (up to 50:1 d.r.). The generality of Michael addition reactions between ketones and nitro olefins promoted by (*S*)-pyrrolidine trifluoromethanesulfonamide **113** was also explored. Excellent levels of enantio- (97% e.e.) and diastereoselectivity (up to 50:1 d.r.) accompanied the reactions of cyclohexanone, but, in contrast, almost no reaction occurred in the cases of five and seven-membered ring cyclic ketones, presumably due to difficulties in the formation of enamines. The electronic nature of the substituents on the nitro olefins

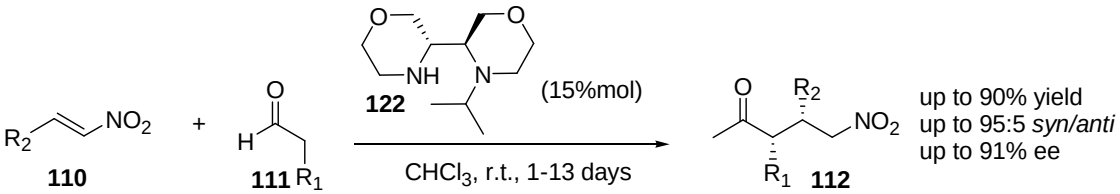
had no effect on stereoselectivity; excellent levels of enantio- (96–99% e.e.) and diastereoselectivity are observed (Scheme 36). More recently, Wang's group has reported[161] the recyclable and reusable fluorous (*S*)-pyrrolidine sulfonamide organocatalyst **119** for promotion of highly enantio- and diastereoselective Michael addition reactions of aldehydes and ketones with nitro olefins in water (Scheme 37).

They demonstrated that fluorous (*S*)-pyrrolidine sulfonamide is a robust catalyst that is effective in water and can be readily separated and reused without significant loss of catalytic activity and stereoselectivity.



Scheme 37: Organocatalyzed Michael reaction of ketones to nitrostyrene promoted by (S)-pyrrolidine sulphonamide **119**

Alexakis and co-workers[162] have developed new 3,3-bimorpholine **122** derivative for asymmetric conjugate additions of various aldehydes to different nitro olefins (Scheme 38).

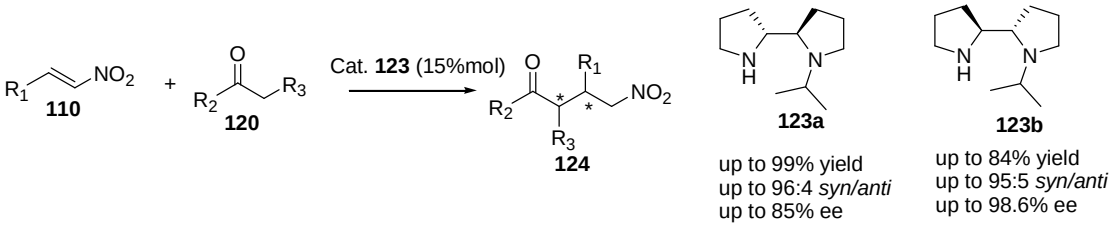


Scheme 38: 1,4-addition of aldehydes t nitro olefins catalyzed by bimorpholine **122**

Interestingly, the nature of aromatic nitro olefins had no influence either on the stereoselectivity or on the yield, but non-aromatic groups on the nitro olefins played a crucial role in terms of reactivity.

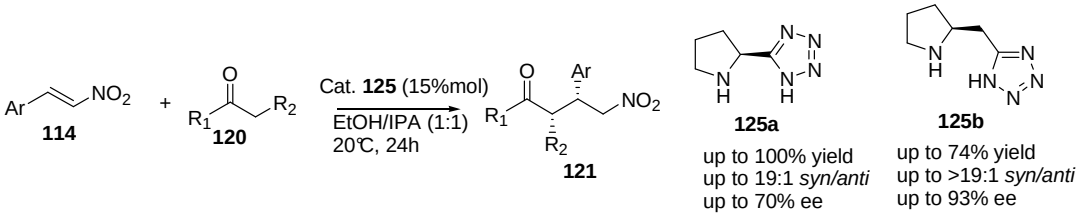
Although the stereoselectivity was maintained, the reactivity, and consequently the yield, decreased dramatically in the case of nitro olefins bearing saturated cyclic systems (e.g. R_2 = *c*Hex).

Furthermore Alexakis et al.[163] reported additions of aldehydes and ketones to nitrostyrene catalyzed by chiral 2,2-bipyrrolidines **123** (Scheme 39). The reaction with acetone gave non-negligible amounts of dinitro adduct, but the addition of a catalytic amount of *p*TSA (0.15 equiv.) completely eliminated the formation of this by-product and also produced an increase in the reaction rate. Catalytic Michael additions of unsymmetrical ketones such as methyl ethyl ketone and methyl propyl ketone raised the issue of regioselectivity.[163a]



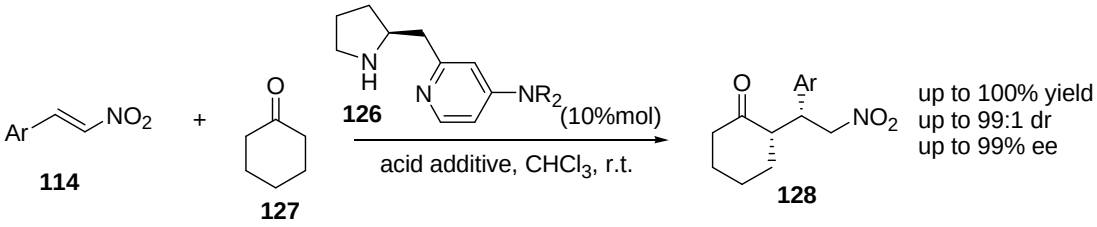
Scheme 39: 1,4-addition of unsymmetrical ketones to nitro olefins catalyzed by bipyrrolidines **123**

When the enamine was formed under kinetic conditions, the less hindered methyl group reacted preferentially, in a low to moderate regioisomeric ratio (43:57 and 33:67, respectively). In the presence of *p*TSA or the hydrochloride catalyst, the formation of the enamine under thermodynamic conditions inverted the regioselectivity to 74:26.[163a] Several advances in asymmetric additions of ketones to nitroolefins with the help of two proline derived tetrazole catalysts **125** have been discovered by Ley et al.[164](Scheme 40).



Scheme 40: Michael reaction between ketones and nitrostyrenes promoted by tetrazole-proline derived **125**

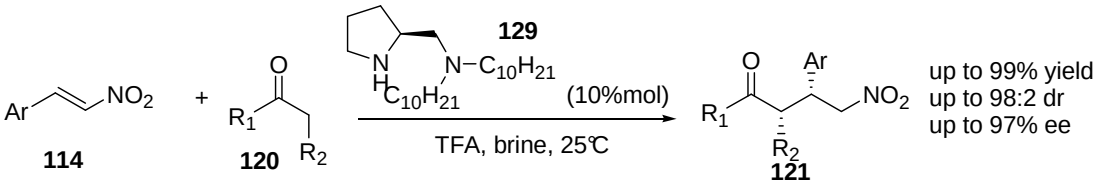
The results are a definite improvement on those previously reported in the literature for this reaction with proline.[158] These organocatalysts outperfom by far L-proline in terms of yield, enantioselectivity, and reaction times. (Scheme 40). Kotsuki et al.[165] have developed a new direct method for asymmetric Michael addition reactions of ketones to nitro olefins in the presence of new pyrrolidine-pyridine catalysts **126**, which are easily prepared from L-prolinol. The reaction was highly efficient in terms of productivity (up to 100% yield), enantioselectivity (up to 99% e.e.), and *syn* diastereoselectivity (up to 99:1 *syn*/*anti*, Scheme 41).



Scheme 41: 1,4-addition of cyclohexanone to nitrostyrene catalyzed by pyrrolidine-pyridine **126**

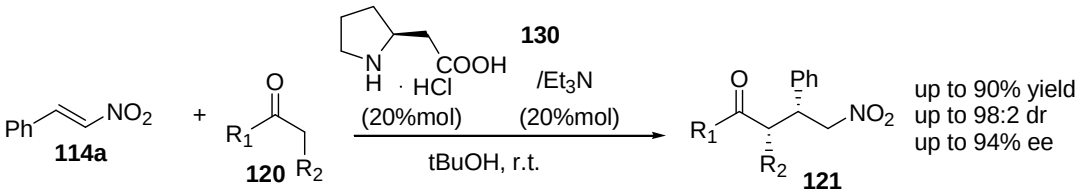
Since several studies had indicated that amine catalysts behave as initiators of polymerization, Barbas III et al.[166] very recently hypothesized that, if the anion intermediate derived from the addition of the amine catalyst to β-nitrostyrene could be stabilized, polymerization should be inhibited and the chemical yield should be improved. They found that the best results were obtained when brine

was used as a solvent, providing excellent yields and high levels of diastereo- and enantioselectivity.



Scheme 42: Michael addition of ketone to nitrostyrene catalyzed by diamine **129** in brine

Addition of TFA to the reaction in brine improved chemical yields by acceleration of enamine formation. The diamine/TFA bifunctional catalyst system **129** thus demonstrated excellent reactivity (up to 99% yield), diastereoselectivity (up to 98:2 d.r.), and enantioselectivity (up to 97% e.e.) in brine (Scheme 42). Oriyama et al.[167] envisaged that (*S*)-homoproline might catalyze highly enantioselective carbon–carbon bond formation and developed asymmetric Michael addition reactions of ketones to β-nitrostyrene and its derivatives in the presence of (*S*)-homoproline as a chiral organocatalyst **130** (Scheme 43). The reaction was highly diastereoselective (up to 98:2 d.r.) and enantioselective (over 90% e.e.).

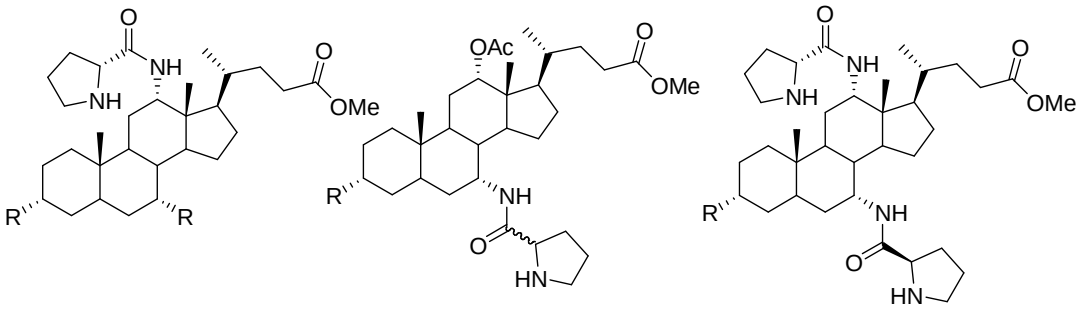


Scheme 43: 1,4-addition of ketone to nitrostyrene in presence of proline salt **130**

5.2 Bile acid derived organocatalysts in Michael reactions

An alternative approach to the achievement of high diastereoisomeric and enantiomeric excesses by means of substrate control can be realized using a proline derivative where the aminoacid moiety is a

part of a chiral cavity where the reaction takes place, as reported in Chapter IV. Since the amine catalyzed Michael addition of ketones to nitro olefins proceeds via an enamine intermediate, as the proline promoted aldol reaction, (see Chapter I) we reasoned that our class of organocatalysts could succeed also in this reaction. To verify this hypothesis cholic acid derived prolinamides and bis-prolinamides were checked as organocatalysts in the asymmetric Michael addition of cyclohexanone to aromatic nitro olefins.



B3a R: OAc, D-Pro
B3b R: OH, D-Pro

B4a (L-Pro) R: OAc
B4b (D-Pro) R: OAc
B4d (D-Pro) R: OH

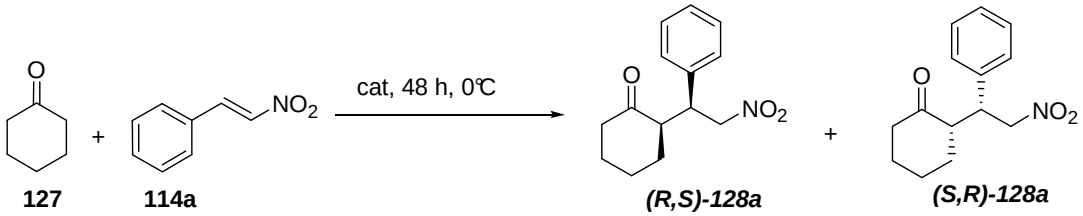
B5 R: OAc
B6 R: OH

These derivatives possess D or L-proline linked to the stereochemically demanding 7 and 12 positions of the cholic acid and free or protected hydroxyl groups to check the effect of these structural features on the outcome of the reaction.

Derivatives **B3-B6** were assayed as organocatalysts in the asymmetric Michael addition of cyclohexanone to β-nitrostyrene and the results obtained are reported in Table 12. The reactions were performed by stirring cyclohexanone and the organocatalyst in the solvent, then adding β-nitrostirene after 1 hour, and were interrupted usually after 48 h. All the reactions proceeded with complete diastereoselectivity in favour of the *syn* diastereoisomer. Derivative **B3a**, bearing a D-

prolinamide moiety linked to the 12-position, afforded, in dichloromethane at room temperature, the reaction product in 44% yield and 41% e.e. (entry 1). Better results were obtained using **B4b**, which possesses the same moiety linked at the 7-position: under the same reaction conditions the Michael adduct was obtained in 94% yield and 61% e.e. (entry 2). Lowering the catalyst loading gave rise to lower yield, without affecting the extent of asymmetric induction (entry 3). The use of toluene as reaction solvent gave a higher e.e. (entry 4) that further increases using lower catalyst loading (entry 5). Unfortunately, this afforded lower yield of the product, suggesting that the use of 10% catalyst loading is mandatory to have satisfactory amount of the Michael adduct. On lowering the temperature the extent of asymmetric induction increased and the product was obtained in 50% yield and 82% e.e. (entry 6). The use of the protic solvent 2-propanol gave poor results both in terms of yield and e.e. (entry 7), suggesting that the Michael addition is favoured by apolar or scarcely polar solvents. Derivative **B4a**, bearing a L-prolinamide moiety at the same position as **B4b**, gave worse results with respect to its diastereoisomer both in terms of yield and e.e. (entry 8), suggesting that the D-prolinamide moiety is in a matched relationship with the cholestanic backbone when linked at the 7-position. The absolute configuration of the prevailing enantiomer of the product depends on the absolute configuration of the prolinamide moiety: as a matter of fact the same prevailing enantiomer was obtained using **B3a** and **B4b**, whereas the sense of asymmetric induction was inverted when the reaction was promoted by **B4a**.

Table 12 Michael addition of cyclohexanone to trans-β-nitrostyrene in the presence of organocatalysts **B3**-**B6**.



Entry[a]	Cat.	Cat. loading (%mol)	Solvent	E.e. (%)[b]	A.C.[c]	Conv. (%)[d]
1	**B3a**	10	DCM	41	*(R,S)*	44
2	**B4b**	10	DCM	61	*(R,S)*	94
3	**B4b**	5	DCM	62	*(R,S)*	38
4	**B4b**	10	PhMe	70	*(R,S)*	80
5	**B4b**	5	PhMe	79	*(R,S)*	28
6	**B4b**	10[e]	PhMe	82	*(R,S)*	50
7	**B4b**	10	i-PrOH	23	*(R,S)*	8
8	**B4a**	10	DCM	34	*(S,R)*	65
9	**B4d**	10	DCM	60	*(S,R)*	50
10	**B4d**	10	PhMe	75	*(S,R)*	36
11	**B3b**	10	DCM	47	*(S,R)*	68
12	**B5**	5	DCM	56	*(R,S)*	97
13	**B5**	5	PhMe	50	*(R,S)*	75
14	**B5**	5	THF	10	*(R,S)*	31
15	**B6**	5	DCM	65	*(S,R)*	53
16	**B5**	5	EtOH	27	*(S,R)*	47
17	**B5**	5	i-PrOH	36	*(S,R)*	19

[a] Reaction conditions: cyclohexanone (2 eq.), trans-β-nitrostyrene (1 eq.), organocatalyst, solvent (1 mL), 48 h, r.t.

[b] Enantiomeric excess of the *syn* diastereoisomer (d.e.>98%, evaluated by ^{1}H NMR) determined by enantioselective HPLC: Chiralcel OJ, Hexane/2-propanol 97:3, 220 nm, 1mL/min. [c] Absolute configuration of the prevailing enantiomer determined by comparison with literature data.

[d] Determined by ^{1}H NMR.

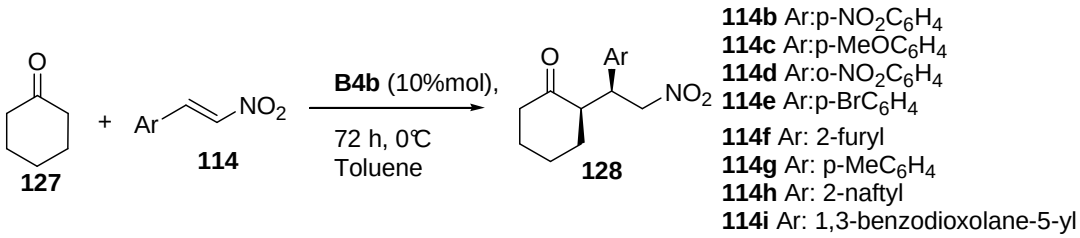
[e] Reaction performed at 0°C for 60 h.

It is to note that the same stereoselectivity inversion is observed using **B4d** (entry 9), which possesses a D-prolinamide moiety linked at the 7-position as **B4b** but has a free hydroxyl group at 3-position. In addition, this organocatalyst gave better results in terms of asymmetric induction with respect to **B4a**, which further improved using toluene as solvent (entry 10). These results suggest that free OH on the cholestanic backbone enters in the transition state leading to the product, changing the asymmetric induction mechanism. It is conceivable that this could happen only if the transition state is developed in the interior of the chiral cavity formed by the cholestanic backbone and the appended prolinamide moiety where the 3-OH group is directed. Changing of the asymmetric induction mechanism depending on the presence of free 3-OH group is observed also using **B3b**, which possesses a D-prolinamide moiety linked at 12-position (entry 11). It gave opposite prevailing enantiomer with respect to **B3a** (entries 1 and 11), although both derivatives possess a D-prolinamide moiety. These results point out the role of the chiral cavity of these organocatalysts in determining the stereochemical outcome of the Michael reaction independently of the position where the prolinamide moiety is located on the cholestanic backbone. Since **B4b** gave the best results both in terms of yield and e.e. of the product and gave the same prevailing enantiomer as **B3a**, we were interested in checking if two D-prolinamide moieties linked at 7-and 12-positions could work in a synergistic way, affording higher asymmetric induction. The bis-prolinamide derivative **B5** gave the reaction product in quantitative yield under a 5% catalyst loading (entry 12). Unfortunately, the e.e.

was slightly lower than that obtained using **B4b**, suggesting that the presence of two prolinamide moieties on the cholestanic backbone does not afford an improved organocatalyst with respect to **B4b**. On the contrary, the higher reaction rate and the very similar asymmetric induction extent suggest that the two moieties work independently as organocatalysts of the reaction. No better results were obtained by changing reaction solvent (entries 13 and 14). The sense of asymmetric induction still changes in passing from **B5** to **B6**, the analogue possessing a free OH group at 3-position (entry 15) and a slight improvement of the e.e. is also observed. The same enantioselectivity inversion is observed when polar protic solvents, such as ethanol or 2-propanol were used (entries 16 and 17): however, the poor results both in terms of yield and e.e. make scarcely interesting these reaction conditions.

