Synthesis, characterization and application of α/β-oligopeptides as bifunctional organocatalysts for the aldol reaction

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Alla mia grande….

…e alla mia piccola

famiglia.

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List of Abbreviations

p-TsOH	*p*-toluenesulfonic acid
DMF	dimethylformamide
DMAP	4-dimethylaminopyridine
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl
DMSO	dimethylsulfoxide
TEA	triethylamine
DCM	dichlormethane
p-TSA	*p*-toluenesulfonic acid
Boc-	*tert*-butoxycarbonyl-
TBDMS-	*tert*-butyldimethylsilyl-
TBS-	*tert*-butylsilyl-
TMS-	trimethylsilyl-
THF	tetrahydrofurane
Cbz-	carbobenzyloxy-
DABCO	1,4-diazabicyclo[2.2.2.]octane
TBDPS	*tert*-butyldiphenylsilyl
β-ACC	β-aminocyclopropanecarboxylic acid
trans-ACHC	*trans*-aminocyclohexanecarboxylic acid
trans-ACPC	*trans*-aminocyclopentanecarboxylic acid
Ph-	phenyl-
EDC hydrochloride	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
Bn-	benzyl-
r. t.	room temperature

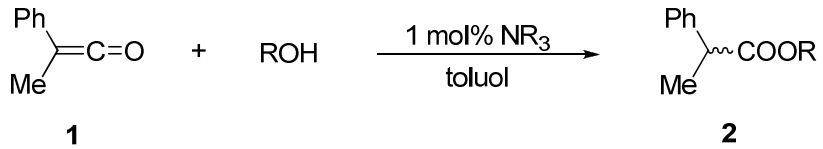
cat	catalyst
Ac-	acetyl-
NOE	nuclear overhauser effect
RDC	residual dipolar coupling
PDMS	polydimethylsiloxane
DNA	deoxyribonucleic acid
MD	molecular dynamics
Et-	ethyl-
Me-	methyl-
d	doublet
m	multiplet
q	quartet
s	singlet
brs	broad singlet
dd	doublet of doublets

INTRODUCTION

During the current decade organocatalysis[1] has met an unprecedented interest. Although the idea of using substoichiometric amounts of organic molecules to promote chemical transformations may look obvious and reminiscent of the natural activity of many enzymes, only few, scattered reports appeared in this field before that *List* and *Barbas*[2] showed proline´s ability to catalyze the asymmetric intermolecular aldol reaction. While up to that date the attention had been largely reserved to metal containing catalysts, it is clear that the use of organocatalysts presents some interesting advantages; as they are more robust and inexpensive than most metal-ligand complexes and they allow us to bypass the toxicity issues that limit the use of metal containing catalysts, for instance, in the pharmaceutical industry.

Organocatalysis, development and classification

In 1960, *Pracejus*[3] was the first to report the use of an enantioselective organocatalyst, showing that 1% of quinidine was able to promote the transformation of ketenes in optically active (*ee*: 60%) *α*-phenyl-propionic acid esters (scheme 1); in the seventies, the remarkable properties of proline as organocatalyst where explored for the first time in the intramolecular aldol reaction by Eder, Sauer, Weichert[4] and Hajos, Parrish[5] (scheme 2).



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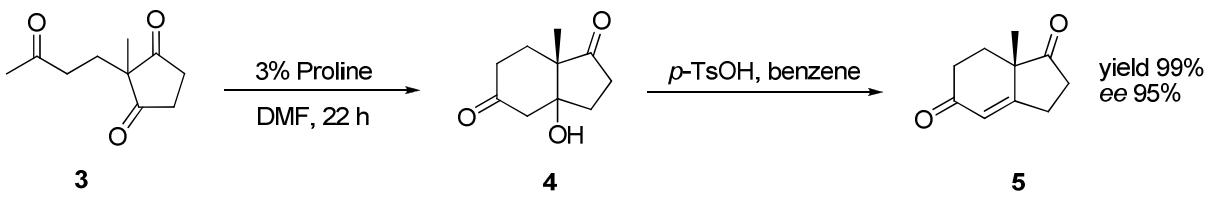
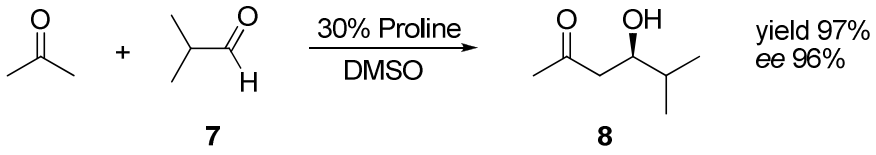
Scheme 1. The asymmetric, organocatalytic, synthesis of *α*-phenyl propionic acid esters proposed by Pracejus in 1960



Scheme 2. The first organocatalytic, proline promoted, aldol reaction.

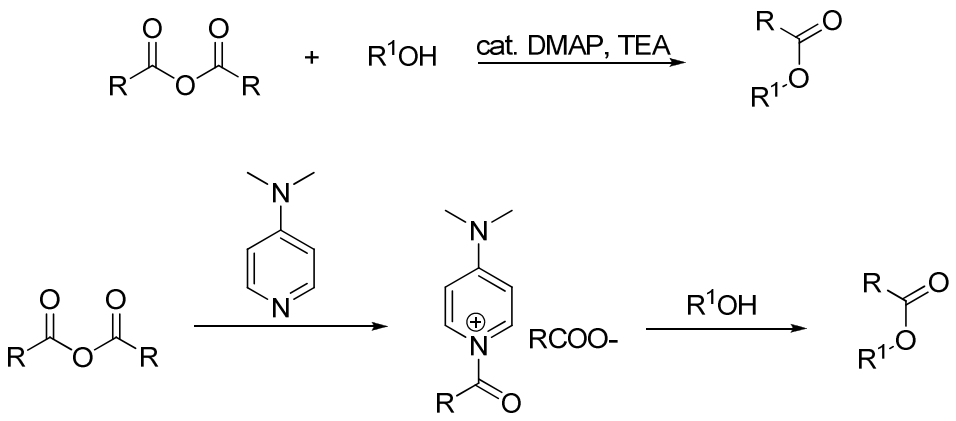
Other examples were reported for the diketopiperazines catalyzed synthesis of cyanhydrins[6], in the Thiazolium catalyzed benzoin and Stetter reactions[7] and in the ketones-catalyzed epoxidation[8]. Some important, non-enantioselective catalysts were developed such as DMAP, for acyl transfer reactions[9] and TEMPO, for alcohol oxidation[10], but it was the discovery of proline as an efficient catalyst for the asymmetric intermolecular aldol reaction that paved the way for organocatalysis to become one of the most challenging and studied fields of this century[2] (scheme 3).



Scheme 3. The Intermolecular aldol reaction is effectively catalyzed by proline

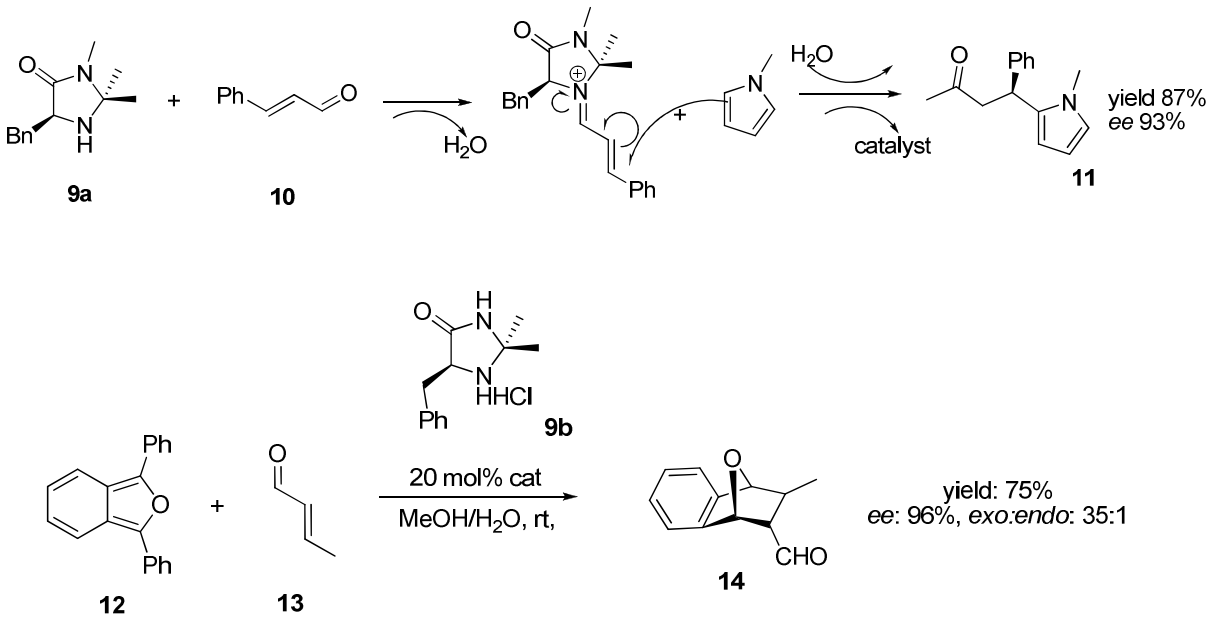
A rational method to classify the different kinds of organocatalysts may be represented by analyzing their mechanism of action; in this way, we can divide them in four families: Lewis base- and Lewis acid catalysts, Brönsted base- and Brönsted acid catalysts.

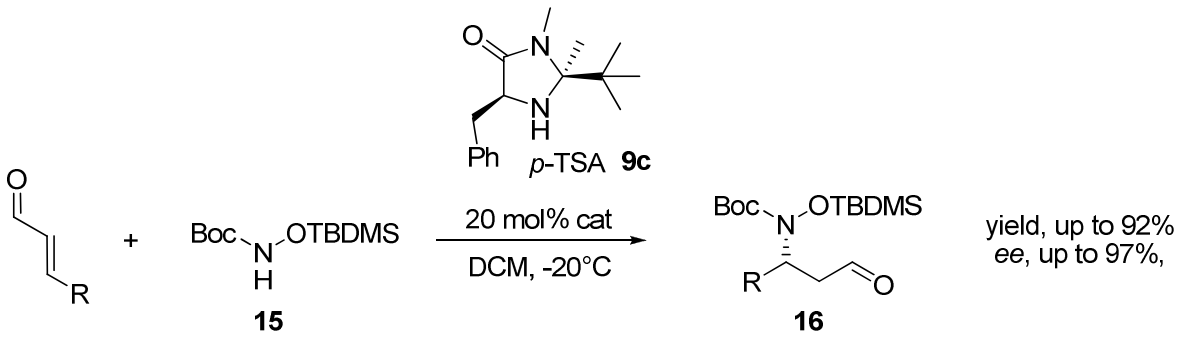
Lewis base catalysts promote the target reaction by building an activated intermediate through a step of nucleophilic addition of the catalyst to the substrate. One important example in this field is represented by the DMAP catalyzed acetylation of alcohols which proceeds through an activated acylpyridium complex[9g] (scheme 4). This discovery prompted the preparation of chiral Lewis base organocatalysts aimed to carry out selective acetylations for the kinetic resolution of alcohols[11], esterification of carboxylic acids[12] or *meso*-anhydride desymmetrization[13].



Scheme 4. DMAP catalyzed alcohols acylation.

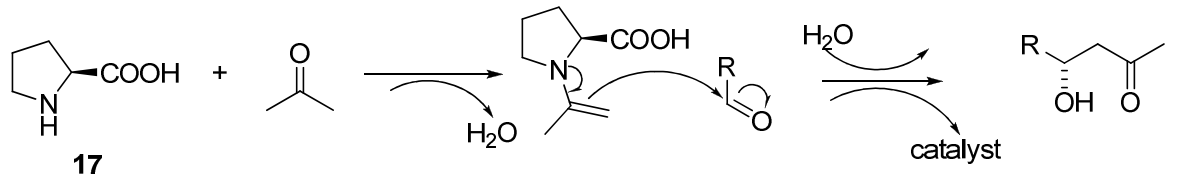
Two other fundamental catalytic processes may be included in this family: imminium- and enamine-based catalysis. In the first case we observe the formation of a reversible imminium intermediate between the catalyst and the substrate, whose enhanced electrophilicity accelerates the reaction with a nucleophile. The catalyst is regenerated by hydrolysis of the imminium intermediate (scheme 5, above). An older example of this kind of process is represented by the Knoevenagel reaction; in the last few years *MacMillan*, *Jorgensen*, *Wang*, *Miller*, *List* and many others showed that chiral amines could work as effective organocatalysts in the Diels-Alder reaction[14], Friedel-Crafts alkylation[15], conjugated addition[16] and cycloadditions[17] (scheme 5, below).





Scheme 5. Mechanism of the imminium catalysis (above); applications of McMillan´s catalyst in the Diels-Alder reaction and in the conjugated addition (below)

Enamine catalysis may be regarded as a special case of the imminium catalysis, in which the imminium intermediate between a secondary amine and a carbonyl compound, having an acid α-proton, evolves to an enamine[18] intermediate which, through a nucleophilic attack on the aldol substrate, leads to the formation of an imminium cation. This is successively hydrolyzed to regenerate the catalyst, as we can see for instance in the case of the proline catalyzed aldol reaction (scheme 6). Enamine catalysis, whose mechanism is reminiscent of the natural activity of the Lys 229 residue in the active site of type I aldolases[19], is of enormous importance for some of the most profusely studied reactions in the last years and will be analyzed later in detail.



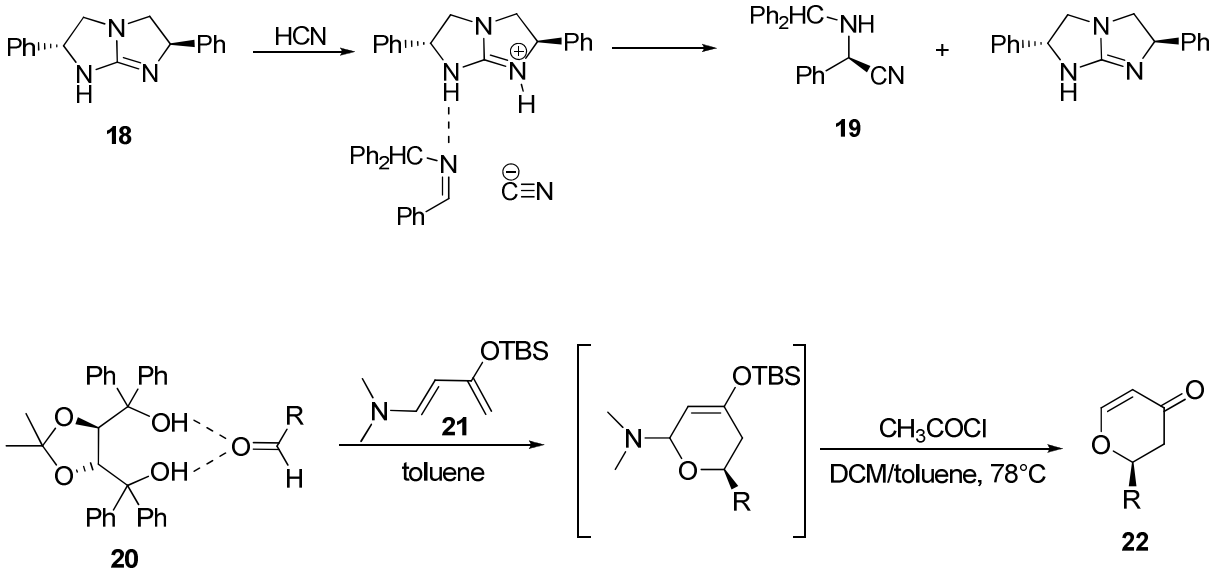
Scheme 6. Mechanism the enamine catalysis.

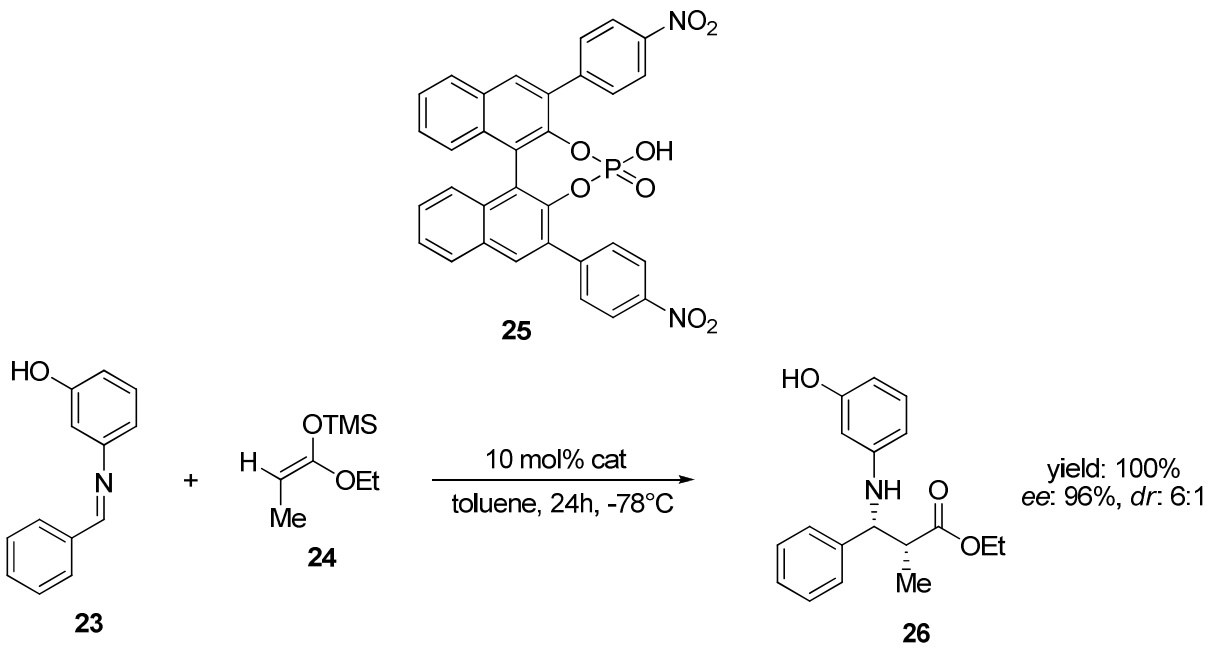
Lewis acid catalysis is generally related to metal containing catalysts, but a good example in the field of organocatalysis is represented by the ketone catalyzed epoxidation of olefines. In this process, the ketone acts as a Lewis acid and following the nucleophilic attack of the anion peroxymonosulfate, a dioxiran is built as oxidizing agent. Similarly, in

the Shi-epoxidation[8], a chiral ketone derived from fructose acts as a selective epoxidation organocatalyst.

Brönsted base catalysis proceeds through a deprotonation step which activates the substrate for the desired reaction. A nice asymmetric organocatalytic approach was proposed by Corey[20] using a guanidine derivative to promote the asymmetric Strecker reaction (scheme 7, above).

The last process of this classification, Brönsted acid catalysis, proceeds through a proton exchange between the catalyst and the substrate or by activation of the substrate through the formation of strong H-bonds. Excellent examples are represented by the use of TADDOL as a metal-free catalyst in the hetero-Diels-Alder reaction[21] (scheme 7, center) or by the thiourea-based organocatalysts in a broad range of transformations[22]. Another example has been presented independently by Akiyama[23] (scheme 7, below) and Terada[24] by developing chiral phosphates that can promote the asymmetric Mannich reaction, or by Sasai[25] who conjugated the Brönsted acid potential of the BINOL unit with a second Lewis base functionality to prepare an effective catalyst for the aza-Morita-Baylis-Hilman reaction.



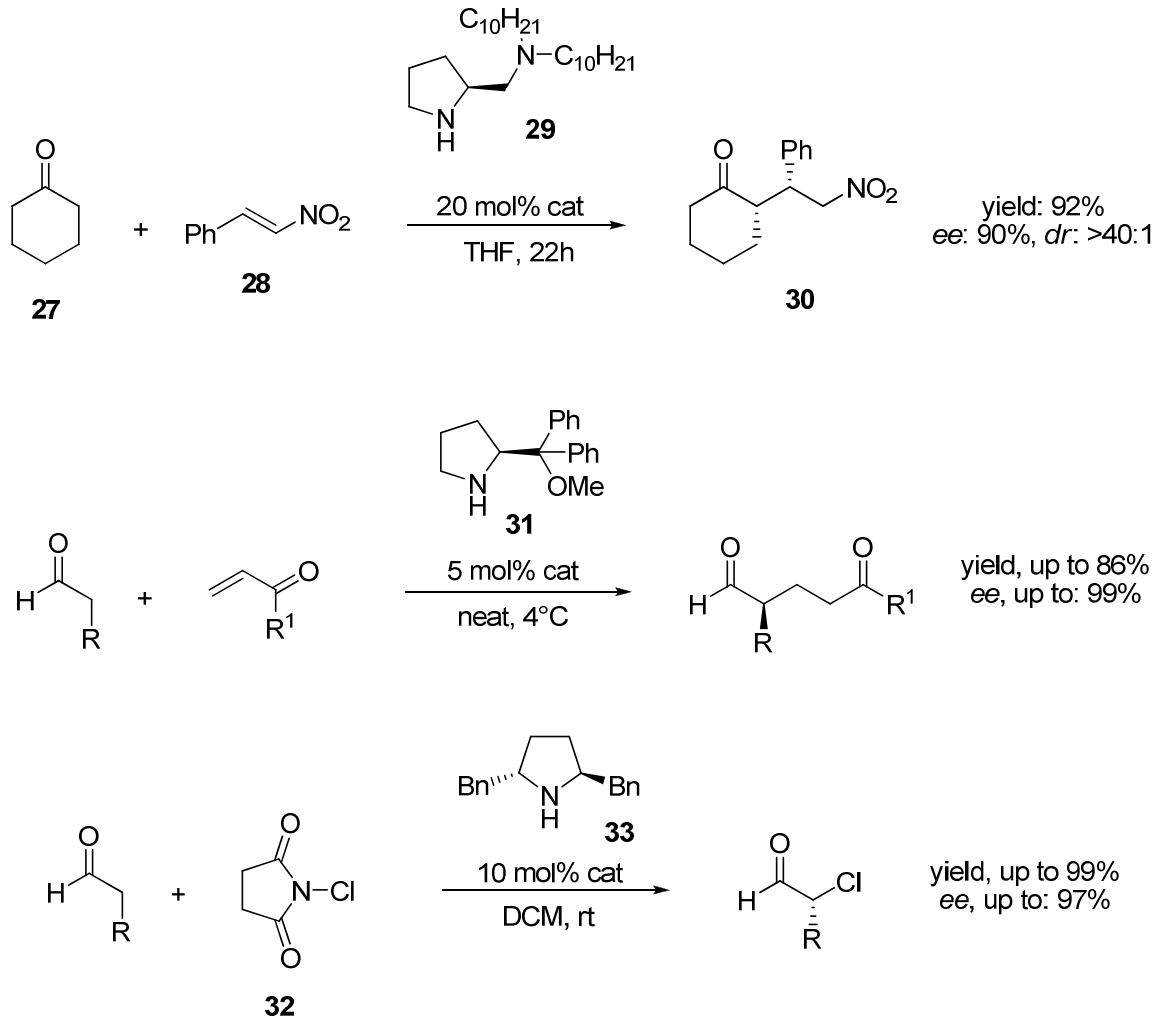


Scheme 7. Brönsted base catalyst proposed by *Corey* (above); Taddol as a Brönsted acid in the hetero-Diels-Alder reaction (center); Brönsted acid catalyst for the Mannich Reaction (below)

In the next chapters of this thesis we will be concerned with reactions following the first mechanism of this classification, in particular we will speak about enamine catalysis.

Enamine catalysis and proline

The reversible formation of an enamine intermediate (scheme 6) represents a convenient, atom-economic catalytic pathway, and finds its roots in the chemistry of the preformed enamines pioneered by Stork[26]; it is comparable to the generation of a carbanion, but the catalyst is freed at the end of the catalytic cycle and does not require a stoichiometric load. Recently, a number of diverse, small molecules able to promote important asymmetric reactions through an enamine-based mechanism have been reported, including asymmetric aldol reaction[1], Michael addition[16f], conjugated addition[16b,16c], Mannich reaction[1a], α-halogenation[27], α-hydroxylation[28] and α-amination of aldehydes[29], aza-Diels-Alder[30] and aza-Morita-Baylis-Hilman[31] (scheme 8).



Scheme 8. Examples of enamine-based organocatalysts.

In 1999 *L*-proline was discovered as an effective catalyst for the intermolecular aldol reaction[2]. Since that time, an extensive study has been carried out to explore scope, limitations, reaction mechanisms and optimization of this catalyst. A deeper analysis of the mechanism[18] of the aldol reaction between acetone and aldehydes, brought about the hypothesis that the catalytic process may involve a chair-like Zimmerman-Traxler transition state in which both functionalities of proline are involved in the step determining the enantioselectivity of the process: while the amino functionality forms a enamine intermediate with acetone, the carboxylic functionality directs the aldol acceptor through an H-bond and takes part to the proton exchange process, like a Brönsted acid co-catalyst. The minor product may arise from the second less stable transition state below or from a transition state in which the R group assumes a less favorable pseudo-axial position (fig. 1).

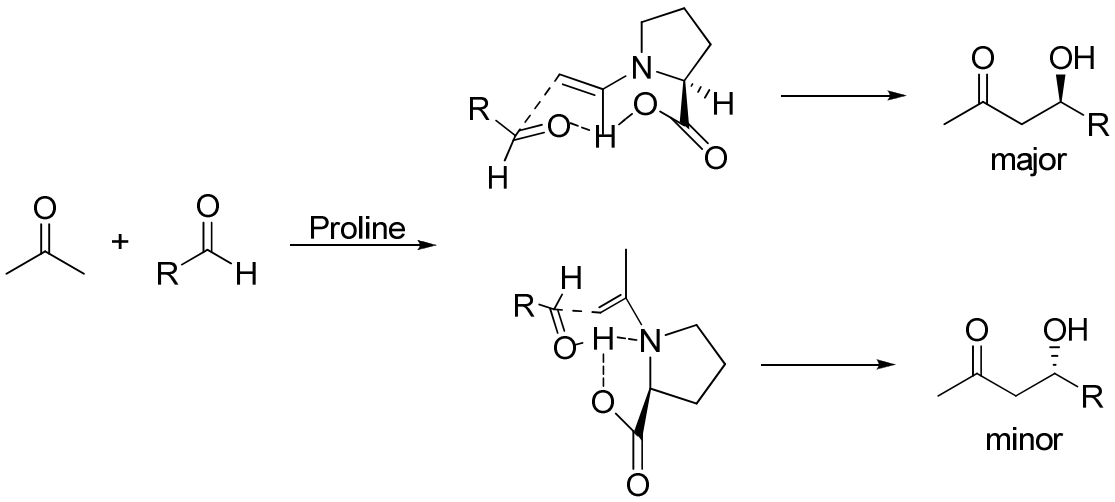
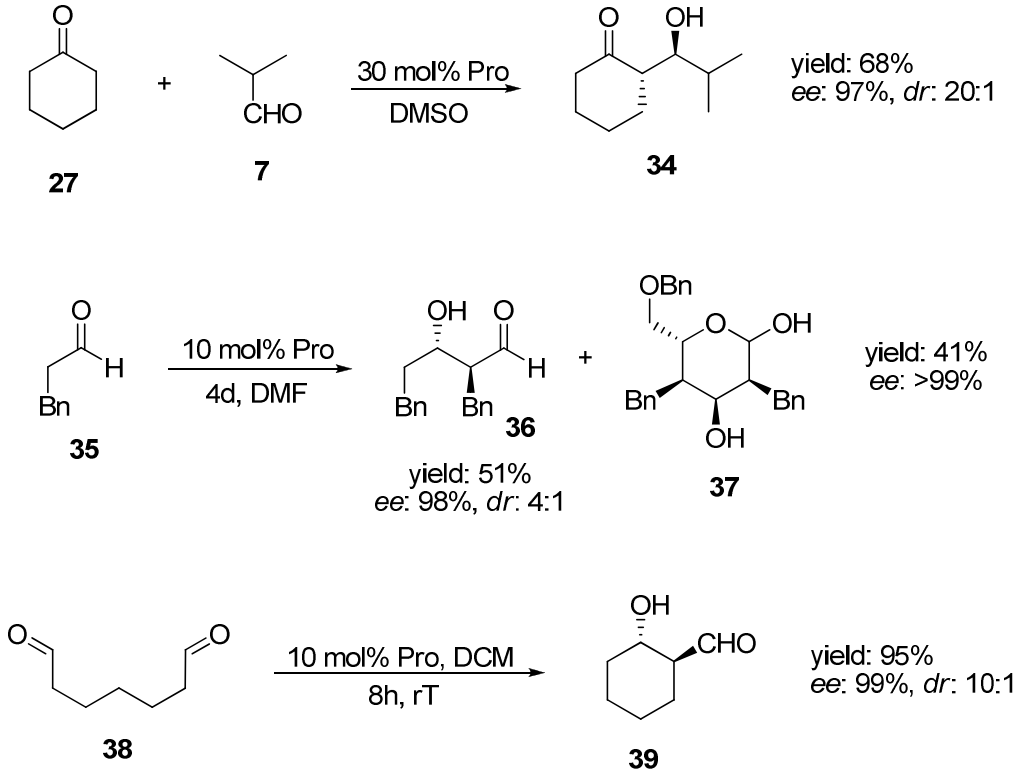


Fig. 1. Transition state models for the proline catalyzed aldol reaction.

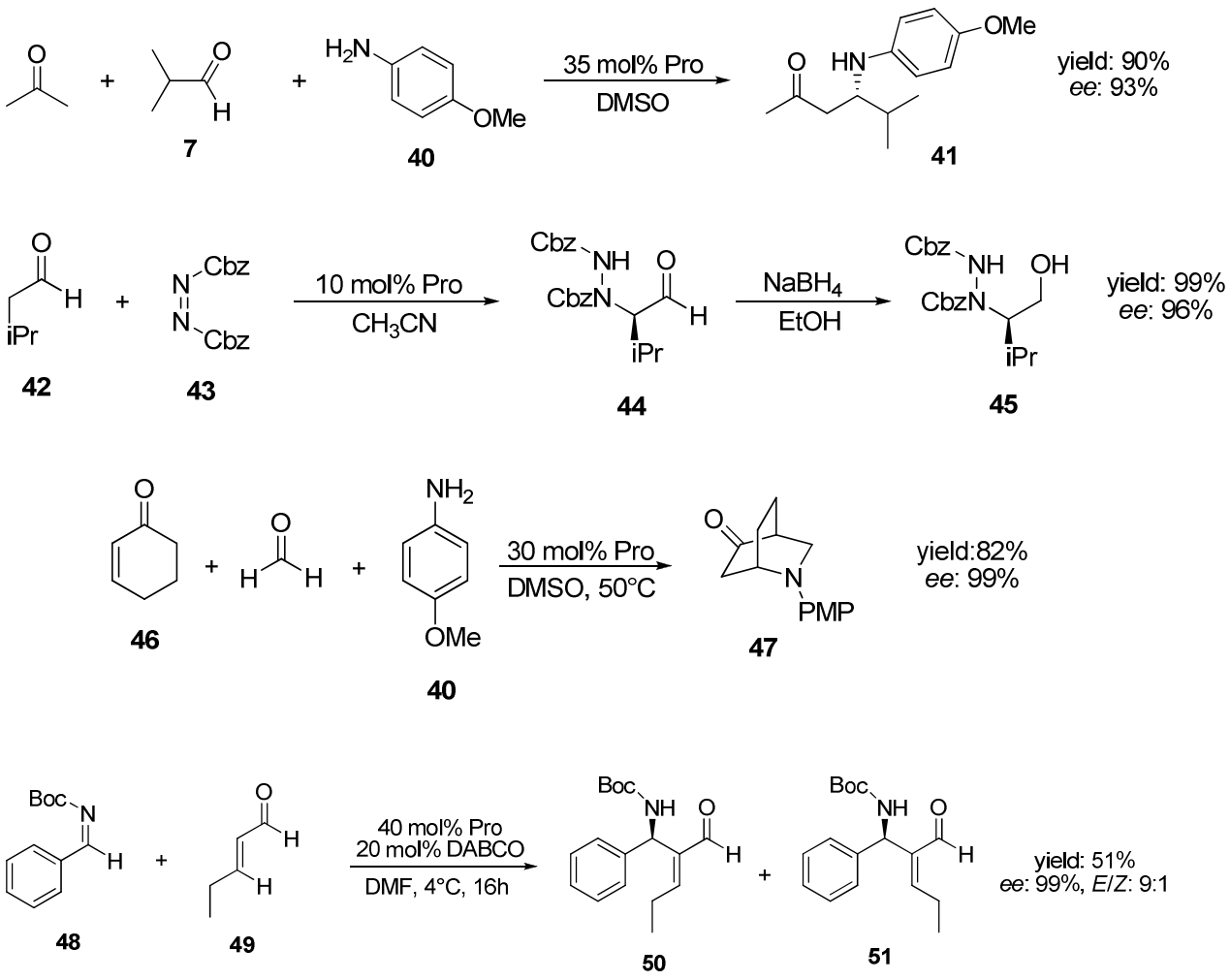
Proline proved to be a formidable catalyst for a number of processes, some of its greatest advantages being the fact that it is extremely versatile, environmentally friendly and also quite inexpensive. A large number of proline catalyzed aldol reactions have been reported[1a,e], some noteworthy examples for the use of this catalyst in the aldol reaction are presented here (scheme 9):



Scheme 9. Examples of inter- and intramolecular aldol reactions effectively catalyzed by proline.

It is interesting to notice how proline allows us to obtain not only a high enantiomeric excess, but an excellent diasteromeric control is often achieved when in the direct intermolecular aldol reaction more then one new stereocenter is generated[32], an effect that can be amplified in the case of the self-aldolization of the α-benzyloxyacetaldehyde to finally yield the hexose Allose with perfect enantioselectivity[33]. It performs well moreover in the *Enolexo* cyclizations[34] as well as in the previously (scheme 2) reported Robinson annulations.

The impressive results obtained in the aldol reaction are accompanied by a number of different other organocatalytic processes such as Mannich reaction[1a], α-amination[29], aza-Diels-Alder[30], aza-Morita-Baylis-Hillman[31], all involving the enamine activation of the substrate in the transition state of the reaction (scheme 10).

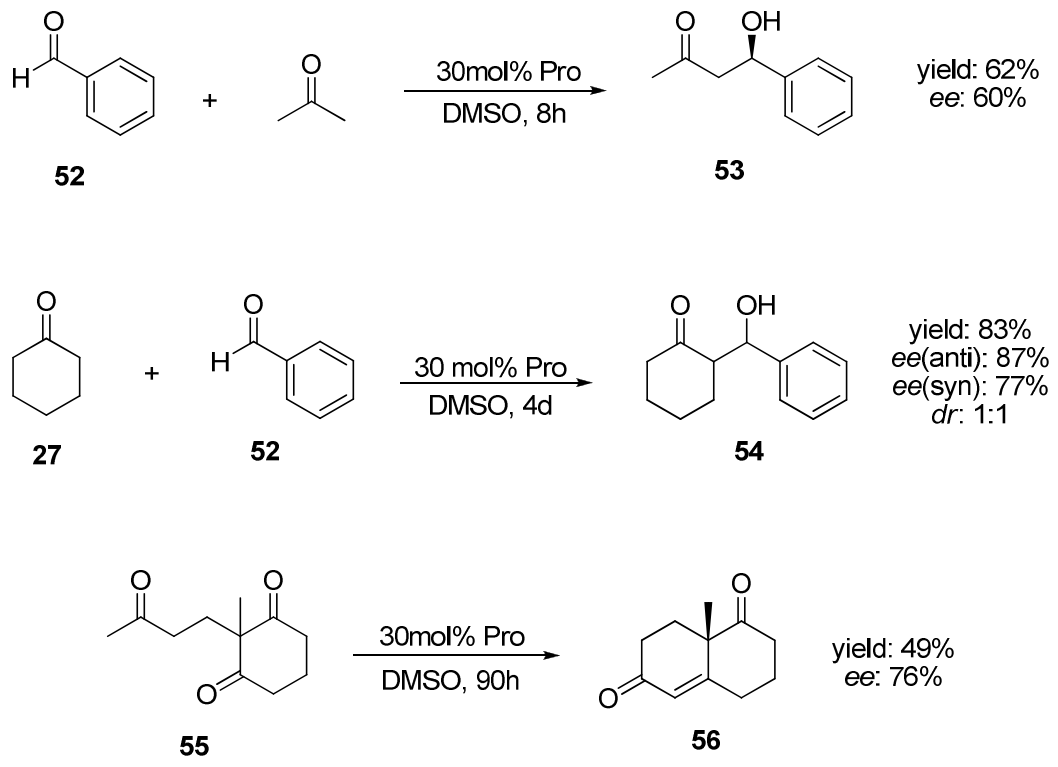


Scheme 10. Proline as a effective organocatalyst for the Mannich reaction, *α*-amination, aza-Diels-Alder and aza-Morita-Baylis-Hillman.