The conditions giving the best results in the asymmetric Michael addition of cyclohexanone and β-nitrostyrene, i.e. 10% of **B4b** as organocatalyst in toluene at 0°C, were used to test the reaction with other aromatic nitro olefins and the results are reported in Table 13.

Table 13. Michael addition of cyclohexanone to aromatic nitroolefins **114** in the presence of organocatalysts **B4b**



Entry[a]	Ar	d.e.(%)[b]	Conv.(%)[b]	e.e.(%)[c]
1	**114b**	98	64	93[d]
2	**114c**	98	55	55[e]
3	**114d**	98	59	49[f]
4	**114e**	62	65	80[g]
5	**114f**	61	68	82[h]
6	**114g**	70	66	79[i]
7	**114h**	71	65	75[j]
8	**114i**	98	26	95[k]

[a] Reaction conditions: cyclohexanone (2 equiv.), nitroolefin (1 equiv.), organocatalyst (10% mol), toluene, 0°C, 72 h.

[b] Determined by ^{1}H NMR.

[c] Enantiomeric excess of the *syn* prevailing diastereoisomer determined by enantioselective HPLC

[d] Chiralcel OJ, 238 nm, 1mL/min, Isoct/Ipa 80:20

[e] Chiralpack AD, 238 nm, 1 mL/min, Hex/Ipa 95:5

[f] Chiralpack AD, 254 nm, 0.5 mL/min, Hex/Ipa 90:10

[g] Chiralpack AS, 238 nm, 1 mL/min, Hex/Ipa 90:10

[h] Chiralpack AD, 254,nm, 0.7 mL/min, Hex/Ipa 90:10

[i] Chiralcel OJ, 220 nm, 1 mL/min, Hex/Ipa 97:3

[j] Chiralpack AS, 254 nm, 0.7 mL/min, Hex/Ipa 55:45

[k] Chiralpack AS, 214 nm, 1 mL/min, Hex/Ipa 97:3

All the reactions were stopped after 72 hours for comparative purposes. Conversions ranging from 55 to 68% were obtained, with the only exception of substrate **114i** that gave poor yield of the

Michael adduct (entry 8), probably because the presence of an electron rich aromatic ring slows down the addition to a greater extent. The reaction is diastereoselective in favour of the *syn* diastereoisomer with all the substrates, but the extent depends on the nature of the nitro olefin. A general trend depending on the electronic character or the substitution pattern of the aromatic ring cannot be found: as a matter of fact complete diastereoselectivity was obtained with both electron-poor and electron-rich substrates (entries 1 and 2) or with substrates bearing substituent on different positions of the aromatic ring (entries 1, 3 and 8). The same considerations can be made with regard to the substrates giving lower diastereoisomeric excesses (entries 4-7). The e.e.s are moderate only for substrates **114c** and **114d** (entries 2 and 3). The other nitro olefins gave the Michael adducts with e.e.s ranging from 75 to 95%. Again a trend depending on electronic characteristics or substitution of the aromatic rings cannot be found and there is no correlation between diastereoselectivity and enantioselectivity. In fact, starting from olefin **114c** a Michael adduct with high diastereoisomeric excess but moderate e.e. was obtained (entry 2), whereas substrate **114f** afforded a product with moderate diastereoselectivity and good e.e. (entry 5).

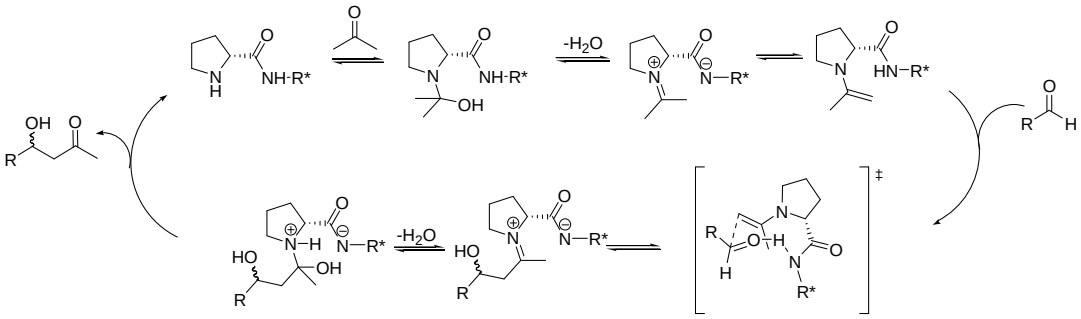
Chapter VI

Studies on B3b and its cyclopentanone enamine

6.1 NMR measurements

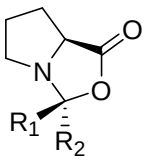
Given that the bile acid derived organocatalysts possess a prolinamide moiety, it can be reasonably assumed that the direct, asymmetric aldol reaction catalyzed by this new class of organocatalysts proceeds through the same mechanism reported in the literature for proline (Scheme 44).[168] According to this mechanism, both a basic site and an acidic proton are required for effective catalysis. In the case of bile acid derived prolinamides the transferred proton must originate from the amide group. This assumption is supported by the better results obtained using bile acid prolinamides with respect proline itself, suggesting that the amide group is able to form a strong hydrogen bond with an aldehyde and, as a result, a tight metal-free Zimmerman-Traxler-like transition state as the carboxylic functionality does. In particular L-proline affords aldol adduct of acetone to p-nitrobenzaldehyde in 24 h with 30-40% of organocatalyst with 68% of yield and 76% of enantiomeric eccess[143] while we obtained with **B3b** the same result in enantioselectivity and complete conversion in the same time with only 5% of catalyst loading (Chapter IV, table 6, entry 8). Good catalytic performance of our system compared to other alkyl amide can be explained by considering the importance of double activation[55] of aldehyde acceptor by hydrogen bonding with 12-NH amide and 7-OH groups attached to the cholestanic backbone. As far as the enantioselectivity is concerned, the presence of the bile acid chiral cavity plays an important role: in fact not only prolinamide bearing additional stereocenters leads to a very high stereocontrol in

aldol reaction,[55] as proposed by Gong et al., but also the development of the transition state at the inner of the cholestanic pocket helps the asymmetric induction process.



Scheme 44: Mechanism of the aldol reaction between acetone and aldehydes in the presence of a chiral prolinamide.

Nevertheless enamines are not the only transient species present in the reaction medium: in fact, several reports have highlighted the importance of the formation of bicyclic oxazolidinones, like **131** and **132** formed between the proline and the aldehyde or ketone.[169]



131 R_1=H, R_2=tBu
132 R_1=R_2=Me

List et al.[169a] established that such oxazolidinone formation leads to a parasitic consumption of the catalyst for the aldol reaction in DMSO, while Blackmond et al. showed that bicyclic oxazolidinone formation in the proline-catalyzed α-aminoxylation and α-amination reactions may actually make the catalyst more active by increasing the solubility of proline or itself can participate as a catalyst in the reaction.[170]

In order to get insights into the enamine formation starting from our organocatalysts and to find the traces of some transient species as an

aza-like oxazolidinone, we carried out ^{1}H-NMR studies on organocatalyst **B3b**

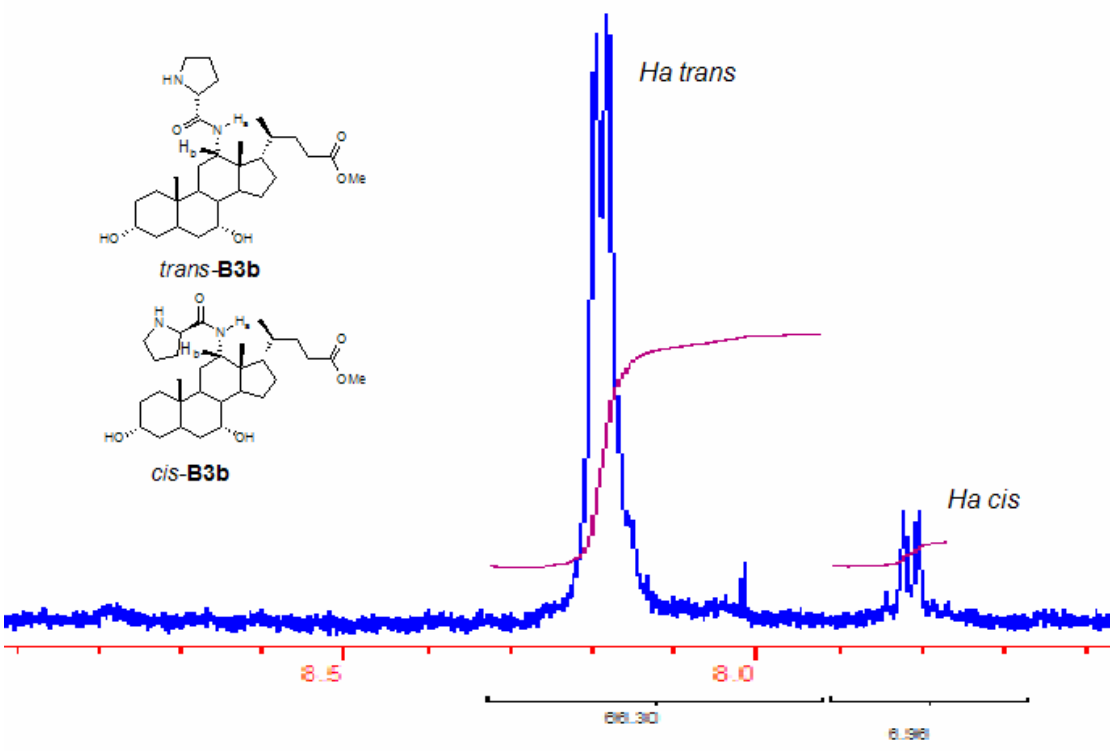


Figure 6: ^{1}H-NMR spectrum of **B3b** (600 MHz,$CDCl_3$), amide region.

The ^{1}H-NMR spectrum of **B3b**, recorded at 600 MHz in $CDCl_3$ as a solvent, shows in region between 7.5 and 9.0 ppm (Figure 6) two doublet signals at 7.8 ppm (J = 9.9 Hz) and at 8.2 ppm (J = 9.9 Hz) corresponding to the resonances of two amide protons. The same coupling constant suggests that they are due to the resonances of the amide protons of **B3b** *trans* and *cis* isomers, present in solution because of the restricted rotation around the amide bond. By comparison with literature data[171] it is possible to attribute the signal at 8.2 ppm to the resonance of the amide proton of the *trans* isomer; the integrated areas of the two signals gives a *trans:cis* 10:1 ratio (Figure 6).

Attempts at studying the formation of enamine in acetone-d_6, as reported by Gryko et al. for prolinethioamide,[76b] were unsuccessful because only the deuterium exchange of amide proton was observed. In addition, no appreciable difference between the spectra of **B3b** recorded in $CDCl_3$ and in acetone-d_6 is observed: this allows the formation of cyclic stable intermediates to be excluded, but, at the same time, suggests the presence of a fast equilibrium between **B3b** and the corresponding enamine, which prevents any NMR study concerning the enamine formation.

Therefore, in order to gain some information about the conformation of the enamines deriving from **B3b** we turned our attention to computational studies on these intermediates. In particular, given that different enantio and diastereoselectivities have been observed in the direct asymmetric aldol reaction between cyclopentanone and arylaldehydes, promoted by **B3b,** depending on the reaction solvent (dichloromethane or water), we were interested in gaining some information about the conformation of **B3b** and its cyclopentanone enamine in these solvents.

6.2 PCM in solvation modeling

In the literature, enamine formation and transition states for organocatalytic reaction have been studied quantitatively and qualitatively by DFT in vacuum.[172] Nowadays, only few pioneer works[173] study the influence of the solvent on the enamine formation,

because of the difficulty in calculating interactions between solvent and transition states.

A good model to introduce solvent effect in DFT calculation is the polarizable continuum model (PCM).[174] This simple approach describes solvation as a process composed by three part:

- Formation of a cavity in the solvent to host a molecule *M*
- Transport of *M* in the cavity
- Interaction between *M* and solvent

The first two points depend on the molecule only in part, being determined exclusively by the molecular geometry and not by its electronic structure. In general, a *cavitation free energy* (ΔG_{cav}), i.e. the isobaric work necessary to form the cavity that hosts the molecule *M*, is assigned to the first part; the second part affects the thermic component of free energy, i.e. the temporary freezing of the degree of translational freedom. The third contribute, which describes the interaction between solute and solvent, is the most important from a chemical point of view.

Solute-solvent interactions are in general classifiable as repulsions, dispersions and purely electrostatic forces: the first two can be described by an exact quantum model because they depend on the electronic exchange and the solute-solvent fast polarization due to electronic correlation; the electrostatic forces can be described by a classical model that takes into account the equilibrium reciprocal polarization between the charge density of a molecule and the charge density of the solvent.

PCM describes the solvent as a polarizable continuum dielectric medium; in particular this model is focused on the solute and consequently considers the solvent as a perturbation that can influence molecular properties. This model solves in a classical manner only the electrostatic part of the solvation problem: the contributes to free energy due to dispersion and repulsion forces and thermal and cavitation phenomena are described by semi-empirical models and, in a first approximation, do not affect solute behaviour. Moreover directional interactions (as H-bond) between solute and solvent are not straightforward to describe with such a method and need more complex techniques.

In contrast, molecular properties are mostly determined by electronic structure of the solute and the classical interaction between its charge density and, with no doubts, solvent is the most important contribution in the calculation of chemical properties (geometry, spectroscopic behaviour,…). In an accurate description it is possible to include in the PCM the first solvation sphere, with a complexity of calculation similar to that used for solute.

PCM divides the space in two different regions: a cavity that contains the molecule and where solvent molecules cannot enter, characterized by a dielectric constant ε_0 in vacuum, the bulky solvent, represented by its dielectric constant, ε. From electrostatic classical theory we know that in vacuum:

$$\nabla \cdot \mathbf{E} = 4\pi\rho_M$$

In a dielectric the equation was modified as follows:

$$\nabla \cdot \mathbf{D} = 4\pi\rho_M \quad \text{with} \quad \mathbf{D} = \varepsilon\mathbf{E}$$

From which derives:

$$\nabla(\varepsilon\mathbf{E}) = 4\pi\rho_M$$

But we have:

$$\mathbf{E} = -\nabla V$$

Then the problem is solved when we know the potential V(**r**), solution of the Poisson equation:

$$-\nabla\cdot(\varepsilon(\mathbf{r})\nabla V(\mathbf{r})) = 4\pi\rho_M(\mathbf{r})$$

This differential equation in the space can be transformed in a integral equation on a surface Γ, the interface between the solvent and the cavity. The integrated form of this equation has a general form like this:

$$\hat{\mathrm{A}}\sigma = \hat{\mathrm{M}}V_M$$

With $\hat{\mathrm{A}}$ and $\hat{\mathrm{M}}$ are two operators. Different forms of $\hat{\mathrm{A}}$ and $\hat{\mathrm{M}}$ give different types of PCM. Solution of the Poisson equation is used to build the perturbation in Hamiltonian of our molecular system:

$$\mathscr{H}_{eff}(\mathbf{R}) = \mathscr{H}_M(\mathbf{R}) + \mathscr{H}_{PCM}(\mathbf{R},\varepsilon) \quad \text{with} \quad \mathscr{H}_{PCM}(\mathbf{R},\varepsilon) = \int_\Gamma d\mathbf{s}\sigma(\mathbf{s})V_M(\mathbf{s})$$

PCM is an iterative method and, starting from a σ obtained by calculations program, it generates a corresponding V_M, calculates the $\mathscr{H}_{PCM}$ and the energy and then, starting from this new energy, it is possible to calculate another σ and so on.

6.3 Computational studies

In order to model qualitatively the *cis* and *trans* isomeric forms of catalyst **B3b,** and the corresponding enamines in dichloromethane and water, the solvents used to perform direct aldol reaction (see Chapter

IV), we carried out calculations on a local development version of the Gaussian03 package[175] at the density functional theory (DFT) level. For calculations in dichloromethane, the organocatalyst was described using B3LYP/6-31G(d,p) and solvent interactions were obtained by using IEF-PCM level[176]. For calculations in water, a hybrid approach[178] was used: six molecules of water were tentatively placed near the functional groups of **B3b** able to form H-bond, choosing a disposition that prevents the water molecules from colliding in a separate cluster. The system constituted by the organocatalyst and the water molecules was described at the Hartree-Fock level of theory with the smaller STO-3G basis, the external H-bond interacting part of the molecule was described, using B3LYP/6-31G(d,p) with ONIOM technique[177] and solvent interactions were obtained by using IEF-PCM. Relative free energies of *cis* and *trans* forms of organocatalyst **B3b** and the corresponding enamines in dichloromethane (DCM) and water are reported in Figure 6. As far as enamines are concerned the calculations were performed using as input geometries both their *syn* and *anti* isomeric forms. Whatever was the starting geometry, *syn* enamine showed the lowest free energy and hence only this one was considered in proposing a reasonable interpretation of experimental data.

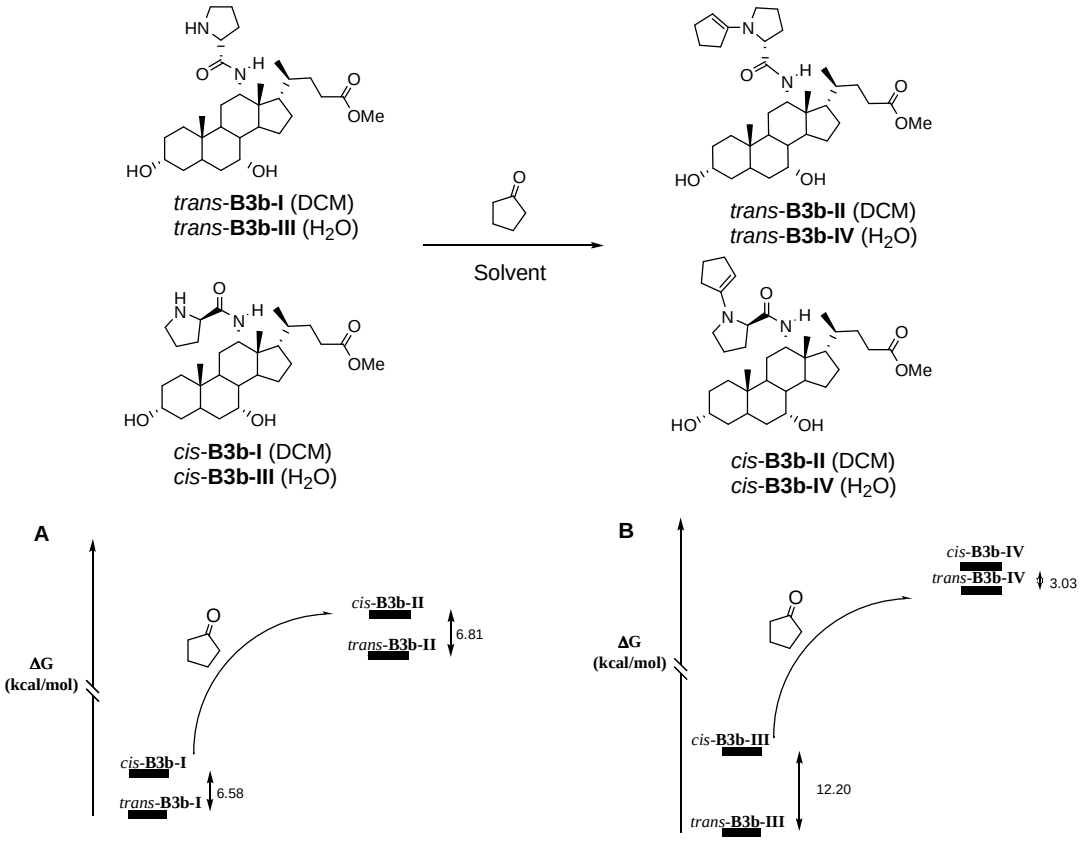


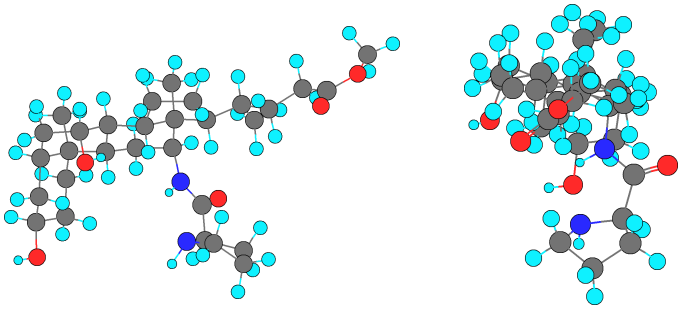
Figure 7: Free energy profiles for formation of enamines with cyclopentanone in DCM (**A**) and water (**B**) for **B3b**.

In accordance to ^{1}H-NMR measurements, calculations showed that the *trans* isomeric form of free organocatalyst **B3b** is favoured both in dichloromethane and in water (*trans*-**B3b-I** and *trans*-**B3b-III)**; the same result was obtained for the corresponding cyclopentanone enamines, *trans*-**B3b-II** and *trans*-**B3b-IV**. In particular, while in DCM the difference between the free energy of *trans* and *cis* forms is very similar for the organocatalyst and the corresponding isomeric enamines (Figure 7A), in the case of water the difference between the free energy of *trans* and *cis* forms is remarkably higher for the organocatalyst than for the corresponding enamines (Figure 7B).

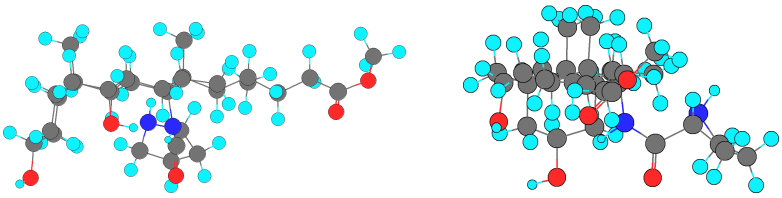
Moreover the free energy of *cis* and *trans* enamines are closer in water than in DCM and it can be reasonably assumed that the *cis* form of enamine was present in a major percentage, at the equilibrium, in the reactions performed in water.

Geometry optimization in DCM and water of both free organocatalysts and enamines gave the results reported in Figures 8 and 9.

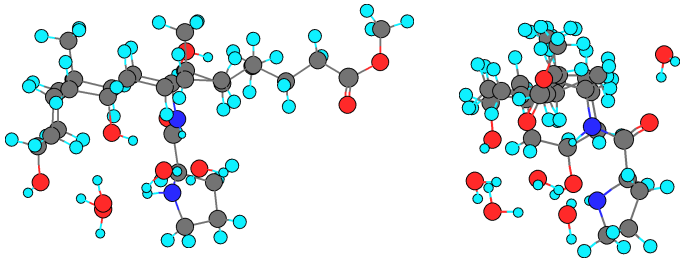
The most noticeable difference in the conformations of the two isomeric forms of the organocatalyst is represented by the different disposition of the proline moiety: *trans* form (*trans*-**B3b-I** and *trans*-**B3b-III**) is closed, with proline inside the semi-cavity of the cholestanic backbone in both solvents, even if in water a large part of this pocket is occupied by the cluster of water; *cis* form (*cis*-**B3b-I** and *cis*-**B3b-III**) is open, with proline moiety outside the pocket.



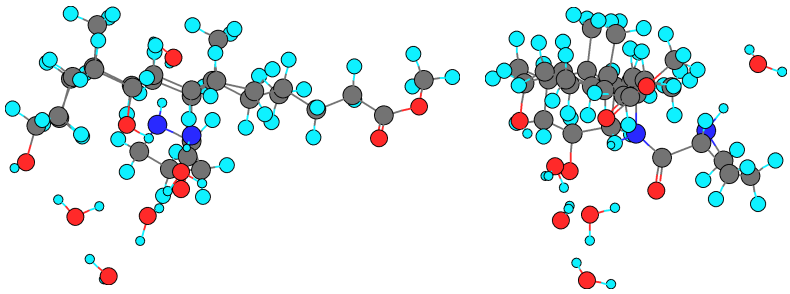
trans-**B3b-I**



cis-**B3b-I**



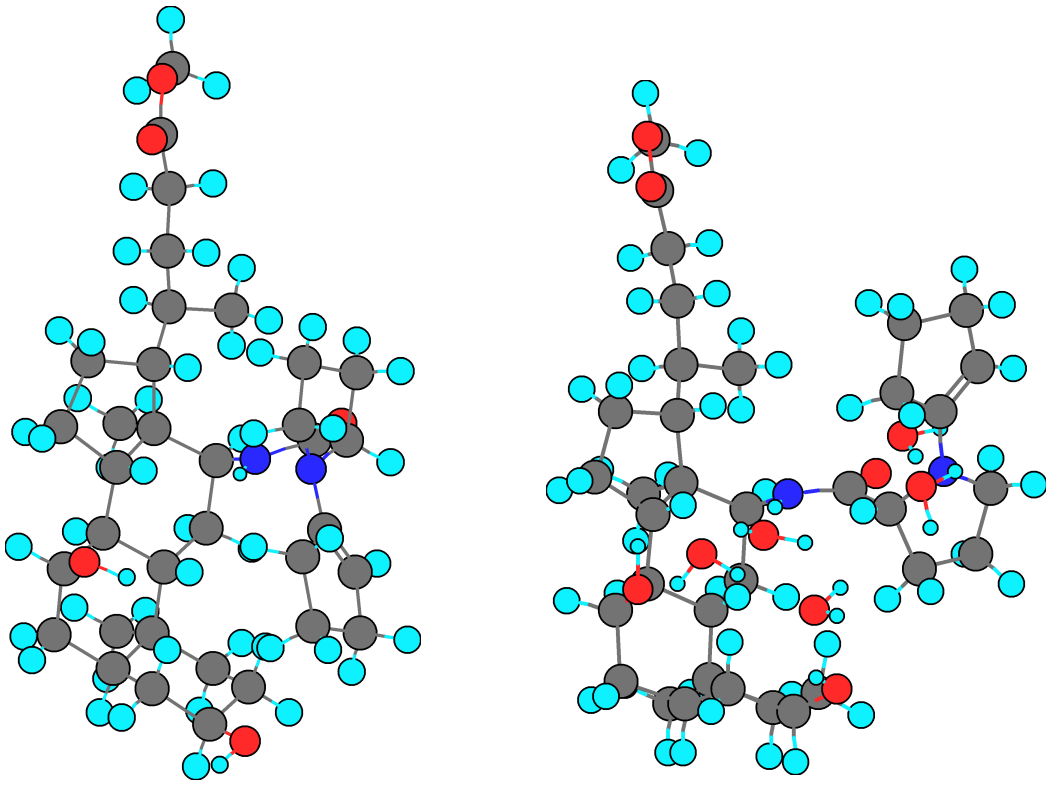
trans-**B3b-III**



cis-**B3b-III**

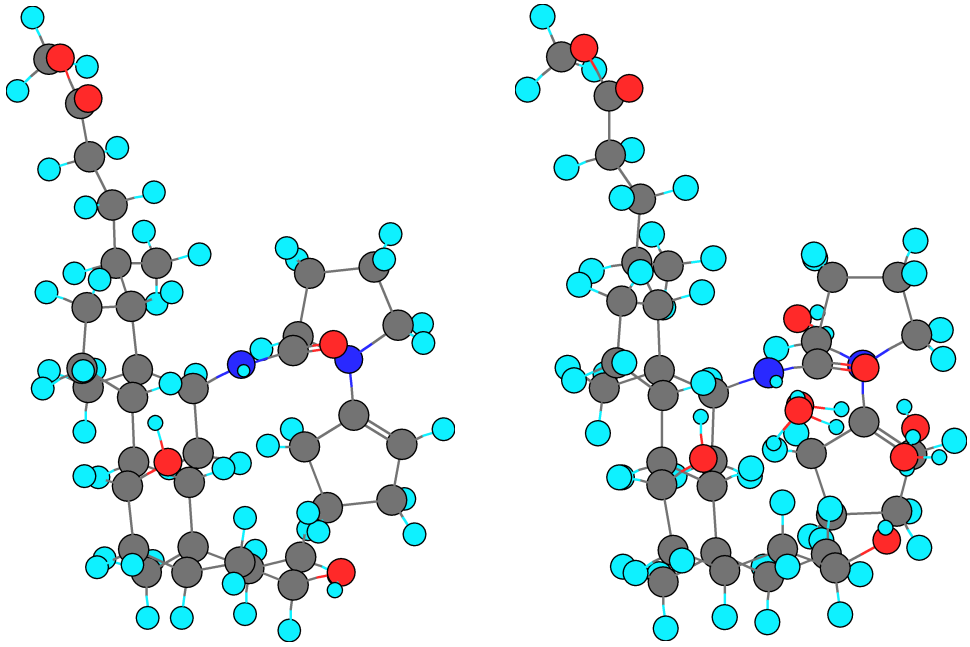
Figure 8: *Trans* and *cis* forms of free organocatalyst **B3b** in DCM (**B3b-I**) and in water with explicit water molecules (**B3b-II**) (C grey, H light blue, N blue, O red).

Enamine intermediates display a very interesting behaviour: *trans*-**B3b-II** in DCM presents the enamine moiety deeply buried in the semi-cavity of cholestanic backbone, with the cyclopentene ring near to 3-OH group, and 12-NH amide and 7-OH group being 3.55 Å apart (Figure 9A). On the contrary, intermediate *trans*-**B3b-IV** in water has a more open structure, with cyclopentene moiety near to carboxylic ester tail, and 12-NH amide and 7-OH group being 3.68 Å apart (Figure 9A). Enamines *cis*-**B3b-II** and *cis*-**B3b-IV** display quite a similar geometry, having an open structure with cyclopentene moiety next to 3-OH group. (Figure 9B)



trans-**B3b-II** *trans*-**B3b-IV**

Figure 9A: Trans isomeric form of enamines derived from organocatalyst **B3b** in DCM (**B3b-II**) and in water with explicit water molecules (**B3b-IV**) (C grey, H light blue, N blue, O red).



cis-**B3b-II** *cis*-**B3b-IV**

Figure 9B: Cis isomeric form of enamines derived from organocatalyst **B3b** in DCM (**B3b-II**) and in water with explicit water molecules (**B3b-IV**) (C grey, H light blue, N blue, O red).

This study on enamine formation can suggest a possible interpretation of the complete inversion of diastereoselectivity in the aldol reaction between cyclopentanone and aromatic aldehydes, on changing solvent from DCM to water, as reported in Chapter IV. The main products of the aldol reactions between aldheydes and cyclopentanone in DCM showed prevalently an *anti* configuration (Chapter IV Table 11): according to this experimental results and with empirical rule proposed by List et al.,[173a] **TS-1** (Figure 10) can be proposed as a reasonable pathway for the aldol reaction in dichloromethane: this transition state is obtained by the interaction between aromatic aldehydes and *trans*-**B3b-II** form of enamine, energetically more accessible, as shown in Figure 6A; both position of the NH and OH groups and steric hindrance of the cholestanic backbone favour the

attack of aromatic aldehydes “from the back” , which gives rise to the *anti* configuration of the final product.

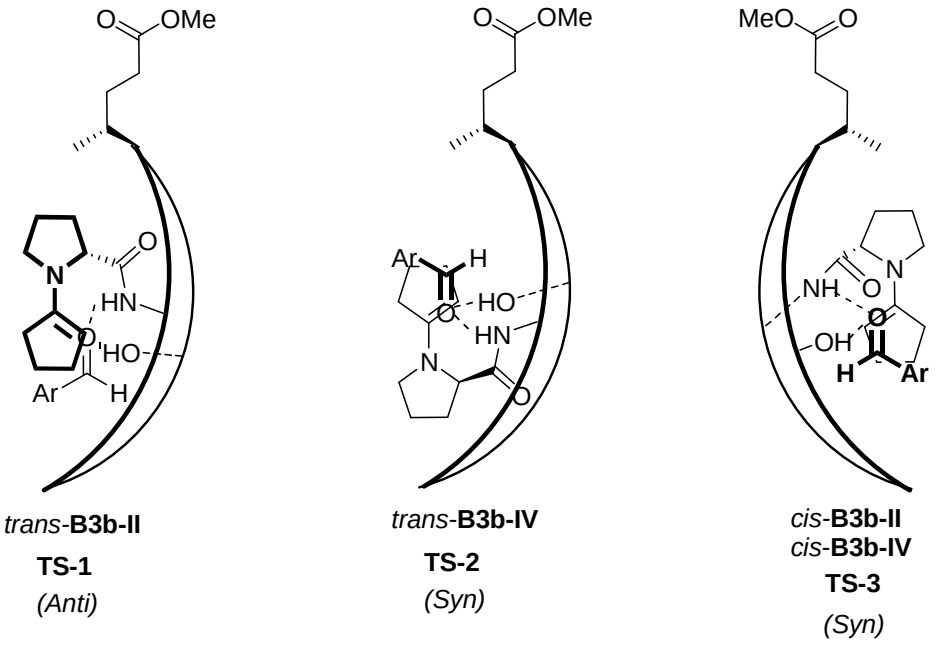


Figure 10: Proposed mechanism for change of diastereoselectivity for cyclopentanone: for clarity, cholestanic backbone was represented as a crescent-shaped system with OH, amide and enamine moiety, and tail as explicit groups. Objects near the observer are represented in bold style.

In order to explain the inverse diastereoselectivity in the case of ortho-substituted aromatic aldehydes in DCM (Chapter IV table 11, entries 6,7) we can hypothesize that for these substrates the energetically disfavoured *cis*-**B3b-II** of organocatalyst, is more active in a catalytic cycle: in fact, even if *trans*-**B3b-II** is the most energetically accessible configuration of the organocatalyst, its geometry is closed and interactions with much sterically demanding 2-chloro- or 2-nitro-benzaldehydes can be prevented because of the little space in the semi-cavity. Space filling representations of *trans*-**B3b-II** show that these aldehydes hardly approach the active site of the organocatalyst, placing the aromatic cycle in the cavity (Figure 11).

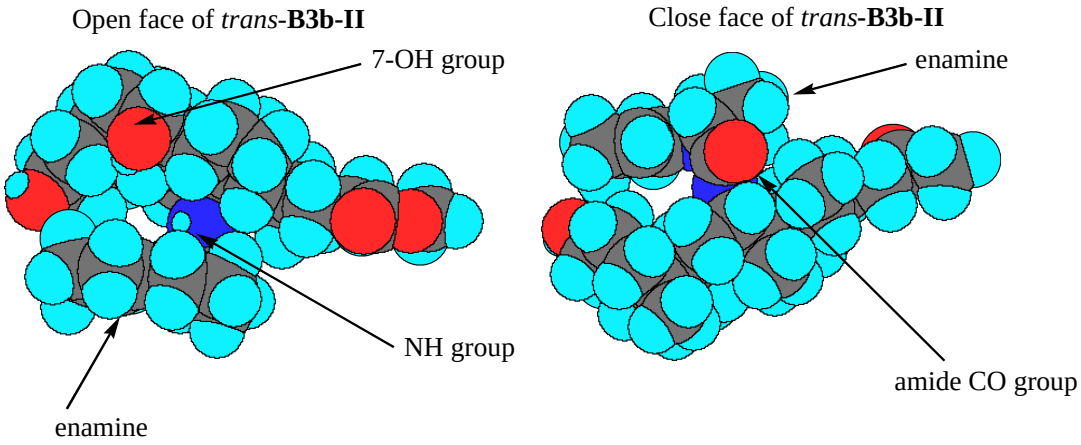


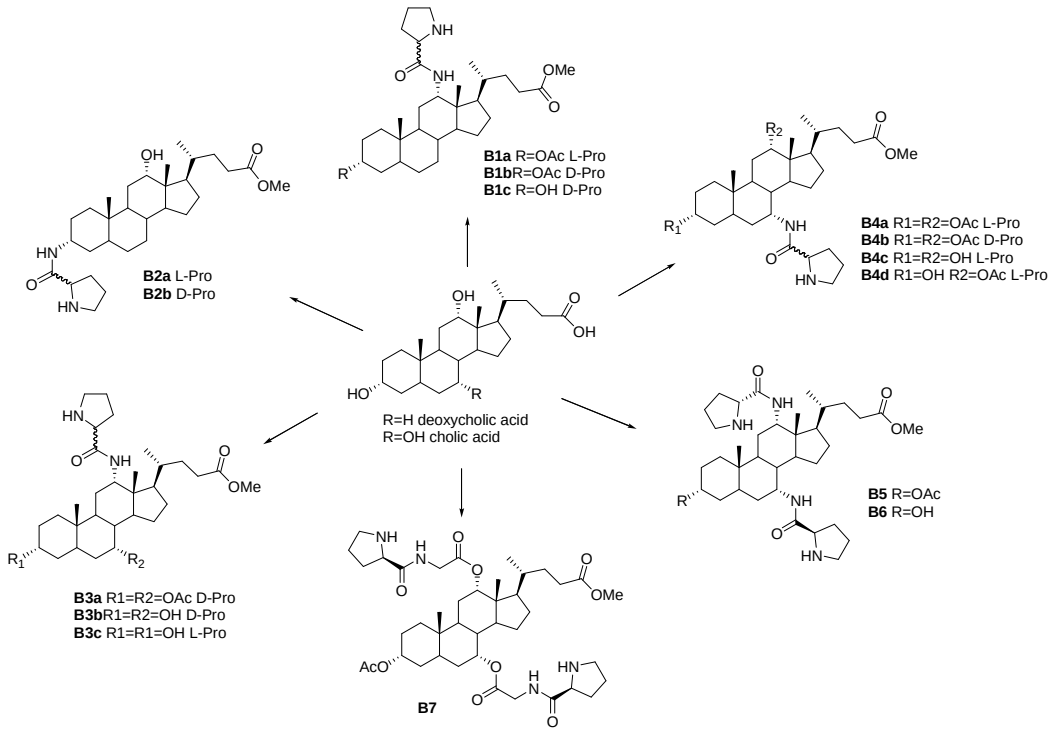
Figure 11: Space filling representations of enamine trans-**B3b-II** (C grey, H light blue, N blue, O red).