Limitations

Despite its advantages and high performances, the use of proline as organocatalyst meets some limitations. The high catalyst load that sometimes reaches 30-35% and is very seldom lower than 20% can surely be justified by its being quite inexpensive, especially if we think that the greatest majority of the organocatalysts developed in the last years as effective alternatives to proline have to be prepared through multistep synthesis procedures, generally involving modifications of the proline moiety itself; although being often more active, they are of course much more expensive than proline. A more stringent problem may be represented by the fact that proline performs better in DMSO and DMF, environmentally unfriendly and expensive solvents which imply generally a not trivial aqueous work up at the end of the reaction, exclude the possibility of recovering the catalyst and are not suitable for the use in a large or industrial scale. Moreover in the last few years, the idea of developing effective organocatalysts that are able to catalyze efficiently the aldol reaction in water, or better, in a heterogeneous water-substrates dispersion, has been blossoming due to the discovery that the organocatalyzed reaction between water-insoluble ketones and aldehydes is feasible, and proceeds well at the interface between the aqueous phase and the organic layer/droplets. In these conditions, and generally in presence of water, proline provides generally racemic products, thus limiting its use for this purpose. Finally, the search for new catalysts has been prompted by the desire of improving the yield, the selectivity and the speed of many processes for which proline resulted to be a unsatisfactory catalyst, some of such reactions, if we just limit ourselves to the field of the aldol reaction are reported in scheme 11.

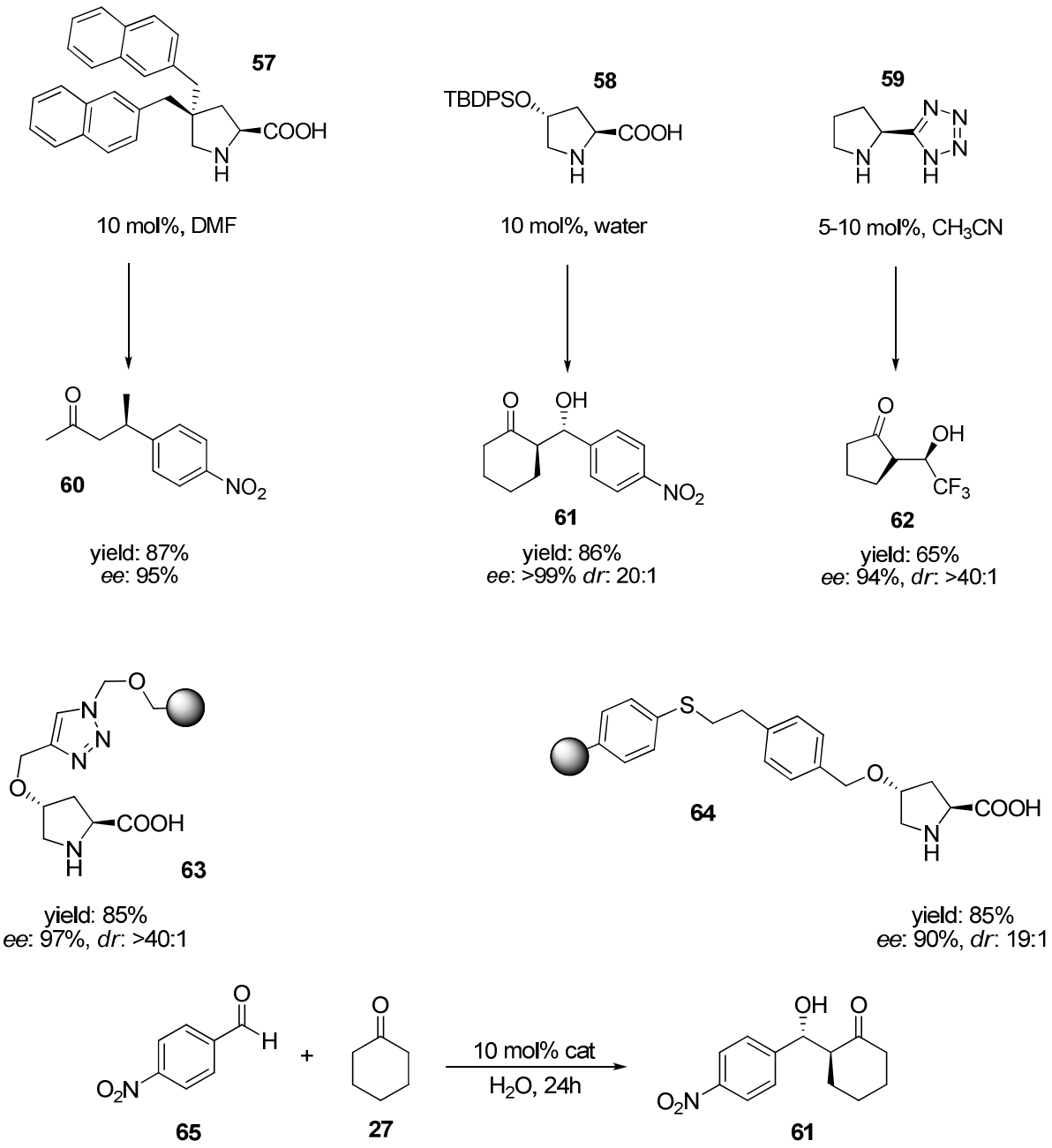
Although proline works generally well in the aldol reaction between aliphatic aldehydes and ketones, the results obtained in the case of aromatic aldehydes[2] can be surely improved. Moreover, in the case of the Wieland-Miescher ketone, the results achieved[4,5,35] were not as brilliant as in the case of its 5-member-ring analogue. Those results prompted a large number of researchers to propose new catalysts and to investigate the full spectrum of the aldol reaction.



Scheme 11. Examples of aldol reactions for which proline did not perform as a satisfactory catalyst.

Development of new catalysts, the aim of this thesis

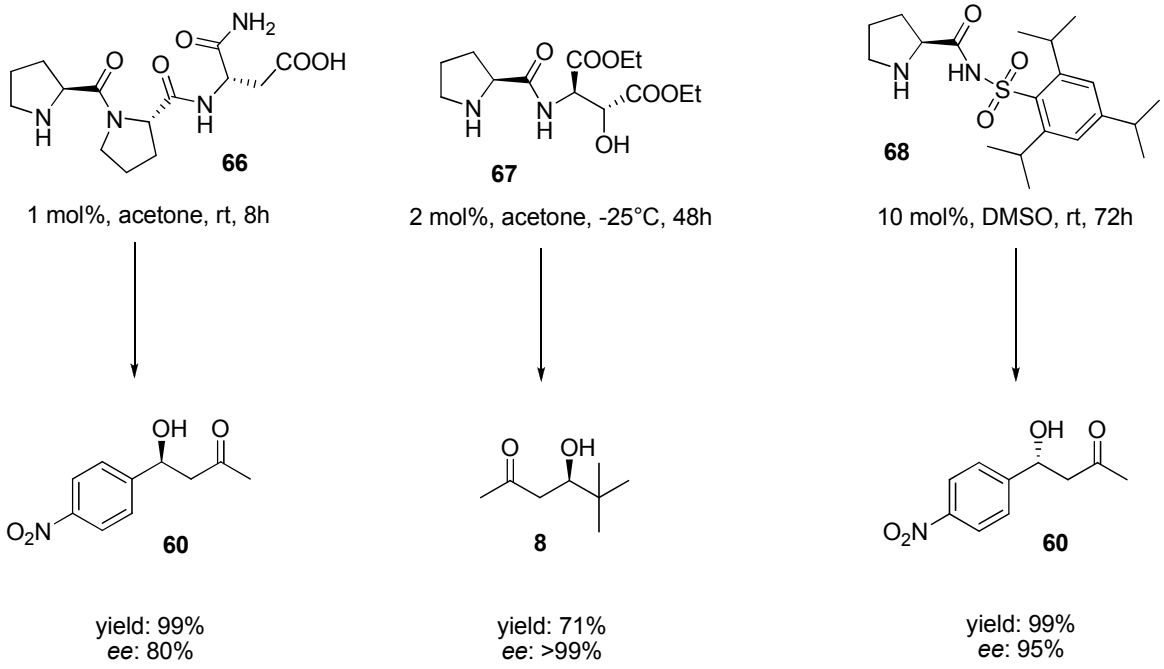
The development of new catalysts has been following some main routes. On one hand attempts have been made to modify the scaffold of proline itself, generally, at the positions 3,3´ of the pyrrolidine ring. In this way, a more demanding steric factor was introduced and, at the same time, an increased solubility of the catalyst in the organic layer of the heterogeneous aldol reaction was obtained[36]. In a comparable way, other authors tried to create easily recoverable catalysts by linking hydroxyproline, for instance, to a polymeric support[1g]. Modification of the proline moiety at the C-terminus has brought to interesting results as well[37] (scheme 12).



Scheme 12. Examples of new catalysts, prepared through modification of the proline moiety (**57**, **58**, **59**) or through its immobilization on a polymeric support (**63** and **64**).

On the other hand, another general way of preparing new catalysts has been represented by coupling proline at its C-terminus with amino acids, amines or amino alcohols in order to build peptides or pseudo-peptides[38]. This idea, relies on the possibility that such catalysts, although maintaining an enamine-based mechanism of action, may present a more suitable spatial arrangement of the amino and carboxylic (or alternative hydrogen

bond donor) functionality in comparison with proline, thus inducing a better selectivity and yield in the aldol reaction (scheme 13).



Scheme 13. Di- and tripeptides prepared as effective catalysts for the aldol reaction.

The development of these catalysts is made extremely challenging by the large number of diverse structures accessible through this strategy. If we simply restrict ourselves to the pool of the standard aminoacid, we may easily see that 20 dipeptides and 4000 tripeptides having proline as N-terminal amino acid are available. An elegant and successful combinatorial approach to this topic (which led to the development of the extremely active catalyst **66**) has been presented[38n,39] (fig. 2), based on the preparation of co-immobilized substrate-tripeptide libraries. This notwithstanding, a more direct approach to catalyst design may be attempted by looking more in detail at the turn inducing properties of the natural and unnatural amino acids. If we compare the plausible transition state[38d] for an already known structure and for a "model" peptide catalyst, (fig. 3) we see how the idea of building a tripeptide catalyst having, for instance, a turn inducing amino acid as a central building block, may look as a rational approach to the preparation of a new class of catalysts.

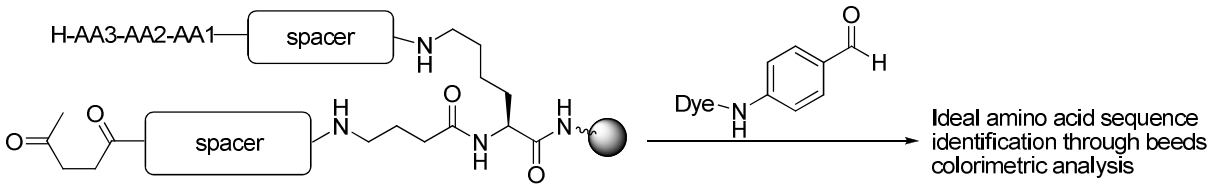


Fig. 2. Identification of an effective catalyst for the aldol reaction through preparation of a co-immobilized substrate-peptide library.

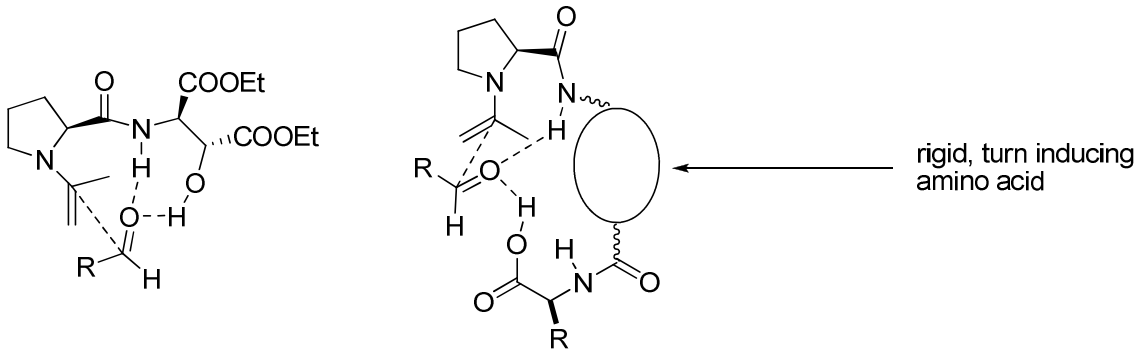
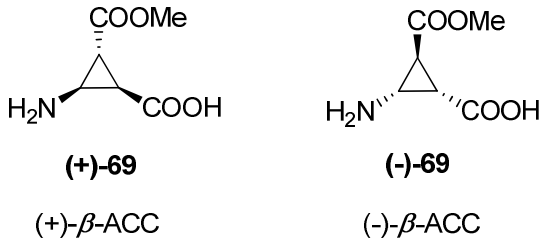


Fig. 3. Transition state for the aldol reaction catalyzed by *Gong´*s dipeptide and by a "model" peptide.

Ideally such building block would induce a turned, H-bond stabilized secondary structure in the catalyst, thus organizing the carboxylic and amino functionalities of the peptide in an ideal spatial arrangement to effectively promote the asymmetric aldol reaction. We would like here to show that the *cis*-*β*-aminocyclopropanecarboxylic acids (*β*-ACC, scheme 14), whose properties, synthesis and behavior in the field of organocatalysis will be the object of the next chapters, represent ideal candidates for the preparation of such catalytic peptides.



(+)-69 **(-)-69**

(+)-*β*-ACC (-)-*β*-ACC

Scheme 14. The two *β*-ACC enantiomers as candidate building block for the synthesis of novel organocatalysts

CHAPTER 1: *β-ACC as a useful building block for the synthesis of bifunctional organocatalysts*

The chemistry of the *β*-ACCs, conformationally constrained *β*-amino acids, (+)-**69** and (-)-**69** scheme 14) has been broadly studied by *Reiser et al.* following the development of convenient strategies for the cyclopropanation of heterocyclic aromatic compounds[40]. It has been found that these *β*-amino acids have, amongst their features, the ability to stabilize secondary structures even in very short peptides or to induce functional *β*-turns. Here we would like to show how these characteristics make these molecules the ideal building blocks for the synthesis of bifunctional organocatalysts.

1.1 β-ACCs- based peptides and foldamers

The use of the *β*-ACCs in the synthesis of new functional peptides has shown the ability of these amino acids to behave as constraining, rigidifying factors. For instance, the substitution of two units of the C-terminal (25-36) portion of neuropeptide Y with two units of (+)-**69** (fig. 4), produced a sequence able to bind with high affinity and selectivity to the receptor subtype Y_1[41]. This effect is probably due to the ability of this building block to impose restraints to the arrangement of the neighboring side chains that are essential for the subtype recognition, obtaining a result comparable to their incorporation in a cyclic structure.

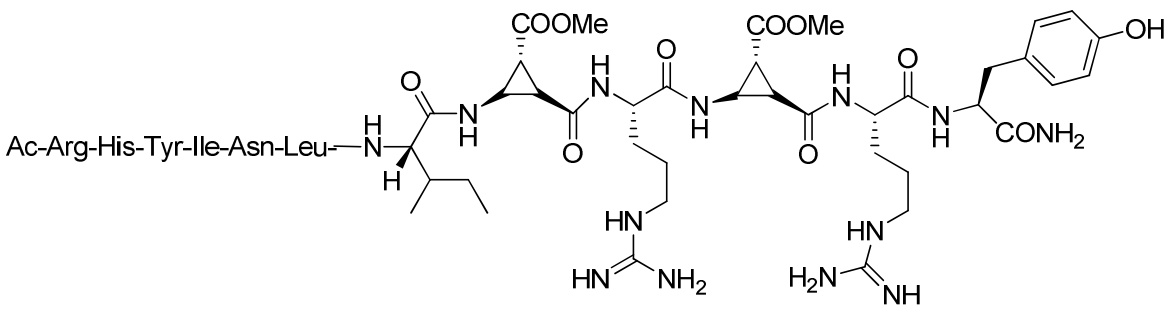


Fig. 4. The (+)-*β*-ACC containing sequence, able to bind with high affinity to the receptor subtype Y_1

Further investigation involved the preparation of foldamers: oligopeptides able to show conformational propensity toward a well defined secondary structure. For many years, chemists and biochemists have been attracted by the exceptional correlations between the secondary structure of proteins and their function in living organisms, which are obviously linked by the way the amino acidic sequences fold to assume their active structures. This generated an intriguing quest toward the possibility of reproducing a folding pattern or even the active site of an enzyme or of an agonist, using a lower number of opportunely chosen amino acids, able to fold in the desired way. As a consequence, we may ask ourselves which is the shortest amino acidic sequence able to fold. Although it is know that 12 natural residues are necessary to obtain a stable secondary structure, during the nineties *Gellman*[42] showed that using the *β*-amino acids *trans*-ACHC or *trans*-ACPC (fig. 5) to build *β*-homooligomers, this number could be reduced to 4-7 units, allowing the preparation of stable 14- or 12-helical conformations in methanol. *Seebach*[43] showed that acyclic *β*-amino acids as well, could be used to induce stable secondary structures in short *β*-hexamers in polar organic solvents (fig. 5).

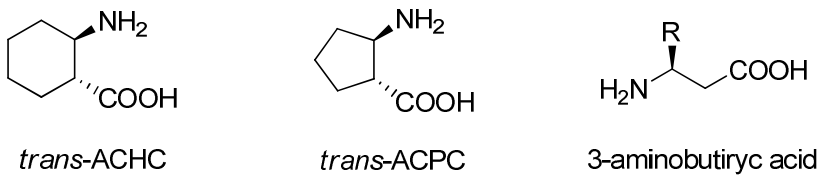


Fig. 5. The *β*-amino acids used by Gellman and Seebach in the preparation of foldamers

In the last few years the attention switched from the homooligomers to mixed sequences of *α*/*β*-amino acids. Such peptides often show a higher propensity to fold in stable helical structures, and allow us to enrich the chemistry of foldamers with the inexpensive, well assorted natural amino acids[44]. *Reiser et al*.[45] reported the first systematic study of such peptides, using methanol as solvent. Several peptides were prepared alternating ▲ or ▼ with L-alanine residues (fig. 6). Looking at the correlation between non-consecutive residues through NOESY spectroscopy, it was documented how all the short peptides analyzed present a certain degree of helical behavior and while the ▲-containing peptides tend to interconvert between mixtures of conformers, the hepta- and nona-

peptides containing ▼ fold in a well defined 3_{13}-helix, confirmed by the presence of various *i-i+3* NOESY contacts.

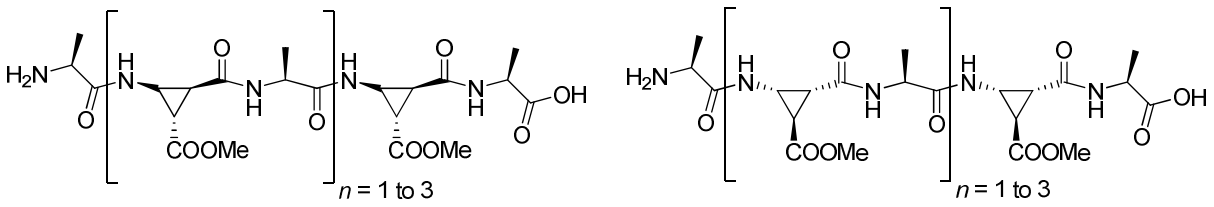


Fig. 6. Short *β*-Acc containing peptides which showed a partial (right) and definite (left) folding propensity.

In a comparable way Gellman[46] used the ACPC unit to induce well defined, or inter-converting *α*-helical structures in mixed *α*/*β*-octamers.

1.1.1 β-ACCs and β-turns

Another remarkable ability of the *β*-ACCs and other *β*-amino acids or *β*-amino acids patterns is the capacity to turn short sequences in to hairpin-shaped peptides, similarly to the way in which anti-parallel *β*-sheets are formed in nature. This is of particular interest in the field of molecular recognition and in the development of this thesis. One interesting example was provided by *Seebach*[47] by synthesizing a *β*-tetramer able to mimic the hormone somatostatin in its binding to the relative human receptor; this *β*-peptide could reproduce the turn crucial for its recognition at the binding site. An example of the *β*-amino acids ability to induce a reverse-turn when slipped between an α-amino acids sequence, was presented again by *Gellman*[48] who used two *β*-units to create a hairpin between complementary natural amino acids (fig. 7a). *Reiser* and *Sewald*[49] reported that the introduction of the *β*-ACC ▲ in a cyclic pentapeptide *cyclo*-(Arg-Gly-Asp-▲-Val) instead of the previously used D-Phe, could confer additional rigidity and a secondary structure stabilization to this cycle, thus enhancing its ability in inhibiting the interaction between RGD containing receptors and integrines. Geometry calculations supported by bi-dimensional NMR studies, show that the *β*-ACC unit resides at the center of a pseudo-*β*-turn (fig. 7b).

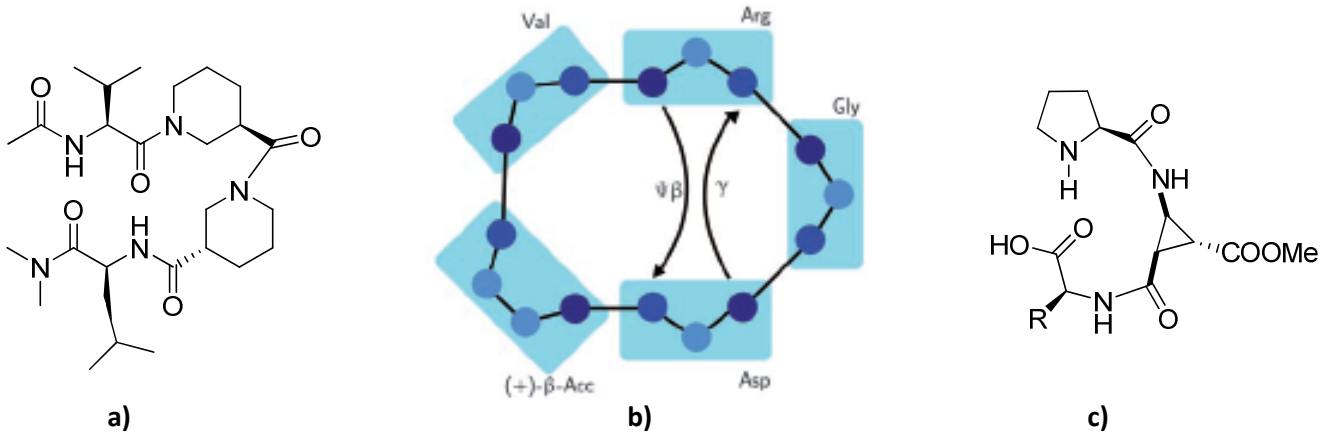


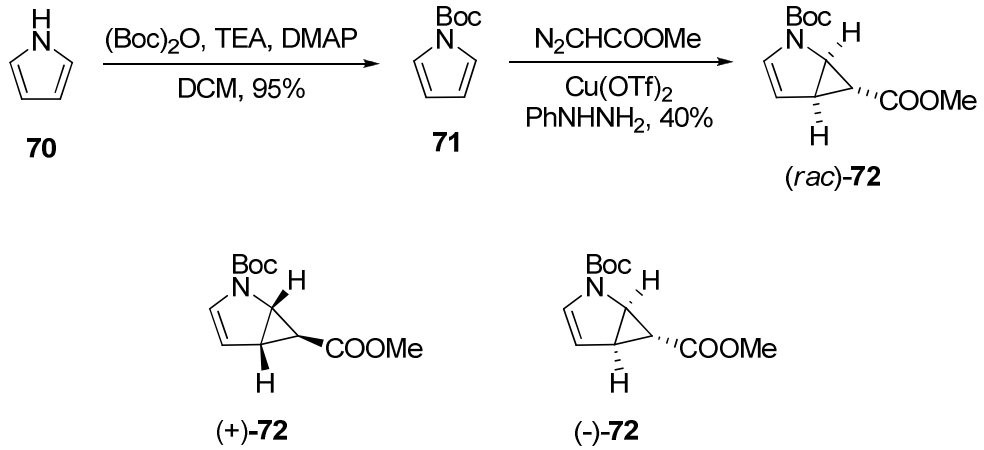
Fig. 7. a) Gellman´s *β*-amino acids based hairpin, **b)** the *β*-ACC residing at the center of a pseudo *β*-turn in the *cyclo*-(Arg-Gly-Asp-▲-Val) peptide and **c)** model, hairpin-like peptide as a candidate organocatalyst.

On the basis of the reported observations it is not with haste to say that the *β*-ACCs may serve as useful building blocks for the synthesis of turned peptides like in fig. 7c, with the functional groups of the proline unit and of a third amino acid able to assume the suitable geometry for catalyzing the asymmetric aldol reaction (fig. 3).

1.2 Synthesis of the cis-β-aminocyclopropanecarboxylic acids (β-ACCs)

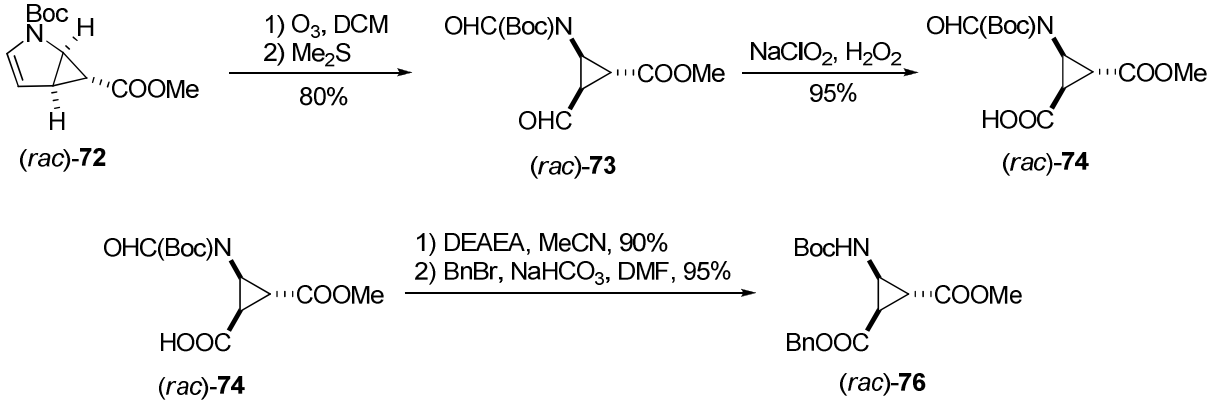
The synthesis of the *β*-ACCs units begins with the cyclopropanation of the inexpensive *N*-Boc-pyrrole[40a,b] (**71**) through its reaction with methyl diazoacetate in the presence of copper (II) triflate and phenylhydrazine (scheme 15). This process is diastereoselective, providing only the *exo* diastereomer, having the methyl group oriented towards the convex face of the bicyclic structure, but it is not enantioselective, yielding a racemic product (***rac*-72**)[40b]. The yield of this process is low, but the starting material, *N*-Boc-pyrrole, may be quantitatively recovered and used for a new cycle, with an overall conversion of 60-70%. The kinetic resolution of this racemate can be successfully carried out on the gram scale using the enzyme lipase L-2[40b] but this did not appear to be a convenient method for a larger scale preparation of the desired building block. A successful approach to the resolution of this mixture in a multigram scale (160 g) was

previously performed[50] on a semi-preparative chiral simulated moving bed chromatographic unit and yielded a large amount of enantiomerically pure (+)-**72** and (-)-**72**. In the development of this work no resolution of this racemic mixture has been carried out, and (*rac*)-**72** has been directly used for the successive steps, with the aim of attempting a resolution of the racemic *β*-ACC at a later stage, through the use of chiral bases or through its incorporation in dipeptides.



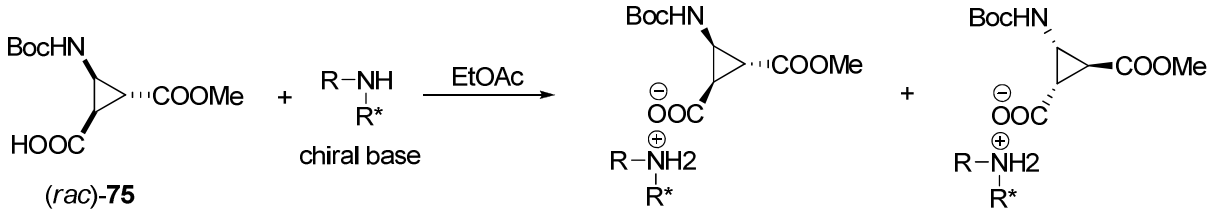
Scheme 15. Boc-Pyrrole cyclopropanation to yield the racemic *exo*- product.

The further steps leading to the target building block include its ozonolysis, oxidation of the aldehyde moiety, deformylation at the *N*-terminus, and benzylation of the free carboxylic functionality, furnishing the opportunely protected building block, in its racemic form (*rac*)-**76** (scheme 16).



Scheme 16. Synthesis of the racemic building block

An attempt to resolve compound (*rac*)-**75** was carried out by reacting it with equivalent amounts of chiral bases in order to achieve a diastereomeric salt crystallization, which represents a common way to resolve chiral mixtures[51] (scheme 17, table 1) of acid or basic organic compounds.



Scheme 17. The diastereomeric salt crystallization attempt of **75**

Table 1. Diastereomeric salt crystallization of **75**

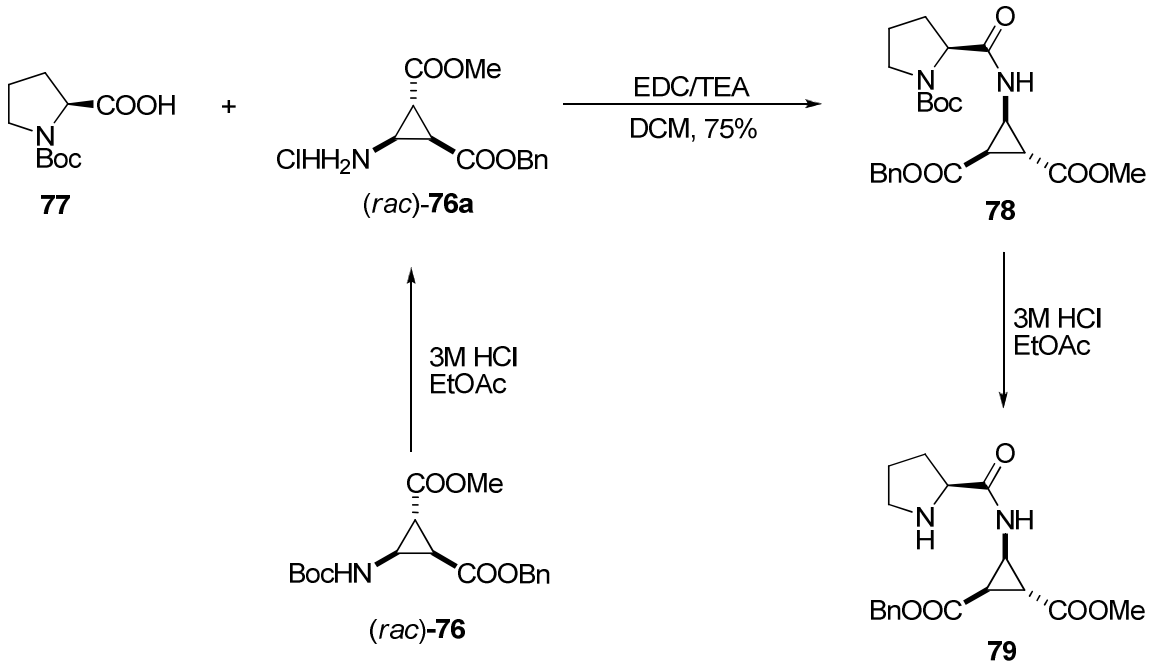
Base	salt aggregation form	precipitate after crystallization[a]	α_D[b] of 75 after workup of the precipitate
(-)-α-methyl benzylamine	oil	-	-
(-)-methyl ephedrine	white solid	oil	-
cinchonidine	white solid	white solid	0

[a]From a Hexane/EtOAc mixture, [b]in MeOH.

Unfortunately this attempt proved to be unsuccessful as only in the case in which cinchonidine was used as a chiral base, a precipitate was obtained from a Hexane/EtOAC mixture , but this salt, after workup, resulted again in the racemic mixture (*rac*)-**75**. For, this reason, we concluded to couple *rac*-**76** with proline and to perform the separation of the two diastereomeric dipeptides.

1.3 Synthesis of dipeptides and diastereomer separation

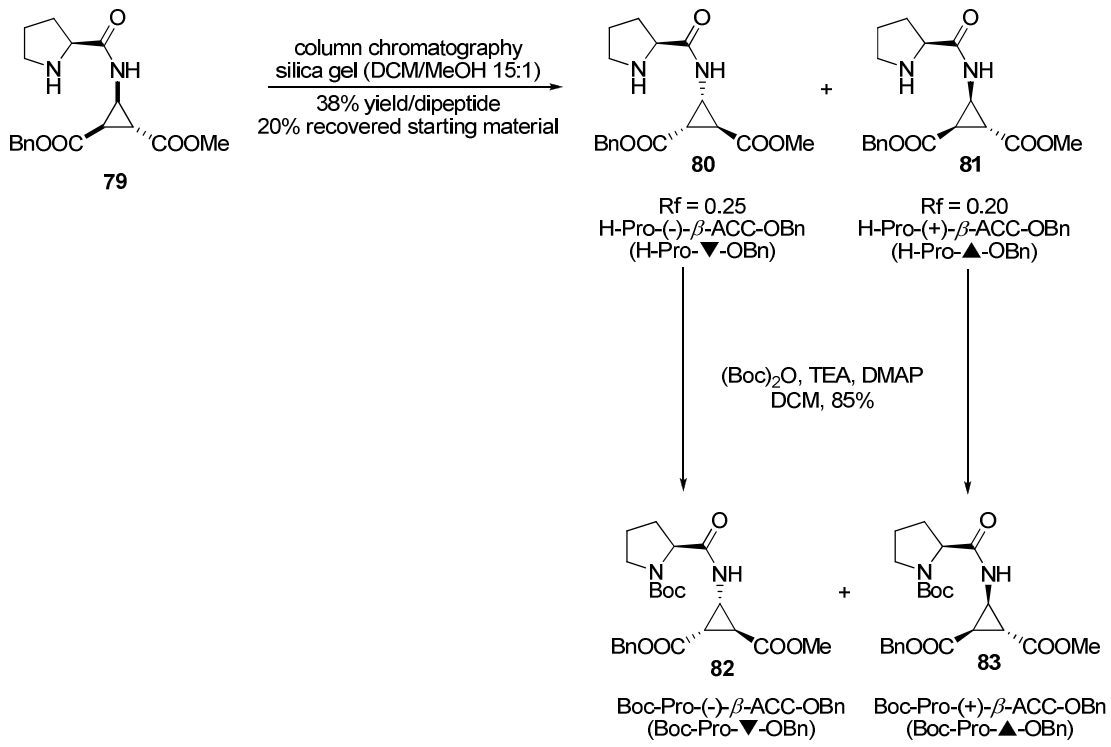
The synthesis of the dipeptide Boc-Pro-*β*-ACC-OBn (**78**) was carried out using the protocol developed[40a,52] by *Reiser et al.* for the coupling of the building block (*rac*)-**76** (scheme 18); the *N*-terminus of the *β*-ACC is deprotected with gaseous HCl and is used as a hydrochloric salt (*rac*)-**76a** to avoid the opening of the cyclopropane ring which takes place for this compound in the presence of the free amino functionality.



Scheme 18. Synthesis of the diastereomeric mixture H-Pro-*β*-ACC-OBn

Although a selective crystallization of **78** from Hexane/EtOAc mixtures was unsuccessful, it was noticed that a column chromatography separation on silica gel (DCM/MeOH 15:1) could be attempted on the Boc-deprotected derivative **79**, leading to two different peptides **80** (Rf = 0.25) and **81** (Rf = 0.20), both isolated with a yield of 35-40%. Interestingly the fractions containing a mixture of the non-separated diastereomers could be recovered and eventually used in a successive diastereomer separation, thus improving the efficiency of the process.

Zwicknagl[53] coupled separately Boc-proline with (+)-**69** and (-)-**69**, being in this way able to prove that dipeptide **80** corresponds to H-Pro-(-)-*β*-ACC-OBn (H-Pro-▼-OBn) and that **81** corresponds to H-Pro-(+)-*β*-ACC-OBn (H-Pro-▲-OBn), see scheme 19 and fig. 8.



Scheme 19. Silica gel separation of the two diastereomers of **79** and their subsequent Boc-protection

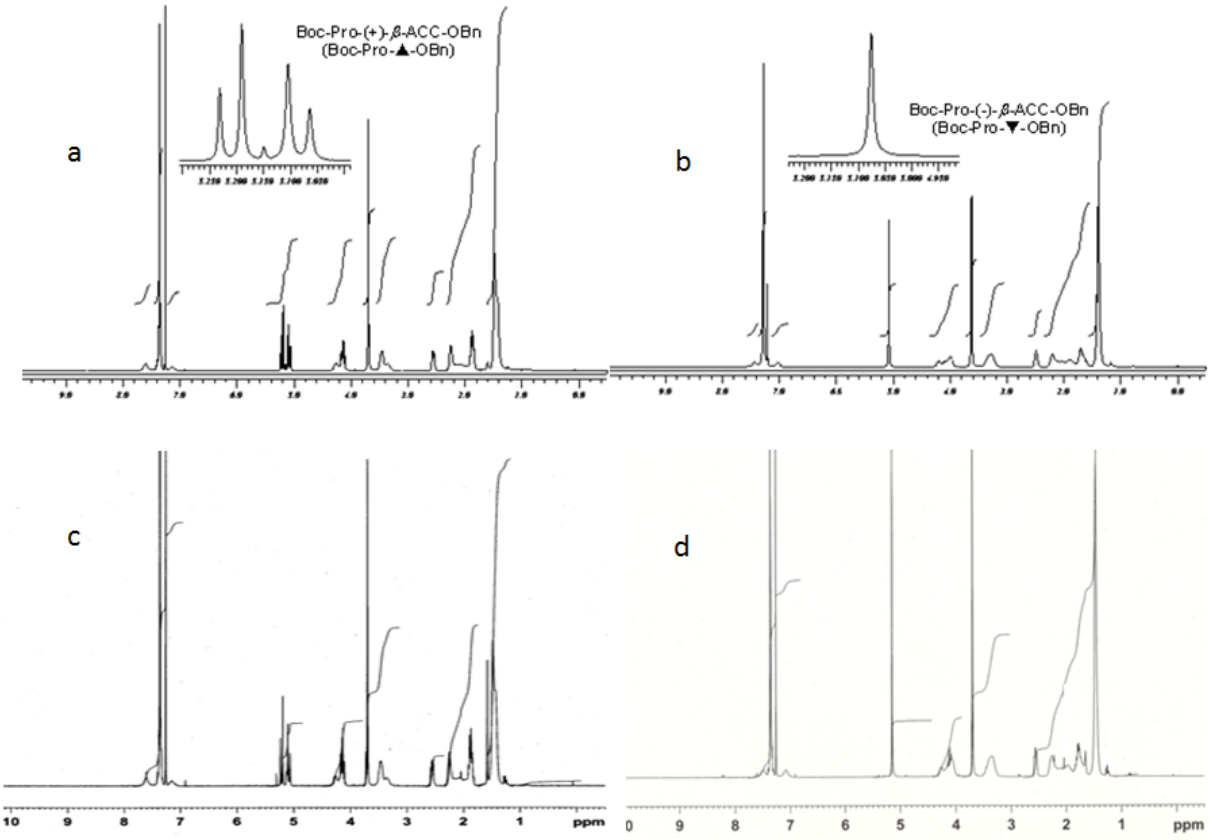
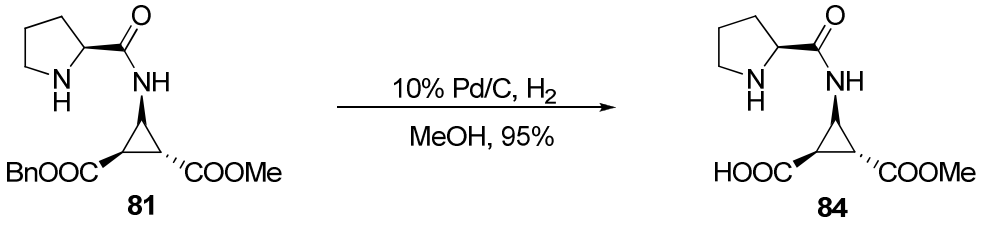


Fig. 8. a), **b)** dipeptides Boc-Pro-▲-OBn and Boc-Pro-▼-OBn, as obtained by *Zwicknagl* after coupling with the enantiomerically pure *β*-ACC, compared to **c)** and **d)** obtained from the Boc-protection of the diastereomers separated through column chromatography on silica gel.

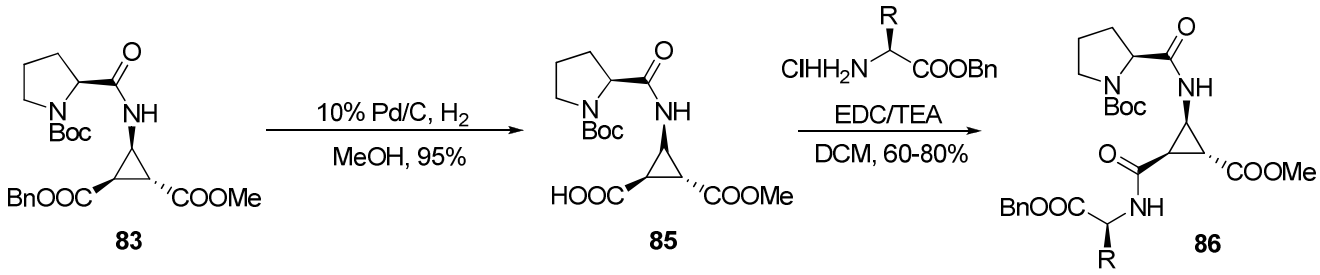
1.4 Synthesis of di- and tripeptides as organocatalysts

As the chromatographic separation of **80** and **81** represented the only way in which it was possible to achieve diastereomerically pure material, all the peptides synthesized proceed from a combination of the Pro-▲ or Pro-▼ patterns with natural amino acids or short peptides. **80** and **81** could be directly deprotected at the carboxylic functionality to furnish dipeptide catalysts **84** and **89** (scheme 20 and fig. 9). The same deprotection step was carried out for peptides **82** or **83** in order to couple them with a third amino acid (scheme 21), thus generating the Boc-Pro-▲-AA-OBn and Boc-Pro-▼-AA-OBn patterns that could be subsequently deprotected to provide a family of catalysts having one of the *β*-ACCs as a central building block.



81 84

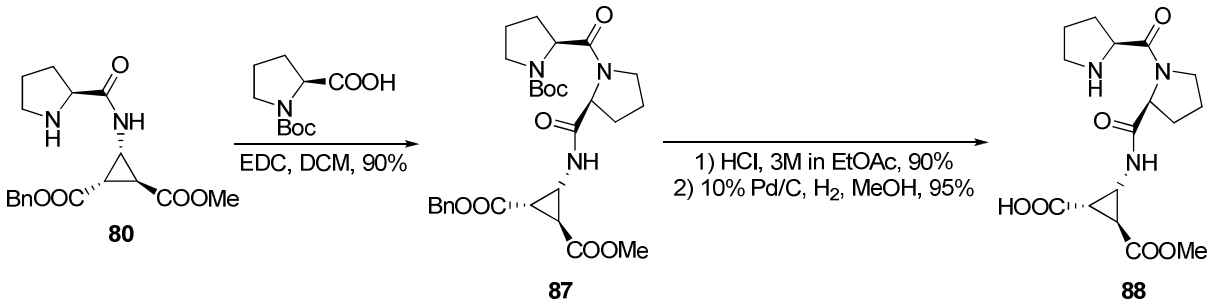
Scheme 20. Dipeptide catalyts are readily available from benzyl deprotection of **81** or **80**



83 85 86

Scheme 21. Synthetic pathway to the tripeptides having a *β*-ACC unit as a central building block

Another possibility is represented by the direct coupling of **80** and **81** at their *N*-terminus with another proline unit, thus generating the tripeptides H-Pro-Pro-▲-OH (**95**, fig. 9) and H-Pro-Pro-▼-OH (**88**, see scheme 22). These peptides diverge from the catalyst design previously suggested (fig. 3 and 7c), but in a similar way, the turn-inducing properties of the Pro-Pro moiety, together with rigidity of the *β*-ACCs were supposed to be able to place the *N*- and *C*- termini of the catalyst in the ideal spatial arrangement to effectively promote the asymmetric aldol reaction.



Scheme 22. Synthesis of the tripeptides having a *β*-ACC unit as *C*-terminal amino acid

A complete overview of the di- and tripeptides synthesized following the above mentioned coupling strategies can be found in fig. 9:

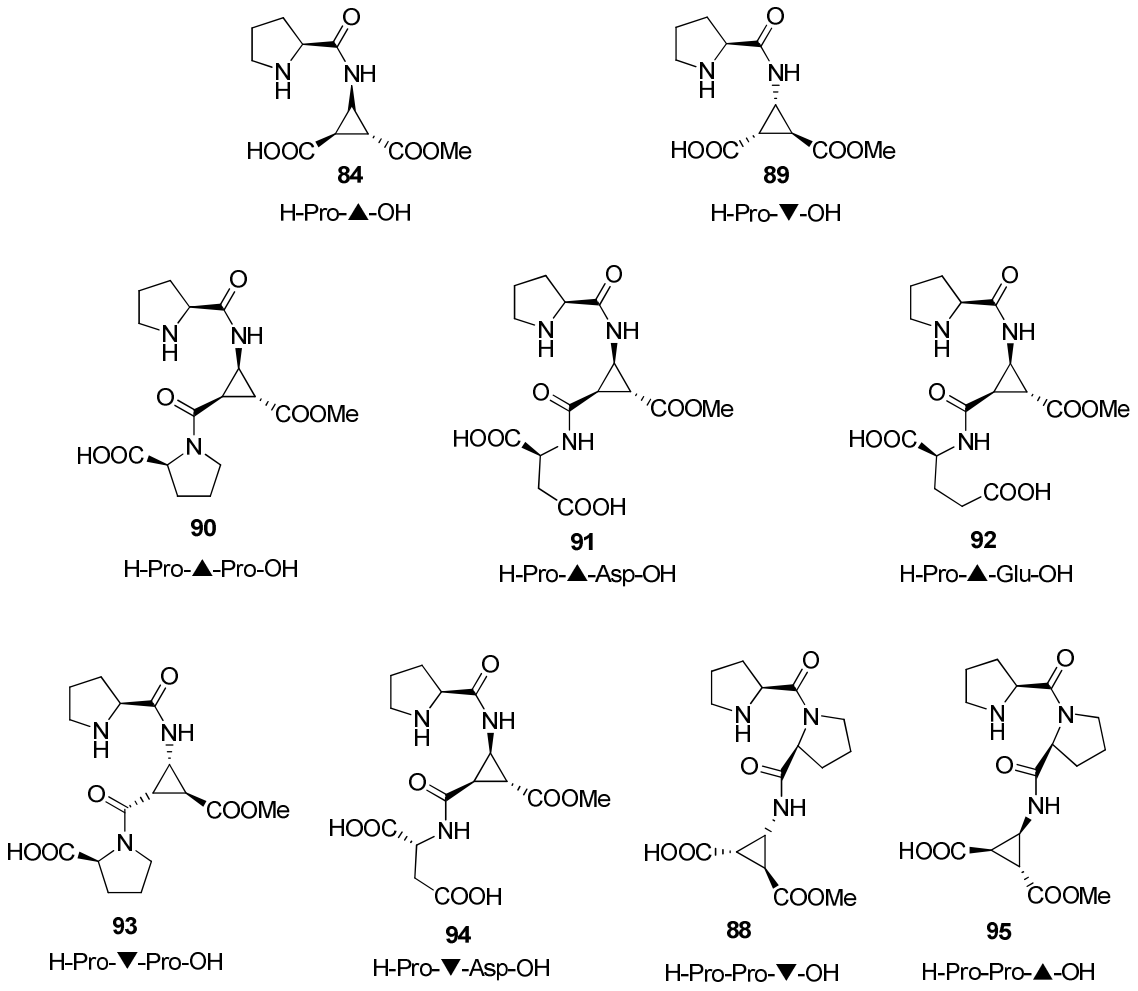
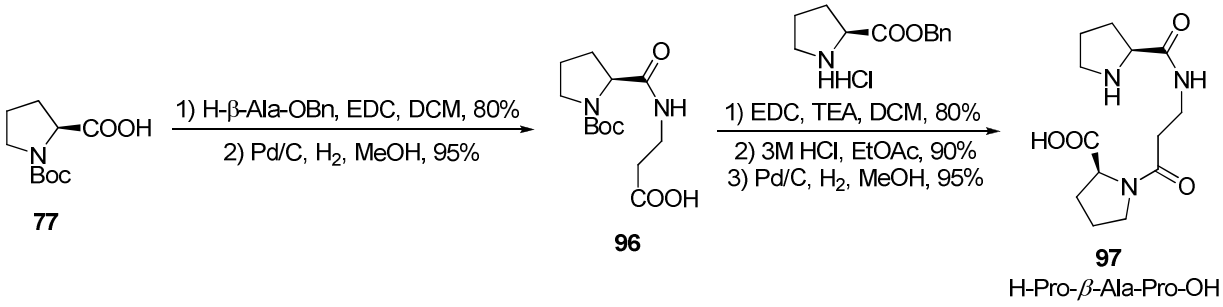


Fig. 9. Di- and tripeptides prepared following the previously mentioned coupling strategies.

The choice of the R side-chain (see scheme 21) in the case of the tripeptides having a *β*-ACC unit as a central amino acid, is not trivial, as according to a pure combinatorial approach, twenty different peptides for each enantiomer of the *β*-ACCs should have been prepared and screened. *Wennemers*[38n] has presented the tripeptide H-Pro-Pro-Asp-NH_2 (**66**) as an effective catalysts for the aldol reaction; further analysis of this catalyst and of its analogs shows that the aspartic acid side-chain represents an ideal compromise between chain flexibility and length, thus playing a pivotal role in the catalytic process[54]. We decided to screen for this purpose the amino acids Asp and Glu as coupling partners for our dipeptide **85** (or its diastereomer containing the ▼ unit), wishing to identify in this way the right sequence able to work as an effective bifunctional catalyst. Moreover, by observing the structure of the model peptide (fig. 7c), we considered that it would have been interesting to prepare a peptide able to assume a *cis* configuration at the amide bond between the *β*-ACC unit and the *C*-terminal amino acid. Bearing in mind that proline, due to the secondary amine nature of its *N*-terminus, is the natural amino acid presenting the highest probability (5 to 50%) to be found in a *cis* peptide bond[55], we decided to include it in the pool of the coupling partners for the synthesis of novel catalysts.

1.5 Tripeptides not containing the β-ACC units

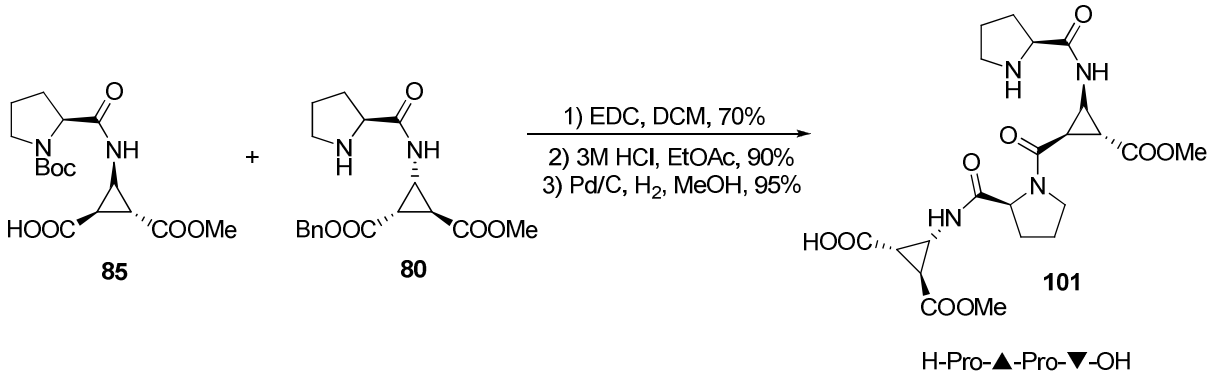
Another tripeptide containing an unnatural amino acid other than the *β*-ACC as a central building block was prepared in order to prove the importance and role of the rigid cyclopropane ring. For this purpose the H-Pro-*β*-Ala-Pro-OH (**97**) sequence was prepared, with *β*-alanine representing the conformationally unlocked equivalent the *β*-ACCs (scheme 23).

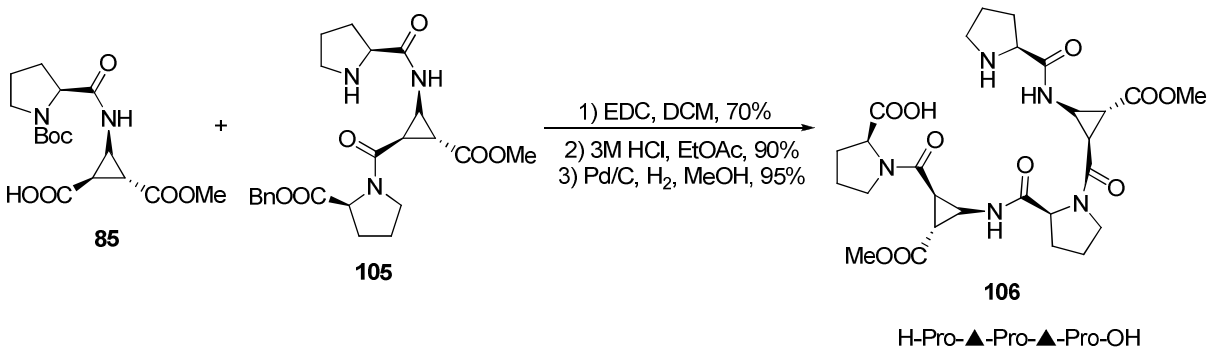


Scheme 23. Synthesis of the tripeptide H-Pro-*β*-Ala-Pro-OH

1.6 Synthesis of tetra- and pentapeptides

The elongation of the tripeptide moiety may be regarded as a way to obtain longer sequences able to assume a well defined secondary structure, thus approaching the concept of foldamers. Although the idea of using a well folded peptide as an organocatalyst may look extremely suggestive (it could be comparable to the preparation of an artificial enzyme) it meets some limitations from a practical point of view: such a catalyst would be much more expensive and laborious to prepare than proline or even than a relatively simple tripeptide. Considering the remarkable results obtained in the field of organocatalysis when simple amino acids and dipeptides have been employed, it would be hard to prove the convenience of such catalysts, even in the case of an outstanding catalytic performance. This notwithstanding, it appeared interesting to look for a correlation between an increased structural rigidity and an improved catalytic behavior. For this reason, two tetrapeptides (H-Pro-▲-Pro-▼-OH (**101**) and the H-Pro-▼-Pro-Pro-OH (**104**) and the pentapeptide H-Pro-▲-Pro-▲-Pro-OH (**106**) were easily prepared, coupling preformed, diastereomerically pure, di- and tripeptides (scheme 24).

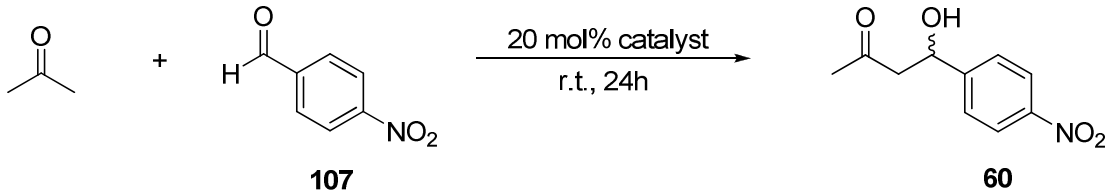




Scheme 24. Synthesis of tetrapeptides **101** and **104** and of pentapeptide **106**, by coupling preformed di- and tripeptides

CHAPTER 2: Organocatalysis

The pool of catalysts prepared according to the methodology described in the former chapter has been tested in the asymmetric aldol reaction, using the classic addition of acetone to *p*-nitrobenzaldehyde (scheme 25) as a benchmark process to evaluate the activity of our peptides. The choice of the right solvent, or solvent mixture, represented a challenging factor in the optimization of the reaction conditions and it had a strong impact on the yields and selectivities. This may look obvious when dealing with short peptides, as changing the reaction environment has a strong impact on the intramolecular H-bonds, thus stabilizing a peculiar conformation of the catalyst more than another, or rendering it completely unstructured. Moreover, the reaction conditions have a strong influence on the kinetics of the reaction and on the evolution of the transition state. It will be shown later in this chapter that the addition of water, in particular, had a dramatic effect on the outcome of the catalytic process.



107 **60**

Scheme 25. Benchmark reaction between acetone and *p*-nitrobenzaldehyde

2.1 Screening of the catalysts in the test aldol reaction

The choice of the solvents used in this first screening process was carried out on the basis of their cost, environmental impact, and of the possibility of recovering the catalyst at the end of the reaction. DMSO and DMF, the solvents which guarantee the best results in the case of proline (for the benchmark reaction a yield of 68% and an *ee* of 76% are obtained in DMSO in 24 hours with 30 mol% proline[1a,1b]), did not look able to satisfy any of the pre-requisites above mentioned and were not employed. *Wennemers*[38n] and *Gong*[38d] have shown that short peptides may perform well using acetone directly as a solvent in the aldol reaction with **107** and other aldehydes. For this reason we concluded to use acetone

in the first test round. The catalyst load was set at 20 mol%, with the intention of reducing it progressively in the case of the most active peptides, while a standard reaction time of 24 hours was adopted, which, in general, corresponded to the time required for a complete, or consistent, conversion of the substrate. L-Proline, was also screened in the same conditions, to allow a comparison between its activity and the activity of our catalysts.

Table 2. Catalysis results for the test reaction in acetone (20 mol% cat, r.t., 24 h)

entry	catalyst	yield (%)	*ee* (%)
1	H-Pro-OH	74	53 (*R*)
2	H-Pro-▲-OH (**84**)	29	64 (*R*)
3	H-Pro-▼-OH (**89**)	56	14 (*R*)
4	H-Pro-▲-Pro-OH (**90**)	99	70 (*R*)
5	H-Pro-▲-Asp-OH (**91**)	55	37 (*R*)
6	H-Pro-▲-Glu-OH (**92**)	30	40 (*R*)
7	H-Pro-▼-Pro-OH (**93**)	80	10 (*R*)
8	H-Pro-▼-Asp-OH (**94**)	45	11 (*R*)
9	H-Pro-Pro-▲-OH (**95**)	65	49 (*S*)
10	H-Pro-Pro-▼-OH (**88**)	98	48 (*S*)
11	H-Pro-▼-Pro-Pro-OH (**104**)	96	58 (*S*)
12	H-Pro-▲-Pro-▼-OH (**101**)	51	70 (*R*)
13	H-Pro-▲-Pro-▲-Pro-OH (**106**)	55	22 (*R*)

Although the catalysts were not initially soluble in acetone, the reactions became homogeneous (excluding the case of the more polar compounds **91**, **92** and **94** having two free carboxylic acid functionalities) as the amino functionality of the peptides screened formed a more soluble enamine intermediate with acetone. At the end of every process the catalysts could be recovered by evaporating the reaction solvent and separating it from the organic products through a simple work up procedure followed by lyophilization of the subsequent aqueous layers. The preliminary data listed in table 2 show that some promising candidate catalysts, dipeptide **84**, tripeptide **90**, and tetrapeptides **104** and **101**, overcome the selectivity offered by proline in the same reaction conditions, and between them, H-Pro-▲-Pro-OH, shows the highest reactivity; it

also overtakes the conversion rate of proline, and approaches its selectivity in DMSO, thus becoming a suitable candidate for further screening and optimization. It is worth noting that although the relative *ee* values leave plenty of room for improvement, some catalysts (**88**, **95**, **104**) are able to induce the formation of the enantiomer of **60** opposite to the one achievable with L-proline or with the other catalysts in our hands, making them potentially useful tools, after the necessary optimization of the reaction conditions. With this aim, we decided to explore other solvents. For instance, using chloroform as the main component of the solvents mixture, instead of neat acetone, we would have limited the formation of intermolecular H-bonds between catalyst and solvent, thus favoring the stabilization of the secondary structure of the catalysts. At the same time, we wanted to check the effect of the addition of water to the organocatalyzed aldol reaction that at that time was still unreported, or in a phase of development. For this reason we tried the pool of catalysts in two new reaction mixtures, 2:1 chloroform/acetone (table 3) and 5:1 acetone/water (table 4).

Table 3. Catalysis results for the test reaction in a 2:1 $CHCl_3$/ acetone mixture (20 mol% cat, r.t., 24 h)

entry	catalyst	yield (%)	*ee* (%)
1	H-Pro-OH	68	61 (*R*)
2	H-Pro-▲-OH (**84**)	25	55 (*R*)
3	H-Pro-▼-OH (**89**)	48	10 (*R*)
4	H-Pro-▲-Pro-OH (**90**)	53	29 (*R*)
5	H-Pro-▲-Asp-OH (**91**)	17	0
6	H-Pro-▲-Glu-OH (**92**)	18	2 (*R*)
7	H-Pro-▼-Pro-OH (**93**)	61	12 (*R*)
8	H-Pro-▼-Asp-OH (**94**)	10	8 (*R*)
9	H-Pro-Pro-▲-OH (**95**)	59	64 (*S*)
10	H-Pro-Pro-▼-OH (**88**)	79	73 (*S*)
11	H-Pro-▼-Pro-Pro-OH (**104**)	76	33 (*S*)
12	H-Pro-▲-Pro-▼-OH (**101**)	21	71 (*R*)
13	H-Pro-▲-Pro-▲-Pro-OH (**106**)	47	11 (*S*)

Table 4. Catalysis results for the test reaction in a 5:1 acetone/water mixture (20 mol% cat, r.t., 24 h)

entry	catalyst	yield (%)	*ee* (%)
1	H-Pro-OH	98	0
2	H-Pro-▲-OH (**84**)	45	65 (*R*)
3	H-Pro-▼-OH (**89**)	47	0 (*R*)
4	H-Pro-▲-Pro-OH (**90**)	72	74 (*R*)
5	H-Pro-▲-Asp-OH (**91**)	70	62 (*R*)
6	H-Pro-▲-Glu-OH (**92**)	59	73 (*R*)
7	H-Pro-▼-Pro-OH (**93**)	77	6 (*R*)
8	H-Pro-▼-Asp-OH (**94**)	89	13 (*R*)
9	H-Pro-Pro-▲-OH (**95**)	48	0
10	H-Pro-Pro-▼-OH (**88**)	83	57 (*S*)
11	H-Pro-▼-Pro-Pro-OH (**104**)	58	0
12	H-Pro-▲-Pro-▼-OH (**101**)	36	74 (*R*)
13	H-Pro-▲-Pro-▲-Pro-OH (**106**)	23	35 (*R*)

In general, the results listed in table 3, show how that use of the 2:1 $CHCl_3$/acetone mixture reduces the reaction rate for each of the catalysts employed and is detrimental to the selectivity of most of the catalysts. The catalysis takes place in a homogeneous environment after the initial stage required for the formation of the enamine derivatives, with the exception of the catalysts bearing two free carboxylic acids (**91**, **92**, **94**) which, being completely insoluble, show even lower catalytic activity. In contrast to this trend, the tripeptides having a *β*-ACC unit as *C*-terminal amino acid, enjoy a clear improvement of their selectivity, thus proposing H-Pro-Pro-▼-OH (**88**) as a candidate for further optimization.

The addition of water (table 4) produced a positive effect for some of the catalysts, in particular, tripeptides **91** and **92** show a high increase in reactivity and selectivity, while the selectivity of **90** increases at expense of a slightly lower activity. In general, a trend emerges making the tripeptides which have ▲ as a central building block far more selective than their ▼ containing diastereomers. Tetrapeptide **101**, shows an interesting

selectivity and a catalytic performance that is nearly independent of the solvent which is used. Unfortunately the yields obtained using this catalyst were always quite low. The addition of water appears to be undesirable in the case of the peptides having the *β*-ACC as a *C*-terminal amino acid.

In conclusion, two tripeptides, **90** and **88**, were able to catalyze the aldol reaction between *p*-nitrobenzaldehyde and acetone in good to high yield and selectivity. The novel peptides containing ▲ as a central building block (**90**, **91**, **92**) are of particular interest as the rigidity of this cyclic amino acid could play an important role in determining their good catalytic behavior. A way to estimate this factor is represented by the evaluation of the catalytic performance of a peptide such as **97** which contains a *β*-alanine unit replacing ▲ at the center of the amino acid sequence. It can be regarded as the conformationally unlocked equivalent of **90** (table 5). **97** did not reach the level of selectivity presented by **90** and interestingly, the relative selectivity changes sign by switching the reaction condition. This is proof that the peculiar, locked structure of the cyclic *β*-amino acid plays an important role in stabilizing the conformation of **90**.

Table 5. Comparison between the organocatalysts **90**, **97** and **100** (20 mol% catalyst, r.t., 24 h)

entry	solvent	catalyst	yield (%)	*ee* (%)
1	5:1 acetone/water	H-Pro-▲-Pro-OH (**88**)	72	74 (*R*)
2	5:1 acetone/water	H-Pro-*β*-Ala-Pro-OH (**97**)	96	51 (*R*)
3	2:1 $CHCl_3$/acetone		66	37 (*S*)

2.1.1 Optimization of the reaction conditions

The further optimization of the reaction conditions for catalysts **88** and **90** can be carried out varying some parameters like the temperature or the composition of the reaction mixture which resulted to be suitable for the given peptide.

Table 6. Effect of the temperature on the performance of the selected catalysts (20 mol% cat, 24h)

entry	solvent	catalyst	T	yield (%)	*ee* (%)
1	5:1 acetone/water	H-Pro-▲-Pro-OH (**90**)	r.t.	72	74 (*R*)
2	5:1 acetone/water	H-Pro-▲-Pro-OH (**90**)	0° C	68	67 (*R*)
3	5:1 acetone/water	H-Pro-▲-Pro-OH (**90**)	40° C	70	72 (*R*)
4	2:1 $CHCl_3$/acetone	H-Pro-Pro-▼-OH (**88**)	r.t.	79	73 (*S*)
5	2:1 $CHCl_3$/acetone	H-Pro-Pro-▼-OH (**88**)	0°C	86	88 (*S*)
6	2:1 $CHCl_3$/acetone	H-Pro-Pro-▼-OH (**88**)	-15 °C	62	88 (*S*)

The variation of the reaction temperature (table 6) plays an important role in the case of tripeptide **88**, which shows a remarkable increase in selectivity by switching from room temperature to 0°C (entries 4, 5) while a further reduction of the reaction temperature, leaves the selectivity unchanged and reduces the yield. This suggests to us that this peptide may exist in $CHCl_3$ as a mixture of conformers with the most energetically stable conformation being the catalytically active. For this reason, reducing the temperature may additionally freeze the structure of the tripeptide thus improving its catalytic performance, up to a point where a further decrease of the temperature has only a detrimental effect on the yield of the reaction, because no further stabilization is achievable. In the case of tripeptide **90**, we do not observe any improvement of the catalytic behavior by varying the temperature, this may suggest, that the catalytically active conformation is not the most energetically stable.

Another attempt to improve the performance of our catalysts can be carried out by changing the composition of the reaction mixtures. In the case of tripeptide **88**, we were interested in a further reduction of the acetone concentration, while in the case of **90**, the effect of a change in the number of equivalents of water employed in the reaction could be an interesting way to optimize the reaction conditions (table 7).

Table 7. Effect of water on the test reaction promoted by catalyst **90** (20 mol% cat, r.t.)

entry	solvent	water/acetone (equiv./equiv.)	time (h)	yield (%)	*ee* (%)
1	acetone	0/130	24	99	70 (*R*)
2	10:1 acetone/water	50/120	24	89	78 (*R*)
3	5:1 acetone/water	90/110	24	72	74 (*R*)
4	3:1 acetone/water	140/100	24	96	71 (*R*)
5	2:1 acetone/water	190/90	24	71	68 (*R*)
6	1:1 acetone/water	280/70	48	53	61 (*R*)
7	1:2 acetone/water	460/20	48	-	-

The reaction tolerates the addition of a large number of equivalents of water, but in correspondence to a 1:1 acetone/water composition, the *p*-nitrobenzaldehyde becomes insoluble in the solvent mixture, thus increasing the reaction time and compromising the overall catalytic performance. As a consequence, for a higher molar fraction of water (entry 7) the reaction is absolutely inhibited. Interestingly, we identified through this optimization procedure an ideal acetone/water ratio (entry 2) which will be the one used in the further exploration of the scope of our catalyst.

Table 8. Effect of the concentration of acetone on the test reaction promoted by catalyst **88** (20 mol% cat, 0°C, 24 h)

entry	solvent	equiv. of acetone	yield (%)	*ee* (%)
1	2:1 $CHCl_3$/acetone	45	86	88 (*S*)
2	3:1 $CHCl_3$/acetone	35	83	88 (*S*)
3	10:1 $CHCl_3$/acetone	10	56	86 (*S*)

In the case of catalyst **88**, a decrease in the acetone concentration (table 8) did not produce a positive effect and tends to be unfavorable for the yield of the process.

As a last step towards the optimization of the process, the possibility to reduce the catalysts load was examined. Considering that the preparation of the *β*-ACCs containing

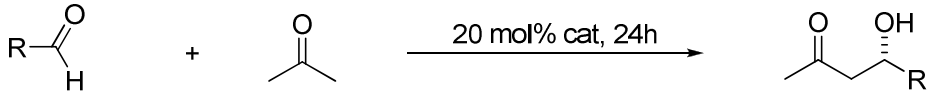
peptides is relatively challenging and that they are much more expensive than proline, a reduction in the amount of catalyst required for an efficient and selective conversion of the substrate, represents a very important goal. The experimental results (table 9) show that the load of the catalysts **88** and **90** can be reduced to 5 and 10 mol% respectively, with small losses in the yields and selectivities.

Table 9. Effect of the catalyst load on the test reaction promoted by catalyst **88** and **90** (24 h)

entry	catalyst	solvent	T	catalyst load (mol %)	yield (%)	*ee* (%)
1				20	89	78 (*R*)
2	**90**	10:1 acetone/water	r. t.	10	74	73 (*R*)
3				5	9	71 (*R*)
4				20	86	88 (*S*)
5	**88**	2:1 $CHCl_3$/acetone	0°C	10	80	88 (*S*)
6				5	75	83 (*S*)

2.1.2 Scope of the catalysts

The selected catalysts were screened in the aldol reaction between acetone and aromatic or aliphatic aldehydes, using the optimized reaction conditions (scheme 26, tables 8 and 9). The results obtained in the case of tripeptide **88**, are compared with the results obtained for proline in the same reaction conditions. The same comparison has not been reported for catalyst **90**, as it was observed during the first screening round (table 4, entry 1) that proline provided only racemic products, in this reaction, in the presence of water. In the case of tripeptide **88**, the reaction temperature has been adjusted for each substrate at the optimal value needed to reach a compromise between reactivity and enantioselectivity (table 10).



Scheme 26. Organocatalyzed aldol reaction between various aldehydes and acetone

Table 10. Catalyst **88** and proline in the reaction of scheme 27 (20 mol% cat, 24h, 2:1 $CHCl_3$/acetone)

entry	catalyst	R	T	yield (%)	*ee* (%)
1	H-Pro-Pro-▼-OH (**88**)	Ph (**52**)	10°C	50	79 (*S*)
	H-Pro-OH		r.t.	28	42 (*R*)
2	H-Pro-Pro-▼-OH (**88**)	*p*-Cl-Ph (**108**)	10°C	42	84 (*S*)
	H-Pro-OH		r.t.	37	50 (*R*)
3	H-Pro-Pro-▼-OH (**88**)	*o*-Cl-Ph (**109**)	5°C	70	80 (*S*)
	H-Pro-OH		r.t.	87	64 (*R*)
4	H-Pro-Pro-▼-OH (**88**)	*o*-Br-Ph (**110**)	5°C	84	82 (*S*)
	H-Pro-OH		r.t.	88	65 (*R*)
5	H-Pro-Pro-▼-OH (**88**)	*p*-NO_2-Ph (**60**)	0°C	86	88 (*S*)
	H-Pro-OH		r.t.	68	61 (*R*)
6	H-Pro-Pro-▼-OH (**88**)	*p*-CF_3-Ph (**111**)	0°C	82	87 (*S*)
	H-Pro-OH		r.t.	65	63 (*R*)
7	H-Pro-Pro-▼-OH (**88**)	*o*-NO_2-Ph (**112**)	r.t.	88	88 (*S*)
	H-Pro-OH		r.t.	58	92 (*R*)
8	H-Pro-Pro-▼-OH (**88**)	*c*-C_6H_{11} (**113**)	r.t.	57	82 (*S*)
	H-Pro-OH		r.t.	55	94 (*R*)

The experimental results prove that **88** can promote the aldol reaction with moderate to high yields and with a high enantioselectivity. In general, it performs remarkably better than proline for what concerns the aromatic aldehydes, either in the same reaction conditions, or in DMSO[2]. Proline, instead, confirms to be an excellent catalyst in the case of the aliphatic substrates.

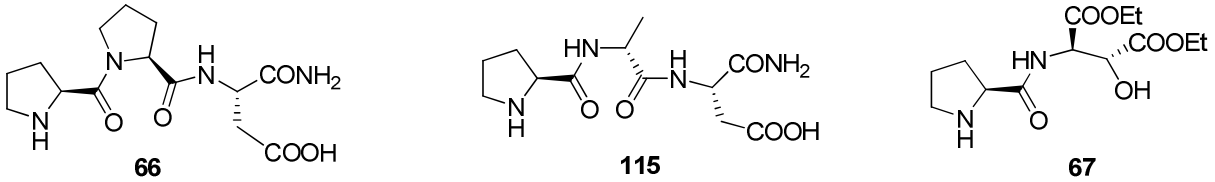
Table 11. Catalyst **90** in the reaction of scheme 27 (20 mol% cat, 24h, 10:1 acetone/water, r.t.)

entry	R	yield (%)	*ee* (%)
1	Ph (**60**)	48	76 (*R*)
2	*p*-Cl-Ph (**108**)	43	80 (*R*)
3	*p*-NO_2-Ph (**60**)	89	78 (*R*)
4	*p*-CF_3-Ph (**111**)	80	80 (*R*)
5	*p*-F-Ph (**114**)	13	72 (*R*)
6	*o*-Br-Ph (**110**)	86	72 (*R*)
7	*o*-Cl-Ph (**109**)	80	68 (*R*)
8	*o*-NO_2-Ph (**112**)	82	91 (*S*)
9	*c*-C_6H_{11} (**113**)	57	41 (*R*)

Tripeptide **90**, proved to be a good catalyst for the aldol reaction between acetone and aromatic aldehydes, with low to moderate yields in the case of the deactivated substrates and very good yields in the case of more reactive aldehydes. The enantioselectivity is generally good to high, slightly lower than what is observed in the case of **88**, but still generally improving the performance of proline in DMSO or in the $CHCl_3$/acetone mixture. Unfortunately **90** did not perform well in the case of the aliphatic aldehydes.