In this case probably only the cavity of *cis*-**B3b-II** possesses enough space for this substrate, as can be seen in **TS-3** (Figure 10), and this fact can explain the prevalence of the *syn* product.

On the contrary, aldol reaction in water shows prevalently *syn* diastereoselectivity (Chapter IV, table 11) and we can assume that the reaction follows the pathway described by transition states **TS-2** and **TS-3**: in fact, in water both *trans*-**B3b-IV** and *cis*-**B3b-IV** present a direction of attack shielded by the cholestanic backbone and the position of NH and OH groups in this case favours the formation of a *syn* product. As obtained by the geometry optimization, we can also try to explain the different behaviour of benzaldehyde (table 11, entry 12), assuming that this little substrate does not suffer the steric effect of the cholestanic backbone and it can attack enamine also on the hindered side, affording prevalently the *anti* product.

Conclusions

In this PhD Thesis, the synthesis of proline derivatives of bile acids as a new class of organocatalysts has been proposed. These organocatalysts were able to promote the direct asymmetric aldol reaction and the asymmetric Michael reaction affording the products in good yield and high enantiomeric excesses. A computational study on the best performing organocatalyst in the aldol reaction was also performed. On the basis of the results obtained the following conclusions can be drawn.



Monoprolinamide and bis-prolinamide of bile acids were obtained in good yield by simple, selective and low cost synthetic methods. In particular, the yield of the reaction between Boc-proline and 12-amino derivatives of bile acids was improved, by using

optimized procedures that overcome problems due to the steric demand of this position.

Both activity and enantioselectivity of this new family of organocatalysts in the asymmetric aldol reaction between cyclic or acyclic ketones and aromatic aldehydes depend not only on the absolute configuration of the proline moiety but also on the position where this one is linked to the cholestanic backbone. As already observed with other kind of chiral auxiliaries derived from bile acids, the derivatives bearing the proline moiety at the position 12 of the steroid skeleton (**B3a-c**) are the most active and enantioselective. In addition, the presence of free OH groups at 3- and 7-positions of cholic acid affords the most efficient organocatalyst, **B3b**, able to promote the asymmetric aldol reaction between acetone and 4-nitrobenzaldehyde, even with a very low catalyst loading (2%), with e.e. up to 80%. These results confirm the central role of the cholestanic chiral pocket on the asymmetric induction process: its concave structure endowed with the proline moiety and hydroxyl groups gives rise to a chiral environment able to host and to orientate substrates in the transition state, determining the formation of a more energetically favourable and stereoselective pathway that results in good enantiomeric excesses.

The use of bis-prolinamide derivatives **B5** and **B7** as organocatalysts in the asymmetric aldol reaction suggests that a synergistic action of the two proline moieties linked to the cholestanic backbone is obtained depending on their distance. As a matter of fact compound **B5** displays low activity and selectivity, quite similar to

proline, suggesting that the proline moieties at 12 and 7 positions are too distant and act independently of each other, because of the rigid structure of cholic acid that does not allow them to cooperate in the transition state of the aldol reaction. On the contrary, organocatalyst **B7,** possessing a spacer between proline and cholestanic backbone that increases mobility of proline moieties and allows them to cooperate in the transition state, gives good results both in terms of yield and enantioselectivity (e.e. up to 67%), carrying out the reaction in organic solvents and in the presence of acid additives.

The bile acid derivative **B3b** is an efficient organocatalyst also for the asymmetric direct aldol reaction of cyclic ketones and aromatic aldehydes, affording aldol products in good yield and e.e.s up to 98%. This organocatalyst showed high versatility, giving good results both in water and in organic solvent, where it works also when used at very low catalyst loading (1%), without loss of stereoselectivity, conditions that rarely afford good results. In addition, organocatalyst **B3b** affords also the highest diastereoisomeric ratios, reported until now, in the aldol reactions of cyclopentanone. The sense of asymmetric induction is determined by the absolute configuration of the proline moiety and, when cyclopentanone is used as donor carbonyl, it depends also on the solvent. This unusual behaviour, which can be likely attributed to a change of asymmetric induction mechanism depending on the solvent, allowed both enantiomers of an aldol product to be obtained starting from the same chiral organocatalyst, simply by changing the reaction solvent.

Screening of different prolinamide and bis-prolinamide derivatives of cholic acid as organocatalysts in the asymmetric Michael addition of cyclohexanone not only allowed us to find a chiral system able to afford satisfactory yields of the products and e.e.s up to 95% (for **B4b** organocatalyst) but also pointed out again the role of the chiral cavity constituted by the cholestanic backbone and the appended prolinamide moieties in determining the stereochemical outcome of the reaction. As a matter of fact, the presence of a free hydroxyl group, which can interact with the substrate only in a transition state developed at the inner of the cavity, gives rise to the change of the sense of asymmetric induction, with respect to the corresponding systems having a protected hydroxyl group. This result has also a practical implication given that deprotection of the OH group is easily realized, so that it is possible to obtain both enantiomers of the Michael adducts starting from the same chiral architecture.

DFT calculations on organocatalyst **B3b** and its cyclopentanone enamine using the polarizable continuum model approach gave the most populated conformations of the two systems in dichloromethane and in water. According to NMR data, calculations found two different isomeric forms of **B3b**, originating from the restricted rotation around the amide bond: the *trans* form is more stable than the *cis* one and the extent of this difference depends on the solvent. Minimum energy conformations of the corresponding enamines obtained starting from *trans* and *cis* forms of organocatalyst **B3b,** display different free energy and the extent of the difference again

depends on the solvent: in DCM the free energy gap between *trans* and *cis* forms of **B3b** and the gap between *trans* and *cis* forms of the enamine is the same, whereas in water this gap is much greater for **B3b** than for enamine. In addition, only the *syn* form of the enamine is energetically favoured in both solvents. On the basis of these calculations a model of the transition state of the aldol reaction between cyclopentanone and arylaldeyde was proposed, which explains the results obtained, in terms of diastereoselectivity, in the asymmetric direct aldol reaction between cyclopentanone and aromatic aldehydes.

Chapter VII

Experimental section

7.1 General procedures and materials

TLC analyses were performed on silica gel 60 sheets; flash chromatography separations were carried out on columns using silica gel 60 (230–400 mesh). Toluene was refluxed over sodium and distilled before the use. THF was refluxed over Na/K alloy and distilled before use. CH_2Cl_2, triethylamine, pyridine and N-methylmorpholine were refluxed over CaH_2 and distilled before use. MeOH was refluxed over magnesium methoxide and distilled before the use. Acetic anhydride was distilled before the use. NBS was recrystallized from water before the use. Zn powder was washed with concentrated HCl, water, acetone, diethylether in that order before the use. MeONa was prepared immediately before the use with dry MeOH and metallic Na at 0°C. Glacial acetic acid was used. Unless otherwise specified, the reagents were used without any purification.

7.2 Instrumentation

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Varian Gemini-300 300 MHz NMR spectrometer, using TMS as external standard. The following abbreviations are used: s= singlet, d=doublet, dd=double doublet, t=triplet, m=multiplet, br=broad. HPLC analyses were performed on a JASCO PU-980 intelligent HPLC pump equipped with JASCO UV-975 detector. Optical rotations were measured with a JASCO DIP-360 digital polarimeter. Melting points were taken using a Kopfler Reichert-Jung apparatus. IR spectra were recorded on a Perkin-Elmer 1710 spectrophotometer.

7.3 Synthesis of monoprolinamide derivatives.

Synthesis of amine 81

Methyl 3α-acetyloxy-12α-hydroxy-5β-cholan-24-oate Hydrated 4-toluenesulphonic acid (500 mg, 3 mmol) was added to a solution of deoxycholic acid **45** (5 g, 12.72 mmol) in AcOMe (150 mL) and water (1.5 mL).The reaction mixture was stirred under reflux for 24 h. The colourless solution was then poured in $NaHCO_3$ 5% (200 mL), the organic product was extracted with CH_2Cl_2 (6x40 mL) and the organic solution dried over anhydrous Na_2SO_4. After removing the solvent under vacuum, the crude product was purified by column chromatography (SiO_2, CH_2Cl_2:acetone 85:15) affording methylester (3.8 g, 67% yield). Chemical-physical and spectroscopic data were identical to those reported in the literature.[119]

Methyl 3α-acetyloxy-12-keto-5β-cholan-24-oate A solution of $K_2Cr_2O_7$ (2.62 g, 8.89 mmol) in water (5 mL) was added to a solution of the previous compound (3.8 g, 8.47 mmol) in acetic acid (170 mL). The mixture was stirred at room temperature for 20 h, then poured into water (350 mL): the solid was filtered, washed with water then dried under vacuum affording chemically pure keton (3,40 g, 90% yield). Chemical- physical and spectroscopic data were identical to those reported in the literature.[119]

Methyl 3α-acetyloxy-12-oxime-5β-cholan-24-oate Sodium acetate trihydrated (5.70 g, 41.86 mmol) and hydroxylamine hydrochloride

(951 mg, 13.70 mmol) were added to a solution of the previous keton (3.40 g, 7.61 mmol) in methanol (70 mL). The reaction mixture was stirred under reflux for 4.5 h. The solvent was removed under vacuum and the residue dissolved in CH_2Cl_2 (30 mL). The organic solution was washed with water (4x20 mL) then dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the crude product was crystallized from methanol affording chemically pure oxime (3.40 g, 88% yield). Chemical-physical and spectroscopic data were identical to those reported in the literature.[119]

Methyl 3α-acetyloxy-12α-amino-5β-cholan-24-oate Hydrated PtO_2 (184 mg, 0.81 mmol) was added to a solution of the previous oxime (3 g, 6.50 mmol) in glacial acetic acid (5 mL) and the mixture was stirred under H_2 (2 bar) at room temperature for six days. The solid was filtered off and powdered Zn (3.48 g, 53.17 mmol) was added to the solution, concentrated under vacuum to 2 mL. The mixture was stirred at room temperature for 12 h then the solid was filtered and washed with acetic acid. After concentration under reduced pressure, water (60 mL) was added and the aqueous solution was made basic with KOH pellets. The organic product was extracted with ethyl acetate (6x30 mL) and the organic extracts dried (Na_2SO_4). The crude product was purified by column chromatography (SiO_2, CH_2Cl_2:acetone 90:10) affording amine **81** (2 g, 70% yield). Chemical-physical and spectroscopic data were identical to those reported in the literature.[119]

Synthesis of amine 82

Methyl 3α,12α-dihydroxy-5β-cholan-24-oate To a solution of deoxycholic acid **45** (5.00 g, 12.24 mmol) in anhydrous THF (25 mL), DBU (1.83 mL, 12.24 mmol) and CH_3I (4.80 mL, 77.11 mmol) were added and the resulting mixture was stirred under reflux for 10 h and at r.t. overnight. The reaction mixture was concentrated under reduced pressure, dissolved in CH_2Cl_2, washed with $NaHCO_3$ 5% (25 mL), $Na_2S_2O_3$ 30% (50 mL, to eliminate iodine) and then with water (3x25 mL); organic phases were dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum affording chemically pure methyl ester (4.39 g, 84% yield). Chemical-physical and spectroscopic data were identical to those reported in the literature.[119]

Methyl-12α-hydroxy-3β-mesyl-5β-cholan-24-oate

Methanesulphonic acid (1.5 mL, 23.0 mmol) was added to a solution of deoxycholic acid methylester (4.39 g, 10.8 mmol) and tryphenylphosphine (8.47 g, 33.0 mmol) in anhydrous THF (40 mL). The solution was stirred at 40°C and DEAD (toluene solution 40%, 15 mL) was added dropwise during 20 min. The resulting mixture was stirred at 40°C for 24 h. After removing solvent under reduced pressure, the crude product was purified by column chromatography (SiO_2, CH_2Cl_2: acetone 92:8) affording the pure product (2.10 g, yield 43%). Chemical-physical and spectroscopic data were identical to those reported in the literature.[119]

Methyl-12α-hydroxy-3α-azido-5β-cholan-24-oate Sodium azide (2.00 g, 31.0 mmol) was added to a solution of the previous mesylate (2.00 g, 4.13 mmol) in DMF (20 mL). The resulting mixture was stirred 40°C for 48 h, then water was added and the reaction was extracted with diethyl ether; the organic phases were washed with water and dried over anhydrous sodium sulphate. After removing solvent under reduced pressure, the crude product was purified by column chromatography (SiO_2, CH_2Cl_2: acetone 93:7), affording pure azide (1.78 g, quantitative yield). Chemical-physical and spectroscopic data were identical to those reported in the literature.[119]

Methyl-12α-hydroxy-3α-amino-5β-cholan-24-oate A mixture containing the previous azide (1.78 g, 4.13 mmol) and Pd/C 5% (190 mg) in ethyl acetate and methanol (1:2) was stirred for 24 h at r.t. under H_2 pressure (8.5 atm). After filtering off catalyst and removing solvent under reduced pressure, the pure amine **82** was obtained (1.52 g, 91% yield). Chemical-physical and spectroscopic data were identical to those reported in literature.[119]

Synthesis of amine 91

Methyl 3α,7α,12α-trihydroxy-5β-cholan-24-oate 83 To a solution of cholic acid **46** (5.00 g, 12.24 mmol) in anhydrous THF (25 mL) DBU (1.83 mL, 12.24 mmol) and CH_3I (4.80 mL, 77.11 mmol) were added and the resulting mixture was stirred under reflux for 10 h and then at r.t. overnight. The reaction mixture was concentrated under

reduced pressure, dissolved in CH_2Cl_2, washed with $NaHCO_3$ 5% (25 mL), $Na_2S_2O_3$ 30% (50 mL, to eliminate the iodine) and then with water (3x25 mL); the organic phases were dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum affording chemically pure **83** (3.3 g, 64% yield). Chemical-physical and spectroscopic data were identical to those reported in the literature.[136h]

Methyl 3α,7α-diacetoxy-12α-hydroxy-5β-cholan-24-oate 84 A solution of methylester **83** (3.6 g, 8.52 mmol), pyridine (5 mL, 62 mmol) and acetic anhydride (5 mL, 53 mmol) in dry toluene (20 mL) was stirred at r.t. for 24 h and then poured into water (100 mL). The organic phase was separated and washed with water (2x50 mL), $NaHCO_3$ 5% (3x50 mL) and again with water and finally dried over anhydrous Na_2SO_4. The crude product was purified by flash chromatography (SiO_2, CH_2Cl_2:acetone 95:5) affording pure **84** (3.1 g, 72% yield). Mp 76-77°C. $[\alpha]^{22}_D$ + 0.34 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.66 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 0.95 (d, 3H, 21-CH_3), 0.90-2.40 (m, 28H, steroidal CH and CH_2), 1.99 (s, 3H, 3-CH_3CO), 2.03 (s, 3H, 7-CH_3CO), 3.63 (s, 3H, CH_3OCO), 3.97 (br t, 1H, 7-CH), 4.55 (m, 1H, 3-CH), 4.86 (br d, 1H, 12-CH), 5.03 (br s, OH). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.7, 17.6, 21.7, 21.9, 22.8, 23.2, 26.9, 27.5, 28.4, 28.8, 31.1, 31.2, 32.5, 34.6, 34.7, 35.0, 35.2, 38.3, 41.2, 42.3, 46.8, 47.4, 51.7, 71.1, 72.9, 74.3, 75.6, 170.6 (acetate C=O), 170.9 (acetate C=O), 174.8 (24 C=O). IR (KBr, cm^{-1}): 2948, 2870, 1773 (C=O), 1733 (C=O), 1711 (C=O), 1655, 1560, 1458,

1380, 1363, 1256,1027, 891. Anal. Calcd for $C_{29}H_{46}O_7$: C 68.64, H 9.15. Found: C 68.70, H 9.11.

Methyl 3α,7α-diacetyloxy-12-keto-5β-cholan-24-oate 85 A solution of $K_2Cr_2O_7$ (1.28 g, 4.35 mmol) in water (5 mL) was added to a solution of methyldiacetylester **84** (2.1 g, 4.14 mmol) in acetic acid (50 mL). The mixture was stirred at room temperature for 20 h, then poured into water (200 mL): the solid was filtered, washed with water then dried under vacuum affording chemically pure keton **85** (1.81 g, 87% yield). Mp 164-165°C. $[\alpha]^{22}_D$ + 0.76 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.80 (d, 3H, 21-CH_3), 0.98 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 0.90-2.55 (m, 28H, steroidal CH and CH_2), 1.97 (s, 3H, 3-CH_3CO), 1.98 (s, 3H, 7-CH_3CO), 3.61 (s, 3H, CH_3OCO), 4.52 (m, 1H, 3-CH), 4.94 (br t, 1H, 7-CH). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 11.7, 18.7, 21.6, 21.6, 22.3, 23.9, 26.7, 27.5, 30.6, 31.4, 31.5, 34.7, 35.1, 35.7, 35.7, 37.8, 38.0, 38.0, 40.6, 46.5, 51.6, 53.3, 57.2, 70.8, 73.7, 170.3 (acetate C=O), 170.8 (acetate C=O), 174.7 (24 C=O) 182.1 (12 C=O). IR (KBr, cm^{-1}): 2948, 2869, 1734 (br C=O), 1700 (C=O),1553, 1470, 1436, 1358, 1245,1228, 1149, 1060, 1021, 964, 937. Anal. Calcd for $C_{29}H_{44}O_7$: C 69.02, H 8.79. Found: C 69.07, H 8.83.

Methyl 3α,7α-diacetyloxy-12-oxime-5β-cholan-24-oate 89 Sodium acetate trihydrate (2.37 g, 17.44 mmol) and hydroxylamine hydrochloride (397 mg, 5.71 mmol) were added to a solution of keton **85** (1.60 g, 3.17 mmol) in methanol (37 mL). The reaction mixture

was stirred under reflux for 4.5 h. The solvent was removed under vacuum and the residue dissolved in CH_2Cl_2 (30 mL). The organic solution was washed with water (4x20 mL) then dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the crude product was crystallized from methanol affording chemically pure oxime **89** (1.60 g, 97% yield). Mp 94-96°C. $[\alpha]^{22}_D$ + 1.30 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.91 (s, 3H, CH_3), 0.92 (d, 3H, 21-CH_3), 1.00 (s, 3H, CH_3), 0.90-2.40 (m, 28H, steroidal CH and CH_2), 2.00 (s, 3H, 3-CH_3CO), 2.03 (s, 3H, 7-CH_3CO), 3.35 (br dd, 1H, NOH), 3.64 (s, 3H, CH_3OCO), 4.57 (m, 1H, 3-CH), 4.92 (br d, 1H, 7-CH). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.2, 19.2, 19.9, 21.6, 21.7, 22.3, 23.8, 26.8, 28.2, 30.7, 31.5, 34.7, 35.2, 35.6, 35.8, 36.0, 38.2, 40.9, 47.0, 49.7, 51.7, 53.7, 70.9, 74.0, 165.9 (C=NOH), 170.5 (acetate C=O), 170.9 (acetate C=O), 174.9 (24 C=O). IR (KBr, cm^{-1}): 2949, 2868, 1734 (br C=O), 1714 (C=O),1557, 1537, 1502, 1472, 1457, 1437, 1376, 1361, 1250, 1230, 1169, 1059, 1023, 963, 917. Anal. Calcd for $C_{29}H_{45}NO_7$: C 67.03, H 8.73, N 2.70. Found: C 67.05, H 8.80, N 2.50.

Methyl 3α,7α-diacetyloxy-12α-amino-5β-cholan-24-oate. **91** Hydrated PtO_2 (69.25 mg, 0.31 mmol) was added to a solution of oxime **89** (1.27 g, 2.44 mmol) in glacial acetic acid (5 mL) and the mixture was stirred under H_2 (2 bar) at room temperature for six days. The solid was filtered off and powdered Zn (1.31 g, 19.96 mmol) was added to the solution, concentrated under vacuum to 2 mL. The mixture was stirred at room temperature for 12 h then the solid was filtered off and washed with acetic acid. After concentration under

reduced pressure, water (60 mL) was added and the aqueous solution was made basic with KOH pellets. The organic product was extracted with ethyl acetate (6x30 mL) and the organic extracts dried (Na_2SO_4). The solvent was evaporated under vacuum affording amine **91** (1 g, 81% yield). Mp 57-58°C. $[\alpha]^{22}_D$ + 0.35 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.70 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.95 (d, 3H, 21-CH_3), 0.90-2.40 (m, 28H, steroidal CH and CH_2), 2.00 (s, 3H, 3-CH_3CO), 2.04 (s, 3H, 7-CH_3CO), 3.16 (br t, 1H, 12-CH), 3.64 (s, 3H, CH_3OCO), 4.56 (m, 1H, 3-CH), 4.86 (br d, 1H, 7-CH). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.7, 17.4, 21.6, 21.8, 22.7, 23.3, 27.0, 27.7, 28.2, 28.9, 31.1, 31.3, 31.5, 34.6, 34.8, 34.9, 35.3, 38.6, 41.2, 42.0, 46.5, 48.0, 51.7, 54.0, 71.1, 74.3, 170.6 (acetate C=O), 170.8 (acetate C=O), 174.7 (24 C=O). IR (KBr, cm^{-1}): 2949, 2868, 1734 (br C=O), 1710 (C=O), 1683, 1653, 1542, 1507, 1472, 1457, 1436, 1376, 1366, 1250, 1235, 1169, 1063, 1023, 928. Anal. Calcd for $C_{29}H_{47}NO_6$: C 68.88, H 9.37, N 2.77. Found: C 68.80, H 9.22, N 2.47.

Synthesis of amine 92

Methyl 3α-acetyloxy-7α,12α-dihydroxy-5β-cholan-24-oate 86
Hydrated 4-toluenesulphonic acid (1 g, 5.9 mmol) was added to a solution of cholic acid **46** (10 g, 24.47 mmol) in AcOMe (300 mL) and water (3 mL).The reaction mixture was stirred under reflux for 24 h. The colourless solution was then poured in $NaHCO_3$ 5% (200 mL), the organic product was extracted with CH_2Cl_2 (6x70 mL) and the organic solution dried over anhydrous Na_2SO_4. After removing the

solvent under vacuum, the crude product was purified by column chromatography (SiO_2, CH_2Cl_2:acetone 70:30) affording **86** (8.5 g, 75% yield). Chemical-physical and spectroscopic data were identical to those reported in the literature.[119]

Methyl 3α-acetyloxy-7-keto-12α-hydroxy-5β-cholan-24-oate 87 N-Bromosuccinimide (4.71 g, 26.48 mmol) was added to a solution of methylester **86** (8.20 g, 17.65 mmol) in a mixture of acetone (200 mL) and water (140 mL); the resulting yellow mixture was stirred for 30 h at r.t. and then concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (100 mL), washed with brine (3x30 mL) and the organic solution dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum affording keton **87** (8.07 g, 97% yield). Chemical-physical and spectroscopic data were identical to those reported in the literature.[119]

Methyl 3α,12α-diacetyloxy-7-keto-5β-cholan-24-oate 88 To a solution of DMAP (385 mg, 3.15 mmol) and keton **87** (7.70 g, 16.64 mmol) in anhydrous THF (70 mL), triethylamine (3.45 mL 24.96 mmol) and Ac_2O (4.71 mL, 49.92 mmol) were added and resulting mixture was stirred at r.t. for 24 h. The solution obtained was concentrated under reduced pressure and the residue dissolved in CH_2Cl_2 (100 mL), washed with HCl 10% (2x30 mL) and $NaHCO_3$ 5% (3x40 mL) and then dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum affording **88** (6.70 g, 88% yield). Chemical-

physical and spectroscopic data were identical to those reported in the literature.[136a]

Methyl 3α,12α-diacetyloxy-7-oxime-5β-cholan-24-oate 90 Sodium acetate trihydrated (5.94 g, 43.62 mmol) and hydroxylamine hydrochloride (992 mg, 14.27 mmol) were added to a solution of **88** (4.00 g, 7.93 mmol) in methanol (70 mL). The reaction mixture was stirred under reflux for 3 h. The solvent was removed under vacuum and the residue dissolved in CH_2Cl_2 (75 mL). The organic solution was washed with water (5x20 mL) then dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the crude product was crystallized from methanol affording chemically pure oxime **90** (3.30 g, 80% yield). Mp 94-96°C. $[\alpha]^{22}_D$ + 0.12 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.75 (s, 3H, CH_3), 0.82 (d, 3H, 21-CH_3), 1.05 (s, 3H, CH_3), 0.90-2.40 (m, 28H, steroidal CH and CH_2), 2.00 (s, 3H, 3-CH_3CO), 2.12 (s, 3H, 12-CH_3CO), 3.10 (br dd, 1H, NOH), 3.66 (s, 3H, CH_3OCO), 4.68 (m, 1H, 3-CH), 5.10 (t, 1H, 12-CH). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.7, 17.9, 21.5, 21.6, 23.2, 24.8, 26.0, 26.5, 27.5, 27.6, 31.0, 31.2, 32.8, 34.1, 34.8, 35.2, 37.4, 42.1, 42.6, 44.6, 45.3, 47.0, 51.7, 73.3, 75.2, 160.6 (C=NOH), 170.7 (acetate C=O), 170.8 (acetate C=O), 174.8 (24 C=O). IR (KBr, cm^{-1}): 2952, 2867, 1735 (br C=O), 1650, 1449, 1382, 1243, 1188, 1018, 957, 860, 732, 605. Anal. Calcd for $C_{29}H_{45}NO_7$: C 67.03, H 8.73, N 2.70. Found: C 67.10, H 8.70, N 2.52.

Methyl 3α,12α-diacetyloxy-7α-amino-5β-cholan-24-oate 92

Hydrated PtO_2 (164 mg, 0.72 mmol) was added to a solution of oxime **90** (3 g, 6.5.77 mmol) in glacial acetic acid (5 mL) and the mixture was stirred under H_2 (2 bar) at room temperature for six days. The solid was filtered off and powdered Zn (5.10 g, 47.24 mmol) was added to the solution, concentrated under vacuum to 2 mL. The mixture was stirred at room temperature for 12 h then the solid was filtered off and washed with acetic acid. After concentration under reduced pressure, water (60 mL) was added and the aqueous solution was made basic with KOH pellets. The organic product was extracted with ethyl acetate (6x30 mL) and the organic extracts dried (Na_2SO_4). The solvent was evaporated under vacuum affording amine **92** (1.60 g, 55% yield). Mp 58-60°C. $[\alpha]^{22}_D$ + 0.68 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.72 (s, 3H, CH_3), 0.79 (d, 3H, 21-CH_3), 0.89 (s, 3H, CH_3), 0.90-2.40 (m, 28H, steroidal CH and CH_2), 2.00 (s, 3H, 3-CH_3CO), 2.08 (s, 3H, 12-CH_3CO), 3.12 (br s, 1H, $CHNH_2$), 3.64 (s, 3H, CH_3OCO), 4.54 (m, 1H, 3-CH), 5.07 (br s, 1H, 12-CH). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.3, 17.7, 21.3, 21.5, 22.1, 22.7, 23.0, 25.3, 26.6, 27.4, 27.5, 30.9, 31.2, 31.4, 34.6, 34.8, 34.9, 37.2, 40.6, 42.8, 45.5, 47.5, 48.8, 51.7, 73.8, 75.0, 170.8 (acetate C=O), 170.9 (acetate C=O), 174.7 (24 C=O), 177.0 (7 $CHNH_2$). IR (KBr, cm^{-1}): 2948, 2870, 1734 (br C=O), 1654, 1559, 1475, 1447, 1374, 1251, 1026, 717, 611, 583. Anal. Calcd for $C_{29}H_{47}NO_6$: C 68.88, H 9.37, N 2.77. Found: C 68.79, H 9.32, N 2.43.

Synthesis of Organocatalysts B1-B4

General procedure for prolinamide synthesis

To a solution of N-Boc protected proline (1.1 equiv.) in anhydrous CH_2Cl_2, N-methylmorpholine (1.22 equiv.) was added and the mixture was cooled to -20°C then isobutyl chloroformate (1.1 equiv.) was added. The reaction temperature was maintained at -20°C for 5 min., then a CH_2Cl_2 solution of amine (1 equiv.) was added dropwise over 15 min at 0°C. The reaction mixture was stirred for 26 h. Finally the reaction mixture was treated with HCl acq., $NaHCO_3$ acq., NaCl acq. and dried over Na_2SO_4. The organic phases were concentrated in vacuo and the residue was purified by column chromatography affording the pure product.

Methyl 3α-acetyloxy-12α-N-(L-Boc-prolinoyl)amino-5β-cholan-24-oate 93a Purified by chromatography (SiO2, CH2Cl2:acetone 92:8). Yield 350 mg, 50%. Mp 68-70°C. $[\alpha]^{22}_D$ + 31.0 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.78 (s, 3H, CH_3), 0.86 (d, 3H, 21-CH_3), 0.90 (s, 3H, CH_3), 1.00-2.05 (m, 28H, steroidal CH and CH_2, and 3' and 4'-CH_2 of Boc-Pro), 1.51 (s, 9H, Boc-3CH_3), 1.99 (s, 3H, CH_3CO), 3.47 (m, 2H, 5'-CH_2 of Boc-Pro), 3.64 (s, 3H, CH_3OCO), 4.16 (br s, 1H, 2'-CH of Boc-Pro), 4.34 (br d, 1H, 12-CH), 4.69 (m, 1H, 3-CH), 7.41 (br s, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.9, 17.3, 21.6, 23.4, 23.9, 24.7, 25.9, 26.3, 26.6, 26.9, 27.6, 28.8 (3 CH_3 Boc), 31.0, 31.3, 32.4, 34.2, 34.9, 35.1, 36.0, 41.8,

44.7, 47.3, 48.5, 50.9, 51.6, 52.9, 60.5, 74.2, 77.4, 80.6, 156.4 (C=O Boc), 170.6 (acetate C=O), 171.0 (amide C=O), 174.9 (24 C=O). IR (KBr, cm^{-1}): 2959, 2858, 1767 (C=O), 1745 (C=O), 1734 (C=O), 1699 (C=O), 1678, 1649, 1633, 1554, 1537, 1520, 1509, 1453, 1245, 1161, 1020. Anal. Calcd for $C_{37}H_{60}N_2O_7$: C 68.91, H 9.38, N 4.34. Found: C 68.99, H 9.33, N 4.30.

Methyl 3α-acetyloxy-12α-N-(D-Boc-prolinoyl)amino-5β-cholan-24-oate 93b Purified by chromatography (SiO_2, CH_2Cl_2:acetone 90:10). Yield 266 mg, 49%. Mp 61-62°C. $[\alpha]^{22}_D$ + 176.0 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.77 (s, 3H, CH_3), 0.79 (d, 3H, 21-CH_3), 0.90 (s, 3H, CH_3), 0.95-2.60 (m, 28H, steroidal CH and CH_2, and 3' and 4'-CH_2 of Boc-Pro), 1.52 (s, 9H, Boc-3CH_3), 2.01 (s, 3H, CH_3CO), 3.34 (m, 2H, 5'-CH_2 of Boc-Pro), 3.65 (s, 3H, CH_3OCO), 4.20 (br d, 1H, 2'-CH of Boc-Pro), 4.37 (br d, 1H, 12-CH), 4.67 (m, 1H, 3-CH), 7.57 (br s, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.8, 17.5, 21.7, 23.6, 24.0, 24.9, 26.2, 26.6, 26.9, 27.2, 27.7, 28.7 (3 CH_3 Boc), 31.2, 31.3, 32.5, 34.5, 35.1, 36.2, 42.2, 44.9, 47.5, 48.8, 50.6, 51.7, 52.2, 60.6, 74.8, 74.2, 77.4, 80.6, 170.9 (acetate C=O), 171.0 (amide C=O), 174.8 (24 C=O) 181.1 (C=O Boc). IR (KBr, cm^{-1}): 2950, 2862, 1738 (br C=O), 1694 (C=O), 1679, 1541, 1526, 1457, 1438, 1398, 1364, 1241, 1167, 1118, 1088, 1024, 980, 886 Anal. Calcd for $C_{37}H_{60}N_2O_7$: C 68.91, H 9.38, N 4.34. Found: C 68.92, H 9.39, N 4.20.

Methyl 12α-hydroxy-3α-N-(L-Boc-prolinoyl)amino-5β-cholan-24-oate 94a Purified by chromatography (SiO_2, CH_2Cl_2:acetone 87:13). Yield 180 mg, 20%. Mp 63-65°C. $[\alpha]^{22}_D$ + 65.0 (c=1.00, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$, δ): 0,69 (s, 3H, CH_3), 0,93 (s, 3H, CH_3), 0,98 (d, 3H, 21-CH_3), 1,00-2,00 (m, 28H, steroidal CH and CH_2, and 3' and 4'-CH_2 of Boc-Pro), 1.51 (s, 9H, Boc-3CH_3), 1.99 (s, 3H, CH_3CO), 2.20 (m, 2H, 5'-CH_2 of Boc-Pro), 3.67 (s, 3H, CH_3OCO), 3.76 (m, 1H, 3-CH), 4.00 (br d, 1H, 12-CH), 4.18 (br s, 1H, 2'-CH of Boc-Pro). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.9, 17.6, 23.5, 23.8, 26.3, 27.1, 27.6, 28.0, 28.6, 28.9, (3 CH_3 Boc), 31.1, 31.3, 33.9, 34.3, 35.3, 36.0, 36.2, 42.6, 46.7, 47.5, 48.6, 49.4, 51.7, 73.4, 80.6, 128.7(C=O Boc), 174.8 (2C methylester C=O, amide C=O). IR (KBr, cm^{-1}): 2956, 2862, 1774 (br C=O), 1730, 1656, 1570, 1566, 1510, 1456, 1450, 1420, 1270, 1190, 1170, 1087, 1069, 1038, 890. Anal. Calcd for $C_{35}H_{58}N_2O_6$: C 68.73, H 9.70, N 4.65. Found: C 68.79, H 9.75, N 4.50.

Methyl 12α-hydroxy-3α-N-(D-Boc-prolinoyl)amino-5β-cholan-24-oate 94b Purified by chromatography (SiO_2, CH_2Cl_2:acetone 87:13). Yield 420 mg, 42%. Mp 69-71°C. $[\alpha]^{22}_D$ + 102.0 (c=1.00, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$, δ): 0,67 (s, 3H, CH_3), 0,90 (s, 3H, CH_3), 0,96(d, 3H, 21-CH_3), 1.00-2.00 (m, 28H, steroidal CH and CH_2, and 3' and 4'-CH_2 of Boc-Pro), 1.52 (s, 9H, Boc-3CH_3), 2.01 (s, 3H, CH_3CO), 2.20 (m, 2H, 5'-CH_2 of Boc-Pro), 3.65 (s, 3H, CH_3OCO), 3.75 (m, 1H, 3-CH), 3.97 (br d, 1H, 12-CH), 4.18 (br d, 1H, 2'-CH of Boc-Pro). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.9, 17.6, 23.5, 23.8, 26.3,

27.2, 27.6, 28.1, 28.6, 28.9 (3 CH_3 Boc), 31.1, 31.3, 34.0, 34.3, 35.3, 35.9, 36.2, 42.6, 46.8, 47.5, 48.6, 49.3, 51.7, 61.3, 73.4, 80.6, 171.7 (Boc C=O), 174.8 (methylester C=O, amide C=O). IR (KBr, cm^{-1}): 2950, 2870, 1753 (br C=O), 1718, 1682, 1655, 1556, 1598, 1507, 1470, 1437, 1371, 1277, 1190, 1169, 1092, 1050, 1025, 898. Anal. Calcd for $C_{35}H_{58}N_2O_6$: C 68.73, H 9.70, N 4.65. Found: C 68.80, H 9.72, N 4.50.