In conclusion, **88** and **90** are efficient catalysts for the aldol reaction, promoting the formation of the aldol products with opposite enantioselectivity. Compared to proline, the increased selectivity of these catalysts is a good tradeoff for an improved catalytic performance in low boiling solvent mixtures (**88**) or in the presence of water (**90**) which allowed the recovery of the catalysts through an aqueous work up. A comparison with the literature results may be carried out by considering *Wennemers´* tripeptides[38n] (**66** and **115**), the first catalytically active tripeptides reported, and *Gong´*s dipeptide[38d] (**67**), clearly the best catalyst for the aldol reactions of acetone (scheme 27, table 11). By comparing the results obtained in the case of some standard substrates (reaction in scheme 27), we are able to see that peptides **88** and **90** reach and generally overcome the level of selectivity, although not the reactivity, of catalysts **66** and **115**, either in the formation of the *S* or the *R* enantiomer. Catalyst **67**, instead, is unrivaled for selectivity

and reactivity, although it requires low reaction temperatures and can promote only the formation of the *R* enantiomer.



Scheme 27. *Wennemers´* (**66**, **115**) and *Gong´*s (**67**) catalysts for the aldol reaction

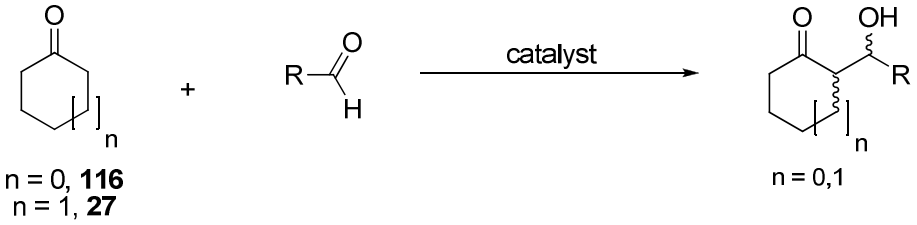
Table 12. Tripeptides **90** and **88** compared with the catalysts **66**, **115** and **67** as reported in literature for the organocatalyzed aldol reaction (Scheme 27, 24h)

entry	catalyst	R	catalyst load (mol %)	T	yield (%)	*ee* (%)
1	H-Pro-Pro-▼-OH (**88**)	*p*-NO_2-Ph	20	0°C	86	88 (*S*)
2	H-Pro-▲-Pro-OH (**90**)		20	r.t.	89	78 (*R*)
3	**66**[a]		1	r.t.	99	80 (*S*)
4	**115**[a]		10	r.t.	73	70 (*R*)
5	**67**[b]		2	-25°C	62	99 (*R*)
6	H-Pro-Pro-▼-OH (**88**)	Ph	20	10°C	50	79 (*S*)
7	H-Pro-▲-Pro-OH (**90**)		20	r.t.	48	76 (*R*)
8	**66**[a]		1	r.t.	69	78 (*S*)
9	**115**[a]		10	r.t.	58	66 (*S*)
10	**67**[b]		2	-25°C	68	98 (*R*)
11	H-Pro-Pro-▼-OH (**88**)	*c*-C_6H_{11}	20	r.t.	57	82 (*S*)
12	H-Pro-▲-Pro-OH (**90**)		20	r.t.	57	41 (*R*)
13	**66**[a]		1	r.t.	66	82 (*S*)
14	**115**[a]		10	r.t.	56	83 (*R*)
15	**67**[b]		5	-25°C	80	99 (*R*)

[a]Taken for refererence 38n. [b]Taken from reference 38d

2.2 Organocatalyzed aldol reaction between cyclic ketones and aldehydes

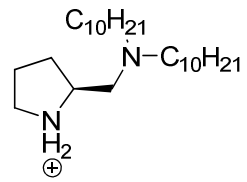
In the case of more complex ketones, we observe some remarkable differences in comparison to the aldol reaction of acetone. In general, these substrates are only partially miscible with water, thus introducing the possibility of carrying out the process in biphasic water-ketone mixtures. Moreover, the increased complexity of the ketone makes it more expensive and less indicated to be used as a solvent or a co-solvent of the reaction, requiring, in this way, the development of new catalytic systems in which it is used in almost equivalent amounts. Among such ketones, cyclic ketones have been attracting a large interest[57] in recent years, with the organocatalyzed aldol reactions of cyclohexanone or cyclopentanone being regarded as useful benchmarks for the development of new catalysts (scheme 28).



Scheme 28. The standard, organocatalyzed aldol reaction between cyclic ketones and aldehydes

It is important to notice that while the use of proline in this reaction is restricted to the case of aliphatic aldehydes (scheme 11), other acyclic amino acids, such as alanine or valine[57e,57f], proved to be extremely selective for a wider range of substrates. Although the solvent used consisted basically of wet DMSO and the reaction times were quite long (3-4 days), this discovery highlighted the potentiality of water to positively influence the organocatalyzed aldol reaction, prompting many research groups to develop catalysts able to promote this reaction in an aqueous medium, that can be seen as an inexpensive, environmentally friendly alternative to the organic solvents. As the solubility of most cyclic ketones in water is generally low, the reaction has to be run in biphasic water/ketones systems or in heterogeneous mixtures consisting of a suspension or emulsion of the reagents in water. This experimental observation led to an interesting debate on whether the catalytic process would take place effectively in water, at the

interface, in the organic layer or in a "concentrated organic phase". *Hayashi*[58] and *Barbas*[57e] were the first to report the development of catalysts able to efficiently perform the aldol reaction (scheme 28) "in water" (water/cyclohexanone 10:1 (v/v)). *Dickerson* and *Janda*[59] argued that the water insoluble ketone would have acted, in this case, as the real solvent for the catalytic process, sequestrating the organic material, together with the enamine intermediate, in hydrophobic pockets which would have excluded the bulk water from the reaction, thus making the term "in water" misleading. This idea can explain why the catalysts that present a faster aldol substrate conversion rate in aqueous media, contain a hydrophobic side-chain able to trigger the inclusion of their enamine derivative in the organic layer (scheme 29) and has been recently supported by *Armstrong*[57d] and by *Gryko*[57c] who conjugated the use of an organocatalyst in the presence of water with the addition of β-cyclodextrin or NaCl. Such additives are able to exalt the formation of concentrated hydrophobic droplets in which the organic reagents come in close contact and are able to react. For the reasons above mentioned, we will refer to this class of transformations as reactions "in the presence of water"[57a,60] rather than as reactions "in water" although water represents the main component of the system. It is interesting to add in this context that it is still not clear why the reaction in the concentrated organic phase that forms in presence of water enjoys experimentally a higher level of enantioselectivity than the same process in a homogeneous, water free environment[57,69]. This may require a further explanation of the role of the water molecules at the interface with the organic phase in coordinating the transition state of the reaction.



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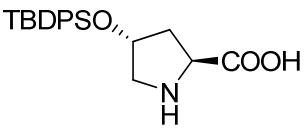

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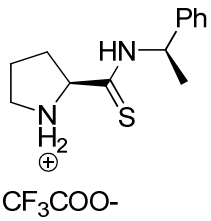

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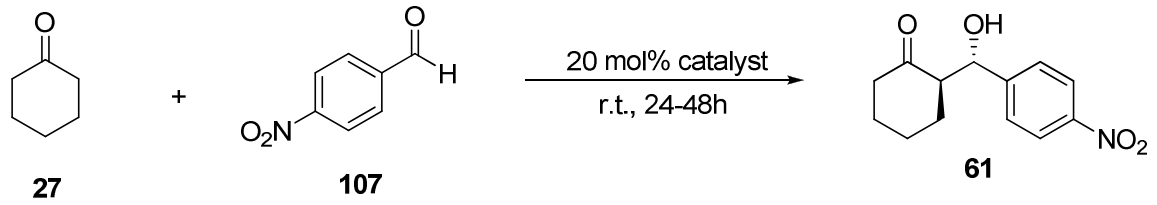
Scheme 29. *Barbas´* (**29**), *Hayashi´*s (**61**) and *Gryko´*s (**117**) effective organocatalysts for the aldol reaction of cyclic ketones in presence of water

2.2.1 β-ACC containing tripeptides as catalysts for the aldol reaction of cyclic ketones

In the search for efficient and versatile organocatalysts we were interested in proving that tripeptides **88** and **90** may serve well in the aldol reaction between cyclic ketones and aldehydes. For this reason, they were tested for the standard reaction in scheme 30 in the conditions that proved to be effective in the case of acetone. As catalyst **90** had proved to be effective in acetone/water mixtures, a 20:1 and a 10:1 cyclohexanone/water mixture were used in the screening process. In the first case, the reduced amount of water allows its dissolution in cyclohexanone, thus making the system monophasic and the subsequent aldol reaction homogeneous. In the latter case a dispersion of small water droplets in cyclohexanone was observed, leading to a biphasic, heterogeneous reaction mixture. In both cases, the aldehyde substrate and the catalysts were completely dissolved in one of the mixture components. In the case of catalyst **88**, which had proved to be extremely effective in a $CHCl_3$/ketone mixture, both $CHCl_3$/cyclohexanone and cyclohexanone/water mixtures were tested. Although it is clearly not convenient from an economical and environmental point of view, we tested the reaction in the neat ketone as well, to better clarify the effect of the addition of water.



27 **107** **61**

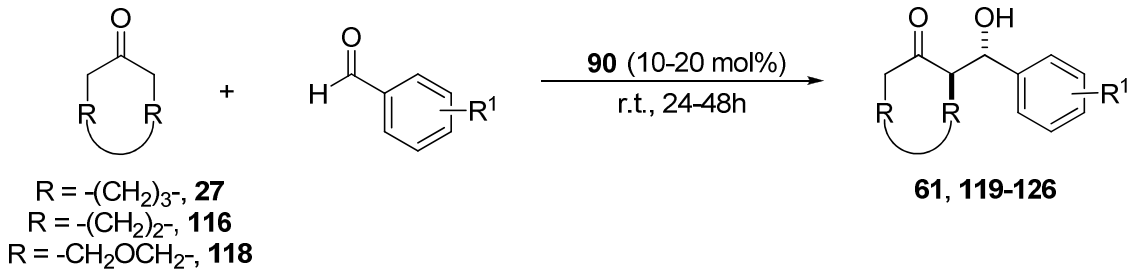
Scheme 30. Benchmark organocatalyzed aldol reaction between *p*-nitrobenzaldehyde and cyclohexanone

Table 13. Catalysts screening for the aldol reaction in scheme 30

entry	catalyst	solvent mixture (v/v)	cyclohexanone/ water equiv.:equiv.	Time (h)	yield (%)	*ee anti* (%)	*ee syn* (%)	*dr* (*anti/syn*)
1	H-Pro-OH	20:1 cyclohexanone/ water	25:5	24	66	88	52	2:1
2	H-Pro-▲-Pro-OH	cyclohexanone	25:0	24	76	66	10	3:1
3	H-Pro-▲-Pro-OH	20:1 cyclohexanone/ water	25:5	24	96	87	73	2:1
4	H-Pro-▲-Pro-OH	10:1 cyclohexanone/ water	25:10	48	75	95	99	6:1
5	H-Pro-▲-Pro-OH	1:10 cyclohexanone/ water	5:150	48	-	-	-	-
6	H-Pro-Pro-▼-OH	cyclohexanone	25:0	24	98	8	2	1:1
7	H-Pro-Pro-▼-OH	2:1 $CHCl_3$/cyclohexa -none	30:0	24	95	0	0	1:1
8	H-Pro-Pro-▼-OH	20:1 cyclohexanone/ water	25:5	24	97	17	11	1:1

Proline is not a good catalyst for the aldol reaction between cyclic ketones and aromatic aldehydes[2b], either in DMSO (see scheme 11) or in cyclohexanone/water mixtures (entry 1, table 13). Tripeptide **88** does not perform efficiently for this process (entries 6 to 8, table 13) either. Catalyst **90**, instead, proved to have an interesting activity in the test reaction, in particular, in the heterogeneous ketone/water mixture (although only in the presence of a high excess of ketone (entry 5). In this condition, we observed a higher enantio- and diastereoselectivity than in the homogeneous mixture, although at the expense of a reduced reaction rate.

Encouraged by this result, catalyst **90** was screened in the aldol reaction between aromatic aldehydes and cyclic ketones, either in the heterogeneous or in the homogeneous reaction conditions (scheme 31, tables 14 and 15).



Scheme 31. The aldol reaction between cyclohexanone (**27**), cyclopentanone (**116**) or tetrahydropyran-4-one (**118**) and aromatic aldehydes, catalyzed by **90**

Table 14. Catalyst **90** in the homogenous aldol reaction between cyclic ketones and aromatic aldehydes (24 h, r.t.)

entry	R^1	R	ketone/water (v/v)	catalyst load (mol %)	yield (%)	*ee anti* (%)	*ee syn* (%)	*dr* (*anti/syn*)
1	*p*-NO_2 (**61**)	$-(CH_2)_3-$	20/1	20	96	87	73	2:1
2	*p*-Cl (**119**)		20/1	20	50	91	66	9:1
3	*o*- NO_2 (**120**)		20/1	20	69	80	-	>60:1
4	*o*-Cl (**121**)		20/1	20	63	90	-	45:1
5	*o*-Br (**122**)		20/1	20	72	93	-	40:1
6	*p*-NO_2 (**123**)	$-CH_2OCH_2-$	20/1	20	71	83	87	1:2
7	*p*-NO_2 (**123**)		3/1	20	80	91	76	3:2
8	*o*-Br (**124**)		20/1	20	35	83	53	1:1
9	*o*-Br (**124**)		3/1	20	70	83	85	3:1
10	*o*- NO_2 (**125**)		3/1	20	79	86	-	4:1
11	*p*-NO_2 (**126**)	$-(CH_2)_2-$	20/1	10	99	56	49	3:1
12	*p*-NO_2 (**126**)		10/1	10	90	61	37	3:1

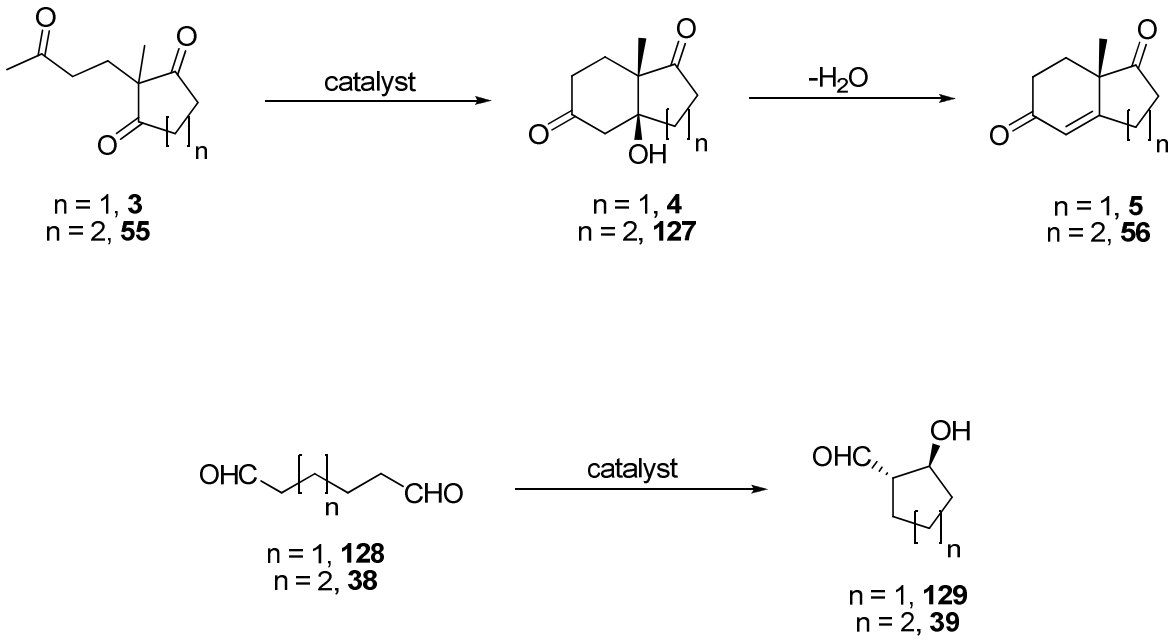
Table 15. Catalyst **90** in the heterogeneous aldol reaction between cyclic ketones and aromatic aldehydes (48 h, r.t.)

entry	R^1	R	ketone/water (v/v)	catalyst load (mol %)	yield (%)	*ee anti* (%)	*ee syn* (%)	*dr* (*anti/syn*)
1	*p*-NO_2 (**61**)		10/1	20	75	95	99	6:1
2	*p*-Cl (**119**)		10/1	20	-	-	-	-
3	*o*-NO_2 (**120**)	-$(CH_2)_3$-	10/1	20	50	98	-	30:1
4	*o*-Cl (**121**)		10/1	20	40	98		70:1
5	*o*-Br (**122**)		10/1	20	60	95		90:1
6	*p*-NO_2 (**126**)	-$(CH_2)_2$-	5/1	10	70	68	40	1:2

The use of catalyst **90** allows the preparation of the aldol product between cyclohexanone and aromatic aldehydes in good yields and with high enantio- and diastereoselectivity (entries 1-5, table 14), which becomes excellent in the case of the heterogeneous reaction conditions (entries 1-5 table 15). This notwithstanding, a remarkable decrease of the overall substrate conversion rate in the biphasic system was observed, which brings the suppression of the reaction between deactivated aldehydes like *p*-chlorobenzaldehyde and cyclohexanone (entry 3, table 15). This effect is probably due to the high water solubility of **90**, which gets trapped in the aqueous layer and is therefore less available for the catalytic process. In the case of the tetrahydropiran-4-one **118**, a ketone which is miscible with water in every proportion, we obtained generally a high enantioselectivity either in the case of the *anti* or of the *syn* isomer (entries 6-10, table 14), although we were are not able to achieve a good diastereomeric control. This reaction shows to be influenced by the amount of water added as we switched from a 20:1 to a 3:1 ketone/water mixture, with the latter granting the best diastereo- and enantioselectivity. Cyclopentanone (**116**) proved to be a very challenging substrate; although its high reactivity allowed the reduction of the catalytic load to only 10 mol%, only moderate enantioselectivity and a low *syn* diastereoselectivity were achieved.

2.3 Intramolecular aldol reactions

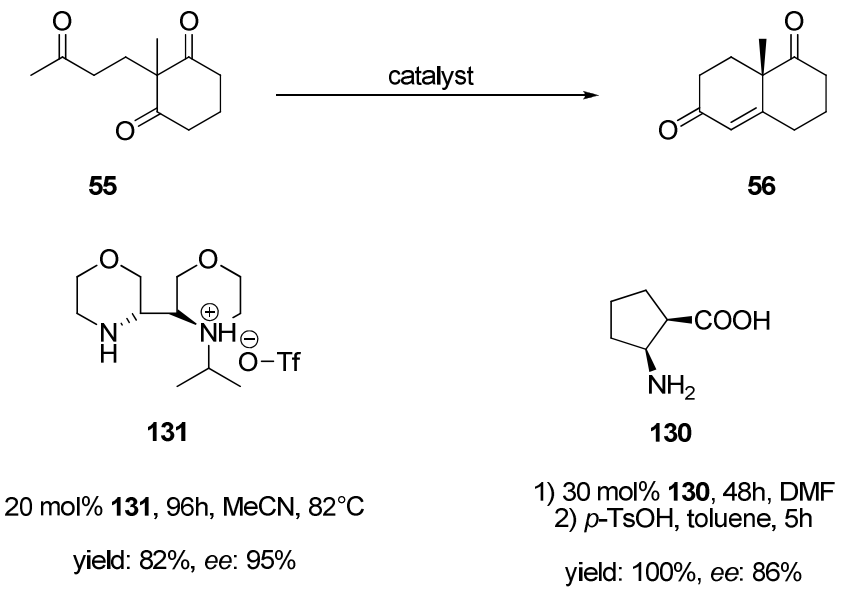
The intramolecular aldol reaction represented the first organocatalytic approach to the aldol reaction (scheme 2)[4,5] and, in general, the first highly enantioselective example of organocatalysis. Usually, the ring closure reactions in scheme 32 represent the most extensively studied processes for this family of transformations and can be classified as "enolendo" or "enolexo" reactions, accordingly to the destiny of the ketone functionality which acts as the aldol donor, to be included in the newly formed ring or to be left outside of it.



Scheme 32. Classic examples for the organocatalyzed "enolendo" (above) and "enolexo" (below) intramolecular aldol reaction

In the seventies, Hajos, Parrish, Eder, Sauer and Weichert[4,5] showed that only 3 mol% of proline in DMF could afford the intermediate alcohol **4**, in 20 hours, in quantitative yield and with an *ee* of 93%; successive dehydration of this intermediate using *p*-TsOH would provide **5**. The intramolecular aldol reaction of triketone **55** proved to be, instead, much more challenging. *Barbas*[35] showed that using 30 mol% of proline in DMSO, a yield of 49% and a selectivity of 76% could be obtained in 90 hours directly from methylvinyl ketone

and 2-methyl-1,3-cyclohexanedione, through a Michael addition-aldol reaction one-pot cascade. Recrystallization of **56** would provide this product in its enantiomerically pure form. The use of antibodies[61] was reported for the enantioselective synthesis of **56**. More recently, it was shown that the unnatural amino acid cispentacine[62] (**130**, scheme 33) is able to catalyze the formation of **127** in DMF, whose subsequent dehydration furnished **56** in quantitative yield and with an *ee* of 86%. Bismorpholine catalyst **131**, has also been reported[63] as an extremely selective catalyst for this reaction.



Scheme 33. cispentacine **130** and bismorpholine **131** are effective catalysts for the intramolecular aldol reaction

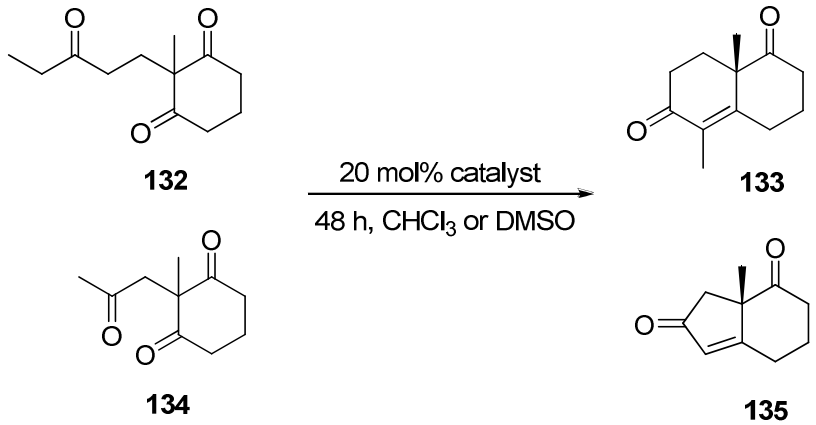
This notwithstanding, it would be useful to develop a catalyst for this process able to promote it as a one-pot reaction at room temperature; moreover, the use of a low boiling solvent would allow to avoid the complicated work up necessary to extract the products from solvents like DMF or DMSO and enhance the possibility of recovering the catalyst. A lower catalyst load and a reduced reaction time, in comparison to the catalysts previously described, are desirable as well. For these reasons, peptides **88**, **90**, **93**, **101** and **104** were tested in this reaction, using chloroform as solvent and only 10 mol% of catalyst (table 16).

Table 16. Catalysts screening for the intramolecular aldol reaction of triketone **55** in $CHCl_3$, r.t., 24 h, 10 mol% catalyst)

entry	catalyst	yield (%)	*ee* (%)
1	H-Pro-OH	27[b]	57
2	H-Pro-▲-Pro-OH (**90**)	88[a]	92
3	H-Pro-▼-Pro-OH (**93**)	11[b]	37
4	H-Pro-Pro-▼-OH (**88**)	10[b]	38
5	H-Pro-▼-Pro-Pro-OH (**101**)	31[b]	61
6	H-Pro-▲-Pro-▼-OH (**104**)	traces	n.d.

[a] isolated yield, [b] estimated conversion through chiral GC.

Interestingly, tripeptide **90** (entry 2, table 16) proved to be once again the most active and selective catalyst in our hands, promoting this reaction with a selectivity superior to any other peptide tested or to proline, either in DMSO or in the same conditions. It approaches the selectivity observed in the case of the bismorpholine catalyst **131**, but in only 24 hours and at room temperature. This result encouraged us to extend the study of the catalytic behavior of **90** to the intramolecular aldol reaction of other triketones (scheme 34) and to explore different reaction conditions (table 17).



Scheme 34. Other interesting intramolecular aldol reactions.

Table 17. Results for the intramolecular aldol reaction promoted by **90** (r.t., 24-48h, 10 mol% catalyst)

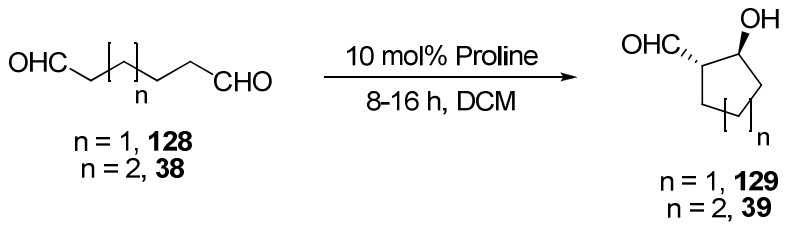
entry	catalyst	triketone	solvent	time (d)	yield (%)	*ee* (%)
1	H-Pro-OH[a]	**3**	$CHCl_3$	5	99	22
2	H-Pro-▲-Pro-OH		$CHCl_3$	5	95	83
3	H-Pro-▲-Pro-OH	**55**	DMSO	2	-	-
4	H-Pro-▲-Pro-OH		DMF	2	-	-
5	H-Pro-▲-Pro-OH		MeCN	2	traces	n.d.
6	H-Pro-▲-Pro-OH		10:1 THF/H_2O	1	49	92
7	H-Pro-OH[a]	**132**	DMSO	2	-	-
8	H-Pro-▲-Pro-OH[a]		$CHCl_3$	2	-	-
9	H-Pro-OH[a]	**134**	DMSO	2	-	-
10	H-Pro-▲-Pro-OH[a]		$CHCl_3$	2	-	-

[a]20 mol% of catalyst were employed

The intramolecular aldol reaction of **3** to provide compound **5** in $CHCl_3$ requires a far longer reaction time than its 6-member ring analogue **55** (entries 1-2, table 17). Five days are required for a complete conversion of the starting material to the cyclized product and although **90** can guarantee quite a higher selectivity than proline in the same reaction conditions, its performance is not comparable to what is observed using 3 mol% of proline in DMF[4,5] (complete conversion of **3** in 20 hours; 93% *ee* after the dehydration of **4**). An attempt to use **90** in more polar solvents resulted in the complete inhibition of the excellent catalytic activity observed in the case of **55** (entries 3-5), a sign that the peculiar structure of this catalyst is disrupted in such environment. The use of a mixture of water and organic solvents (in this case THF) which proved to be helpful in the case of the intermolecular aldol reaction, only had the effect to reduce the activity of the catalyst (entry 6). Finally we tried tripeptide **90** and proline in the annulations of **132** and **134**; while the first of them is reported to undergo the selective intramolecular aldol reaction using as much as one equivalent of phenylalanine in harsh reaction conditions[64], the second cyclization is still unreported. Unfortunately either proline or **90** failed in providing the desired products in $CHCl_3$ and in DMSO.

2.3.1 "Enolendo" intramolecular aldol reaction

The organocatalytic *"enolendo"* cyclizations were reported by *List*[34]. Proline (scheme 35) resulted to be an effective catalyst in the case of the heptanediale **38**, but was less suitable for the enantioselective synthesis of **129**.



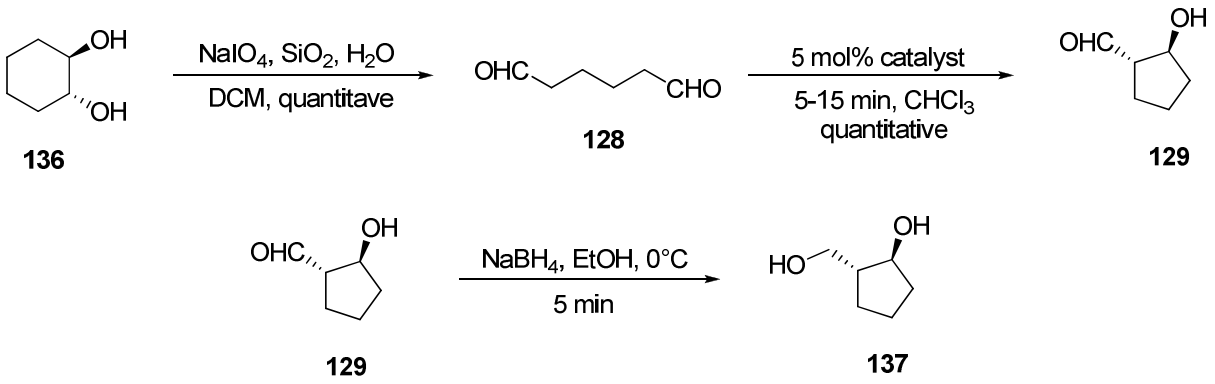
n = 1, yield: n.r., *ee*(*anti*): 79%; *ee*(*syn*): 37%, *dr*: 2:1

n = 2, yield: 95%, *ee*(*anti*): 99%, *dr*: 10:1

Scheme 35. The *enolendo* intramolecular aldol reaction catalyzed by proline

The peptides in our hands (fig. 9 and scheme 24) were tested as organocatalysts in the intramolecular aldol reaction of **128**, as no organocatalyst has been reported to effectively promote this process. To prepare compound **128** we carried out an oxidative ring opening of *trans*-1,2-cyclohexanediol with $H_2O/SiO_2/NaIO_4$, while **129** was immediately treated with $NaBH_4$ in EtOH, as it undergoes complete dehydration within a few hours, with the consequent loss of the chiral information (scheme 36). In this way, this process can be regarded as the synthesis of diol **137** starting from diol **136**. Interestingly, 5 mol% of any of the peptides tested can catalyze the fast and complete cyclization of **128**, but generally, with low enantio- and diastereoselectivity. Dipeptide **84** (entry 2, table 18) is the only one able to provide an enantioselectivity superior to the one obtained with proline in the same reaction conditions, but with almost no *anti*/*syn* selectivity. Tetrapeptide **104** (entry 6, table 18) promotes the conversion of the starting material in only 5 min and with good diastereoselectivity obtaining racemic **137** in its *trans*-isomer. A slightly higher diastereoselectivity (9:1 anti/syn) has been obtained only by *Lee*[65] using a $HgCl_2$ catalyzed reduction of 2-hydroxymethylcyclopentanone in 12 hours in the presence of 10 equivalents of Mg. Pentapeptide **106** and tripeptide **88** were able

tead to promote the formation of an excess of the *syn*-isomer of **129**, although with low or no chiral induction.



Scheme 36. Synthesis the diol **137** as final product of the organocatalytic ring closure of **128**

Table 18. Results for the peptide catalyzed intramolecular aldol reaction of **128** ($CHCl_3$, at r.t., with 5 mol% of catalyst, conversion of the starting material was always complete)

entry	catalyst	conversion time (min)	*ee anti* (%)	*ee syn* (%)	*dr anti/syn*
1	H-Pro-OH[a]	30	54	-	2:1
2	H-Pro-▲-OH (**84**)	15	71	-	3:2
3	H-Pro-▼-OH (**89**)	15	39	-	3:2
4	H-Pro-▲-Pro-OH (**90**)	15	6	-	3:2
5	H-Pro-Pro-▼-OH (**88**)	15	5	-	1:2
6	H-Pro-▼-Pro-Pro-OH (**104**)	5	-	-	7:1
7	H-Pro-▲-Pro-▼-OH (**101**)	15	-	-	1:1
8	H-Pro-▲-Pro- Pro-▲-OH (**106**)	15	26	-	1:2

[a]10 mol% of proline were employed

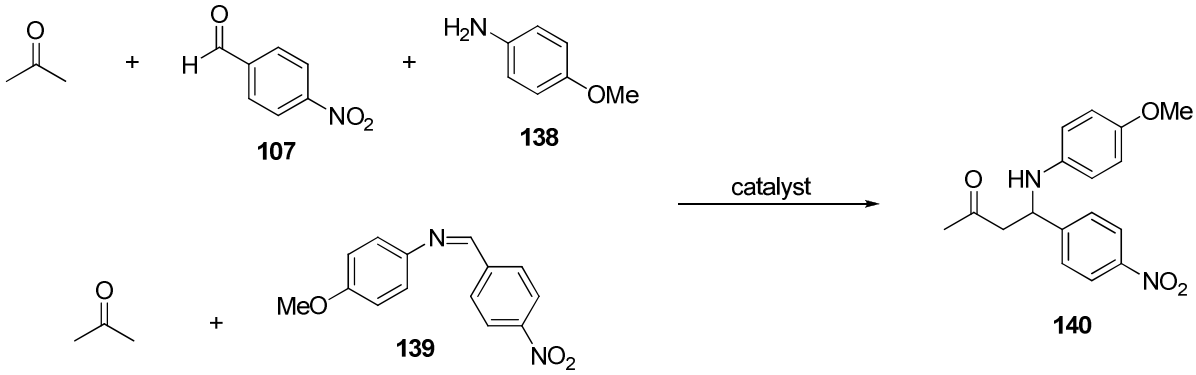
2. 4 Other Reactions

In the search for efficient and versatile organocatalysts, it was of our interest to explore the behavior of the peptides synthesized, in other common C-C bond forming reactions.

In particular, other processes able to proceed through the formation of an enamine intermediate can represent useful benchmarks in this case.

2.4.1 Mannich Reaction

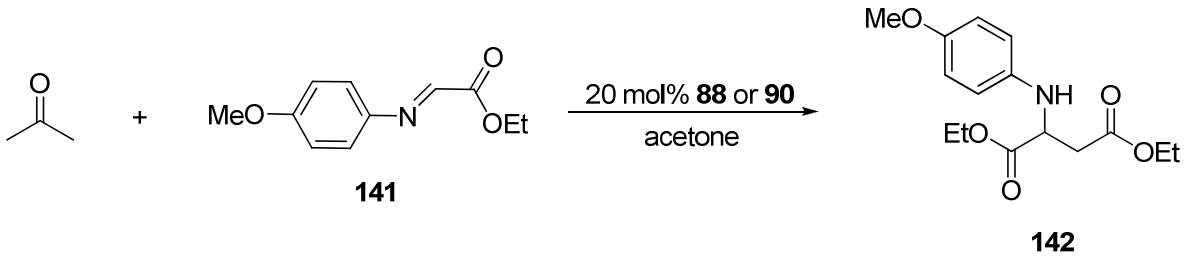
The three component Mannich reaction (scheme 37) was regarded as a possible candidate to expand the field of application of peptides **88** and **90**. List[1a,66] reported that 35 mol% of proline, in DMSO, are able to catalyze this process with excellent enantioselectivity, although only moderate yields were reported, due to the concurrent formation of the aldol product **60** between acetone and the aldehyde substrate. As previously reported, DMSO was not regarded by us as a convenient solvent and the Mannich reaction was carried out directly in acetone, or in a $CHCl_3$/acetone using catalysts **88** and **90** (20 mol%). To our great disappointment, although traces of the Mannich product were observed, the aldol product resulted to be by far the main product of the process, independent of the reaction conditions or of the kind of aldehyde employed.



Scheme 37. The organocatalyzed three component Mannich reaction (above) and the related two component process involving the preformed imine (below)

Switching from the classic Mannich reaction to the addition of acetone to the preformed imine **139**, was regarded as a way to avoid the formation of the undesired aldol product. Unfortunately, no formation of the Mannich product was observed under these conditions and, over time, the decomposition of **139** brought again the formation of the

aldol product. An analogous reaction (scheme 38), involves the addition of acetone to the preformed imine between ethyl glyoxylate and an aromatic amine[67].

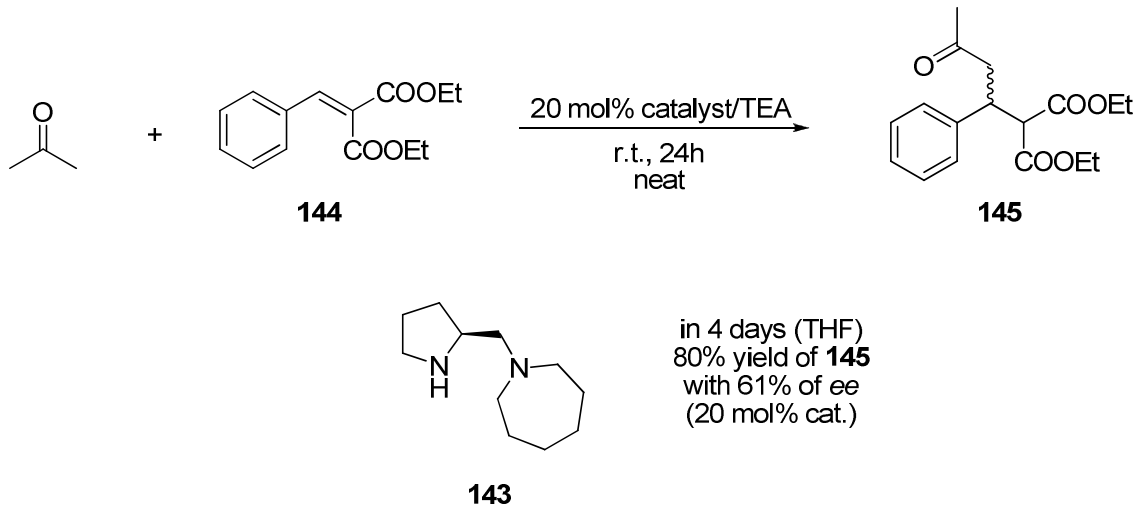


Scheme 38. The reaction between acetone, ethyl glyoxylate and *p*-methoxyaniline, proceeding through the preformed imine **141**

This process did provide only traces of racemic **142**, either using catalyst **88** or **90**. We concluded that these peptides are not suitable catalysts for the Mannich reaction.

2.4.2 Michael addition

Michael addition represents another important C-C bond forming reaction. The process in scheme 39 has been reported by *Barbas*[68] and was used as a benchmark reaction. While proline did not show to be an active catalyst for this process, diamine **143** showed a promising activity[68]. Similarly to proline, the peptides synthesized (Fig. 9 and scheme 24) did not prove to be able to promote this reaction, unless a catalytic amount of a base is added to deprotonate them at the carboxylic functionality. Triethylamine, which is not an active catalyst for this process (table 19, entry 13) was employed, and the reactions were carried out in neat acetone.



Scheme 39. Organocatalyzed Michael addition between acetone and **144** (above) and *Barbas´* diamine catalyst **143** (below)

Table 19. Catalysts screening for the Michael addition (in acetone, at r.t., 24 h, 20 mol% catalyst + 20 mol% TEA)

entry	catalyst	yield (%)	α_D ($CHCl_3$)	calculated *ee*[a] (%)
1	H-Pro-OH	-	-	-
2	H-Pro-OH/TEA	62	+1.0	6
3	H-Pro-▲-OH/TEA	25	0	0
4	H-Pro-▼-OH/TEA	55	0	0
5	H-Pro-▲-Asp-OH/TEA	45	-1.0	6
6	H-Pro-▼-Asp-OH/TEA	46	0	0
7	H-Pro-▲-Glu-OH/TEA	50	+6.1	36
8	H-Pro-▲-Pro-OH/TEA	37	0	0
9	H-Pro-▼-Pro-OH/TEA	56	0	0
10	H-Pro-Pro-▼-OH/TEA	60	+2.7	17
11	H-Pro-▼-Pro-Pro-OH/TEA	65	0	0
12	H-Pro-▲-Pro-▼-OH/TEA	33	-1.2	7
13	TEA	traces	n.d.	-

[a] On the base of the value reported by *Barbas*[68] (α_D = 10.2 for **145** (61% *ee*))

Unfortunately, although in comparison with catalysts **143** some of the peptides employed in the screening process could provide moderate yields in only 24 hours (entries, 4, 9, 7, 11) the enantioselectivity observed for this reaction was generally very low.

In conclusion, the screening process of our peptides in the aldol reaction between acetone and *p*-nitrobenzaldehyde (scheme 25) and the further optimization procedure, allowed us to identify two efficient catalysts, H-Pro-▲-Pro-OH (**90**) and H-Pro-Pro-▼-OH (**88**) able to promote the aldol reaction between different aldehydes and acetone in high yields and selectivity. Catalyst **90** showed later to be the most versatile between the peptides synthesized, performing well in the aldol reaction between cyclic ketones and aromatic aldehydes and in the intramolecular aldol reaction; this notwithstanding, some issues connected to its catalytic activity, like the large excess of ketone required and the slow conversion rate in the heterogeneous aldol reaction, can be addressed only by modifying the structure of our catalyst and will be examined in the next chapter.

Chapter 3: Development and applications of new catalysts

3.1 Development of new catalysts

In the previous chapter we showed that the unnatural *β*-amino acids ▲, (+)-**69**, and ▼, (-)-**69**, can be used as useful building blocks for the synthesis of "reasonable" and sometimes excellent catalysts like **90** and **88**. Naturally the results obtained leave plenty of room for improvement. In particular, **90** represents in our opinion a very interesting molecule from a structural point of view (see chapter 4) and is quite versatile, performing extremely well in the intramolecular aldol reaction and providing excellent selectivities in the heterogeneous reaction between cyclohexanone and aromatic aldehydes. In this case it would be useful to compare the catalytic activity of **90** with some interesting examples reported in literature during the last few years[57,69] (fig. 10).

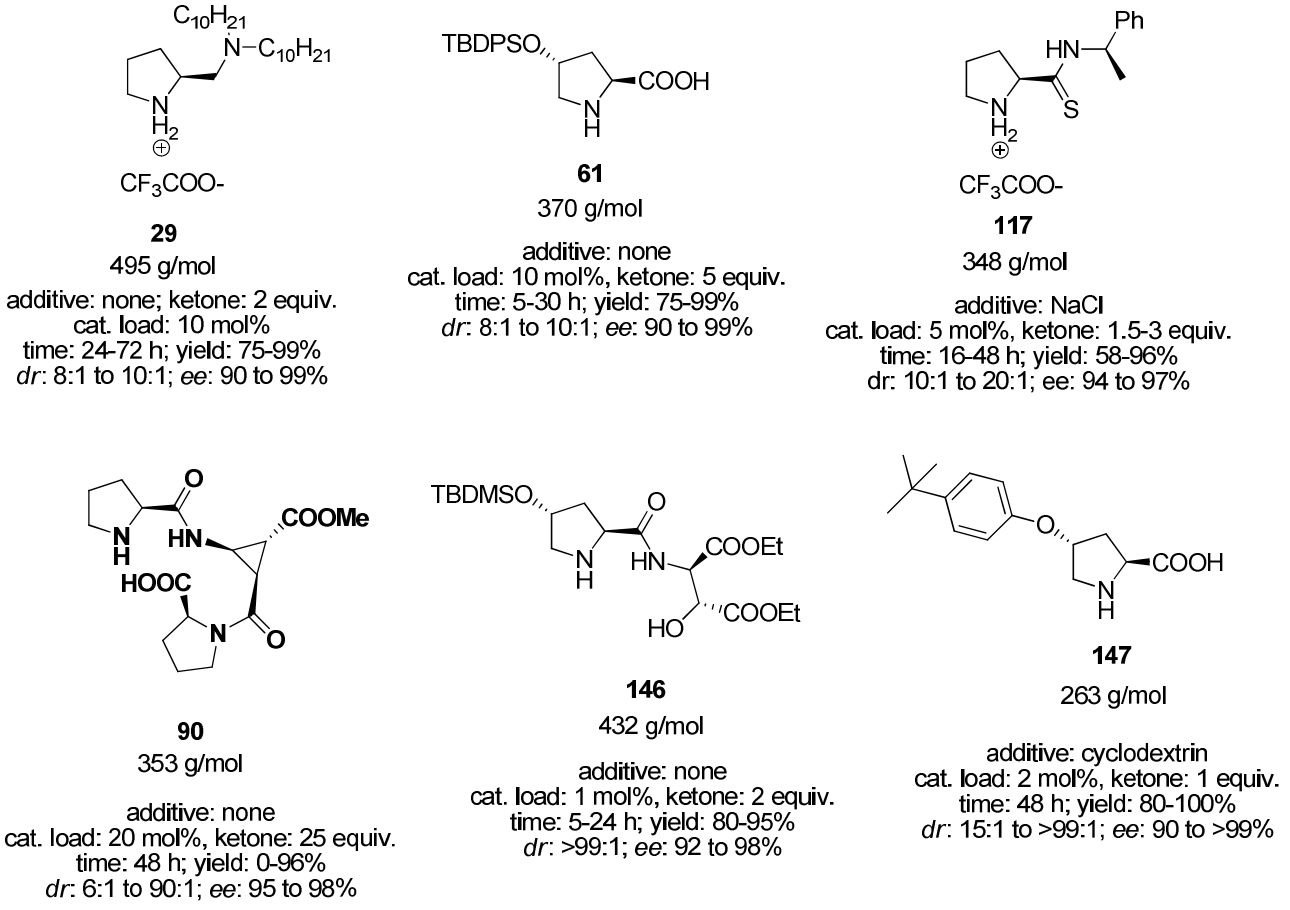


Fig. 10. Catalysts comparison for the aldol reaction between cyclohexanone and aromatic aldehydes in aqueous media

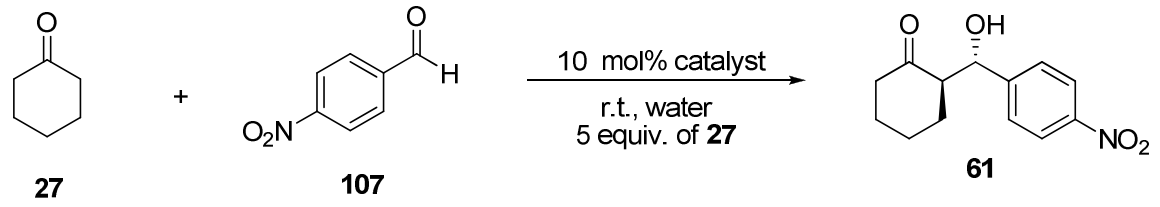
While the selectivities and the reaction times observed for **90** are comparable to the case of the excellent catalysts reported above, some other parameters are for sure unsatisfactory. Although the procedure that enables us to quantitatively recover **90** at the end of the process may upset the high catalytic load required, more remarkable is the fact that a very high excess of ketone is needed for an efficient conversion of the substrates, and this notwithstanding, deactivated aldehydes do not react at all (in heterogeneous conditions) in the presence of **90** (see table 15).

A plausible explanation for this drawback may be found analyzing the structures reported in fig. 10; it is easy to notice that only **90** misses a lipophilic side chain which would favor its inclusion in the organic phase of the heterogeneous aldol reaction. For this reason, while the other catalysts are able to interact with the organic material suspended in water, **90** ends up to be sequestrated in the aqueous phase of the reaction. Under such conditions, we can achieve good reactivity only adding large amounts of ketone and using more reactive aldehydes.

A similar observation has been reported by *Gong*[57b] who reported that catalyst **67** performs outstandingly in the homogeneous aldol reaction between acetone and aldehydes[38c], but it is less suitable for heterogeneous processes. This behavior was similarly explained on the base of its increased hydrophilicity. Its analog **146**, that is basically the same catalyst with an “apolar arm”, is a extraordinary catalyst for the aldol reaction of cyclohexanone in the presence of water.

In the search for an improved catalytic system, we did not believe that the use of addictives, like in the case of **117**[57c] or **147**[57d], would have led to any particular improvement. The latter catalysts are already able to work in absence of a high excess of ketone and the addition of a “salting-in” compound has the only effect of improving the aggregation of the organic material in concentrated droplets, thus accelerating the reaction. The ability of the catalyst to pass into the organic phase after the formation of the enamine intermediate is still required. Moreover, such hydrosoluble addictives would have made the recovery of the pure tripeptide impractical, thus being unsuitable for a catalyst which requires a high load.

For these reasons, we concluded that the most practical way to improve our catalyst would be "attaching" a hydrophobic side chain to **90**; this could be carried out by substituting the *N*-terminal proline with a suitably alkylated *trans*-hydroxyproline. We desired to keep the molecular weight of the resulting catalyst as low as possible and we needed at the same time to introduce a group which would have been stable under the protection-deprotection conditions used during the synthesis of our peptides; for this reason, we excluded from the pool of the available side-chains the bulky silylated substituents used by *Hayashi*[57a] (**61**) and *Gong*[57b] (**146**) or long alkyl chains (see catalyst **29**)[57e] and settled on a simple benzyl group (scheme 41). To have a more comprehensive overview of the role of this substituent a preliminary screening to evaluate the catalytic properties of the benzylated *trans*-hydroxyproline H-Hyp(OBn)-OH (**148**) was carried out in comparison with proline and with the proline-based catalysts **61**, in the same conditions used by *Hayashi*[57a] (table 20, scheme 40).



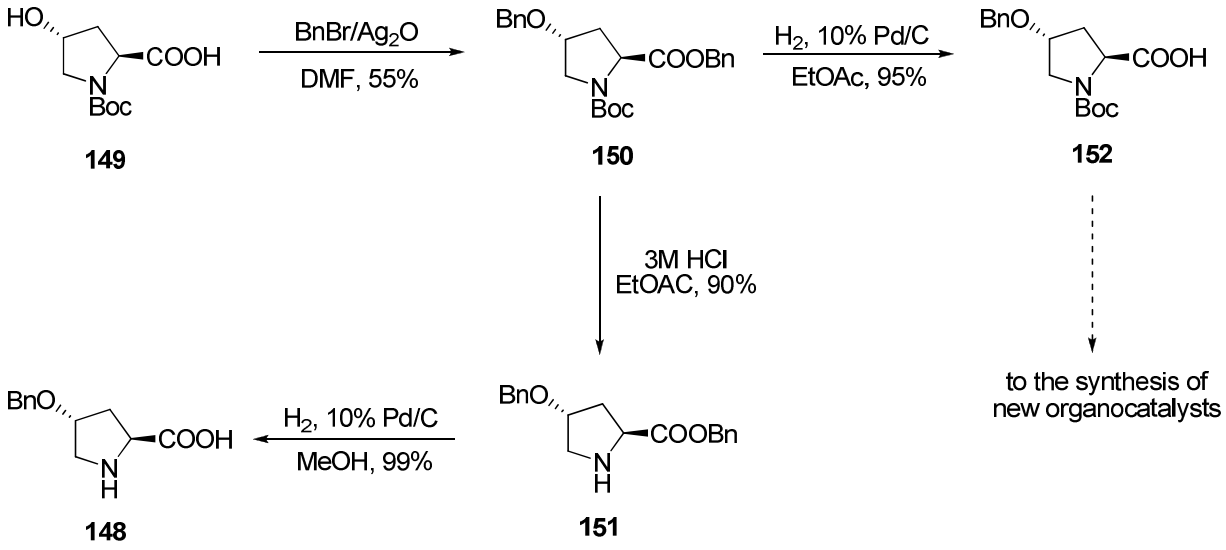
Scheme 40. The organocatalyzed aldol reaction between **27** and **107** in presence of water and of 5 equiv. of ketone

Table 20. Preliminary screening of catalyst **148** for the aldol reaction in scheme 40 and its comparison with analogous catalysts (10 mol% cat., r.t., 5 equiv. of **27** in presence of water, 16-18 equiv.)

entry	catalyst	time (h)	yield (%)	*dr* (*anti*/*syn*)	*ee anti* (%)
1	H-Pro-OH	24	traces	-	-
2	H-Hyp(OBn)-OH[a]	12	85	1:1	≈0
3	H-Hyp(OTBDPS)-OH	5	86	20:1	>99

[a]Taken from reference 57a

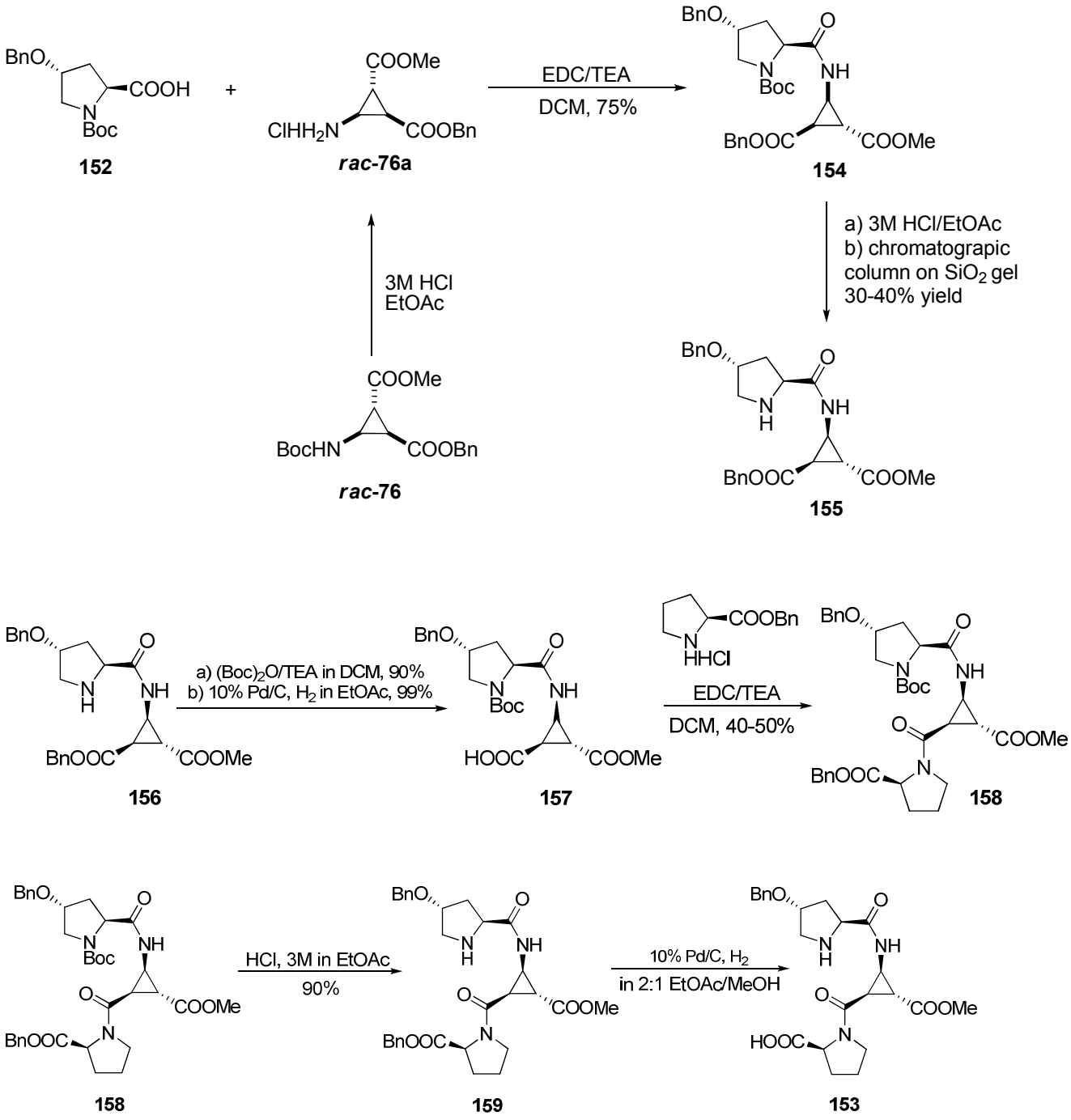
As expected (entry 1), a small, highly water soluble molecule like proline is not able to catalyze the heterogeneous aldol reaction in presence of a large excess of water; this notwithstanding, the introduction of a relatively small, apolar, side chain, like in the case of **148** (entry 2), resulted in a radical change of the catalytic behavior, allowing the inclusion of the enamine intermediate in the organic material droplets and obtaining a reactivity comparable to the one reported by *Hayashi* for the bulky TBDPS substituent (entry 3). At the same time, catalyst **148** provided only racemic aldol product, thus showing that the steric hindrance provided by the larger substituent is necessary for the pyrrolidine moiety to work as a selective catalyst in presence of water. Although this may appear to be in conflict with the choice to adopt the benzylic side-chain, we relayed on the intrinsic capability of the alkylated derivative of **90** to efficiently carry out the catalytic process, due to its peculiar turned structure, and we hypothesized that the benzyl group would have simply favored its access into the organic layer.



Scheme 41. Synthesis of the new building block **152** and of catalyst **148**

The hydroxyproline derivative **152**, the new alkylated building block for the synthesis of new catalysts, was readily synthesized through the complete benzylation of Boc-Hyp-OH (**149**) using silver (I) oxide; the yield of this step is moderate but the product of monobenzylation at the carboxylic acid moiety can be recovered in about 30 to 40% yield

and reused in a new set-up. The debenzylation of **150** at room temperature, using a H_2 balloon, in EtOAc, is selective at the carboxylic functionality and provides the desired building block. Moreover, catalyst **148** was easily obtained by diverting part of the intermediate compound **150** to the alternative synthetic pathway. The synthesis of tripeptide **153** can be completed in a way similar to the preparation of catalyst **90** (scheme 42).



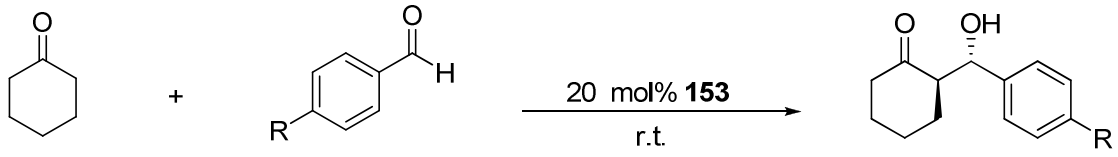
Scheme 42. Synthesis of catalyst **153**

An important operative difference between the preparation of catalysts **90** and **153** is observed during the selective debenzylation step which leads to the synthesis of intermediate **157**. In this case, we were compelled to use EtOAc as solvent, instead of methanol, to avoid the complete deprotection of both benzyl groups. The same effect is observed in the preparation of **152** from **150**. This notwithstanding, in the final deprotection step of tripeptide **159**, it was noticed that the presence of a certain amount of methanol was required for the reaction to proceed. For this reason, a 2:1 EtOAc/methanol mixture was successfully used to obtain selectively peptide **153**. In general, the presence of the Boc-group on the *N*-atom of the pyrrolidine ring activates the debenzylation of the protected hydroxyl moiety which can be turned off reducing the polarity of the solvent used. The successful separation of **155** from its diastereomer H-Hyp(OBn)-▼-OBn (**160**) through chromatography on silica gel, allowed the preparation of dipeptides (**161**) and (**162**), as additional candidate organocatalysts, after the opportune deprotection at the *C*-terminus (fig. 11).

Fig. 11. Dipeptides **161** and **162** were easily accessible after the diastereomeric separation of **155** and **160**.

3.2 Catalysis

The novel catalyst **153** was screened in the aldol reaction between cyclohexanone and aromatic aldehydes. Initially, the same reaction conditions used in the case of **90**, either for the homogeneous or for the heterogeneous process were adopted. Although such conditions are not the most advantageous, due to the large excess of ketone employed, we considered it as a possibility to directly compare the activity of the catalysts **90** and **153** (tables 21 and 22).



27

Table 21, homogeneous reaction (20:1 cyclohexanone/water)
Table 22, heterogeneous reaction (10:1 cyclohexanone/water)

Table 21. Catalyst **90** and **153** in the homogenous aldol reaction between **27** and aromatic aldehydes (r.t., 20:1 cyclohexanone/water, ca. 25 equiv. of **27** and 5 equiv. of water)

entry	R^1	catalyst	time (h)	yield (%)	*ee anti* (%)	*dr* (*anti/syn*)
1	*p*-NO_2 (**61**)	H-Pro-▲-Pro-OH (**90**)	12	55	87	67:33
2		H-Hyp(OBn)-▲-Pro-OH (**153**)	12	72	91	90:10
3	*p*-Cl (**119**)	H-Pro-▲-Pro-OH (**90**)	24	50	91	90:10
4		H-Hyp(OBn)-▲-Pro-OH (**153**)	24	72	92	91:9

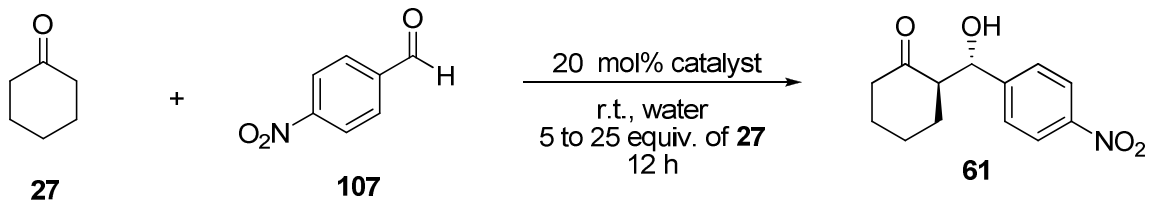
Table 22. Catalyst **90** and **153** in the heterogeneous aldol reaction between **27** and aromatic aldehydes (r.t., 10:1 cyclohexanone/water, ca. 25 equiv. of **27** and 10 equiv. of water)

entry	R^1	catalyst	time (h)	yield (%)	*ee anti* (%)	*dr* (*anti/syn*)
1	*p*-NO_2 (**61**)	H-Pro-▲-Pro-OH (**90**)	24	44	95	86:14
2		H-Hyp(OBn)-▲-Pro-OH (**153**)	12	64	92	86:14
3	*p*-Cl (**119**)	H-Pro-▲-Pro-OH (**90**)	48	-	-	-
4		H-Hyp(OBn)-▲-Pro-OH (**153**)	24	60	94	91:9

Two substrate aldehydes were employed in the comparison between **90** and **153**: the activated *p*-nitrobenzaldehyde and the much less reactive *p*-chlorobenzaldehyde. As expected, the difference in reaction speed observed using the two different catalysts is larger when we consider the heterogeneous reaction conditions. In particular, while the

reaction between *p*-chlorobenzaldehyde and cyclohexanone catalyzed by **90** is completely turned off in the heterogeneous system, it proceeds smoothly in presence of **153** (table 22, entries 3 and 4); moreover, a higher selectivity is generally observed in the case of our new catalyst. Based on the enhanced activity of **153** in the heterogeneous aldol reaction, a reduction of the number of equivalents of ketone employed was attempted (table 23).

Table 23. Effect of the concentration of **27** on the heterogeneous aldol reaction catalyzed by **153** (r.t., 20 mol% of catalyst, 12 h, ca. 150 equiv. of water)



27 **107** **61**

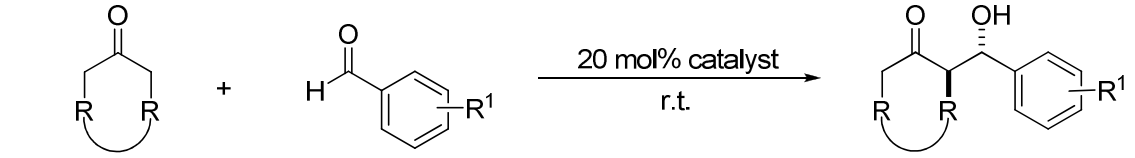
entry	equiv. of ketone	yield (%)	*ee anti* (%)	*dr* (*anti/syn*)
1	25	64	92	86:14
2	10	74	94	91:9
3	5	72	93	91:9
4[a]	5	92	94	91:9
5	2	66	91	83:17

[a]In 24 hours

It is interesting to observe that **153** retains a good activity using just two equivalents of the aldol donor **27** (entry 5). At the same time, we observed under such conditions a slight decline of the catalytic activity. It is possible to conclude that the conditions in entries 3 and 4, with five equivalents of ketone, represent the optimal compromise in this case. It is remarkable that in the same conditions tripeptide **90** did not show any catalytic activity (entry 5, table 13), confirming the hypothesis that an apolar side chain is required to favor the inclusion of the catalyst into the organic layer.

Having identified the optimal reaction conditions, the scope of catalyst **153** was extended to the heterogeneous aldol reaction between cyclic ketones and aromatic aldehydes (table 24).

Table 24. Catalyst **153** in the heterogenous aldol reaction between cyclic ketones and aromatic aldehydes (20 mol% catalyst, r.t., water ca. 150 equiv.)



R = -$(CH_2)_3$-, **27**
R = -$(CH_2)_2$-, **116**
R = -CH_2OCH_2-, **118**

61, **119-126**, **163**

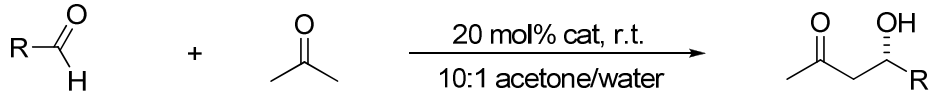
entry	R^1	R	equiv. of ketone	time (h)	yield (%)	*ee anti* (%)	*ee syn* (%)	*dr* (*anti/syn*)
1			5	12	72	94	-	91:9
2	*p*-NO_2 (**61**)		5	24	92	94	-	91:9
3			2	12	66	91	-	83:17
4			5	12	76	97	-	97:3
5	*o*- NO_2 (**120**)	-$(CH_2)_3$-	2	12	72	97	-	95:5
6	*p*-Cl (**119**)		5	24	72	96	-	94:6
7	*o*-Cl (**121**)		5	12	60	97	-	>99:1
8	*o*-Br (**122**)		5	12	71	95	-	97:3
9	H (**163**)		5	24	60	93	-	90:10
10			5	24	traces	-	-	-
11	*p*-NO_2 (**123**)	-CH_2OCH_2-	10	24	62	88	83	40:60
12	*o*-Br (**124**)		10	24	48	85	0	67:33
13	*o*- NO_2 (**125**)		10	24	85	87	-	80:20
14[a]			5	8	81	72	60	20:80
15[a]	*p*-NO_2 (**126**)	-$(CH_2)_2$-	2	8	74	70	63	23:77

[a]With only 10 mol% of catalyst

The experimental data show that **153** is an excellent catalyst for the aldol reaction between cyclohexanone and aromatic aldehydes (entries 1-9), obtaining good yields and very high enantio- and diastereoselectivity in 12 to 24 hours. The excess of ketone could be reduced to two equivalents for the most reactive aldehydes, although the best catalytic performance is generally observed in presence of five equivalents of cyclohexanone. Cyclopentanone (entries 14, 15) proved to be an extremely reactive aldol donor, allowing the reduction the reaction time to 8 hours and the catalyst load to only 10 mol%. Unfortunately, the enantioselectivity and the *syn* diastereoselectivity were just moderate. In the case of the tetrahydropiranone **118**, we obtained moderate to high yields and high enantioselectivity for the *anti* isomer, but the diastereoselectivity observed was generally low. It is interesting to notice that this ketone is miscible with water in any proportion and in presence of five equivalents of **118**, the aldehyde substrates did not dissolve at all in the ketone/water mixture, thus inhibiting the reaction. For this reason the ketone load was increased to ten equivalents.

To complete the study concerning the intermolecular aldol reaction catalyzed by **153** in the presence of water, we explored its behavior (together with the novel peptides **161** and **162**) in the reaction between acetone and aromatic aldehydes (scheme 27) under the same conditions which had been previously optimized for catalyst **90**. It is useful to remember that similarly to what observed for the processes involving cyclic ketone **118**, this reaction proceeds in a homogeneous acetone/water mixture. For this reason, no substantial difference between the newly synthesized catalyst **153** and the parent tripeptide **90** was expected in this case (table 25).

Table 25. the aldol reaction between acetone and aromatic aldehydes catalyzed by **90**, **153**,



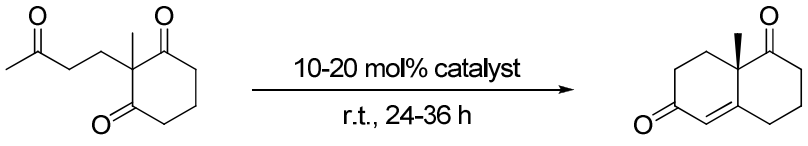
entry	catalyst	R	time (h)	yield (%)	*ee* (%)
1	H-Pro-▲-Pro-OH (**90**)	*p*-NO_2-Ph (**60**)	24	89	78 (*R*)
2	H-Hyp(OBn)-▲-Pro-OH (**153**)		6	85	88 (*R*)
3	H-Pro-▲-Pro-OH (**90**)	*p*-CF_3-Ph (**111**)	24	80	80 (*R*)
4	H-Hyp(OBn)-▲-Pro-OH (**153**)		4	90	80 (*R*)
5	H-Pro-▲-Pro-OH (**90**)	*p*-Cl-Ph (**108**)	24	43	80 (*R*)
6	H-Hyp(OBn)-▲-Pro-OH (**153**)		12	60	78 (*R*)
7	H-Pro-▲-Pro-OH (**90**)	*o*-Cl-Ph (**109**)	24	80	68 (*R*)
8	H-Hyp(OBn)-▲-Pro-OH (**153**)		18	80	73 (*R*)
9	H-Pro-▲-Pro-OH (**90**)	*o*-Br-Ph (**110**)	24	86	72 (*R*)
10	H-Hyp(OBn)-▲-Pro-OH (**153**)		8	87	70 (*R*)
11	H-Pro-▲-OH (**84**)	*p*-NO_2-Ph (**60**)	24	47	65 (*R*)
12	H-Hyp(OBn)-▲-OH (**161**)		24	90	51 (*R*)
13	H-Pro-▼-OH (**89**)	*p*-NO_2-Ph (**60**)	24	47	-
14	H-Hyp(OBn)-▼-OH (**162**)		24	80	48 (*R*)

Surprisingly, also in the case of this process catalyst **153** showed a higher activity than catalyst **90** (entries 1-8), although the gap between their catalytic behavior is not as dramatic as in the case of the heterogeneous systems. Given the homogeneity of the reaction mixture, the reason for this reaction rate acceleration is not clear. The *ee* values obtained using **153** and **90** are comparable and range from good to high; the best selectivity was obtained in the case of the activated aromatic aldehydes. Dipeptides **161** and **162** show a strong change in their catalytic performance, when compared to the related catalysts **84** or **89**. Again, we observed higher yields for the benzylated catalysts and, in the case of **162**, also a radical increase in enantioselectivity. This notwithstanding the overall level of selectivity provided by the two dipeptides is unsatisfactory.

3.2.1 Intramolecular aldol reaction

Finally, we tested tripeptide **153** for the intramolecular aldol reaction towards the synthesis of the Wieland-Meschier ketone, **56**. Chloroform had shown to be a suitable solvent for this cyclization in the case of catalyst **90**. At the same time, the enhanced catalytic activity of **153** in the heterogeneous intermolecular aldol reaction, prompted us to explore the intramolecular process in water and in presence of water (table 26) which, to our best knowledge, is still unreported.

Table 26. Results for the intramolecular aldol reaction promoted by **90** and **153** (r.t., 24-36h, 10-20 mol% catalyst)



55 **56**

entry	catalyst	catalyst load	solvent	time (h)	yield (%)	*ee* (%)
1	H-Pro-▲-Pro-OH	10 mol%	$CHCl_3$	24	88	92
2	H-Hyp(OBn)-▲-Pro-OH	10 mol%	$CHCl_3$	24	56[a]	96
3	H-Hyp(OBn)-▲-Pro-OH	20 mol%	$CHCl_3$	24	>99	96
4	H-Pro-▲-Pro-OH	10 mol%	5:1 water/$CHCl_3$	36	traces	-
5	H-Hyp(OBn)-▲-Pro-OH	20 mol%	5:1 water/$CHCl_3$	36	75[a]	74
6	H-Pro-▲-Pro-OH	10 mol%	water	48	-	-
7	H-Hyp(OBn)-▲-Pro-OH	20 mol%	water	48	<20%	-

[a]Determined through chiral GC

Catalyst **153** provided the product of the intramolecular aldol reaction in excellent yield and selectivity, allowing the complete conversion of the starting material in only 24 hours using 20 mol% of catalyst (entry 3). In comparison with catalyst **90** (entry 1), a slight increase in enantioselectivity and a moderately lower reaction speed were observed. As **153** can be quantitatively recovered at the end of this process using the same procedure

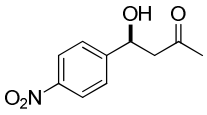
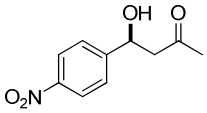
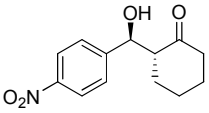
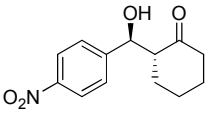
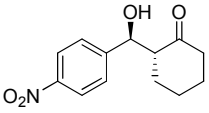
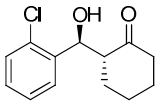
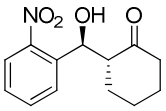
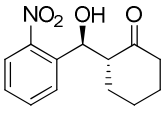
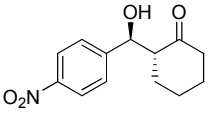
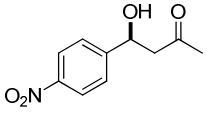
employed in the intermolecular aldol reaction the catalyst load employed was raised to 20 mol% (entries 1-3), obtaining a smooth reaction which did not require any purification of the final product, other than a aqueous work up to recover the catalyst.

The use of a mixture of water and chloroform represented an interesting way to carry out this reaction in heterogeneous conditions (entries 4-7). Once again, tripeptide **153** showed to be a suitable catalyst for the biphasic water/organic solvent systems, affording a good level of conversion in the water/$CHCl_3$ mixture in 36 hours. Unfortunately, the overall catalytic performance in water or in presence of water, proved to be inferior to the results obtained in neat $CHCl_3$, with no conversion at all in the organic solvent free condition (entries 6-7). This notwithstanding, we reported here the first enantioselective approach to the organocatalyzed intramolecular reaction in presence of water.

3.2.2 Catalyst Recovery

The quantitative catalyst recovery strategy developed for **90** was extended to **153** and the behavior of this tripeptide within a cycle of ten reactions was tested. 20 milligrams of tripeptide were used for the first round of the reactions cycle and were recovered at the end of each process by separating the catalyst from the products through an aqueous work up. The subsequent removal of water through lyophilization provided **153** for the next stage of the catalytic cycle. The data collected show that **153** can be successfully reused at least ten times, without any significant loss in yield and selectivity. At the end of the overall cycle 17 milligrams of catalyst were still in our hands, resulting in an average tripeptide recovery of about 98% for each step. This positive outcome can upset the negative aspect of the high catalyst load employed, as only the 1.5% of the initial amount of **153** was lost per reaction.