Methyl 3α,7α-diacetyloxy-12α-N-(D-Boc-prolinoyl)amino-5β-cholan-24-oate 95a Purified by flash chromatography (SiO_2, CH_2Cl_2:acetone 95:5). Yield 236 mg, 33%. Mp. 63-64°C $[\alpha]^{22}_D$ + 113.0 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.78 (s, 3H, CH_3), 0.79 (d, 3H, 21-CH_3), 0.95 (s, 3H, CH_3), 0.95-2.60 (m, 28H, steroidal CH and CH_2, and 3' and 4'-CH_2 of Boc-Pro), 1.52 (s, 9H, Boc-3CH_3), 2.06 (s, 3H, 3-CH_3CO), 2.19 (s, 3H, 7-CH_3CO), 3.36 (m, 2H, 5'-CH_2 of Boc-Pro), 3.68 (s, 3H, CH_3OCO), 4.28 (br d, 1H, 2'-CH of Boc-Pro), 4.47 (br d, 1H, 12-CH), 4.57 (m, 1H, 3-CH), 4.94 (br d, 1H, 7-CH) 8.04 (br s, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.3, 17.2, 21.6, 21.6, 22.9, 23.2, 24.9, 26.7, 27.1, 27.4, 28.5 (3 CH_3 Boc), 28.8, 30.8, 30.9, 31.5, 34.5, 34.7, 35.0, 38.3, 41.1, 43.8, 44.6, 47.4, 48.2, 51.6, 51.7, 59.8, 70.7, 74.3, 80.6, 156.4 (C=O Boc), 170.5 (acetate C=O), 170.6 (acetate C=O), 170.8 (amide C=O), 174.6 (24 C=O). IR (KBr, cm^{-1}): 2961, 1738, 1703, 1698, 1682, 1537, 1457, 1400, 1365, 1260, 1167, 1118, 1024, 935, 802. Anal. Calcd for $C_{39}H_{62}N_2O_9$: C 66.64, H 8.89, N 3.99. Found: C 66.70, H 8.90, N 3.60.

Methyl 3α,7α-diacetyloxy-12α-N-(L-Boc-prolinoyl)amino-5β-cholan-24-oate 95b

Purified by flash chromatography (SiO_2, CH_2Cl_2:acetone 93:7). Yield 250 mg, 36 %. Mp. 57-59°C $[\alpha]^{22}_D$ +28.3 (c=1.00, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$, δ): 0.80 (s, 3H, CH_3), 0.89 (d, 3H, 21-CH_3), 0.93 (s, 3H, CH_3), 1.00-2.60 (m, 28H, steroidal CH and CH_2, and 3' and 4'-CH_2 of Boc-Pro), 1.48 (s, 9H, Boc-3CH_3), 1.98 (s, 3H, 3-CH_3CO), 2.10 (s, 3H, 7-CH_3CO), 3.47 (m, 2H, 5'-CH_2 of Boc-Pro), 3.64 (s, 3H, CH_3OCO), 4.25 (br d, 1H, 2'-CH of Boc-Pro), 4.34 (br d, 1H, 12-CH), 4.57 (m, 1H, 3-CH), 4.91 (br d, 1H, 7-CH) 6.71 (br s, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.7, 17.4, 21.6, 21.8, 22.8, 23.4, 24.5, 26.3, 26.9, 27.4, 28.7 (3 CH_3 Boc), 29.5, 30.8, 31.4, 34.6, 34.8, 34.9, 38.2, 40.9, 44.6, 47.4, 48.5, 51.5, 51.6, 61.1, 71.1, 73.8, 80.6, 155.4 (C=O Boc), 170.3 (amide C=O), 170.7 (acetate C=O), 170.9 (acetate C=O), 174.8 (24 C=O). IR (KBr, cm^{-1}): 2950, 2872, 1738 (C=O), 1654, 1516, 1431, 1364, 1246, 1180, 1070, 921, 852. Anal. Calcd for $C_{39}H_{62}N_2O_9$: C 66.64, H 8.89, N 3.99. Found: C 66.74, H 8.83, N 3.50.

Methyl 3α,12α-diacetyloxy-7α-(L-Boc-Prolinoyl)amino-5β-cholan-24-oate 96a Purified by flash chromatography (SiO_2, CH_2Cl_2:acetone 90:10). Yield 260 mg, 50%. Mp 160-161°C. $[\alpha]^{22}_D$ + 23.0 (c=1.00, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$, δ): 0.72 (s, 3H, CH_3), 0.79 (d, 3H, 21-CH_3), 0.93 (s, 3H, CH_3), 0.90-2.40 (m, 28H, steroidal CH and CH_2 and 3' and 4'-CH_2 of Boc-Pro), 1.49 (s, 9H, Boc-3CH_3), 2.02 (s, 3H, 3-CH_3CO), 2.15 (s, 3H, 12-CH_3CO), 3.39 (br

s, 1H, 7-CH), 3.64 (s, 3H, CH_3OCO), 4.27 (br s, 1H, 2'-CH of Boc-Pro), 4.52 (m, 1H, 3-CH), 5.11 (br s, 1H, 12-CH) 6.93 (br s, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.5, 17.8, 21.6, 21.7, 23.1, 25.8, 27.3, 27.4, 28.7 (3 CH_3 Boc), 29.2, 29.5, 31.0, 31.4, 34.8, 34.9, 35.0, 36.8, 41.5, 44.2, 45.3, 47.5, 47.9, 51.7, 74.2, 75.5, 170.5 (acetate C=O), 170.8 (acetate C=O), 171. (amide C=O), 174.7 (24 C=O), 181.1 (C=O Boc). IR (KBr, cm^{-1}): 2948, 2353, 1733 (br C=O), 1677, 1654, 1560, 1520, 1509, 1452, 1408, 1357, 1250, 1167, 1121, 1021, 802, 611. Anal. Calcd for $C_{39}H_{62}N_2O_9$: C 66.64, H 8.89, N 3.99. Found: C 66.65, H 8.93, N 3.72.

Methyl 3α,12α-diacetyloxy-7α-(D-Boc-Prolinoyl)amino-5β-cholan-24-oate 96b Purified by flash chromatography (SiO_2, CH_2Cl_2:acetone 90:10). Yield 170 mg, 32%. Mp 67-68°C. $[\alpha]^{22}_D$ + 103.0 (c=1.00, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$, δ): 0.76 (s, 3H, CH_3), 0.81 (d, 3H, 21-CH_3), 0.96 (s, 3H, CH_3), 0.90-2.40 (m, 28H, steroidal CH and CH_2 and 3' and 4'-CH_2 of Boc-Pro), 1.54 (s, 9H, Boc-3CH_3), 2.03 (s, 3H, 3-CH_3CO), 2.18 (s, 3H, 12-CH_3CO), 3.32 (br s, 1H, 7-CH), 3.67 (s, 3H, CH_3OCO), 3.94 (br s, 1H, 2'-CH of Boc-Pro), 4.36 (br s, 1H, 12-CH) 4.54 (m, 1H, 3-CH), 6.93 ppm (br s, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.4, 17.6, 21.6, 22.7, 23.0, 25.8, 27.0, 27.2, 28.6, 29.3, 30.8, 31.0, 34.6, 34.8, 36.6, 41.3, 43.9, 45.3, 46.6, 47.3, 47.6, 51.6, 74.2, 75.4, 80.4, 170.4, 170.7, 171.2, 174.6, 198.1 ppm. IR (KBr, cm^{-1}): 2960, 2871, 1738 (br C=O), 1703 (C=O), 1679, 1654, 1526, 1506, 1457, 1437, 1398, 1368, 1245, 1162,

1118, 1083, 1022, 960, 887. Anal. Calcd for $C_{39}H_{62}N_2O_9$: C 66.64, H 8.89, N 3.99. Found: C 66.66, H 8.95, N 3.84.

General procedure for deacetylation

Boc protected diacetylated derivative (1 equiv.) was treated with a 10% MeONa solution in MeOH (2.2 equiv.) for 6 h at r.t. Reaction was quenched in HCl acq., extracted with CH_2Cl_2 and dried over Na_2SO_4. Organic phases were concentrated in vacuo and chromatographated over silica gel.

Methyl 3α,7α-dihydroxy-12α-N-(Boc-D-Prolinoyl)amino-5β-cholan-24-oate 97a Purified by chromatography (SiO_2, CH_2Cl_2/acetone 1:1). Yield 210 mg, 46%. Mp 97-98°C. $[\alpha]^{22}_D$ + 97.4 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.77 (s, 3H, CH_3), 0.83 (d, 3H, 21-CH_3), 0.84 (s, 3H, CH_3), 0.90-2.40 (m, 28H, steroidal CH and CH_2, and 3' and 4'-CH_2 of Boc-Pro), 1.45 (s, 9H, Boc-3CH_3), 3.37 (m, 2H, 5'-CH_2 of Pro), 3.44 (m, 1H, 3-CH) 3.63 (s, 3H, CH_3OCO), 3.83 (br s, 1H, 12-CH), 4.27 (br d, 1H, 7-CH), 7.10 (br d, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.7, 17.6, 19.5, 22.7, 23.4, 24.7, 26.1, 27.7, 27.9, 28.8, 30.5, 31.1, 31.1, 34.7, 35.0, 35.6, 39.7, 39.9, 41.6, 44.5, 44.6, 47.8, 48.7, 51.7, 52.2, 61.1, 68.2, 71.9, 81.0, 171.0 (Boc C=O), 174.7 (24 C=O). IR (KBr, cm^{-1}): 2954, 2870, 1772 (br C=O), 1762, 1650, 1569, 1457, 1366, 1258, 1166, 1118, 1078, 1020, 982. Anal. Calcd for $C_{35}H_{58}N_2O_7$: C 67.93, H 9.45, N 4.53. Found: C 67.99, H 9.60, N 4.32.

Methyl 3α,7α-dihydroxy-12α-N-(Boc-L-Prolinoyl)amino-5β-cholan-24-oate 97b

Purified by chromatography (SiO_2, CH_2Cl_2/acetone 6:4). Yield 66 mg, 39% . Mp 95-98°C. $[\alpha]^{22}_D$ +15.3 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.78 (s, 3H, CH_3), 0.87 (d, 3H, 21-CH_3), 0.89 (s, 3H, CH_3), 1.10-2.60 (m, 28H, steroidal CH and CH_2, and 3' and 4'-CH_2 of Boc-Pro), 1.47 (s, 9H, Boc-3CH_3), 3.40 (m, 2H, 5'-CH_2 of Pro), 3.49 (m, 1H, 3-CH) 3.64 (s, 3H, CH_3OCO), 3.84 (br s, 1H, 12-CH), 4.27 (br d, 1H, 7-CH), 7.15 (br d, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.5, 17.4, 22.6, 23.4, 24.3, 26.0, 27.5, 28.6 (3 CH_3 Boc), 29.4, 30.8, 30.9, 31.3, 34.7, 34.8, 35.1, 35.3, 39.6, 40.0, 41.5, 44.1, 44.4, 47.3, 48.7, 51.5, 51.9, 60.6, 68.2, 71.9, 80.8, 155.7 (C=O Boc), 171.2 (amide C=O), 174.7 (24 C=O). IR (KBr, cm^{-1}): 2955, 2871, 1774 (br C=O), 1761, 1650, 1569, 1457, 1380, 1243, 1133, 1118, 1078, 1019, 954. Anal. Calcd for $C_{35}H_{58}N_2O_7$: C 67.93, H 9.45, N 4.53. Found: C 67.96, H 9.40, N 4.38.

Methyl 3α,12α-dihydroxy-7α-N-(Boc-L-Prolinoyl)amino-5β-cholan-24-oate) **98a** Yield 180 mg, 93%. Mp 104-108°C. $[\alpha]^{22}_D$ – 3.6 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.66 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.96 (d, 3H, 21-CH_3), 0.90-2.40 (m, 28H, steroidal CH and CH_2, and 3' and 4'-CH_2 of Boc-Pro), 1.46 (s, 9H, Boc-3CH_3), 3.37 (m, 2H, 5'-CH_2 of Pro), 3.49 (m, 1H, 3-CH) 3.65 (s, 3H, CH_3OCO), 3.92 (br s, 1H, 12-CH), 3.92 (br s, 1H, 2'-CH of Pro), 4.18 (br d, 1H, 7-CH), 7.18 (br d, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.5, 17.3, 22.5, 23.2, 27.4, 27.7, 28.2, 28.5, 30.0, 30.9, 31.3, 32.3,

34.8, 35.4, 36.8, 39.9, 41.6, 42.2, 46.1, 46.6, 47.0, 47.2, 51.5, 60.0, 71.8, 73.1, 80.8, 171.2 (Boc C=O), 174.7 (24 C=O). IR (KBr, cm^{-1}): 2963, 2880, 1780 (br C=O), 1702, 1684, 1539, 1401, 1370, 1260, 1162, 1118, 1087, 1048, 920. Anal. Calcd for $C_{35}H_{58}N_2O_7$: C 67.93, H 9.45, N 4.53. Found: C 67.89, H 9.53, N 4.30.

General procedure for Boc-deprotection

A solution of amide in CH_2Cl_2 (typically 5 mL) was treated with a large excess of TFA (typically 2 mL) and stirred at r.t. for 15'. The resulted mixture was washed with 5% $NaHCO_3$ to remove the excess of acid and extracted with CH_2Cl_2 (typically 3x30 mL). Organic phase was then washed with brine (3x50 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum affording chemical pure product.

Methyl 3α-acetyloxy-12α-N-(L-prolinoyl)amino-5β-cholan-24-oate B1a Yield 250 mg, 94%. Mp 55-57°C. $[\alpha]^{22}_D$ + 73.0 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.79 (s, 3H, CH_3), 0.82 (d, 3H, 21-CH_3), 0.89 (s, 3H, CH_3), 0.90-2.40 (m, 28H, steroidal CH and CH_2, and 3' and 4'-CH_2 of Pro), 1.98 (s, 3H, CH_3CO), 3.05 (m, 2H, 5'-CH_2 of Pro), 3.64 (s, 3H, CH_3OCO), 3.82 (br m, 1H, 2'-CH of Pro), 4.16 (br d, 1H, 12-CH), 4.68 (m, 1H, 3-CH), 8.18 (br d, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.8, 17.5, 21.6, 23.3, 24.00, 26.3, 26.4, 26.6, 26.9, 27.7, 31.1, 31.3, 32.3, 34.3, 34.9, 35.1, 36.0, 41.9, 44.8, 47.6, 49.0, 51.1, 51.7, 51.8, 60.8, 74.2, 170.7 (acetate C=O),

173.1 (amide C=O), 174.8 (24 C=O). IR (KBr, cm^{-1}): 2940, 2867, 1733 (br C=O), 1655, 1517, 1450, 1383, 1361, 1244, 1194, 1166, 1100, 1022, 978, 805. Anal. Calcd for $C_{32}H_{52}N_2O_5$: C 70.55, H 9.62, N 5.14. Found: C 70.59, H 9.60, N 5.02.

Methyl 3α-acetyloxy-12α-N-(D-prolinoyl)amino-5β-cholan-24-oate B1b Yield 174 mg, quantitative. Mp 75-77°C. $[\alpha]^{22}_D$ + 171.0 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.79 (s, 3H, CH_3), 0.81 (d, 3H, 21-CH_3), 0.90 (s, 3H, CH_3), 0.90-2.40 (m, 28H, steroidal CH and CH_2, and 3' and 4'-CH_2 of Pro), 2.02 (s, 3H, CH_3CO), 3.09 (m, 2H, 5'-CH_2 of Pro), 3.65 (s, 3H, CH_3OCO), 3.95 (br s, 1H, 2'-CH of Pro), 4.17 (br d, 1H, 12-CH), 4.67 (m, 1H, 3-CH), 8.14 (br d, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.9, 17.4, 21.7, 23.5, 24.0, 26.2, 26.3, 26.7, 26.9, 27.1, 27.8, 31.1, 31.2, 32.5, 34.3, 34.9, 35.1, 36.2, 42.0, 44.8, 47.7, 48.7, 51.0, 51.8, 52.1, 60.9, 74.5, 170.8 (acetate C=O), 172.8 (amide C=O), 175.0 (24 C=O). IR (KBr, cm^{-1}): 2941, 2872, 1737 (br C=O), 1664, 1649, 1556, 1511, 1447, 1383, 1359, 1256, 1228, 1167, 1093, 1025, 975, 758. Anal. Calcd for $C_{32}H_{52}N_2O_5$: C 70.55, H 9.62, N 5.14. Found: C 70.60, H 9.57, N 5.08.

Methyl 12α-hydroxy-3α-N-(L-prolinoyl)amino-5β-cholan-24-oate B2a Yield 150 mg, quant. Mp 68-72°C. $[\alpha]^{22}_D$ + 36.0 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0,67 (s, 3H, CH_3), 0,91 (s, 3H, CH_3), 0,96 (d, 3H, 21-CH_3), 1,00-2,00 (m, 28H, steroidal CH and CH_2, and 3' and 4'-CH_2 of Pro), 1.98 (s, 3H, CH_3CO), 2.90 (m, 2H, 5'-CH_2 of Pro), 3.66 (s, 3H, CH_3OCO), 3.75 (m, 1H, 3-CH), 3.98 (br

d, 1H, 12-CH). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.9, 17.6, 23.5, 23.8, 26.3, 27.2, 27.6, 28.0, 29.0, 30.8, 31.0, 33.5, 34.0, 34.3 35.3 36.0, 36.2 42.6 46.7 47.4, 47.6, 48.6, 48.9, 51.6 (OCH_3), 60.7 (C3), 73.4 (C12), 173.7 (amide C=O), 174.9 (24 C=O). IR (KBr, cm^{-1}): 2939, 2858, 1734 (br C=O), 1713, 1678, 1648, 1633, 1628, 1537, 1522, 1507, 1456, 1431, 1376, 1260, 1189, 1169, 1099, 1069, 1038, 802. Anal. Calcd for $C_{30}H_{50}N_2O_4$: C 71.67, H 10.02, N 5.57. Found: C 71.69, H 10.09, N 5.42.

Methyl 12α-hydroxy-3α-N-(D-prolinoyl)amino-5β-cholan-24-oate B2b Yield 360 mg, quantitative. Mp 80-85°C. $[\alpha]^{22}_D$ + 54.0 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.64 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.94 (d, 3H, 21-CH_3), 1.00-2.00 (m, 28H, steroidal CH and CH_2, and 3' and 4'-CH_2 of Pro), 2.02 (s, 3H, CH_3CO), 2.95 (m, 2H, 5'-CH_2 of Pro), 3.67 (s, 3H, CH_3OCO), 3.73 (m, 1H, 3-CH), 3.96 (br d, 1H, 12-CH). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.9, 17.5, 23.5, 23.8, 26.2, 27.2, 27.6, 28.0, 29.0, 30.8, 31.0, 33.5, 34.0, 34.2, 35.2, 35.9, 36.1, 42.6, 46.7, 47.4, 48.7, 48.9, 51.6 (OCH_3), 60.7 (C3), 73.4 (C12), 173.6 (amide C=O), 174.8 (24 C=O). IR (KBr, cm^{-1}): 2939, 2858, 1734 (br C=O), 1718, 1678, 1648, 1638, 1632, 1542, 1522, 1507, 1457, 1437, 1371, 1260, 1189, 1169, 1099, 1069, 1038, 802. Anal. Calcd for $C_{30}H_{50}N_2O_4$: C 71.67, H 10.02, N 5.57. Found: C 71.75, H 9.99, N 5.32.