Table 27. Recovery and reuse of catalyst **153** (r.t., 12 h, 20 mol% catalyst)

entry	Reaction product	yield (%)	*ee anti* (%)	*dr* (*anti*/*syn*)
1[a]		95	87	-
2[a]		91	88	-
3[b]		70	93	91:9
4[b]		69	94	90:10
5[b]		75	94	91:9
6[b]		58	96	>99:1
7[b]		78	94	97:3
8[b]		70	97	98:2
9[b]		70	94	91:9
10[a]		93	87	-

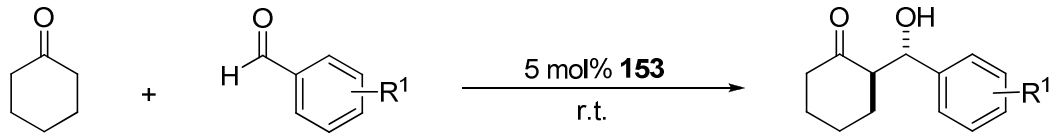
[a]In 10:1 acetone/water; [b] In water, with 5 equiv. of ketone.

3.2. 3 NaCl as a useful additive for the heterogeneous aldol reaction

Although a well established catalyst recovery procedure has been developed, we were still interested in the possibility of a drastic reduction of the catalyst load employed. *Gryko* and *Saletra*[57c] showed that the addition of NaCl and in general of "salting in"

compounds could accelerate the organocatalyzed aldol reaction in water by favoring the aggregation of the organic material in concentrated hydrophobic pockets. In this way, it was possible for them to reduce the catalyst load (to 5 mol%) and the number of equivalents of cyclohexanone employed in the process (fig. 10). The results exposed in table 28 show that by substituting the aqueous phase of the heterogeneous aldol reaction with the same volume of a saturated NaCl solution in water, a remarkable acceleration of the reaction was obtained. Such achievement suggested the possibility of reducing the amount of catalyst employed to only 5 mol%.

Table 28. Catalyst **153** in the heterogenous aldol reaction between cyclohexanone and aromatic aldehydes in brine (r.t., 5 equiv. of ketone, water ca. 150 equiv, 5 mol% of **153**)



27 **61**, **119-122**

entry	R^1	time (h)	yield (%)	*ee anti* (%)	*dr* (*anti/syn*)
1	*p*-NO_2 (**61**)	1[a]	95	94	91:9
2		12	80	94	91:9
3	*o*- NO_2 (**120**)	12	85	92	97:3
4	*p*-Cl (**119**)	24	70	96	92:8
5	*o*-Cl (**121**)	12	72	98	96:4
6	*o*-Br (**122**)	12	75	95	97:3

[a]20 mol% of catalyst

Within moderate reaction times, good to high yields are reported, together with very high enantio- and diastereoselectivity. It is worth noticing that under such reaction conditions it is still possible to use our standard procedure to recover the catalyst, although a mixture of NaCl and our peptide is obtained, rather than the pure catalyst. Further investigation should be carried out to check out the possibility of recycling the aqueous

solution containing the NaCl/catalyst mixture at the end of the reaction and reuse it for a new catalytic cycle, after extraction of the organic material.

In conclusion, the addition of a lipophilic side chain to the scaffold of catalyst **90**, resulted in tripeptide **153**, that performed clearly better in the heterogeneous aldol reaction between cyclic ketones and aromatic aldehydes. The new molecule also reproduced or surpassed the activity of **90** for the homogeneous intermolecular process or in the intramolecular aldol reaction and can be recovered, through a well established extraction/lyophilization procedure. Alternatively, the aldol reaction promoted by **153** can be successfully carried out in a saturated aqueous NaCl solution; this has the effect of accelerating the process and allowed us to reduce the catalyst load to only 5 mol%.

Chapter 4: Structural analysis

The development of organocatalysis has been accompanied by a strong interest in understanding the mechanism of the catalytic process and the related transition state. Proline-catalyzed reactions have been studied in an exhaustive way in the last few years[18], strengthening the hypothesis that the enamine complex formed between the catalyst and the aldol donor represents the decisive step to the enantioselective formation of the aldol products (fig. 1, introduction). Also in the case of the peptide-catalyzed aldol reactions an enamine transition state has been proposed[77]. In our case, the geometry of the activated complex is strictly connected to the ability of the *β*-ACC to stabilize short amino acidic sequences. The investigation of the structural role of both enantiomers (▲ and ▼) of this cyclic amino acid has represented an active, intriguing research topic in the last few years[45,49,71,75] and in the course of this work.

4.1 NMR studies

Although both diastereomers of the *β*-ACC can provide a high degree of stabilization to the secondary structure of short peptides[41,45,49], we noticed that the catalysts we prepared are definitely too short to possess a unique, well defined secondary structure and they exist as a mixture of more conformers. This notwithstanding, the accurate structural analysis carried out up to date[71] on tripeptides H-Pro-▼-Pro-OBn (**165**) and H-Pro-Pro-▼-OBn (**167**) suggests that the presence of the rigid *β*-ACC units (fig. 17 and 19) has the effect of rigidifying the overall structure and results in a main conformation prevailing over the others, making of these molecules some excellent examples of short, constrained peptides. The rate at which the different rotamers interconvert is slow compared to the NMR time scale and this enables us to individuate the main conformations through ^{1}H-NMR and ^{13}C-NMR. A nice example of the existence of multiple conformations can be observed in the case of tetrapeptide H-Pro-▲-Pro-▼-OBn (**164**, precursor of catalyst **101**, fig. 12, above). In this case, the splitting of the amide proton signals in two sets of doublets, suggests us that a major and a minor conformation are present in $CDCl_3$ at room temperature in a ratio of 8:1.

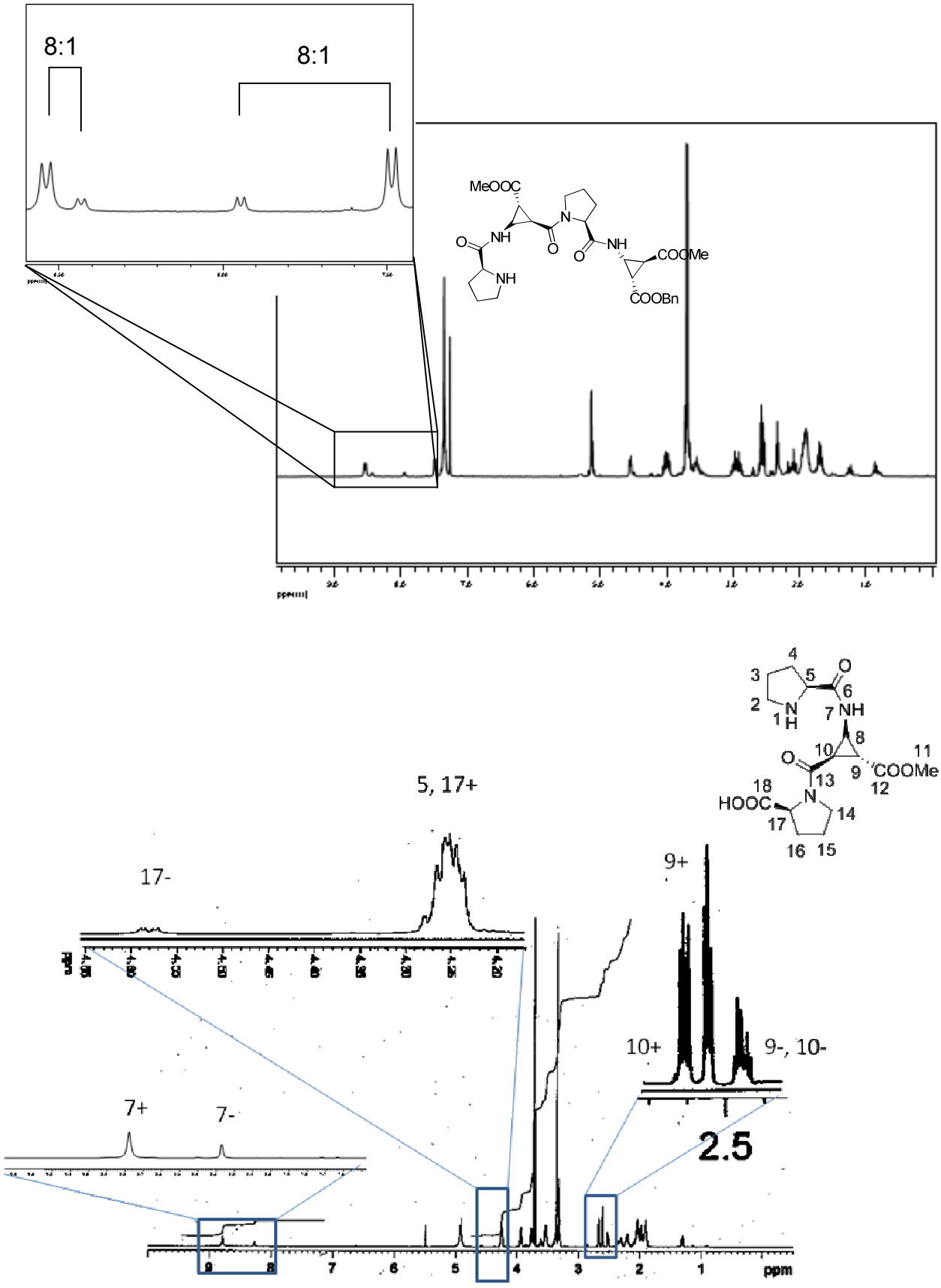


Fig. 12. (above) Signal doubling for the amide protons of tetrapeptide H-Pro-▲-Pro-▼-OBn and for the signals of tripeptide H-Pro-▲-Pro-OH (below).

A similar behavior is observed in the case of catalyst **90**. The ^{1}H-NMR signals of this tripeptide have been unequivocally assigned in CD_3OH by mean of bidimensional NMR spectroscopy (HSQC, HMBC, NOEs). It is evident that some signals (7, 9, 10, 17) double because of the presence of two different populations of conformers (fig. 12, below).

This effect was confirmed by studying the behavior of the amide proton (7) at different temperatures (fig. 13). As expected, cooling the probe to 0°C results in an increase of the 7+/7- (8:1) ratio, as the structure of **90** tends to be frozen in its most energetically stable conformation. At about 27°C the ratio between the two populations decreases to 3:1 and is just 2:1 at 40°C, sign that a higher degree of rotational freedom is available at higher temperatures.

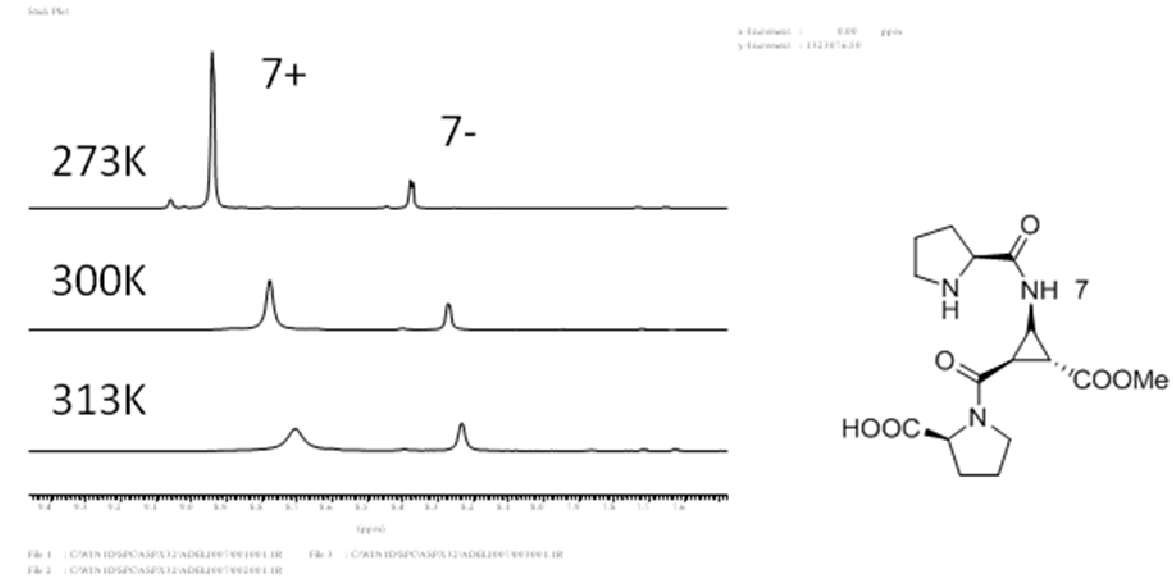


Fig. 13. ^{1}H-NMR spectra for the imidic proton of catalyst **90** at 273K, 300K, 313K (CD_3OH)

4.1.1 Cis/trans isomerism

It is reasonable to suppose that in catalysts such as **90** or **153**, an important source of conformational isomerism would be generated by the partially hindered rotation around the imidic peptide bond placed at *N*-terminus of proline[55]. This phenomenon leads to the formation of *cis*- and *trans*-conformers (fig. 14).

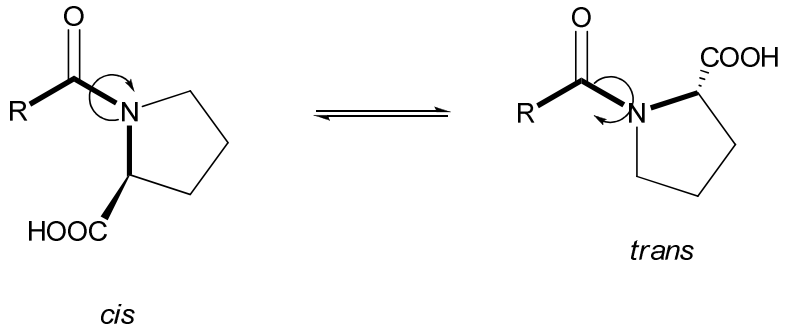


Fig. 14. *Cis-trans* isomerism for the imidic peptide bond of proline

Kessler and *Schmitt*[70] reported that bidimensional NMR techniques such as NOESY or ROESY can be used to identify and distinguish the *cis* and *trans* isomers by looking at the cross peak intensities between the *α* proton of the antecedent residue and the α and δ protons of proline respectively. In particular, the presence of a long range coupling between the two α protons is typical for a *cis* conformer. In the *trans* conformation this coupling is not present and a strong NOE signal between the *δ* protons of proline and the *α* proton of the preceding amino acid is observed.

In the case of tripeptide **90** we noticed an excellent agreement between the NOESY spectra we recorded in CD_3OH and the predicted long range interactions mentioned above. If we look at the magnified sections of the NOESY spectrum in fig. 15 (left), it is possible to notice that in the minor conformation (-) there is a cross peak between the *α* proton of the *β*-ACC unit (10-) and the *α* proton of the C-terminal proline (17-). In the same picture, we can see that there is not such coupling in correspondence of the major conformer. In the box at the right side we observe that only for the main conformer a strong coupling between the *α* proton of the *β*-ACC unit (10+) and the *δ* protons of the C-terminal proline (14) exists. On the basis of these data we can finally conclude that the main conformation corresponds to the *trans* isomer, while the rotation of the proline moiety around the amide bond generates a minor conformer with a *cis* configuration. It is possible to estimate, through integration of the ^{1}H-NMR spectrum signals, that a 3:1 *trans*/*cis* ratio is found in CD_3OD, at room temperature, for tripeptide **90**.

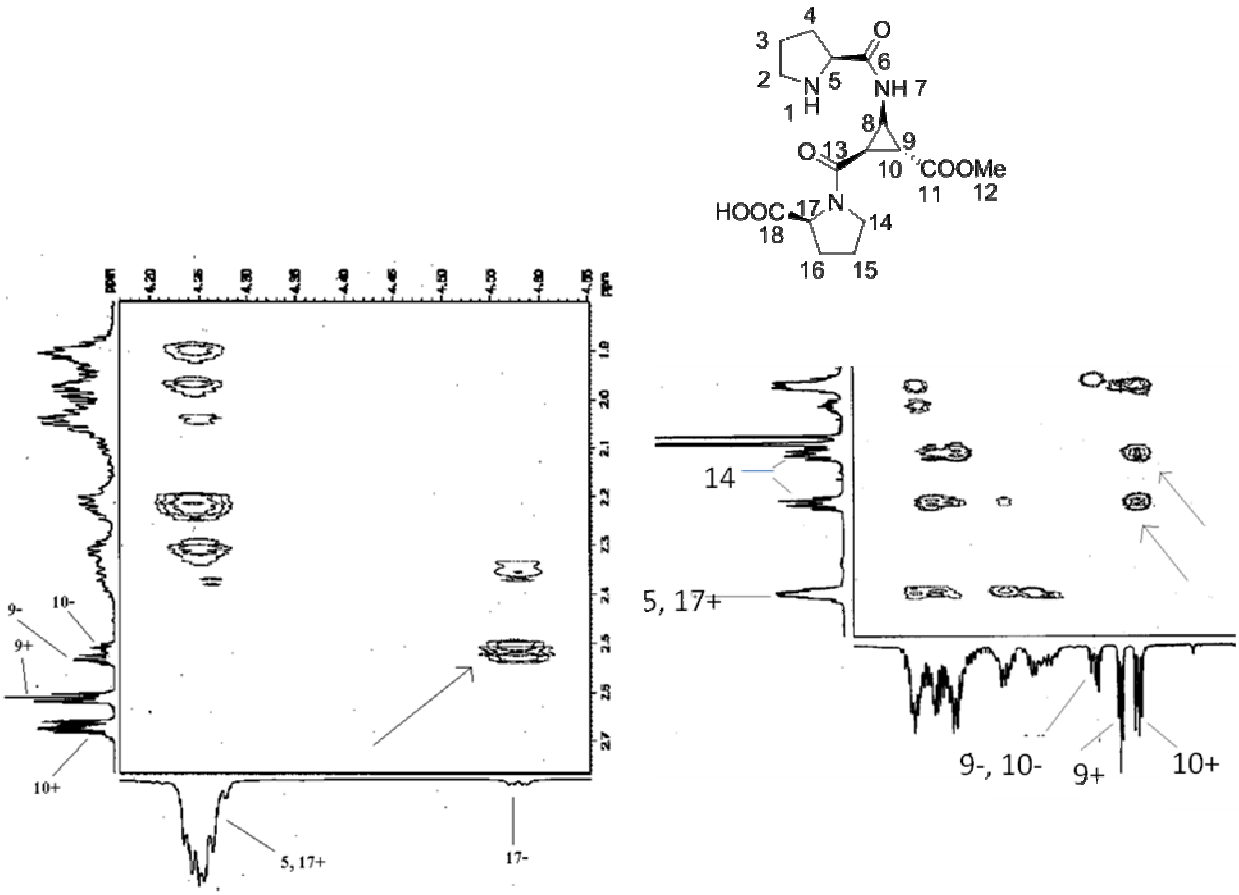


Fig. 15. Magnified sections of the NOESY spectrum of **90** (CD_3OD) showing the cross peaks relevant to the identification of the *cis*/*trans* conformers

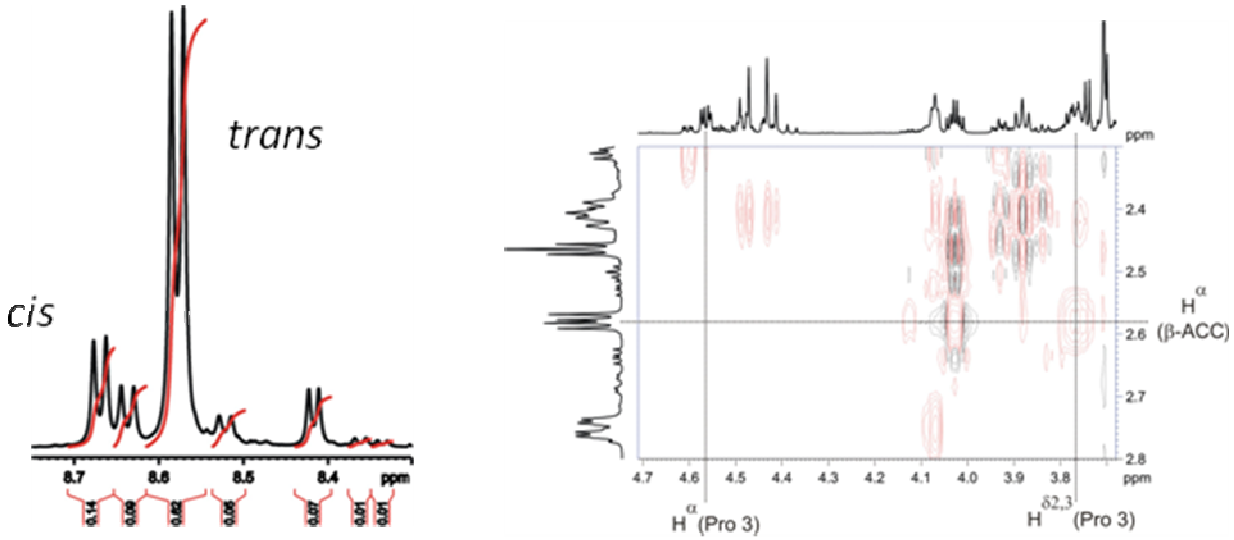
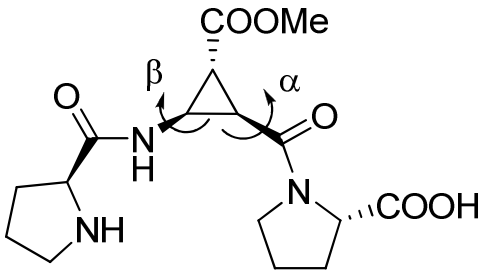


Fig. 16. amide proton region and NOE spectrum ($CDCl_3$) for H-Hyp(OBn)-▲-Pro-OBn

In the case of tripeptide H-Hyp(OBn)-▲-Pro-OBn (fig. 16) a similar isomerism is observed, with a *trans*/*cis* ratio of 4.5:1 and the presence of some other minor conformations.

In CHAPTER 1, an hairpin-shaped model catalyst (fig. 7) in which the amino and the carboxylic functionalities of the molecule were placed in a suitable spatial disposition to take simultaneously part to the catalytic process, was proposed. We also postulated that proline would be an ideal *C*-terminal amino acid, aimed at inducing, at least in part, such *cis* conformation. The discovery of the existence of a stable *cis*-configuration in methanol represents an important step forward in demonstrating the validity of this assertion. This notwithstanding, the NMR analysis above reported does not tell us any definitive information about the structure of the remaining fragments of the molecule and about their ability to rotate freely or being relatively fixed in space. In particular, it would be interesting to know if the rotation around the α and β angles in fig. 17 is hindered to any extent as this would be extremely relevant in understanding the role of the *β*-ACC unit in stabilizing the structure of our catalyst. It would also give us a hint about the conformation of the transition state of the aldol reaction promoted by **90**. An outstanding contribution to this field has been provided by *Schmid*, *Fleischmann*, *Gronwald* and *Gschwind*[71] through NOE and RDC spectroscopic analysis of tripeptides H-Pro-▼-Pro-OBn and H-Pro-Pro-▼-OBn. This study was accompanied by molecular dynamic calculations based on the restraints obtained through the bidimensional NMR techniques. Their results are exposed in the next paragraph.



90

Fig. 17. Important bond rotations in tripeptide **90**

4.1.2 Residual dipolar coupling[72]

The intensity of the spin dipolar coupling between two nuclei of a molecule depends on their distance and on their orientation relative to the static magnetic field applied. In isotropic media, the molecules tumble randomly and, as a consequence, the orientational term averages to zero in solution. Carrying out the NMR spectra in media that can partially order the molecules allows us to observe a part of the orientational contribution to the spin coupling. This can be achieved by measuring the NMR spectra in orientating media like liquid crystals or polymer gels within NMR tubes. Stretching or compressing such gels creates anisotropic cavities that allow a partial alignment of the molecules (SAG: strain-induced alignment in a gel). The spin-spin coupling measured under such conditions contains an additional component which can be calculated by subtracting the scalar spin-spin coupling (measured in an isotropic solution), thus generating the residual dipolar coupling. RDCs are of great importance in modern spectroscopy as they contain information about the inter-nuclear distance and the orientation of the vector between the two nuclei relative to the applied magnetic field (B_0). In a similar way, NOEs contacts provide useful distance information for structural investigations but are hampered by spin diffusion and are referred to distance restraints without directional reference, while RDCs provide orientational restraints referenced to the external magnetic field. Moreover, RDCs can be observed for longer inter-atomic distances, as the intensity of this coupling is inversely proportional to r^3 (r = inter-nuclear distance) while NOEs depends on r^{-6}. An interesting example of the ability of this modern spectroscopic technique to provide excellent structural information can be seen in fig. 18. *Zhou*[73] showed that using RDC data, an excellent structure of the B-form of DNA could be obtained (right), while NOE spectroscopy is effective only at a shorter range (left).

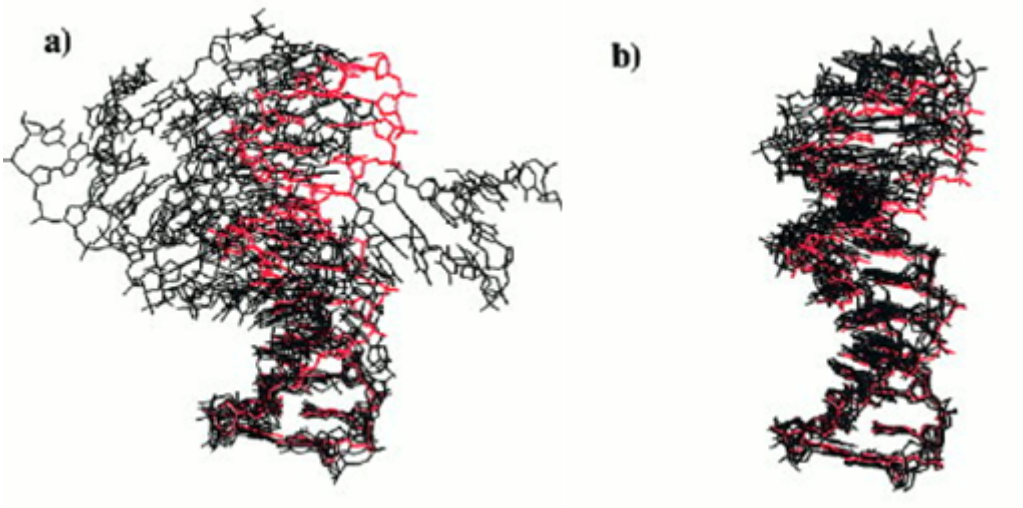


Fig. 18. Structures of the B-form of DNA as obtained by *Zhou* using NOE restraints (a) or RDC restraints (b)

Gschwind et al.[71] showed that even in the case of simple tripeptides, RDCs can represent a useful tool to derive structural information. For this purpose, H-Pro-▼-Pro-OBn (**165**) was studied either ^{1}H-NMR, ^{1}H, ^{13}C-HSQC and P.E.HSQC spectroscopy in $CDCl_3$ and in a strained PDMS/$CDCl_3$ gel. Both MD calculations, based on NOE or RDC restraints, lead to very similar structures in which the α and β angles of **165** show an unexpected stability, with the values based on the RDC analysis being identified between 175°-200° and 160°-180° respectively (see fig. 19). These data are corroborated by the calculated *Karplus* curve for $^{3}J(H^{N}\text{-}H^{\beta})$ that resulted to assume the unusual value of 9.69 Hz at 240°C and of 8.89 Hz at room temperature, sign that we are observing here a dominant molecular conformation. The relevant RDC and NOE restraints were used for MD studies that confirm a remarkable stability for tripeptide **165** for what concerns the rotation around the α and β angles, with an extremely narrow level of uncertainty for the RDC-calculated structure (fig. 19, below).

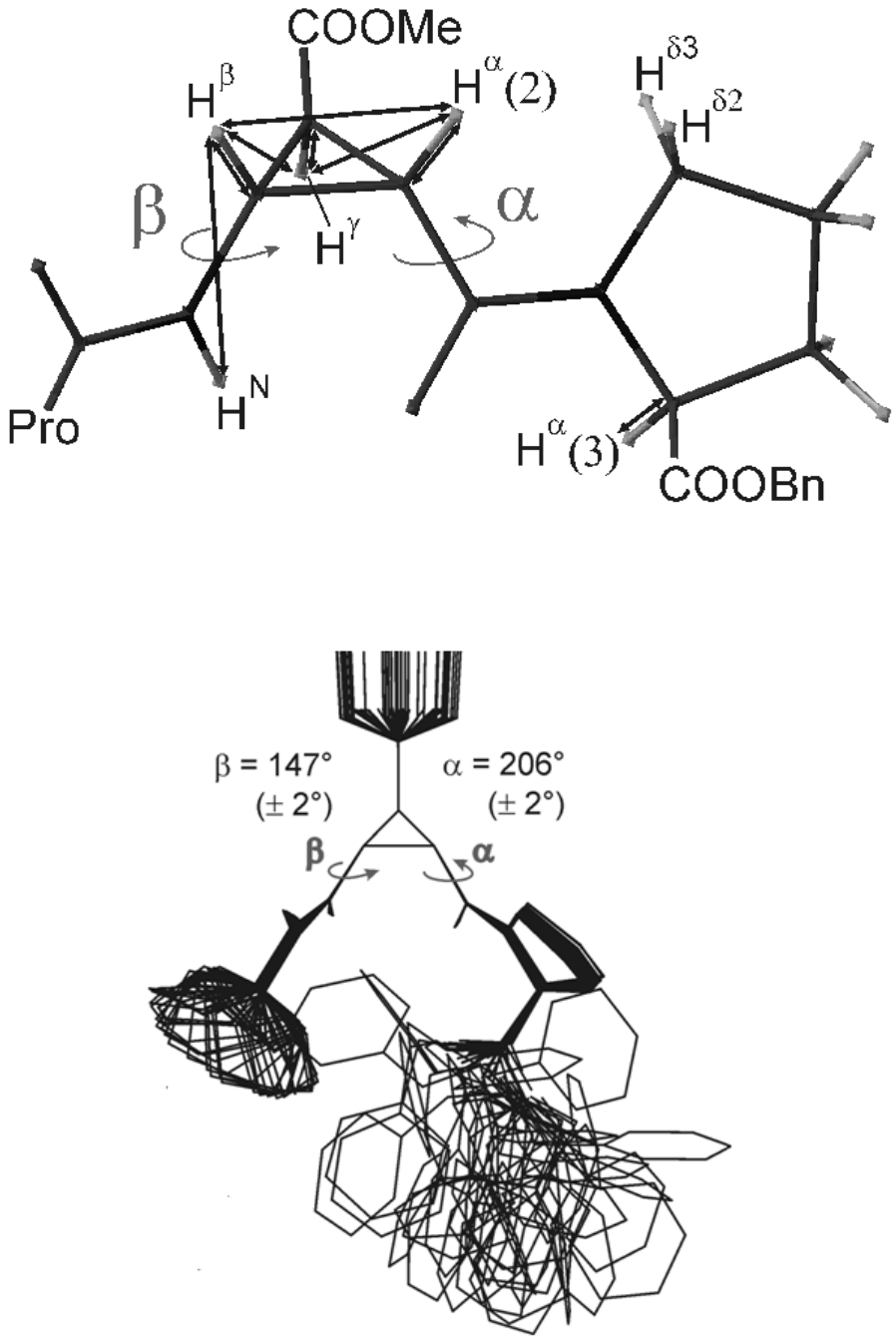


Fig. 19. NMR analysis of tripeptide H-Pro-▼-Pro-OBn, with the relevant RDCs (above) and MD-derived structures based on the RDC contacts (below).

The structure obtained on the base of the RDC data fits the MD calculations carried out using the set of coordinates for the hydrogen atoms of the cyclopropane ring which were attained from the crystal structure of **166** (fig. 20, right). This represents a further proof of the possibility to use this method to achieve useful and precise structural data and as an effective tool to validate the parametrization method employed.

An important part of this study concerned the structural and computational investigation of tripeptide H-Pro-Pro-▼-OBn (**167**). In the case of this molecule it was not possible to measure the RDC spectra, as it degrades with the time to the corresponding diketopiperazine. This notwithstanding, it was possible to obtain a large number of useful NOE restraints that were used for MD calculations. The picture of **167** which emerges from this study shows a turned, conformationally stable peptide.

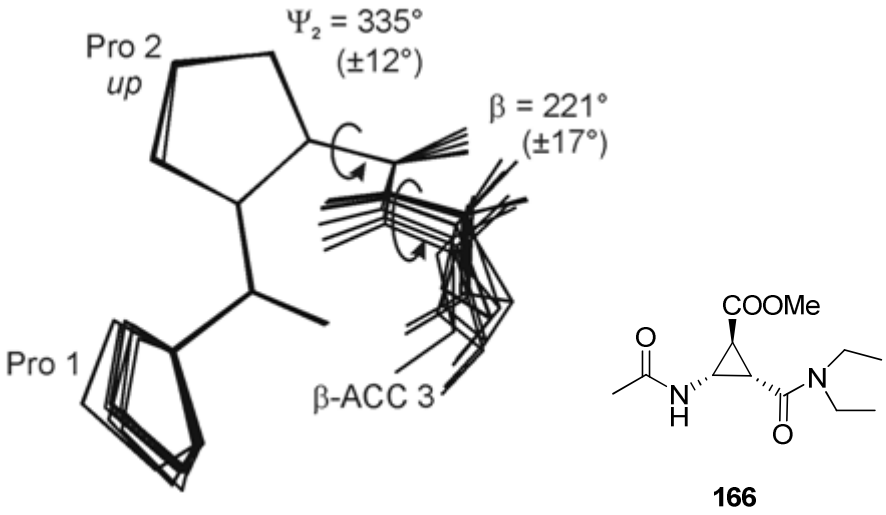


Fig. 20. The structure of H-Pro-Pro-▼-OBn obtained by *Gschwind et al.* (left) and the derivate of ▼ used to obtain the correct parametrization (right)

4.2 IR studies

IR spectroscopy represents an important instrument for the investigation of the structure of proteins and peptides. It is known[74] that the existence of intramolecular H-bonds, which are extremely relevant to the folding of the amino acidic sequence, can be detected on the basis of the value of the amide proton absorption. An IR absorption at frequencies lower than 3400 cm^{-1} is indicative of the presence of a H-bonded amide proton. *Zorn*[75] used this concept to estimate the number of H-bonded amide protons for a number of short peptides (fig. 21) and was able to complete the structural information provided by bidimensional NMR spectroscopy, CD and MD. Another useful observation produced during this study was the presence of an intra-residual H-bond in **166** (IR

absorption at 3364 cm^{-1}). For this molecule, although the value of the dihedral NH---O angle is far from the optimum of 180°, the good acceptor ability of the tertiary amide overcomes the disadvantage of the non-ideal bond geometry.

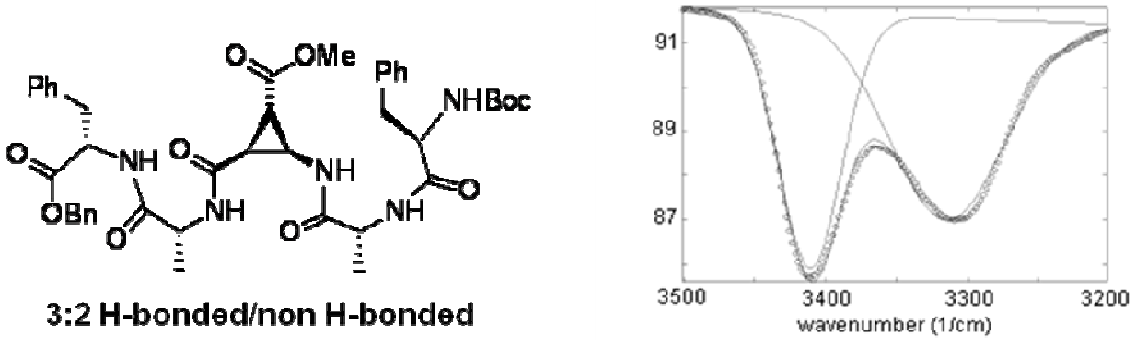


Fig. 21. Integration of the IR spectrum allows to quantify the ratio between H-bonded and non H-bonded amide protons

The exceptional structural stability showed by tripeptide **165** (fig. 19) as a result of the NMR studies, suggests that the hindered rotation around the angles α and β may involve the existence of a stable intra-residual H-bond for the *β*-ACC unit of this molecule as well. This may be true also for the compounds having ▲ at the center of the amino acidic sequence. For this purpose, we measured the FT-IR spectra of H-Pro-▲-Pro-OBn and H-Pro-▼-Pro-OBn in CH_2Cl_2, a solvent unable to disrupt intramolecular H-bonds. The concentration of the probe was 10 mM, which can guarantee the absence of intermolecular interactions[75]. In both cases the amide proton absorption was observed at about 3320 cm^{-1}, within the limits assumed for H-bonded protons.