Methyl 3α,7α-diacetyloxy-12α-(D-Prolinoyl)amino-5β-cholan-24-oate B3a Yield 30 mg, quantitative. Mp 70-71°C. $[\alpha]^{22}_D$ + 69.5

(c=1.00, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$, δ): 0.77 (s, 3H, CH_3), 0.82 (d, 3H, 21-CH_3), 0.90 (s, 3H, CH_3), 0.90-2.40 (m, 28H, steroidal CH and CH_2, and 3' and 4'-CH_2 of Pro), 2.01 (s, 3H, 3-CH_3CO), 2.05 (s, 3H, 7-CH_3CO), 2.99 (m, 2H, 5'-CH_2 of Pro), 3.62 (s, 3H, CH_3OCO), 3.78 (dd , 1H, 2'-CH of Pro), 4.16 (br d, 1H, 12-CH), 4.52 (m, 1H, 3-CH), 4.88 (d, 1H, 7-CH), 8.19 (br d, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.4, 17.4, 21.6, 22.8, 23.4, 26.4, 26.6, 27.1, 27.5, 28.9, 30.8, 31.1, 31.4, 34.6, 34.8, 35.0, 35.3, 38.2, 41.0, 44.3, 44.7, 47.7, 48.7, 51.1, 51.6, 61.0, 70.9, 74.3, 170.2 (acetate C=O), 170.6 (acetate C=O), 173.8 (amide C=O), 174.6 (24 C=O). IR (KBr, cm^{-1}): 2954, 2875, 1740 (br C=O), 1662, 1652, 1516, 1438, 1365, 1249, 1172, 1099, 1066, 1024, 968, 892. Anal. Calcd for $C_{34}H_{54}N_2O_7$: C 67.74, H 9.03, N 4.65. Found: C 67.69, H 9.09, N 4.42.

Methyl 3α,7α-dihydroxy-12α-(D-Prolinoyl)amino-5β-cholan-24-oate B3b Yield 100 mg, quant. Mp 98-100°C. $[\alpha]^{22}_D$ + 79.9 (c=1.00, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$, δ): 0.81 (s, 3H, CH_3), 0.84 (d, 3H, 21-CH_3), 0.89 (s, 3H, CH_3), 0.90-2.40 (m, 28H, steroidal CH and CH_2, and 3' and 4'-CH_2 of Pro), 2.98 (m, 2H, 5'-CH_2 of Pro), 3.45 (m, 1H, 3-CH) 3.66 (s, 3H, CH_3OCO), 3.78 (dd , 1H, 2'-CH of Pro), 3.87 (br s, 1H, 12-CH), 4.19 (br d, 1H, 7-CH), 8.17 (br d, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.7, 17.5, 23.0, 23.5, 26.6, 27.8, 28.0, 30.9, 31.3, 34.8, 35.2, 39.8, 41.6, 44.7, 47.7, 48.9, 51.7, 61.0, 68.2, 72.1, 173.9 (Boc C=O), 174.8 (24 C=O). IR (KBr, cm^{-1}): 2957, 2925, 2869, 1739 (br C=O), 1648, 1523, 1448, 1382, 1261, 1198, 1170,

1078, 992, 801. Anal. Calcd for $C_{30}H_{50}N_2O_5$: C 69.46, H 9.72, N 5.40. Found: C 69.49, H 9.79, N 5.33.

Methyl 3α,7α-dihydroxyl-12α-(L-Prolinoyl)amino-5β-cholan-24-oate B3c

Purified by chromatography (SiO_2, AcOEt/MeOH 7:3).Yield 25 mg, 38%. Mp 103-105 °C. $[\alpha]^{22}_D$ +28.3 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.79 (s, 3H, CH_3), 0.83 (d, 3H, 21-CH_3), 0.88 (s, 3H, CH_3), 0.90-2.70 (m, 28H, steroidal CH and CH_2, and 3' and 4'-CH_2 of Pro), 3.12 (m, 2H, 5'-CH_2 of Pro), 3.45 (m, 1H, 3-CH), 3.65 (s, 3H, CH_3OCO), 3.87 (br d, 1H, 12-CH), 4.00 (d, 1H, 2'-CH Pro), 4.25 (d, 1H, 7-CH), 8.32 (br d, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.4, 17.5, 22.6, 23.4, 25.6, 26.6, 27.6, 30.8, 31.1, 31.4, 34.8, 34.9, 35.0, 35.2, 39.3, 39.6, 41.5, 43.8, 44.6, 47.2, 48.7, 51.5, 52.1, 60.6 68.2, 70.3, 71.9, 171.8 (amide C=O), 174.6 (24 C=O). IR (KBr, cm^{-1}): 2944, 2864, 1734 (C=O), 1714, 1658, 1648, 1623, 1573, 1543, 1508, 1437, 1382, 1261, 1166, 1075, 985. Anal. Calcd for $C_{30}H_{50}N_2O_5$: C 69.46, H 9.72, N 5.40. Found: C 69.52, H 9.74, N 5.29.

Methyl 3α,12α-diacetyloxy-7α-(L-Prolinoyl)amino-5β-cholan-24-oate B4a Yield 120 mg, 74%. Mp 78-79°C. $[\alpha]^{22}_D$ + 84.0 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.73 (s, 3H, CH_3), 0.80 (d, 3H, 21-CH_3), 0.94 (s, 3H, CH_3), 0.90-2.40 (m, 28H, steroidal CH and CH_2 and 3' and 4'-CH_2 of Pro), 2.03 (s, 3H, 3-CH_3CO), 2.13 (s, 3H, 12-CH_3CO), 3.08 (m, 2H, 5'-CH_2 of Pro) 3.60 (br s, 1H, 7-CH), 3.65 (s, 3H, CH_3OCO), 3.96 (br s, 1H, 2'-CH of Pro), 4.53 (m, 1H, 3-CH),

5.10 (br s, 1H, 12-CH) 7.95 (br s, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.5, 17.8, 21.6, 21.7, 23.0, 23.2, 25.7, 26.0, 26.1, 27.2, 27.3, 29.2, 31.0, 31.3, 32.0, 34.8, 34.9, 35.0, 35.7, 36.9, 41.5, 44.4, 45.2, 46.0, 47.5, 47.9, 51.7, 60.6, 74.3, 75.5, 76.5, 170.4 (acetate C=O), 170.5 (acetate C=O), 171.1 (amide C=O), 174.7 (24 C=O). IR (KBr, cm^{-1}): 2925, 2861, 1739, 1734, 1730, 1718, 1700, 1696, 1684, 1675, 1669, 1663, 1653, 1647, 1636, 1559, 1534, 1517, 1499, 1457, 1437, 1395, 1375, 1026, 804. Anal. Calcd for $C_{34}H_{54}N_2O_7$: C 67.74, H 9.03, N 4.65. Found: C 67.79, H 9.01, N 4.47.

Methyl 3α,12α-diacetyloxy-7α-(D-Prolinoyl)amino-5β-cholan-24-oate B4b Yield 121 mg, quantitative. Mp 80-82°C. $[\alpha]^{22}_D$ +131.0 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.76 (s, 3H, CH_3), 0.83 (d, 3H, 21-CH_3), 0.96 (s, 3H, CH_3), 0.90-2.40 (m, 28H, steroidal CH and CH_2 and 3' and 4'-CH_2 of Pro), 2.02 (s, 3H, 3-CH_3CO), 2.15 (s, 3H, 12-CH_3CO), 3.08 (m, 2H, 5'-CH_2 of Pro), 3.67 (s, 3H, CH_3OCO), 3.95 (br s, 1H, 7-CH) 3.96 (br s, 1H, 2'-CH of -Pro), 4.57 (m, 1H, 3-CH), 5.13 (br s, 1H, 12-CH) 8.12 (br s, NH amide)^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.3, 17.6, 21.4, 22.8, 25.5, 25.9, 26.9, 27.2, 29.0, 30.9, 31.1, 31.9, 34.6, 34.8, 35.4, 36.7, 41.3, 44.2, 45.1, 45.9, 47.2, 47.6, 51.6, 60.5, 74.2, 75.3, 170.4 (acetate C=O), 170.5, 188.6 (amide C=O), 174.6 (24 C=O). IR (KBr, cm^{-1}): 2964, 2923, 2856, 1743, 1722, 1650, 1637, 1623, 1565, 1544, 1525, 1511, 1479, 1463, 1442, 1380, 1263, 1091, 883, 802. Anal. Calcd for $C_{34}H_{54}N_2O_7$: C 67.74, H 9.03, N 4.65. Found: C 67.71, H 9.10, N 4.50.

Methyl 3α,12α-dihydroxy-7α-(D-Prolinoyl)amino-5β-cholan-24-oate B4c Yield 100 mg, quantitative. Mp 128-130°C. $[\alpha]^{22}_{D}$ + 3.7 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.67 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 0.96 (d, 3H, 21-CH_3), 0.90-2.40 (m, 28H, steroidal CH and CH_2 and 3' and 4'-CH_2 of Pro), 3.28 (m, 2H, 5'-CH_2 of Pro), 3.64 (s, 3H, CH_3OCO), 3.92 (br s, 1H, 2'-CH of -Pro), 3.95 (br s, 1H, 7-CH) 4.89 (m, 1H, 3-CH), 5.45 (br d, 1H, 12-CH) 8.89 (br s, NH amide)^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.3, 17.4, 22.7, 23.6, 26.2, 27.9, 28.1, 28.2, 29.9, 31.0, 31.3, 31.4, 31.7, 32.6, 35.5, 36.0, 36.3, 37.1, 41.4, 42.2, 42.4, 46.9, 47.7, 48.1, 48.7, 51.7, 60.0, 72.5, 73.5, 166.9 (amide C=O), 174.8 (24 C=O). IR (KBr, cm^{-1}): 2960, 2923, 2864, 1740, 1727, 1698, 1661, 1631, 1562, 1556, 1497, 1463, 1454, 1381, 1260, 1196, 1161, 1098, 1051, 1023, 804. Anal. Calcd for $C_{30}H_{50}N_2O_5$: C 69.46, H 9.72, N 5.40. Found: C 69.48, H 9.68, N 5.33.

General procedure for 3-Hydroxy deprotection

A solution of the 3-acetyl derivative (1 equiv.) in MeOH (typically 8 mL) was treated with a large excess of concentrated HCl (typically 0.2 mL) and stirred at r.t. for 24 h. The resulting mixture was washed with $NaHCO_3$ 5% to remove the excess of acid and extracted with CH_2Cl_2 (3x30 mL). Organic phase was then washed with brine (3x50 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum affording chemically pure product

Methyl 3α-hydroxy-12α-N-(D-prolinoyl)amino-5β-cholan-24-oate B1c Yield 160 mg, quantitative. Mp 79-80°C. $[\alpha]^{22}_{D}$ +69.6 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.81 (s, 3H, CH_3), 0.85 (d, 3H, 21-CH_3), 0.91 (s, 3H, CH_3), 0.90-2.40 (m, 28H, steroidal CH and CH_2, and 3' and 4'-CH_2 of Pro), 3.04 (m, 2H, 5'-CH_2 of Pro), 3.62 (m, 1H, 3-CH), 3.67 (s, 3H, CH_3OCO), 3.84 (br s, 1H, 2'-CH of Pro), 4.18 (br d, 1H, 12-CH), 8.21 (br d, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.7, 17.4, 23.4, 23.9, 26.2, 26.3, 26.6, 27.1, 27.7, 29.8, 30.6, 30.8, 31.1, 31.2, 34.2, 34.9, 35.1, 36.1, 36.3, 42.1, 44.7, 47.6, 48.8, 51.1, 51.6, 51.8, 60.9, 71.7, 173.3 (24 C=O), 174.8 (amide C=O). IR (KBr, cm^{-1}) 2923, 2862, 1741, 1654, 1647, 1560, 1522, 1449, 1583, 1310, 1261, 1167, 1097, 1034, 802. Anal. Calcd for $C_{30}H_{50}N_2O_4$: C 71.67, H 10.02, N 5.57. Found: C 71.62, H 10.03, N 5.49.

Methyl 3α-hydroxy-12α-acetyloxy-7α-(D-Prolinoyl)amino-5β-cholan-24-oate B4d Yield 92 mg, quantitative. Mp 92-93°C. $[\alpha]^{22}_{D}$ + 132.6 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.76 (s, 3H, CH_3), 0.84 (d, 3H, 21-CH_3), 0.96 (s, 3H, CH_3), 0.90-2.40 (m, 28H, steroidal CH and CH_2), 2.14 (s, 3H, 12-CH_3CO), 3.04 (br m, 2H, 3'-CH_2 of 7-Pro), 3.49 (br s, 1H, 7-CH), 3.67 (s, 3H, CH_3OCO), 3.77 (br s, 1H, 2'-CH of Boc-Pro), 5.13 (br s, 1H, 12-CH) 3.99 (m, 1H, 3-CH), 8.20 ppm (br s, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.4, 17.6, 21.6, 22.7, 23.0, 25.8, 27.0, 27.2, 28.6, 29.3, 30.8, 31.0, 34.6, 34.8, 36.6, 41.3, 43.9, 45.3, 46.6, 47.3, 47.6, 51.6, 74.2, 75.4, 80.4, 170.4, 170.7, 171.2, 174.6, 198.1 ppm. IR (KBr, cm^{-1}): 2960, 2871, 1738 (br C=O), 1703 (C=O), 1679, 1654, 1526, 1506, 1457, 1437, 1398, 1368,

1245, 1162, 1118, 1083, 1022, 960, 887. Anal. Calcd for $C_{32}H_{52}N_2O_6$: C 68.54, H 9.35, N 5.00. Found: C 68.52, H 9.33, N 4.97.

7.4 Synthesis of bis-prolinamide derivatives

Methyl 3α-acetyloxy-12α,7α-diketo-5β-cholan-24-oate 99

A solution of $K_2Cr_2O_7$ (2.62 g, 8.89 mmol) in water (5 mL) was added to a solution of **84** (3.8 g, 8.47 mmol) in acetic acid (170 mL). The mixture was stirred at room temperature for 20 h, then poured into water (350 mL): the solid was filtered, washed with water then dried under vacuum affording chemically pure keton **99** (3,40 g, 90% yield). Chemical-physical and spectroscopic data were identical to those reported in the literature. [179]

Methyl 3α-acetyloxy-12α,7α-dioxime-5β-cholan-24-oate 100

Sodium acetate trihydrated (5.70 g, 41.86 mmol) and hydroxylamine hydrochloride (951 mg, 13.70 mmol) were added to a solution of keton **99** (3.40 g, 7.61 mmol) in methanol (70 mL). The reaction mixture was stirred under reflux for 4.5 h. The solvent was removed under vacuum and the residue dissolved in CH_2Cl_2 (30 mL). The organic solution was washed with water (4x20 mL) then dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the crude product was crystallized by methanol affording chemically pure oxime **100** (3.40 g, 88% yield). Chemical-physical and spectroscopic data were identical to those reported in the literature.[179]

Methyl 3α-acetyloxy-12α,7α-diamino-5β-cholan-24-oate 101 Hydrated PtO_2 (120 mg, 12,5 mol%) was added to a solution of dioxime **100** (2.07 g, 4.22 mmol) in glacial acetic acid (9 mL) and the mixture was stirred under H_2 (2 bar) at room temperature for six days. The solid was filtered off and powdered Zn (4.5 g, 33.72 mmol) was added to the solution, concentrated under vacuum to 2 mL. The mixture was stirred at room temperature for 12 h then the solid was filtered off and washed with acetic acid. After concentration under reduced pressure, water was added and the aqueous solution was made basic with KOH pellets. The organic product was extracted with ethyl acetate and the organic extracts dried (Na_2SO_4). The solvent was evaporated under vacuum affording pure amine **101**. Yield 1.11 g, 57%. M.p. 94-95°C. . $[\alpha]^{22}_D$ + 35.0 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0,73 (s, 3H, CH_3), 0,92 (s, 3H, CH_3), 0,97 (d, 3H, 21-CH_3), 1,00-2,60 (m, 28H, steroidal CH and CH_2), 2.01 (s, 3H, CH_3CO), 3.10 (br t, 1H, 12-CH), 3.16 (br t, 1H, 7-CH), 3.67 (s, 3H, OMe), 4.56 (m, 1H, 3-CH). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.7, 17.3, 21.6, 22.8, 23.7, 26.0, 26.8, 27.7, 28.4, 31.0, 31.2, 34.8, 35.1, 35.1, 35.3, 36.1, 39.7, 41.7, 42.0, 46.2, 47.7, 47.9, 51.6, 54.0, 74.5, 170.9 (acetate C=O), 174.6 (24 C=O). IR (KBr, cm^{-1}): 2961, 1735, 1654, 1636, 1560, 1458, 1382, 1261, 1164, 1096, 1033, 804. Anal. Calcd for $C_{27}H_{46}N_2O_4$: C 70.09, H 10.02, N 6.05. Found: C 70.01, H 10.03, N 6.00.

Methyl 3α-acetyloxy-7α,12α-bis(Boc-D-Prolinoyl)amino-5β-cholan-24-oate 102 To a solution of N-Boc protected D-proline (512

mg, 2.38 mmol) in anhydrous CH_2Cl_2, N-methylmorpholine (290 μL, 2.63 mmol) was added and the mixture was cooled to -20°C, then isobutyl chloroformate (340 μL, 2.38 mmol) was added. The reaction temperature was maintained at -20°C for 5 min., then a CH_2Cl_2 solution of diamine **101** (500 mg, 1.08 mmol) was added dropwise over 15 min at 0°C. The reaction mixture was stirred for 26 h. Finally the reaction mixture was treated with HCl acq., $NaHCO_3$ acq., NaCl acq. and dried over Na_2SO_4. The organic phases were concentrated in vacuo and the residue was purified by column chromatography (SiO_2, AcOEt/ Hex 1:1) affording pure **102**. Yield 500 mg, 54%. M.p. 66-67°C. . $[\alpha]^{22}_D$ + 79,5 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0,77 (s, 3H, CH_3), 0,90 (d, 3H, 21-CH_3), 0,94 (s, 3H, CH_3), 1,00-2,40 (m, 28H, steroidal CH and CH_2 and 8H 3' and 4'-CH_2 of Boc-Pro), 1.46 (s, 18H, Boc 6CH_3) 2.03 (s, 3H, CH_3CO), 3.35 (br m, 1H, 2'-CH of 7-Boc-Pro), 3.37 (br m, 4H, 3'-CH_2 of 7 and 12-Boc-Pro) 3.66 (s, 3H, OMe), 4.01 (br m, 1H, 2'-CH of 12-Boc-Pro), 4.27 (br d, 1H, 12-CH), 4.37 (br d, 1H, 7-CH), 4.56 (m, 1H, 3-CH), 7.60 (br d, 1H, 7 and 12-NH amide) . ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.6, 14.3, 17.0, 21.6, 23.1, 23.4, 24.5, 24.7, 26.9, 27.1, 27.4, 28.6 (3C, Boc), 28.6 (3C, Boc), 29.4, 31.0, 31.4, 31.9, 34.8, 35.0, 35.5, 37.1, 41.5, 44.8, 45.0, 46.1, 47.2, 47.3, 48.4, 51.5, 51.9, 59.9, 60.2, 60.5, 74.6, 79.9, 80.4, 170.4 (acetate C=O), 171.0 (7 and 12 Boc C=O), 171.5 (7 and 12 amide C=O), 174.6 (24 C=O). IR (KBr, cm^{-1}): 2962, 2874, 1739, 1679, 1528, 1401, 1365, 1260, 1164, 1120, 1088, 1026, 802. Anal. Calcd for $C_{47}H_{76}N_4O_{10}$: C 65.86, H 8.94, N 6.54. Found: C 65.82, H 8.97, N 6.37.

Methyl 3α-acetyloxy-12α,7α-bis(D-prolinoyl)amino-5β-cholan-24-oate B5 A solution of amide **102** (568 mg, 0.66 mmol) in CH_2Cl_2 (5 mL) was treated with a large excess of TFA (5 mL) and stirred at r.t. for 30 min. The resulting mixture was washed with $NaHCO_3$ 5% to remove the excess of acid and extracted with CH_2Cl_2 (3x30 mL). The collected organic extracts were washed with brine (3x50 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum affording chemically pure **B5**. Yield 435 mg, quantitative. M.p. 102-103°C. $[\alpha]^{22}_D$ + 43.4 (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0,82 (s, 3H, CH_3), 0,84 (d, 3H, 21-CH_3), 0,96 (s, 3H, CH_3), 1,00-2,60 (m, 28H, steroidal CH and CH_2 and 8H 3' and 4'-CH_2 of Pro), 1.99 (s, 3H, CH_3CO), 2.99 (br m, 1H, 2'-CH of 7-Pro), 3.09 (br m, 4H, 3'-CH_2 of 7 and 12-Pro) 3.66 (s, 3H, OMe), 3.78 (br m, 1H, 2'-CH of 12-Pro), 3.88 (br d, 1H, 12-CH), 4.24 (br d, 1H, 7-CH), 4.55 (m, 1H, 3-CH), 8.18 (br d, 1H, 7 and 12-NH amide) . ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.6, 17.5, 21.4, 23.1, 23.2, 26.2, 26.5, 27.0, 27.4, 29.2, 30.7, 30.9, 31.0, 31.8, 34.7, 34.9, 35.8, 37.0, 41.2, 44.6, 45.3, 45.6, 47.3, 47.7, 48.9, 51.0, 51.6, 60.8, 61.1, 74.1, 170.3 (acetate C=O), 173.6 (7 and 12 amide C=O), 174.7 (24 C=O). IR (KBr, cm^{-1}): 2961, 2871, 1735, 1654, 1648, 1560, 1541, 1508, 1458, 1448, 1381, 1364, 1260, 1170, 1098, 1025, 801. Anal. Calcd for $C_{37}H_{60}N_4O_6$: C 67.65, H 9.21, N 8.53. Found: C 67.62, H 9.23, N 8.47.

Methyl 3α-hydroxy-12α,7α-bis(D-prolinoyl)amino-5β-cholan-24-oate B6

A solution of **B5** (96 mg, 0.15 mmol) in MeOH (2 mL) was treated with a large excess of HCl concentrated (0.2 mL) and stirred at r.t. for 24 h. The resulted mixture was washed with $NaHCO_3$ 5% to remove the excess of acid and extracted with CH_2Cl_2 (3x10 mL). Organic phases were washed with brine (3x10 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum affording chemically pure **B6**. Yield 90 mg, quantitative. M.p. 133-135°C. $[\alpha]^{22}_D$ + 112,7° (c=1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0,79 (s, 3H, CH_3), 0,85 (d, 3H, 21-CH_3), 0,92 (s, 3H, CH_3), 1,00-2,40 (m, 28H, steroidal CH and CH_2 and 8H 3' and 4'-CH_2 of Pro), 3.03 (br m, 1H, 2'-CH of 7-Pro), 3.46 (br m, 4H, 3'-CH_2 of 7 and 12-Pro) 3.63 (s, 3H, OMe), 3.82 (br m, 1H, 2'-CH of 12-Pro), 3.85 (br d, 1H, 12-CH), 4.22 (br d, 1H, 7-CH), 4.36 (br d, 1H, 3-CH), 8.08 (br d, 1H, NH amide) 8.28 (br d, 1H, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.6, 17.7, 22.7, 23.1, 23.2, 26.6, 25.0, 26.2, 26.4, 26.9, 27.5, 29.4, 29.9, 30.6, 31.2, 31.7, 32.1, 34.8, 35.1, 36.0, 36.9, 37.1, 38.6, 39.9, 41.7, 41.9, 44.8, 45.5, 46.1, 46.8, 47.4, 47.7, 51.3, 51.7, 59.8, 60.8, 61.3, 62.0, 70.5, 71.8, 72.5, 172,5 (amide C=O), 173.5 (amide C=O), 174.8 (24 C=O). IR (KBr, cm^{-1}): 2937, 2862, 1737, 1669, 1538, 1458, 1433, 1371, 1340, 1309, 1247, 1197, 1167, 1089, 1068, 1012, 919, 820. Anal. Calcd for $C_{35}H_{58}N_4O_5$: C 68.37, H 9.51, N 9.11. Found: C 68.32, H 9.53, N 9.01.

Methyl-3α-acetyloxy-7α,12α-bis(Boc-glycyloxy)cholate 103

To a solution of **84** (2.0 g, 4.3 mmol) in 32 mL of CH_2Cl_2 1.9 g of Boc-glycine (10.8 mmol) and 0.132 g of DMAP (1.08 mmol) were added; the resulting mixture was cooled to 0°C and 2.28 g of DCC (11.05 mmol) in 15 mL of dry CH_2Cl_2 were added in 15'. The reaction was stirred at 0°C for 15' and then a r.t. overnight. Solid formed during the reaction was removed by filtration and organic phase was washed with 10% HCl, 10% $NaHCO_3$ and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was purifed by flash chromatography (SiO_2, CH_2Cl_2:acetone 90:10). Yield 3.41 g, quantitative. Mp 72-75°C. $[\alpha]^{22}_D$ +45.5 (c=1.00, CH_2Cl_2). ^{1}H-NMR (300 MHz, $CDCl_3$, δ): 0.72 (s, 3H, CH_3), 0.79 (d, 3H, CH_3 21), 0.91 (s, 3H, CH_3) 1.00-2.40 (m, 43H, CH and CH_2 steroidal, Boc, and CH_3 acetyl), 3.65 (s, 3H, $COCH_3$), 3.80-4.02 (m, 4H, CH_2 Boc-glycine), 4.53 (m, 1H, 3-CH), 5.01 (m, 1H, 7-CH), 5.11 (d, 2H, NH), 5.17 (m, 1H, 12-CH). ^{13}C-NMR (75 MHz, $CDCl_3$, δ): 12.3, 17.6, 21.6, 22.6, 22.9, 25.0, 25.7, 27.0, 27.2, 28.6 (6C Boc), 30.7, 30.9, 31.5, 32.7, 34.0, 34.4, 34.7, 34.9, 37.9, 40.9, 42.9, 43.2, 45.3, 47.4, 49.2, 51.6, 72.2, 73.9, 80.1, 155.6 (C=O Boc), 157.3 (C=O Boc), 169.7 (C=O glycine), 170.0 (C=O glycine), 170.9 (C=O acetyl), 174.6 (24 C=O). IR (KBr, cm^{-1}): 3395, 2964, 2118, 1734, 1559, 1521, 1456, 1383, 1367, 1260, 1164, 1054, 1029, 965, 802. Anal. Calcd for $C_{41}H_{66}N_2O_{12}$: C 63.22, H 8.54, N 3.60. Found: C 63.25, H 8.51, N 3.50.

Methyl-3α-acetyloxy-7α,12α-bis(glycyloxy)cholate 104

A solution of **103** (3 g, 3.8 mmol) in CH_2Cl_2 (7.5 mL) was treated with a large excess of TFA (2.6 mL) and stirred at r.t. for 15'. A 5% solution of Na_2CO_3 was then added dropwise and the reaction was extracted with CH_2Cl_2. The organic phases were washed with brine and then dried on anhydrous Na_2SO_4. The solvent was removed under reduced pressure affording pure **104** without further purification. Yield 2 g, 92%. Mp 53-56°C. $[\alpha]^{22}_D$ +58.9 (c=1.00, CH_2Cl_2). ^{1}H-NMR (300 MHz, $CDCl_3$, δ): 0.72 (s, 3H, CH_3), 0.78 (d, 3H, CH_3 21), 0.90 (s, 3H, CH_3) 1.00-2.40 (m, 25H, CH and CH_2 steroidal, and CH_3 acetyl), 3.30-3.57 (m, 4H, CH_2 glycine), 3.65 (s, 3H, $COCH_3$), 4.53 (m, 1H, 3-CH), 4.96 (d, 1H, 7-CH), 5.14 (m, 1H, 12-CH). ^{13}C-NMR (75 MHz, $CDCl_3$, δ): 12.3, 17.7, 21.6, 22.6, 22.9, 25.0, 25.7, 27.0, 27.2, 28.9, 30.8, 30.9, 31.4, 34.0, 34.4, 34.7, 37.9, 40.9, 43.4, 44.2, 44.3, 45.3, 47.5, 49.1, 51.6, 71.5, 73.9, 76.0, 170.7 (C=O acetyl), 173.5 (C=O glycine), 173.7 (C=O glycine), 174.5 (24 C=O). IR (KBr, cm^{-1}): 3386, 3327, 2950, 2871, 1734, 1653, 1624, 1576, 1436, 1392, 1365, 1260, 1196, 1071, 1026, 964, 891. Anal. Calcd for $C_{31}H_{50}N_2O_8$: C 64.34, H 8.71, N 4.84. Found: C 64.32, H 8.75, N 4.77.

Methyl-3α-acetyloxy-7α,12α-bis(L-Boc-prolylglycyloxy)cholate 105

To a solution of N-Boc L-proline (1.5, 7.4 mmol) in anhydrous CH_2Cl_2 (24 mL), N-methylmorpholine (1.1 mL, 9 mmol) was added and the mixture was cooled to -20°C, then isobutyl chloroformate (1.1 mL, 7.4 mmol) was added. The reaction temperature was maintained

at -20°C for 5 min., then a CH_2Cl_2 solution of **104** (2 g, 3.4 mmol) was added dropwise over 15 min at 0°C. The reaction mixture was stirred for 26 h. Finally the reaction mixture was treated with 10% HCl, 5% $NaHCO_3$, brine and dried over Na_2SO_4. The organic phases were concentrated in vacuo affording product **105** without further purification. Yield 1,3 g, 40%. Mp 94-96°C. $[\alpha]^{22}_D$ -8.63 (c=1.00, CH_2Cl_2). ^{1}H-NMR (300 MHz, $CDCl_3$, δ): 0.73 (s, 3H, CH_3), 0.79 (d, 3H, CH_3 21), 0.91 (s, 3H, CH_3) 1.00-2.40 (m, 51H, CH and CH_2 steroidal, CH_2 3' and 4' Boc-proline, 3 CH_3 Boc, and CH_3 acetyl), 3.47 (m, 2H, CH 5' Boc-proline), 3.65 (s, 3H, $COCH_3$), 3.80-4.35 (m, 6H, CH_2 glycine, CH_2 2' Boc-proline), 4.57 (m, 1H, 3-CH), 5.01 (d, 1H, 7-CH), 5.17 (m, 1H, 12-CH), 6.58 (bs, 1H, NH amide), 7.30 (bs, 1H, NH amide). ^{13}C-NMR (75 MHz, $CDCl_3$, δ): 12.4, 17.7, 21.7, 22.7, 23.0, 23.9, 24.5, 25.1, 25.8, 25.9, 27.1, 27.3, 27.8, 28.3, 28.6, 29.1, 29.7, 30.8, 31.1, 31.6, 34.0, 34.5, 34.8, 38.0, 41.0, 43.5, 45.6, 46.5, 47.3, 47.6, 49.4, 51.7, 53.6, 59.2, 73.9, 76.0, 80.9, 149.2 (C=O), 153.6 (C=O Boc), 167.4 (C=O proline), 167.9 (C=O proline), 169.3 (C=O glycine), 169.4 (C=O glycine), 171.0 (C=O acetyl), 174.7 (24 C=O). IR (KBr, cm^{-1}): 3334, 2953, 2876, 1734, 1701, 1560, 1534, 1395, 1249, 1162, 1124, 1026, 965, 891, 856, 774. Anal. Calcd for $C_{51}H_{80}N_4O_{14}$: C 62.94, H 8.29, N 5.76. Found: C 62.92, H 8.33, N 5.61.

Methyl-3α-acetyloxy-7α,12α-bis(L-prolylglycyloxy)cholate B7

A solution of **105** (1 g, 1.0 mmol) in CH_2Cl_2 (2.5 mL) was treated with a large excess of TFA (2.6 mL) and stirred at r.t. for 15'. A 5%

solution of Na_2CO_3 was then added dropwise and the reaction was extracted with CH_2Cl_2. The organic phases were washed with brine and then dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure affording pure **B7** without further purification. Yield 0.64 g, 84%. Mp 74-78°C. $[\alpha]^{22}_D$ +13.41 (c=1.00, CH_2Cl_2). ^{1}H-NMR (300 MHz, $CDCl_3$, δ): 0.73 (s, 3H, CH_3), 0.81 (d, 3H, CH_3 21), 0.91 (s, 3H, CH_3), 1.00-2.40 (m, 33H, CH and CH_2 steroidal, CH_2 3' and 4' proline and CH_3 acetyl), 3.00 (m, 6H, CH 2' and CH_2 5' proline), 3.65 (s, 3H, $COCH_3$), 3.80-4.35 (m, 4H, CH_2 glycine), 4.55 (m, 1H, 3-CH), 5.04 (d, 1H, 7-CH), 5.17 (m, 1H, 12-CH), 8.16 (m, 2H, NH amide). ^{13}C-NMR (75 MHz, $CDCl_3$, δ): 12.4, 17.7, 21.7, 22.7, 23.0, 25.1, 25.9, 26.4, 27.1, 27.3, 29.1, 30.9, 31.1, 31.6, 34.2, 34.6, 34.8, 35.0, 38.9, 41.1, 41.5, 43.5, 45.5, 47.5, 47.6, 49.3, 51.6, 60.7, 60.8, 72.2, 74.1, 76.7, 169.5 (C=O glycine), 169.7 (C=O glycine), 170.8 (C=O acetyl), 174.6 (24 C=O), 175.8 (C=O proline), 175.8 (C=O proline). IR (KBr, cm^{-1}): 3334, 2946, 2871, 1734, 1668, 1521, 1437, 1382, 1364, 1249, 1194, 1100, 1062, 1026, 964, 891, 802, 604. Anal. Calcd for $C_{41}H_{64}N_4O_{10}$: C 63.71, H 8.35, N 7.25. Found: C 63.72, H 8.33, N 7.11.

7.5 General procedure for aldol reactions

The organocatalyst (usually 5%) was stirred in 0.5 mL of solvent with keton (0.5 mmol) for 1 h. Aromatic aldehyde (0.25 mmol) was added and the mixture was stirred at the desired temperature for the desired time. This solution was quenched in aqueous NH_4Cl (1 mL) and

extracted with AcOEt (1 mL), then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified over silica gel (AcOEt:hexane 1:1) and after concentration analyzed with HPLC and ^{1}H NMR. All the aldol adducts matched the reported characteristics.

2-(Hydroxy-(p-fluoro)methyl)cyclopentan-1-one

Syn diastereoisomer ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.28-2.52 (m, 6H, CH_2 cyclopentanone), 2.71 (dd, J_1 =11.4 Hz, J_2 = 4.4 Hz 1H, C*H*CHOH), 4.63 (s, 1H, CHCHO*H*), 5.29 (d, 1H, *J* = 2.8 Hz, CHC*H*OH) 7.04 (t, *J* = 7.8 Hz, 2H, aromatic), 7.30 (dd, J_1 = 6.0 Hz, J_2 = 2.6 Hz, 2H, aromatic). Enantiomeric excess was determined by HPLC with a Chiralpak AD column (95:5 hexane:2-propanol), r.t., 220 nm, 0.5 mL/min; *syn* diastereoisomer: t_R = 22.55 min, t_R = 27.47 min; *anti* diastereomer: t_R = 31.69 min, t_R = 35.72 min.

2-(Hydroxy-(p-chloro)methyl)cyclopentan-1-one

Syn diastereoisomer ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.22-2.38 (m, 6H, CH_2 cyclopentanone), 2.90 (t, *J* = 13.2 Hz 1H, C*H*CHOH), 4.63 (s, 1H, CHCHO*H*), 5.23 (*br* s, 1H, CHC*H*OH) 7.32 (dd, J_1 = 22.8 Hz, J_2 = 8.4 4H, aromatic). Enantiomeric excess was determined by HPLC with a Chiralpak AS column (90:10 hexane:2-propanol), r.t., 220 nm, 1 mL/min; *syn* diastereoisomer: t_R = 12.35 min, t_R = 14.65 min; *anti* diastereisomer:t_R= 16.85 min, t_R = 21.11 min.

7.6 General procedure for Michael reactions

The organocatalyst (5 or 10 mol%) was stirred in the solvent with cyclohexanone (0.5 mmol) for 1 h. Trans-β-nitrostyrene (0.25 mmol) was added and the mixture was stirred at desired temperature, monitoring the reaction by TLC (SiO_2 ethyl acetate: hexane 30:70). The reaction was stopped evaporating the solvent and the crude product was analyzed by HPLC on chiral stationary phase and ^1H NMR.

7.7 Computational details

For the qualitative purposes of this work all the calculations were carried out at the density functional theory (DFT) level, using the B3LYP hybrid functional in conjunction with the 6-31G(d) basis set. The largest calculations were performed at the Hartree-Fock level of theory with the smaller STO-3G basis set as regards the rigid skeleton of the molecule ad at the B3LYP/6-31G(d,p) as regards the external, H-Bond interacting part of the molecule, using the ONIOM technique. The simulations in dichloromethane were carried out treating the solvent by means of the IEF-PCM model, while the effect of water was described with an hybrid approach: a small cluster made by the molecule and a few water molecules, placed where H-bond formation was possible, was described at the ONIOM level, as said before, while the bulk effect of the solvent was described at the IEF-PCM level. All the geometries were fully optimized using analytical gradients. All

computations were performed with a local development version of the Gaussian03 package.

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Chapter I

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