4.3 Models for the transition state

The studies presented in the former paragraphs represent important advances in understanding the structure of the *β*-ACC containing peptides in $CHCl_3$. In the future, the development of this method can provide useful information in investigating the structure of the unprotected form of catalyst **90** and **153** and their mechanism of action. This notwithstanding, the nature of the transition state for the aldol reaction catalyzed by **88**,

90 or **153** is still unclear, as the structure of their enamine derivatives in the medium used for the reaction should be determined. This is particularly challenging in the case of the heterogeneous process catalyzed by **90** and **153**, which takes place in a dispersion of organic material in water. Based on the calculations carried out by *Tang*[76] and on the stereochemistry of the product of the catalytic process, other authors[77,57b] have adopted model transition states for their catalytically active pseudo-peptides that explain the *anti* selectivity observed in the case of organocatalyzed reaction between cyclohexanone and aldehydes. An analogous transition state can be suggested in the case of **90** and **153**. In this case we assumed that the *cis* conformation observed in the case of **90** in MeOH represents the catalytically active configuration of our peptide, as this is the conformation in which the amino and carboxylic functionalities are placed in the suitable orientation to simultaneously take part to the catalytic event (fig. 22). In a similar way, the formation of the *S*-enantiomer in the aldol reaction between acetone and aldehydes catalyzed by **88** can be explained through the transition state reported in fig. 23.

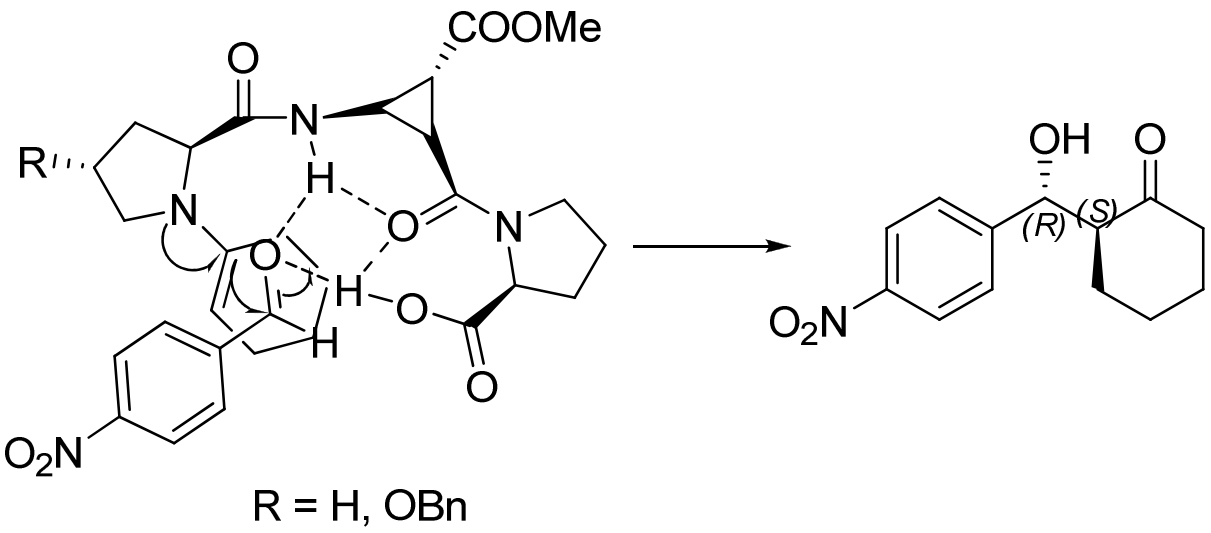


Fig. 22. Model transition state for **90** and **153** in the aldol reaction between cyclohexanone and *p*-nitrobenzaldehyde

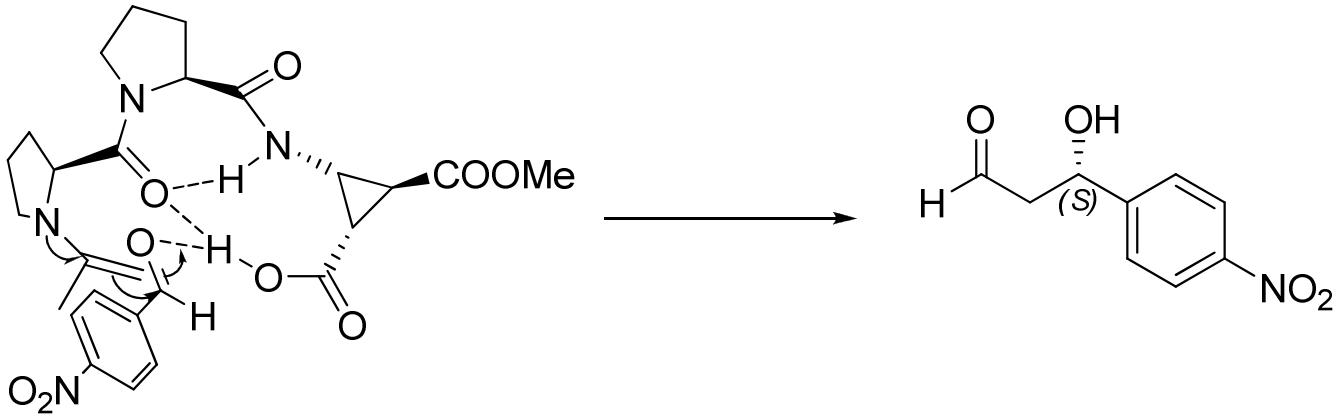


Fig. 23. Model transition state for the aldol reaction between acetone and *p*-nitrobenzaldehyde promoted by **88**

4.4 Conclusion

Short peptides were synthesized coupling proline with the racemic mixture of the two enantiomers of the *β*-ACC, ▲ and ▼, following standard amino acid coupling protocols. Chromatographic separation of the diastereomeric mixtures of the resulting peptides provided us with diastereomerically pure material and enabled the synthesis of tri- and tetrapeptides that were tested as organocatalyst for the aldol reaction. Since from the first screening rounds, the tripeptides having ▲ as a central building block (and in particular **90**) or ▼ as the *C*-terminal unit (**88**), emerged as the most interesting and efficient structures. In particular, **90** showed to be a versatile catalyst performing well in the intramolecular aldol reaction as well as in the intermolecular aldol reaction, either in homogeneous or heterogeneous water/ketone mixtures. Further developments saw the modification of the structure of **90** in order to produce a more lipophilic specie (**153**) able to carry out the heterogeneous aldol reaction in the presence of water between cyclic ketones and aromatic aldehydes with higher efficiency. This improvement allowed us to report shorter reaction times, to use a lower excess of ketone and to reduce the catalyst load to only 5 mol% in the case in which NaCl is added to the reaction mixture. A well established catalyst recovery procedure, through an easy extraction/lyophilization methodic, was applied to recuperate quantitatively tripeptides **90** and **153** at the end of the reaction, allowing their recycling without any loss in efficiency. Structural spectroscopic studies were carried out by *Fleischmann*, *Schmid* and *Gschwind*, showing that the presence of the *β*-ACC units confers an extraordinarily and unexpected

secondary structure stabilization to the tripeptides containing the ▼ amino acid as a central building block (**165**) or at its *C*-terminus (**167**). Further investigation will be aimed at determining the structure of the most active and versatile catalysts (**90** and **153**) and their mechanism of action. A concise view of the results obtained using catalysts **88**, **90** and **153** is presented in the following scheme (fig. 24).

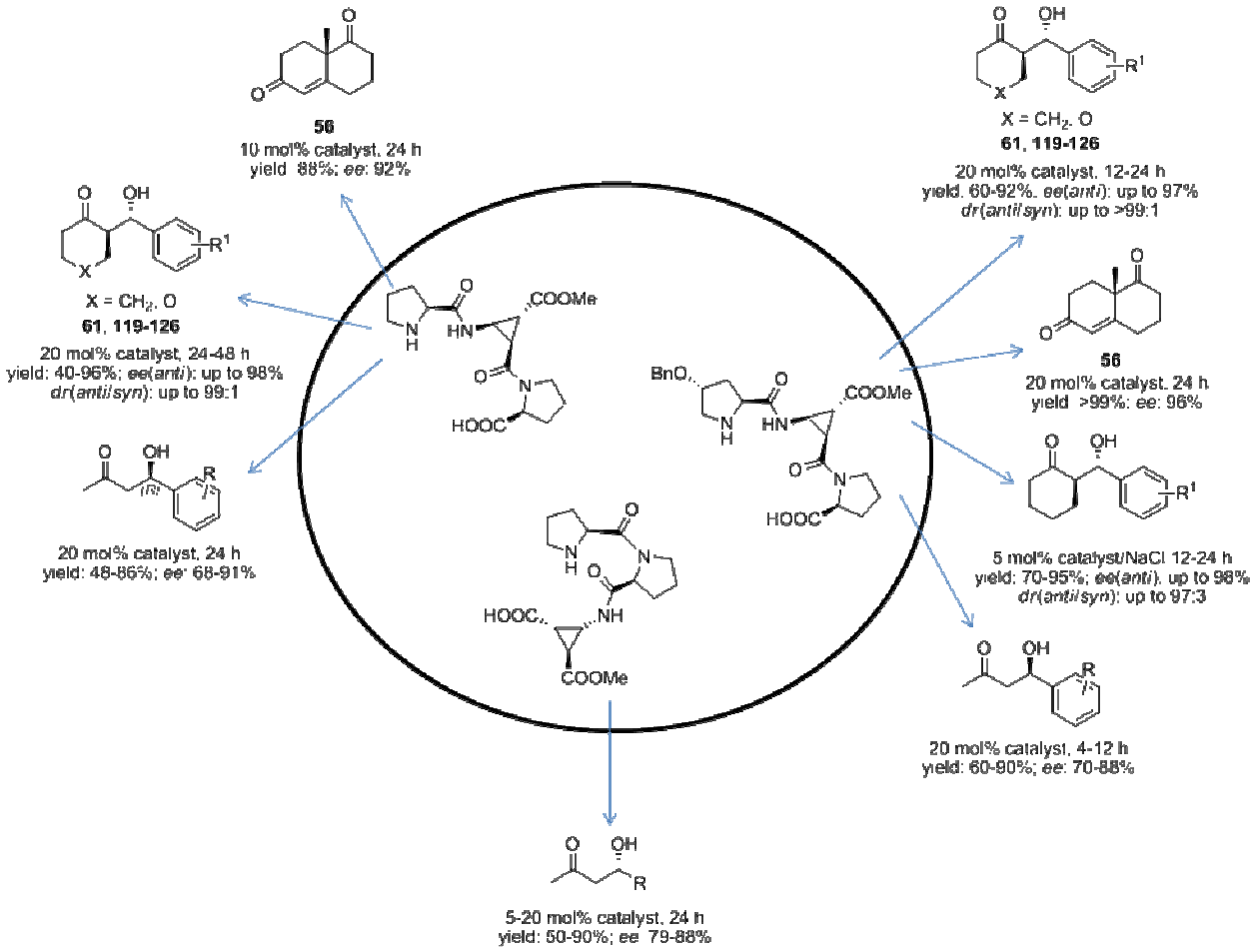


Fig. 24. Comprehensive view for the most relevant catalysis results obtained using tripeptides **88**, **90** and **153**

References

1) Reviews: a) Mukherjee, S.; Yang, J. W.; Hoffmann, S.; List, B. *Chem. Rev.* **2007**, *107*, 5471. b) List, B. *Chem. Commun.* **2006**, 819. c) Xu, L.-W.; Lu, Y. *Org. Biomol. Chem.* **2008**, *6*, 2047. d) Guillena, G.; Najera, C.; Ramon, D. J. *Tetrahedron: Asymm.* **2007**, *18*, 2249. Pellissier, H. *Tetrahedron*, **2007**, *63*, 9267. e) Seayad, J.; List, B. *Org. Biomol. Chem.* **2005**, *3*, 719. f) Buckley, B. R. *Annu. Rep. Prog. Chem., Sect. B,* **2007**, *103*, 90. g) Grottadauria, M.; Giacalone, F.; Noto, R. *Chem. Soc. Rev.* **2008**, *37*, 1666. h) Dalko, P. I.; Moisan, L. *Angew. Chem. Int. Ed.* **2004**, *43*, 5138.

2) a) List, B.; Lerner, A. R.; Barbas, C. F. III, *J. Am. Chem. Soc.* **2000**, *122*, 2395. b) Sakthivel, K.; Notz, W.; Bui, T.; Barbas, C. F. III, *J. Am. Chem. Soc.* **2001**, *123*, 5260.

3) Praecejus, H.; *Justus Liebigs Ann. Chem.* **1960**, *9*, 634.

4) Eder, U.; Sauer, G. R.; Weichert, *Angew. Chem. Int. Ed.* **1971**, *10*, 496.

5) Hajos, Z. G.; Parrish, D. R. *J. Org. Chem.* **1974**, *39*, 1615.

6) Kenzo, T.; Mori, A.; Inoue, S. *J. Org. Chem.* **1990**, *55*, 181.

7) Enders, D.; Balensiefer, T. *Acc. Chem. Res.* **2004**, *37*, 534.

8) Wang, Z.-X.; Tu, Y.; Frohn, M.; Zhang, J.-R.; Shi, Y. *J. Am. Chem. Soc.* **1997**, *119*, 11224.

9) a) Steglich, W.; Höfle, G. *Angew. Chem. Int. Ed.* **1969**, *81*, 1001. b) Höfle, G.; Steglich, W.; Vorbrüggen, H. *Angew. Chem. Int. Ed.* **1978**, *17*, 569. c) Litvinenko, L. M.; Kirichenko, A. I. *Dokl. Akad. Nauk. SSSR,* **1967**, *176*, 97. d) Hassner, H.; Krepski, L. R.; Alexanian, V. *Tetrahedron* **1978**, *34*, 2069. e) Spivey, A. C.; Arseniyadis, S. *Angew. Chem. Int. Ed.* **2004**, *43*, 5436. f) Heinrich, M. R.; Klisa, H. S.; Mayr, H.; Zipse, H. *Angew. Chem. Int. Ed.* **2003**, *42*, 4826. g) Xu, S.; Held, I.; Kempf, B.; Mayr, H.; Steglich, W.; Zipse, H. *Chem. Eur. J.* **2006**, *11*, 4751.

10) Nooy, A. E. J.; De Besemer, A. C.; van Bekkum, H. *Synthesis*, **1996**, 1153.

11) a) Ruble, J. C.; Tweddell, J.; Fu, G. C. *J. Org. Chem.* **1998**, *63*, 2794. b) Vedejs, E.; Jure, M. *Angew. Chem. Int. Ed.* **2005**, *44*, 3971.

12) a) Takeda K.; Akiyama, A.; Nakamura, H.; Takizawa, S.; Mizuno, Y.; Takayanagi, H.; Harigaya, Y. *Synthesis* **1994**, 1063. b) Gooßen, L. J.; Döhring, A. *Synlett* **2004**, 263. c) Gooßen, L. J.; Döhring, A. *Adv. Synth. Catal.* **2003**, *345*, 943. d) Held, I.; von den Hoff, P.; Stephenson, D. S.; Zipse, H. *Adv. Synth. Catal.* **2008**, *350*, 1891.

13) a) Bolm, C.; Schiffers, I.; Dinter, C. L.; Gerlach, A. *J. Org. Chem.* **2000**, *65*, 6984. b) Chen, Y.; Tian, S.-K.; Deng, L. *J. Am. Chem. Soc.* **2000**, *122*, 9542.

14) Ahrendt, K. A.; Borths, C. J.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2000**, *122*, 4243.

15) Paras, N. A.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2001**, *123*, 4370.

16) a) Brown, S. P.; Goodwin, N. C.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2003**, *125*, 1192. b) Prieto, A.; Halland, N.; Jorgensen, K. A. *Org. Lett.* **2005**, *7*, 3897. c) Halland, N.; Hazell, G.; Jorgensen, K. A. *J. Org. Chem.* **2002**, *67*, 8331. d) Peelen, T. J.; Chi, Y. G.; Gellman, S. H. *J. Am. Chem. Soc.* **2005**, *127*, 11598. e) Wang, J.; Li, H.; Duan, W.; Zu, L.; Wang, W. *Org. Lett.* **2005**, *7*, 4713. f) Halland, N.; Hansen, T.; Jorgensen, K. A. *Angew. Chem. Int. Ed.* **2003**, *42*, 4955. g) Wang, J.; Li, H.; Zu, L.; Jiang, W.; Xie, H.; Duan, W.; Wang, W. *J. Am. Chem. Soc.* **2006**, *128*, 12652. h) Guerin, D. J.; S. J. Miller *J. Am. Chem. Soc.* **2002**, *124*, 2134. i) Paras, N. A.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2002**, *124*, 7894. j) Austin, J. F.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2002**, *124*, 1172. k) Hechavarria Fonseca, M. T.; List B. *Angew. Chem. Int. Ed.* **2004**, *43*, 3958. l) Hayashi, Y.; Gotoh, H.; Tamura, T.; Yamaguchi, H.; Masui, R.; Shoji, M. *J. Am. Chem. Soc.* **2005**, *127*, 16028. m) Tuttle, J. B.; Ouellet, S. G.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2006**, *128*, 12662. n) Chen, Y. K.; Yoshida, M.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2006**, *128*, 12662. o) Kunz, R. K.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2005**, *127*, 3240.

17) a) Northrup, A. B.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2002**, *124*, 2458. b) Wilson, R. M.; Jen, W. S.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2005**, *127*, 11616.

18) a) Bahmanyar, S.; Houk, K. N.; Martin, J. H.; List, B. *J. Am. Chem. Soc.* **2003**, *125*, 2475. b) Hoang, L.; Bahmanyar, S.; Houk, K. N.; List, B. *J. Am. Chem. Soc.* **2003**, *125*, 16. c) Fu, A.; Li, H.; Tian, F.; Yuan, S.; Si, H.; Duan, Y. *Tetrahedron: Asymm.* **2008**, *19*, 1288. d) Hammar, P.; Cordova, A.; Himo, F. *Tetrahedron: Asymm.* **2008**, *19*, 1617.

19) Machajewski, T. D.; Wong, C.-H. *Angew. Chem. Int. Ed.* **2000**, *39*, 1352.

20) Corey, E. J.; Grogan, M. J. *Org. Lett.* **1999**, *1*, 157.

21) Huang, Y.; Unni, A. K.; Thadani, A. N.; Rawal, V. H. *Nature*, **2003**, *424*, 146.

22) Review: Connon, S. J. *Chem. Eur. J.* **2006**, *12*, 5418.

23) Akiyama, T.; Itoh, J.; Yokota, K.; Fuchibe, k. *Angew. Chem. Int. Ed.* **2004**, *43*, 1566.

24) Uraguchi, D.; Terada, M. *J. Am. Chem. Soc.* **2004**, *126*, 5356.

25) a) Matsui, K.; Tanaka, K.; Horii, A.; Takizawa, S.; Sasai, H. *Tetrahedron: Asymm.* **2006**, *17*, 578. b) Matsui, K.; Takizawa, S.; Sasai, H. *Synlett* **2006**, *5*, 761.

26) a) Stork, G.; Brizzolara, A.; Landesman, H.; Szmuszkovicz, J.; Terrell, R. *J. Am. Chem. Soc.* **1963**, *85*, 207. b) Stork, G.; Saccomanno, N. A.; *Tetrahedron Lett.* **1987**, *28*, 2087.

27) a) Brochu, M. P.; Brown, S. P.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2004**, *126*, 4108. b) Halland, N.; Braunton, A.; Bachmann, S.; Marigo, M.; Jorgensen, K. A. *J. Am. Chem. Soc.* **2004**, *126*, 4790.

28) a) Zhong, Z. *Angew. Chem. Int. Ed.* **2003**, *42*, 4247. b) Brown, S. P.; Brochu, M. P.; Sinz, C. J.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2003**, *125*, 10808.

29) List, B. *J. Am. Chem. Soc.* **2002**, *124*, 5656.

30) Sunden, H.; Ibrahem, I.; Eriksson, L.; Cordova, A. *Angew. Chem. Int. Ed.* **2005**, *44*, 4877.

31) Vesely, J.; Dziedzic, P.; Cordova, A. *Tetrahedron Lett.* **2007**, *48*, 6900.

32) List, B.; Pojarliev, P.; Castello C. *Org. Lett.* **2001**, *3*, 573.

33) a) Casas, J.; Engqvist, M.; Ibrahem, I.; Kaynak, B.; Cordova, A. *Angew. Chem. Int. Ed.* **2005**, *44*, 1343. b) Reyes, E.; Cordova, A. *Tetrahedron Lett.* **2005**, *46*, 6605. c) Northrup, A. B.; MacMillan, D. W. C. *Science*, **2004**, *305*, 1752. d) Northrup, A. B.; Mangion, I. K.; Hettche, F.; MacMillan, D. W. C. *Angew. Chem. Int. Ed.* **2004**, *43*, 2152. e) Sorensen, E. J.; Sammis, G. M.; *Science*, **2004**, *305*, 1725. f) Kazmaier, U. *Angew. Chem. Int. Ed.* **2005**, *44*, 2186. g) Mangion, I. K.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2005**, *127*, 3696. h) Cordova, A.; Engqvist, M.; Ibrahem, I.; Casas, J.; Sunden, H. *Chem. Commun.* **2005**, 2047. i) Cordova, A.; Ibrahem, I.; Casas, J.; Sunden, H.; Engqvist, M.; Reyes, E. *Chem. Eur. J.* **2005**, *11*, 4772.

34) Pidathala, C.; Hoang, L.; Vignola, N.; List, B. *Angew. Chem. Int. Ed.* **2003**, *42*, 2785.

35) Bui, T.; Barbas, C. F. III *Tetrahedron Lett.* **2000**, *41*, 6951.

36) a) Gu, L.; Yu, M.; Wu, X.; Zhang, Y.; Zhao, G. *Adv. Synth. Catal.* **2006**, 3*48*, 2223. b) Aratake, S.; Itoh, T.; Okano, T.; Nagae, N.; Sumiya, T.; Shoji, M.; Hayashi, Y. *Chem. Eur. J.* **2007**, *13*, 10246. c) Huang, J.; Zhang, X.; Armostrong, D. W. *Angew. Chem. Int. Ed.* **2007**, *46*, 9073.

37) a) Mase, N.; Nakai, Y.; Ohara, N.; Yoda, H.; Takabe, K.; Tanaka, F.; Barbas, C. F. III. *J. Am. Chem. Soc.* **2006**, *128*, 734. b) Cobb, A. J. A.; Shaw, D. M.; Ley, S. V. *Synlett*, **2004**, 558. c) Torii, H.; Nakadai, M.; Ishihara, K.; Saito, S.; Yamamoto, H. *Angew. Chem. Int. Ed.* **2004**, *43*, 1983. d) Hartikka, A.; Arvidsson, P. I. *Tetrahedron: Asymm.* **2004**, *15*, 1831. e) Hartikka, A.; Arvidsson, P. I. *Eur. J. Org. Chem.* **2005**, 4287. f) Lacoste, E.; Landais, Y.; Schenk, K.; Verlhac, J.-B.; Vincent, J.-M. *Tetrahedron Lett.* **2004**, *45*, 8035.

38) a) Tang, Z.; Cun, L.-F.; Cui, X.; Mi, A.-Q.; Jiang, Y.-Z.; Gong, L.-Z. *Org. Lett.* **2006**, *8*, 1263. b) Wang, W.; Li, H.; Wang, J. *Tetrahedron Lett.* **2005**, *46*, 5077. c) Tang, Z.; Jiang, F.; Yu, L.-T.; Cui, X.; Gong, L.-Z.; Mi, A.-Q.; Jiang, Y.-Z.; Wu, Y.-D. *J. Am. Chem. Soc.* **2003**, *125*, 5262. d) Tang, Z.; Yang, Z.-H.; Chen, X.-H.; Cun, L.-F.; Mi, A.-Q.; Jiang, Y.-Z.; Gong, L.-Z. *J. Am. Chem. Soc.* **2005**, *127*, 9285. e) He, L.; Tang, Z.; Cun, L.-F.; Mi, A.-Q.; Jiang, Y.-Z.; Gong, L.-Z. *Tetrahedron*, **2006**, *62*, 346. f) Berkessel, A.; Koch, B.; Lex, J. *Adv. Synth. Catal.* **2004**, *346*, 1141. g) Cobb, A. J. A.; Shaw, D. M.; Longbottom, D. A.; Gold, J. B.; Ley, S. V. *Org. Biomol. Chem.* **2005**, *3*, 84. h) Bellis,

E.; Vasilatou, K.; Kokotos, G. *Synthesis*, **2005**, 2407. i) Chen, J.-R.; Lu, H.-H.; Li, X.-Y.; Cheng, L.; Wan, J.; Xiao, W.-J. *Org. Lett.* **2005**, *7*, 4543. j) Samanta, S.; Liu, J.; Dodda, R.; Zhao, C.-G. *Org. Lett.* **2005**, *7*, 5321. k) Raj, M.; Vishnumaya, Ginotra, S. K.; Singh, V. K. *Org. Lett.* **2006**, *8*, 4097. l) Griko, D.; Lipinski, R. *Adv. Synth. Catal.* **2005**, *347*, 1948 and *Eur. J. Org. Chem.* **2006**, 3864. m) Cheng, C.; Sun, J.; Wang, C.; Zhang, Y.; Wei, S.; Jiang, F.; Wu, Y. *Chem Commun.* **2006**, 215. n) Krattiger, P.; Kovasy, R.; Revell, J. D.; Ivan, S.; Wennemers, H. *Org. Lett.* **2005**, *7*, 1101.

39) Krattiger, P.; McCarthy, C.; Pfaltz, A.; Wennemers, H. *Angew. Chem. Int. Ed.* **2003**, *42*, 1722.

40) a) Bubert, C.; Cabrele, C.; Reiser, O. *Synlett*, **1997**, 827. b) Böhm, C.; Schinnerl, M.; Bubert, C.; Zabel, M.; Labahn, T.; Parisini, E.; Reiser, O. *J. Org. Chem.* **2000**, *65*, 8960. c) Beumer, R.; Reiser, O. *Tetrahedron*, **2001**, *57*, 6497. d) Gnad, F.; Reiser, O. *Chem. Rev.* **2003**, *103*, 1603. e) Chhor, R. B.; Nosse, B.; Sörgel, S.; Böhm, C.; Seitz, M.; Reiser, O. *Chem. Eur. J.* **2003**, *9*, 260. f) Schinnerl, M.; Böhm, C.; Seitz, M.; Reiser, O. *Tetrahedron: Asymm.* **2003**, *14*, 765. g) Gnad, F.; Poleschak, M.; Reiser, O. *Tetrahedron Lett.* **2004**, 45, 4277.

41) Koglin, N.; Zorn, C.; Beumer, R.; Cabrele, C.; Bubert, C.; Sewald, N.; Reiser, O.; Beck-Sickinger, A. G. *Angew. Chem. Int. Ed.* **2003**, *42*, 202.

42) Gellman, S. H.; *Acc. Chem Res.* **1998**, *31*, 173. Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1994**, *116*, 1054. Appella, D. H.; Christianson, L. A.; Karle, I. L.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 13071. d) Appella, D. H.; Christianson, L. A.; Klein, D. A.; Powell, D. R.; Huang, X.; Barchi, J. J.; Gellman, S. H. *Nature*, **1997**, *387*, 381. e) Krauthäuser, S.; Christianson, L. A.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1997**, *119*, 11719.

43) a) Seebach, D.; Overhand, M.; Kühnle, F. N. M.; Martinoni, B.; Oberer, L.; Hommel, U.; Widmer, H.; *Helv. Chim. Acta*, **1996**, *79*, 913. b) Seebach, D.; Ciceri, P. E.; Overhand, M.; Jaun, B.; Rigo, D.; Oberer, L.; Hommel, U.; Armstuzt, R.; Widmer, H.; *Helv. Chim. Acta*, **1996**, *79*, 2043. c) Hintermann, T.; Seebach, D. *Synlett*, **1997**, 437. d) Hintermann, T.; Seebach, D. *Chimia*, **1997**, *51*, 244. e) Guichard, G.; Seebach, D. *Chimia*, **1997**, *51*, 315.

44) Horne, W. S.; Gellman, S. H. *Acc. Chem. Res.* **2008**, *41*, 1399.

45) De Pol, S.; Zorn, C.; Klein, C. D.; Zerbe, O.; Reiser, O. *Angew. Chem. Int. Ed.* **2004**, *43*, 511.

46) Hayen, A.; Schmitt, M. A.; Ngassa, F. N.; Thomasson, K. A.; Gellman, S. H. *Angew. Chem. Int. Ed.* **2004**, *43*, 505.

47) Gademann, K.; Kimmerlin, T.; Hoyer, D.; Seebach, D. *J. Med. Chem.* **2001**, *44*, 2460.

48) Huck, B. R.; Fisk, J. D.; Gellman, S. H. *Org. Lett.* **2000**, *2*, 2607.

49) Urman, S.; Gaus, K.; Yang, Y.; Strijowski, U.; Sewald, N.; De Pol, S.; Reiser, O. *Angew. Chem. Int. Ed.* **2007**, *46*, 3976.

50) Gheorghe, A. *Dissertation*, **2006**, Regensburg.

51) Lam, W. H.; Ng, K. M. *AIChE J.* **2007**, *53*, 429.

52) Zorn, C.; Gnad, F.; Salmen, S.; Herpin, T.; Reiser, O. *Tetrahedron Lett.* **2001**, *42*, 7049.

53) Zwicknagl, H. *Diplomarbeit*, **2005**, Regensburg.

54) Revell, J. D.; Wennemers, H. *Adv. Synth. Catal.* **2008**, *350*, 1046.

55) Fischer, G. *Chem. Soc. Rev.* **2000**, *29*, 119.

56) Bordessa, *Dissertation*, **2008**, Regensburg.

57) a) Aratake, S.; Itoh, T.; Okano, T.; Nagae, N.; Sumiya, T.; Shoji, M.; Hayashi, Y. *Chem. Eur. J.* **2007**, *13*, 10246. b) Zhao, J.-F.; Long, H.; Jiang, J.; Tang, Z.; Cun, L.-F.; Gong, L.-Z. *Tetrahedron Lett.* **2008**, *49*, 3372. c) Gryko, D.; Saletra, W. J. *Org. Biomol. Chem.* **2007**, *5*, 2148. d) Huang, J.; Zhang, X.; Armstrong, D. W. *Angew. Chem. Int. Ed.* **2007**, *46*, 9073. e) Mase, N.; Nakai, Y.; Ohara, N.; Yoda, H.; Takabe, K.; Tanaka, F.; Barbas, C. F. III. *J. Am. Chem. Soc.* **2006**, *128*, 734. f) Cordova, A.; Zou, W.; Ibrahem, I.; Reyes, E.; Engqvist, M.; Liao, W.-W. *Chem. Commun.* **2005**, 3586. g) Cordova, A.; Zou, W.; Dziedzic, P.; Ibrahem, I.; Reyes, E.; Xu, I. *Chem. Eur. J.* **2006**, *12*, 5383. h) Amedjkouhm, M. *Tetrahedron: Asymm.* **2005**, *16*, 1411.

58) Hayashi, Y.; Sumiya, T.; Takahashi, J.; Gotoh, H.; Urushima, T.; Shoji, M. *Angew. Chem.* **2006**, *118*, 972.

59) Brogan, A. P.; Dickerson, T. J.; Janda, K. D. *Angew. Chem. Int. Ed.* **2006**, *45*, 8100.

60) Hayashi, Y. *Angew. Chem. Int. Ed.* **2006**, *45*, 8103.

61) Zhong, C.; Hoffmann, T.; Lerner, R. A.; Danishefsky, S.; Barbas, C. F. III, *J. Am. Chem. Soc.* **1997**, *119*, 8131.

62) a) Kriis, K.; Kanger, T.; Laars, M.; Kailas, T.; Müürisepp, A.-M.; Pehk, T.; Lopp, M. *Synlett*, **2006**, 1699. b) Kanger, T.; Kriis, K.; Laars, M.; Kailas, T.; Müürisepp, A.-M.; Pehk, T.; Lopp, M. *J. Org. Chem.* **2007**, *72*, 5168.

63) Davies, S. G.; Sheppard, R. L.; Smith, A. D.; Thomson, J. E. *Chem. Commun.* **2005**, 3802.

64) Nagamine, T.; Inomata, K.; Endo, Y.; Paquette, L. A. *J. Org. Chem.* **2007**, *72*, 123.

65) Kim, J. Y.; Kim, H. D.; Seo, M. J.; Kim, H. R.; No, Z.; Ha, D.-C.; Lee, G.H. *Tetrahedron Lett.* **2006**, *47*, 9.

66) a) List, B. *Synlett* **2001**, 1675. b) List, B. *J. Am. Chem. Soc.* **2000**, *122*, 9336.

67) a) Wang, W.; Wang, J.; Li, H. *Tetrahedron Lett.* **2004**, *45*, 7243. b) Cobb, A. J. A.; Shaw, D. M.; Longbottom, D. A.; Gold, J. B.; Ley, S. V. *Org. Biomol. Chem.* **2005**, *3*, 84. c) Notz, W.; Tanaka, F.; Barbas, C. F. III, *Acc. Chem. Res.* **2004**, *37*, 580.

68) Betancort, J. M.; Sakthivel, K.; Thayumanavan, R.; Tanaka, F.; Barbas, C. F. III, *Synthesis*, **2004**, 1509.

69) D´Elia, V.; Zwicknagl, H.; Reiser, O. *J. Org. Chem.* **2008**, *73*, 3262.

70) Kessler, H; Schmitt, W. In *Encyclopedia of Nuclear Magnetic Resonance*; Grant, D. M.; Harris, R. K., Eds.; John Wiley & Sons: New York, **1996**, pp 3527.

71) Schmid, M. B.; Fleischmann, M.; D´Elia, V.; Reiser, O.; Gronwald, W.; Gschwind, R. M. *ChemBioChem.* **2009**, accepted.

72) a) Grishaev, A.; Bax, A. *Curr. Opin. Struct. Biol.* **2005**, *15*, 563. b) Lipsitz, R. S.; Tjandra, N. *Annu. Rev. Biophys. Biomol. Struct.* **2004**, *33*, 387. c) Gschwind, R. M. *Angew. Chem. Int. Ed.* **2005**, *44*, 4666.

73) Zhou, H.; Vermeulen, A.; Jucker, F. M.; Pardi, A. *Biopolymers*, **1999-2000**, *52*, 168.

74) a) Gellman, S. H.; Dado, G. P.; Liang, G.-B.; Adams, B. R. *J. Am. Chem. Soc.* **1991**, *113*, 1164. b) Gellman, S. H.; Dado, G. P. *J. Am. Chem. Soc.* **1993**, *115*, 4228.

75) Zorn, C. *Dissertation*, Regensburg, **2001**, pp 31.

76) Fu, Y.-Q.; Li, Z.-C.; Ding, L.-N.; Tao, J.-C.; Zhang, S. H.; Tang, M.-S.; *Tetrahedron: Asymm.* **2006**, *17*, 3351.

77) a) Sathapornvajana, S.; Vilaivan, T. *Tetrahedron*, **2007**, *63*, 10253. b) Chen, F.; Huang, S.; Zhang, H.; Liu, F.; Peng, Y. *Tetrahedron*, **2008**, *64*, 9585.

EXPERIMENTAL

E.1 General Information

Reactions were carried out in closed 10 mL vials following the procedure subsequently reported; distilled, water free solvents have been employed in all the reactions that did not involve the presence of water. Dry DCM for peptide coupling has been obtained from a molecular sieves solvent purification system. Analysis grade ketones have been employed in the catalysis involving water containing mixtures. Aldehyde substrates have been distilled before use, if liquid at room temperature. Silica gel 60 (0.063-0.200 mm) was used for the column chromatography. TLC analysis was carried out on silica gel 60 F_{254} coated on aluminium sheets. Ninhydrine coloration was employed to check the reaction progress during peptide synthesis, vanilline was employed to follow the formation of the products of catalysis. ^{1}H (300 MHz) and ^{13}C (75.5 MHz) NMR spectra were recorded on a 300 MHz Spectrometer in $CDCl_3$ (7.27 ppm for ^{1}H, 77 ppm for ^{13}C) or CD_3OD (3.31 ppm for ^{1}H, 49.1 ppm for ^{13}C). IR spectra were recorded using a *golden gate*, single reflection, ATR system. The *ee* was determined by chiral HPLC on a Chiralpak-AS column or by chiral GC on a CP Chiralsil-Dex CB column (injector temperature, 250°C, detector temperature, 250°C). In the case of the products of the intermolecular aldol reaction, the GC analysis was carried out always after trimethylsilylation of the product. The trimethylsilylation was carried out directly on the GC sample, dissolved in DCM by addition of one drop of pure trimethylsilylimidazole. The absolute configuration was assigned by comparison of the optical rotation for the isolated compounds, with the values reported in literature. The yields reported are referred to the isolated compounds; in the case in which a mixture of *syn* and *anti* isomers was formed, the yields reported in this work are referred to the total amount of the isolated diastereomeric mixture.

E.2 General procedure for catalytic asymmetric aldol reaction

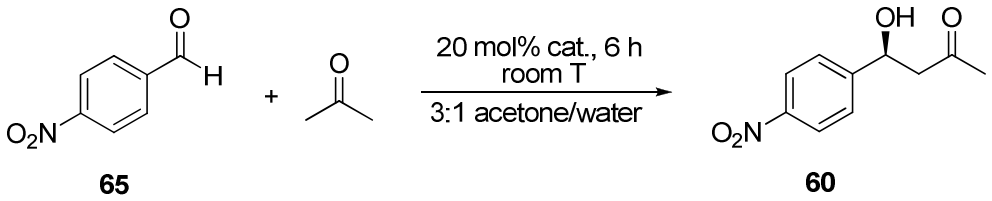
E.2.1 Intermolecular aldol reactions of acetone

Water free conditions: 0.01 to 0.04 mmol (5 to 20% catalyst) of the selected catalyst were added to 0.2 mmol of aldehyde and dissolved under N_2 atmosphere in 2 ml of dry acetone or $CHCl_3$/acetone mixture in a 10 ml vial. The reaction was stirred for the time indicated, subsequently, the solvent was evaporated and 5 mL of EtOAc and 2 mL of water were added to the crude material. The organic phases were extracted again with 1 mL of water, dried (Na_2SO_4), concentrated and purified on silica (3:1 hexanes/EtOAc) to yield the desired aldol product.

Catalyst recovery: the combined water layers were extracted with Et_2O (2 mL) and frozen at –20°C. Lyophilization of the sample allowed the recovery of the catalyst (90-95%), which could be reused without any loss in performance.

Homogeneous acetone/water mixture: *p*-nitrobenzaldehyde (38 mg, 0.25 mmol) and catalyst (H–Pro-▲-Pro–OH) (17.7 mg, 0.05 mmol) were dissolved in 0.75 mL of a 3:1 acetone/water (molar ratio 1:30:42 aldehyde/acetone/water), and the reaction mixture was stirred for 6 hours at room temperature. Acetone was evaporated under reduced pressure and EtOAc (5 mL) and water (2 mL) were added to the resulting suspension. The organic layers were separated, extracted with water (1 mL), dried (Na_2SO_4) and evaporated. The residue was purified on silica (3:1 hexanes/EtOAc) to yield **60** (50 mg, 95% yield, 71% ee).

Catalyst recovery: the combined aqueous layers were extracted with Et_2O (2 mL) and frozen at –20°C. Lyophilization allowed the recovery of the catalyst (90-95%), which could be reused without any loss in performance. The following table illustrates an example of catalyst recycling through three catalytic processes.



65 **60**

cycle	*time*	*Yield*	*ee*	*catalyst recovery*
1	6h	95%	71%	90%
2	6h	92%	71%	95%
3	6h	96%	71%	93%

Table S-1: catalytic cycles with fresh (entry 1) and recovered (entries 2,3) catalyst.

E.2.2 Intermolecular aldol reactions of cyclohexanone

Reaction between aromatic aldehydes and cyclohexanone (homogeneous): in a 5 mL vial, 0.03 mmol of H–Pro-▲-Pro–OH were added to 0.15 mmol of aldehyde, followed by 20 µL of water and 0.4 mL of ketone; the reaction was left stirring for 24 h. After this period 3 mL of EtOAc and 1 mL of water were added to the crude material. The organic phases were extracted with 1 mL of water and dried (Na_2SO_4), evaporated and purified on silica (4:1 hexanes/EtOAc) to furnish the desired aldol product.

Catalyst recovery: the combined water layers were extracted with 2 mL Et_2O and frozen at –20°C. Lyophilization of the sample allowed the recovery of 90-95% of the catalyst.

Reaction between aromatic aldehydes and cyclohexanone (heterogeneous):

a) in a 5 mL vial, 0.03 mmol of H–Pro-▲-Pro–OH were added to 0.15 mmol of aldehyde, followed by 40 µL of water and 0.4 mL of ketone; the reaction mixture was stirred for 48 h. After this period 3 mL of EtOAc and 1 mL of water were added to the crude material. The organic phases were extracted with 1 mL of water and dried (Na_2SO_4), evaporated and purified on silica (4:1 hexanes/EtOAc) to furnish the desired aldol product.

Catalyst recovery: the combined water layers were extracted with 2 mL Et_2O and frozen at -20°C. Lyophilization of the sample allowed the recovery of 90-95% of the catalyst.

b) in a 5 mL vial, 0.03 mmol of H–Hyp(OBn)-▲-Pro–OH were added to 0.15 mmol of aldehyde, followed by 0.4 mL of water and 5 equivalents of cyclohexanone (80 µL); the reaction mixture was stirred for 12-24 h. After this period 3 mL of Et_2O and 1 mL of water were added to the crude material. The organic phases were extracted with 1 mL of water and dried (Na_2SO_4), evaporated and purified on silica (4:1 hexanes/EtOAc) to furnish the desired aldol product.

Catalyst recovery: the combined water layers were extracted with 2 mL Et_2O and frozen at -20°C. Lyophilization of the sample allowed the recovery of 90-95% of the catalyst.

c) in a 5 mL vial, 0.03 mmol of H–Hyp(OBn)-▲-Pro–OH were added to 0.15 mmol of aldehyde, followed by 0.4 mL of brine and 5 equivalents of cyclohexanone (80 µL); the reaction mixture was stirred for 12 h. After this period 3 mL of Et_2O and 1 mL of water were added to the crude material. The organic phases were extracted with 1 mL of water and dried (Na_2SO_4), evaporated and purified on silica (4:1 hexanes/EtOAc) to furnish the desired aldol product.

E.2.3 Intramolecular aldol reaction

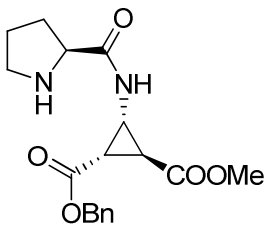
Water free: 0.02 mmol of **90** or 0.04 mmol of **153** were added to 0.2 mmol of **55** in 1 mL of $CHCl_3$. The reaction mixture was stirred at room temperature. After 24 hours the solvent was evaporated under reduced pressure and the crude material was dissolved in 5 mL of EtOAc and extracted with 2 mL of water. The organic phases were dried (Na_2SO_4), evaporated and, if necessary, purified through chromatography on silica gel (2:1 hexanes/EtOAc).

Catalyst recovery: the water layer was washed with 2 mL Et_2O and frozen at –20°C. Lyophilization of the sample allowed the recovery of 90-95% of catalyst that could be reused without any loss of catalytic activity.

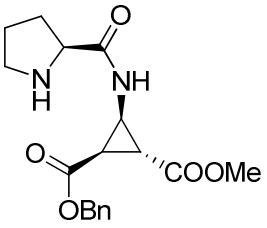
In presence of water: 0.03 mmol of **153** were added to 0.15 mmol of **55** in 0.5 mL of water and 0.1 mL of $CHCl_3$. The reaction mixture was stirred at room temperature. After 36 hours 5 mL of EtOAc 2 mL of water were added. The organic phases were dried over Na_2SO_4, evaporated and the conversion of the starting material was estimated through chiral GC.

Catalyst recovery: the water layer was washed with 2 mL Et_2O and frozen at –20°C. Lyophilization of the sample allowed the recovery of 90-95% of catalyst that could be reused without any loss of catalytic activity.

E.3 Synthesis of peptides



80



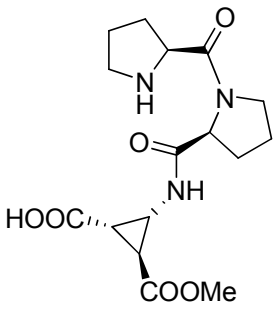
81

Synthesis of H-Pro-▼-OBn (80) and H-Pro-▲-OBn (81): Racemic building block ***rac*-76** (2.0 g, 5.7 mmol, 1.0 equiv.) was dissolved in a saturated solution of HCl in EtOAC (c≈3 mol/L), 20 mL, at 0°C. After stirring for 2 hours the acid was removed under reduced pressure, leaving a white/pinkish solid as a residue. To this deprotected building block ***rac*-76a** was added a solution of *N*-Boc-L-proline (1.5 g, 7.1 mmol, 1.25 equiv.) and EDC·HCl (1.4 g, 7.1 mmol, 1.25 equiv.) in DCM (25 mL) that had been stirred beforehand for 30 min. Using a dropping funnel TEA (0.87 mL, 6.8 mmol, 1.2 equiv.) in DCM (20 mL) was added over a period of 1.5 h. The mixture was stirred overnight. Then the reaction was quenched with water (20 mL) and the solution acidified to pH 3 using $KHSO_4$ solution

(1 M), then extracted with DCM. The organic phase was extracted successively with $NaHCO_3$ solution and brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. Purification by chromatography on silica gel (1:1 hexanes/EtOAc) yielded 1.78 g (70%) of the mixture of diastereomers **78**. To this diastereomeric mixture (2.0 g, 4.5 mmol) was added at 0°C a saturated solution of HCl in EtOAC (15 mL, c≈3 mol/L). After stirring for 1.5 hours the acid was removed under reduced pressure leaving a white wax as a residue. It was redissolved in water (20 mL) and extracted with Et_2O. The aqueous phase was then adjusted to basic pH using $NaHCO_3$ (saturated solution) and then extracted with DCM (3x20 mL). The organic phase was dried over Na_2SO_4, filtrated and evaporated under reduced pressure to yield **79** (1.52 g, 4.4 mmol, 98%). The two diastereomers **80** and **81** were separated using column chromatography (DCM/MeOH 15:1) to give pure **80** (570 mg, 38% R_f=0.25) and pure **81** (570 mg, 38%, R_f=0.20) along with the recovered mixture **79** (380 mg).

H-Pro-▼-OBn (80), ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 8.45 (d, 1H); 7.35 (m, 5H); 5.15 (q, 2H); 4.04 (m, 1H); 3.70 (m, 1H); 3.69 (m, 3H); 2.94 (m, 1H); 2.76 (m, 1H); 2.57 (m, 1H); 2.37 (m, 1H) 2.04 (m, 1H) 1.80 (m, 1H); 1.59 (m, 1H). ^{13}C (75.5 MHz, $CDCl_3$) δ: 176.3, 170.1, 169.4, 135.3, 128.6, 128.5, 128.3, 67.2, 60.5, 52.4, 47.2, 36.1, 30.7, 28.5, 26.7, 26.1. (ES-MS) for 346.15, $C_{18}H_{22}N_2O_5$, (MH^+) was found: 347.1; High resolution mass (EI-MS) for 346.1529, $C_{18}H_{22}N_2O_5$, 346.1529 was found. FT-IR (film) ν_{max}: 3322.0, 2975.3, 2955.5, 1723.4, 1673.5, 1508.4, 1451.4, 1309.9, 1177.1; mp: 114°C.

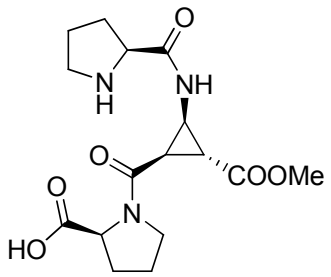
H-Pro-▲-OBn (81), ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 8.42 (brs, 1H); 7.35 (m, 5H); 5.15 (q, 2H); 4.10 (m, 1H); 3.72 (m, 1H); 3.68 (s, 3H); 3.66 (m, 1H); 2.96 (m, 1H); 2.85 (m, 1H); 2.58 (m, 1H); 2.37 (m, 1H); 1.93 (m, 1H); 1.67 (m, 2H). ^{13}C (75.5 MHz, $CDCl_3$) δ: 26.3, 26.6, 28.6 30.8, 36.2, 47.3, 52.5, 60.6, 67.4, 128.5, 128.6, 128.8, 135.4, 169.7, 170.2, 176.4; (ES-MS) for 346.15, $C_{18}H_{22}N_2O_5$, (MH^+) was found: 347.1; High resolution mass (EI-MS) for 346.1529, $C_{18}H_{22}N_2O_5$, 346.1528 was found (δ = 0.3 ppm). FT-IR (film) ν_{max}: 3325.0, 2968.3, 2957.4, 1723.8, 1667.6, 1510.5, 1407.3, 1300.0, 1179.6; oil.



88

Synthesis of H-Pro-Pro-▼-OH (88): *N*-Boc-L-proline (300 mg, 1.4 mmol) was dissolved in DCM (10 mL) and EDC·HCl (295mg, 1.54 mmol, 1.1 equiv.) was added. After 30 min **80** (580 mg, 1.68 mmol, 1.2 equiv.) was added and the reaction was stirred overnight. Then DCM (10 mL) and water (10 mL) were added and the pH was adjusted to 4 by addition of small amounts of $KHSO_4$ solution (1M). The organic phase was extracted and then washed with $NaHCO_3$ solution (sat.) (10 mL) and brine (10 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give **87** (495 mg, 0.91 mmol, 65%). The preparation of **88** was completed by de-protecting the amino and carboxylic acid functionalities of this intermediate. Boc-group deprotection of **87** (495 mg, 0.91 mmol) was performed as reported for **78** (360 mg, 90%). Debenzylation was carried out by dissolving **167** (200 mg, 0.45 mmol) in MeOH and adding Pd/C (10 m%, 20 mg). Stirring under H_2 atmosphere was continued for 1.5 hours. After this period the Pd/C residue was filtered through celite and the collected solvent was evaporated to provide **88** (160 mg, quantitative).

H-Pro-Pro-▼-OH (88), ^{1}H-NMR (300 MHz; CD_3OD) δ: 4.59-4.14 (m, signal doubling because of rotamers, 2H); 3.69 (s, 3H + m, 1H); 3.64 (m, 1H); 3.48 (m, 1H); 3.78 (m, 2H); 2.49 (m, 1H); 2.34 (m, 1H); 2.24 (m, 2H); 2.08 (m, 6H). ^{13}C (75.5 MHz, CD_3OD) δ: (major conformer) 175.2, 174.3, 172.98, 169.2, 62.5, 60.6, 52.8, 47.5, 46.2, 36.2, 33.0, 30.4, 29.6, 29.0, 26.0, 25.3. (ES-MS) for 353.1, $C_{16}H_{23}N_3O_6$, (MH^+) was found: 354.1; High resolution mass (EI-MS) for 353.1587, $C_{16}H_{23}N_3O_6$, 353.1584 was found (δ = 0.9 ppm). FT-IR (solid) ν_{max}: 2961.5, 1725.1, 1615.5, 1517.6, 1437.8, 1409.2, 1296.5, 1195.8, 1171.2, 1045.8; mp: 169-171°C. This product is not stable after prolonged storage.

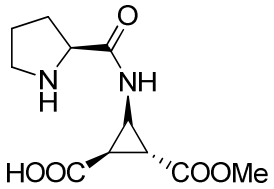


90

Synthesis of H-Pro-▲-Pro-OH (90): **81** (500 mg, 1.44 mmol) was dissolved in DCM (10 mL) and then Boc_2O (330 mg, 1.5 mmol, 1.5 equiv.) was added. A mixture of TEA (0.15 mL, 1.1 mmol, 1.1 equiv.) and DMAP (18 mg, 0.15 mmol, 0.15 equiv.) in DCM (10 mL) was then added dropwise into the mixture over a period of 1 h, and stirring continued for 10 h. The reaction was quenched with water and $KHSO_4$ solution (1 M) was used to acidify the solution, which was then extracted with DCM. The combined organic phases were extracted with brine, dried over Na_2SO_4, evaporated under reduced pressure to give **83** (580 mg, 90%). The following debenzylation was performed according to the procedure described above for the synthesis of **88** and proceeded in quantitative yield, affording 460 mg of **85**. Dipeptide **85** (360 mg, 1.0 mmol) was then dissolved in DCM (15 mL) and EDC·HCl (200 mg, 1.05 mmol, 1.05 equiv.) was added to the solution. After stirring for 30 min NH(HCl)-Pro-OBn (290 mg, 1.2 mmol, 1.2 equiv.) followed by TEA (0.17 mL, 1.2 mmol, 1.2 equiv.) were added and the mixture was stirred for 24 h. DCM (10 mL) and water (10 mL) were added and the pH of the aqueous phase was adjusted to 4 using a $KHSO_4$ solution (1M). The organic phase was washed with $NaHCO_3$ solution (sat.) (10 mL) and brine (10 mL). Then it was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give the protected form of **90** (380 mg, 70%). Deprotection of this intermediate to furnish the final peptide catalyst has been carried out as described above for **78** (Boc-deprotection) and **88** (debenzylation), leading to **90** (240 mg, quantitative).

H-Pro-▲-Pro-OH (**90**), ^{1}H-NMR (300 MHz; CD_3OH) δ: 8.79-8.27 (bs, signal doubling because of rotamers, 1H); 4.24 (m, 2H); 3.93 (m, 1H); 3.78 (m, 1H); 3.73 (s, 3H); 3.48-3.65 (m, 2H); 3.38 (m 1H); 2.50-2.69 (m, 2H); 2.28 (m, 1H); 2.20 (m, 1H); 1.97 (m, 3H); 1.90 (m, 3H). ^{13}C (75.5 MHz, CD_3OD) δ: (major conformer) 177.0, 172.3, 171.3, 166.6, 60.9, 53.0, 50.0, 48.8, 47.6, 36.3, 31.3, 30.9, 30.4, 26.7, 25.7, 25.0. (ES-MS) for 353.1, $C_{16}H_{23}N_3O_6$,

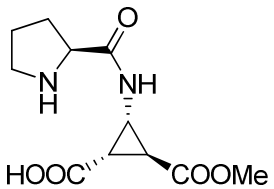
(MH^+) was found: 354.1; High resolution mass (EI-MS) for 353.1587, $C_{16}H_{23}N_3O_6$, 353.1593 was found (δ = 1.7 ppm). FT-IR (solid) ν_{max}: 2963.4, 1725.1, 1616.7, 1438.9, 1299.1, 1195.8, 1176,3; mp: 184-186°C.



84

Synthesis of H-Pro-▲-OH (84): Debenzylation of **81** was carried out by dissolving it (200 mg, 0.6 mmol) in MeOH and adding Pd/C (10 m%, 20 mg). Stirring under H_2 atmosphere was continued for 1.5 hours. After this period the Pd/C residue was filtered through celite and the collected solvent was evaporated to provide **84** (150 mg, quantitative).

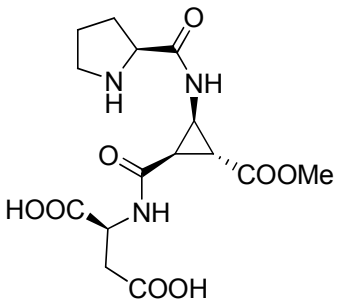
H-Pro-▲-OH (84), ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 4.27 (m, 1H); 3.72 (s, 3H); 3.57 (dd, *J* = 4.6; 7.9 Hz, 1H); 3.35 (m, 2H); 2.42 (m, 1H); 2.35-2.00 (m, 5H). ^{13}C (75.5 MHz, $CDCl_3$) δ: 172.3, 172.2, 170.9, 61.2, 53.0, 47.4, 36.3, 31.0, 29.6, 28.1, 24.9. High resolution mass (EI-MS) for 256.1059, $C_{11}H_{16}N_2O_5$, 256.1054 was found (δ = 0.2 ppm). FT-IR (solid) ν_{max}: 3439, 3207, 2955, 1678, 1639, 1543, 1146; mp: 130-132°C.



89

Synthesis of H-Pro-▼-OH (89): Debenzylation of **80** was carried out by dissolving it (200 mg, 0.6 mmol) in MeOH and adding Pd/C (10 m%, 20 mg). Stirring under H_2 atmosphere was continued for 1.5 hours. After this period the Pd/C residue was filtered through celite and the collected solvent was evaporated to provide **89** (150 mg, quantitative).

H-Pro-▼-OH (89), ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 4.27 (m, 1H); 3.72 (s, 3H); 3.62 (dd, *J* = 5.1; 7.4 Hz, 1H); 3.35 (m, 2H); 2.38 (m, 1H); 2.27 (m, 2H); 2.05 (m, 3H). ^{13}C (75.5 MHz, $CDCl_3$) δ: 174.2, 172.0, 170.0, 61.0, 52.8, 47.2, 36.3, 30.8, 30.6, 28.5, 25.1. High resolution mass (EI-MS) for 256.1059, $C_{11}H_{16}N_2O_5$, 256.1053 was found (δ = 0.2 ppm). FT-IR (solid) ν_{max}: 3379, 3241, 3059, 1732, 1686, 1447, 1167; mp: 132-134°C.

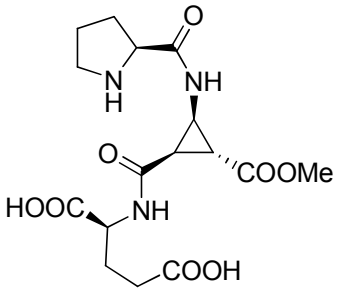


91

Synthesis of H-Pro-▲-Asp-OH (91): Dipeptide **85** (360 mg, 1.0 mmol) was dissolved in DCM (15 mL) and EDC·HCl (200 mg, 1.05 mmol, 1.05 equiv.) was added to the solution. After stirring for 30 min NH_2(HCl)-Asp(OBn)-OBn (420 mg, 1.2 mmol, 1.2 equiv.) followed by TEA (0.17 mL, 1.2 mmol, 1.2 equiv.) were added and the mixture was stirred for 24 h. DCM (15 mL) and water (15 mL) were added and the pH of the aqueous phase was adjusted to 4 using a $KHSO_4$ solution (1M). The organic phase was washed with $NaHCO_3$ solution (sat.) (10 mL) and brine (10 mL). Then it was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give the protected form of **91** (380 mg, 70%). Deprotection of this intermediate to furnish the final peptide catalyst has been carried out as described above for **78** (Boc-deprotection) and **88** (debenzylation), leading to **91** (250 mg, quantitative).

H-Pro-▲-Asp-OH (91), ^{1}H-NMR (300 MHz; CD_3OD) δ: 4.62 (m, 1H); 3.85 (m, 1H); 3.77 (s, 3H); 3.67-3.42 (m, 2H); 3.04 (m, 1H); 2.82 (m, 1H); 2.70 (m, 1H); 2.63-2.38 (m, 3H); 2.08 (m, 3H). ^{13}C (75.5 MHz, D_2O) δ: 174.6, 174.4, 173.3, 170.6, 59.8, 52.8, 51.2, 46.4, 34.7, 34.5, 30.7, 29.5, 29.0, 27.1, 23.7. (ES-MS) for 371.2, $C_{15}H_{21}N_3O_8$, (MH^+) was found: 372.3; High resolution mass (PI-LSIMS) for 372.1408, $C_{15}H_{21}N_3O_8$, 372.1412 was found (δ = 1.1

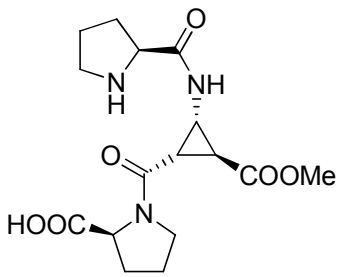
ppm). FT-IR (solid) ν_{max}: 2967.3, 2864.3, 1648.3, 1580.4, 1442.5, 1190.8. mp: decomposition at 240-245°C.



92
H-Pro-▲-Glu-OH

Synthesis of H-Pro-▲-Asp-OH (92): Dipeptide **85** (360 mg, 1.0 mmol) was dissolved in DCM (15 mL) and EDC·HCl (200 mg, 1.05 mmol, 1.05 equiv.) was added to the solution. After stirring for 30 min NH_2(HCl)-Glu(OBn)-OBn (440 mg, 1.2 mmol, 1.2 equiv.) followed by TEA (0.17 mL, 1.2 mmol, 1.2 equiv.) were added and the mixture was stirred for 24 h. DCM (15 mL) and water (15 mL) were added and the pH of the aqueous phase was adjusted to 4 using a $KHSO_4$ solution (1M). The organic phase was washed with $NaHCO_3$ solution (sat.) (10 mL) and brine (10 mL). Then it was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give the protected form of **92** (380 mg, 70%). Deprotection of this intermediate to furnish the final peptide catalyst has been carried out as described above for **78** (Boc-deprotection) and **88** (debenzylation), leading to **92** (260 mg, quantitative).

H-Pro-▲-Glu-OH (92), ^{1}H-NMR (300 MHz; CD_3OD) δ: 4.41 (m, 1H); 4.20 (m, 1H); 3.71 (s, 3H); 3.52-3.71 (m, 3H); 2.52 (m, 2H); 2.40 (m, 2H); 2.15 (m, 1H); 2.02 (m, 5H). ^{13}C (75.5 MHz, D_2O) δ: 178.3, 174.1, 173.0, 170.6, 59.8, 54.2, 52.3, 46.4, 34.8, 31.1, 30.1, 29.5, 27.1, 26.0, 23.7. (ES-MS) for 385.2, $C_{16}H_{23}N_3O_8$, (MH^+) was found: 386.0; High resolution mass (PI-LSIMS) for 386.1563, $C_{16}H_{23}N_3O_8$, 386.1567 was found (δ = 1.0 ppm). FT-IR (solid) ν_{max}: 2965.3, 2856.3, 1643.4, 1622.0, 1444.5, 1195.3. decomposition at 245-250°C.

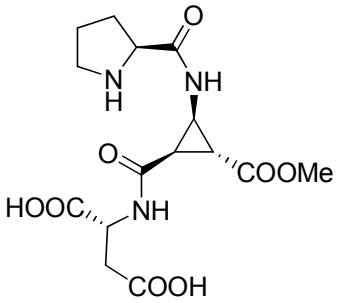


93

Synthesis of H-Pro-▼-Pro-OH (93): **80** (500 mg, 1.44 mmol) was dissolved in DCM (10 mL) and then Boc_2O (330 mg, 1.5 mmol, 1.5 equiv.) was added. A mixture of TEA (0.15 mL, 1.1 mmol, 1.1 equiv.) and DMAP (18 mg, 0.15 mmol, 0.15 equiv.) in DCM (10 mL) was then added dropwise into the mixture over a period of 1 h, and stirring continued for 10 h. The reaction was quenched with water and $KHSO_4$ solution (1 M) was used to acidify the solution, which was then extracted with DCM. The combined organic phases were extracted with brine, dried over Na_2SO_4, evaporated under reduced pressure to give **82** (580 mg, 90%). The following debenzylation was performed according to the procedure described above for the synthesis of **88** and proceeded in quantitative yield, affording 460 mg of Boc-Pro-▼-OH (**102**). Dipeptide **102** (360 mg, 1.0 mmol) was then dissolved in DCM (15 mL) and EDC·HCl (200 mg, 1.05 mmol, 1.05 equiv.) was added to the solution. After stirring for 30 min NH(HCl)-Pro-OBn (290 mg, 1.2 mmol, 1.2 equiv.) followed by TEA (0.17 mL, 1.2 mmol, 1.2 equiv.) were added and the mixture was stirred for 24 h. DCM (10 mL) and water (10 mL) were added and the pH of the aqueous phase was adjusted to 4 using a $KHSO_4$ solution (1M). The organic phase was washed with $NaHCO_3$ solution (sat.) (10 mL) and brine (10 mL). Then it was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give the protected form of **93** (385 mg, 71%). Deprotection of this intermediate to furnish the final peptide catalyst has been carried out as described above for **78** (Boc-deprotection) and **88** (debenzylation), leading to **93** (240 mg, quantitative).

H-Pro-▼-Pro-OH (93), ^{1}H-NMR (300 MHz; CD_3OD) δ: 4.58-4.32 (m, 1H); 4.18 (m, 1H); 3.90 (m, 1H); 3.71 (s, 3H); 3.55 (m, 1H); 3.34 (m, 3H); 2.71 (m, 1H); 2.51 (m, 1H); 2.38 (m, 1H); 2.18 (m, 1H); 2.02 (m, 6H). ^{13}C (75.5 MHz, CD_3Cl) δ: (major conformer) 172.5, 171.7, 170.6, 166.2, 61.1, 54.9, 52.9, 47.2, 36.0, 31.0, 30.8, 30.5, 26.5, 25.3, 25.1. (ES-MS) for 353.1, $C_{16}H_{23}N_3O_6$, ($M\text{-}H^+$) was found: 352.1; High resolution mass (EI-MS) for 353.1587,

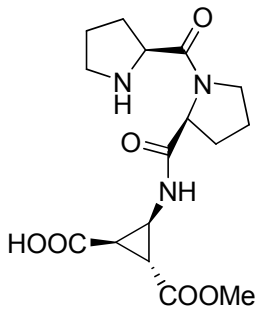
$C_{16}H_{23}N_3O_6$, 353.1581 was found (δ = 1.7 ppm). FT-IR (solid) ν_{max}: 2977.5, 1723.1, 1668.7, 1615.6, 1438.1, 1409.2, 1293.5, 1196.4, 1169.4; mp: 169-171°C.



94

Synthesis of H-Pro-▼-Asp-OH (94): Dipeptide **102** (360 mg, 1.0 mmol) was dissolved in DCM (15 mL) and EDC·HCl (200 mg, 1.05 mmol, 1.05 equiv.) was added to the solution. After stirring for 30 min NH_2(HCl)-Asp(OBn)-OBn (420 mg, 1.2 mmol, 1.2 equiv.) followed by TEA (0.17 mL, 1.2 mmol, 1.2 equiv.) were added and the mixture was stirred for 24 h. DCM (15 mL) and water (15 mL) were added and the pH of the aqueous phase was adjusted to 4 using a $KHSO_4$ solution (1M). The organic phase was washed with $NaHCO_3$ solution (sat.) (10 mL) and brine (10 mL). Then it was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give the protected form of **94** (380 mg, 70%). Deprotection of this intermediate to furnish the final peptide catalyst has been carried out as described above for **78** (Boc-deprotection) and **88** (debenzylation), leading to **94** (250 mg, quantitative).

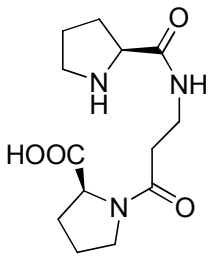
H-Pro-▼-Asp-OH (94), ^{1}H-NMR (300 MHz; CD_3OD) δ: 4.62 (m, 1H); 3.85 (m, 1H); 3.77 (s, 3H); 3.67-3.42 (m, 2H); 3.04 (m, 1H); 2.82 (m, 1H); 2.70 (m, 1H); 2.63-2.38 (m, 3H); 2.08 (m, 3H). ^{13}C (75.5 MHz, D_2O) δ: 174.6, 174.4, 173.3, 170.6, 59.8, 52.8, 51.2, 46.4, 34.7, 34.5, 30.7, 29.5, 29.0, 27.1, 23.7. (ES-MS) for 371.2, $C_{15}H_{21}N_3O_8$, (MH^+) was found: 372.3; High resolution mass (PI-LSIMS) for 372.1408, $C_{15}H_{21}N_3O_8$, 372.1412 was found (δ = 1.1 ppm). FT-IR (solid) ν_{max}: 2967.3, 2864.3, 1648.3, 1580.4, 1442.5, 1190.8. mp: decomposition at 240-245°C.



95

Synthesis of H-Pro-Pro-▲-OH (95): *N*-Boc-L-proline (300 mg, 1.4 mmol) was dissolved in DCM (10 mL) and EDC·HCl (295mg, 1.54 mmol, 1.1 equiv.) was added. After 30 min **81** (580 mg, 1.68 mmol, 1.2 equiv.) was added and the reaction was stirred overnight. Then DCM (10 mL) and water (10 mL) were added and the pH was adjusted to 4 by addition of small amounts of $KHSO_4$ solution (1M). The organic phase was extracted and then washed with $NaHCO_3$ solution (sat.) (10 mL) and brine (10 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give Boc-Pro-Pro-▲-OBn (**95a**, 495 mg, 0.91 mmol, 65%). The preparation of **95** was completed by de-protecting the amino and carboxylic acid functionalities of this intermediate. Boc-group deprotection of **95a** (495 mg, 0.91 mmol) was performed as reported for **78** (360 mg, 90%). Debenzylation was carried out by dissolving the intermediate generated during the former step (200 mg, 0.45 mmol) in MeOH and adding Pd/C (10 m%, 20 mg). Stirring under H_2 atmosphere was continued for 1.5 hours. After this period the Pd/C residue was filtered through celite and the collected solvent was evaporated to provide **95** (160 mg, quantitative).

H-Pro-Pro-▲-OH (95), ^{1}H-NMR (300 MHz; CD_3OD) δ: 4.59 (m, 1H); 4.47 (m, 1H); 3.76 (s, 3H + m, 1H); 3.68 (m, 3H); 3.44 (m, 1H); 2.55 (m, 1H); 2.38 (m, 2H); 2.26 (m; 2H); 2.05 (m, 5H). ^{13}C (75.5 MHz, CD_3OD) δ: (major conformer) 175.1, 174.6, 172.8, 169.0, 79.6, 62.3, 52.9, 47.6, 46.3, 36.4, 30.7, 29.67, 28.6, 26.0, 25.3, 24.2; (ES-MS) for 353.1, $C_{16}H_{23}N_3O_6$, (MH^+) was found: 354.1; High resolution mass (EI-MS) for 353.1587, $C_{16}H_{23}N_3O_6$, 353.1583 was found (δ = 1.1 ppm). FT-IR (solid) ν_{max}: 2956.5, 1723.1, 1617.5, 1514.6, 1437.9, 1410.2, 1299.5, 1194.8, 1171.2; wax. This product is not stable under prolonged storage.

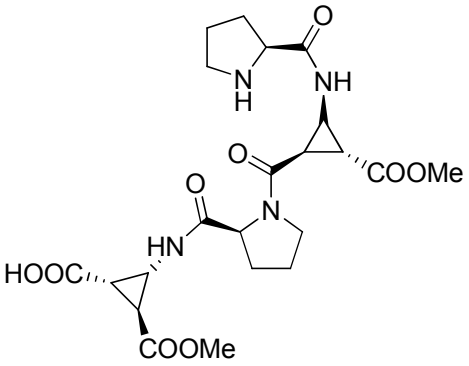


97

Synthesis of H-Pro-β-Ala-Pro-OH (97): *N*-Boc-L-proline (300 mg, 1.4 mmol) was dissolved in DCM (10 mL) and EDC·HCl (295 mg, 1.54 mmol, 1.1 equiv.) was added. After 30 min H-*β*-Ala-OBn (300 mg, 1.68 mmol, 1.2 equiv.) was added and the reaction was stirred overnight. Then DCM (10 mL) and water (10 mL) were added and the pH was adjusted to 4 by addition of small amounts of $KHSO_4$ solution (1M). The organic phase was extracted and then washed with $NaHCO_3$ solution (sat.) (10 mL) and brine (10 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give Boc-Pro- *β*-Ala-OBn (395 mg, 1.05 mmol, 75%). Debenzylation of this intermediate to provide **96** was carried out as reported for **88** (290 mg, quantitative). **96** (290 mg, 1.05 mmol) was dissolved in DCM (10 mL) and EDC·HCl (220 mg, 1.16 mmol, 1.1 equiv.) was added. After 30 min NH(HCl)-Pro-OBn (305 mg, 1.26 mmol, 1.2 equiv.) was added and the reaction was stirred overnight. Then DCM (15 mL) and water (15 mL) were added and the pH was adjusted to 4 by addition of small amounts of $KHSO_4$ solution (1M). The organic phase was extracted and then washed with $NaHCO_3$ solution (sat.) (10 mL) and brine (10 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give Boc-Pro- *β*-Ala-Pro-OBn (400 mg, 0.84 mmol, 80%). Deprotection of this intermediate to furnish the final peptide has been carried out as described above for **78** (Boc-deprotection) and **88** (debenzylation), leading to **97** (230 mg, quantitative).

H-Pro-*β*-Ala-Pro-OH (97), ^{1}H-NMR (300 MHz; CD_3OD) δ: 4.35 (m, 1H); 4.20 (m, 1H); 3.62 (m, 1H); 3.53 (m, 3H); 3.34 (m, 2H); 2.66 (m, 1H); 2.49 (m, 1H); 2.38 (m, 1H); 2.24 (m, 1H); 2.02 (m, 5H); 1.88 (m, 1H). ^{13}C (75.5 MHz, CD_3OD) δ: 177.4, 171.8, 169.8, 62.8, 61.2, 47.4, 36.7, 34.0, 32.8, 31.0, 30.7, 25.7, 25.2. (ES-MS) for 283.1, $C_{13}H_{21}N_3O_4$, (MH^+) was found: 284.1; High resolution mass (EI-MS) for 283.1532, $C_{13}H_{21}N_3O_4$, 283.1531 was found (δ =

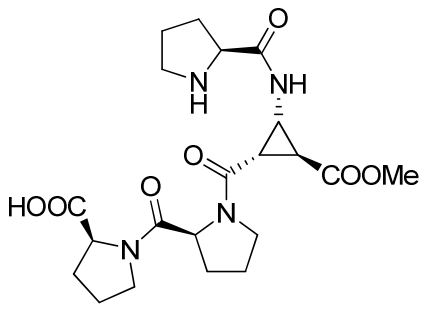
0.3 ppm). FT-IR (solid) ν_{max}: 2958.3, 2744.4, 1622.7, 1556.6, 1445.7, 1303.5, 1195.3, 1169.4; wax.



101

Synthesis of H-Pro-▲-Pro-▼-OH (101): To a solution of **85** (300 mg, 0.84 mmol) in 15 mL of DCM, EDC·HCl (180 mg, 0.93 mmol, 1.1 equiv.) was added. After 30 min **80** (350 mg, 1 mmol, 1.2 equiv.) was added and the reaction was stirred overnight. Then DCM (10 mL) and water (10 mL) were added and the pH was adjusted to 4 by addition of small amounts of $KHSO_4$ solution (1M). The organic phase was extracted and then washed with $NaHCO_3$ solution (sat.) (10 mL) and brine (10 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give Boc-Pro-▲-Pro-▼-OBn (480 mg, 0.7 mmol, 70%). Deprotection of this intermediate to furnish the final peptide has been carried out as described above for **78** (Boc-deprotection) and **88** (debenzylation), leading to **101** (300 mg, 85%).

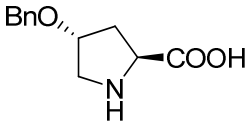
H-Pro-▲-Pro-▼-OH (101), ^{1}H-NMR (300 MHz; CD_3OD) δ: 4.20 (m, 2H); 3.77 (m, 1H); 3.73 (s, 3H); 3.71 (s, 3H); 3.48 (m, 2H); 3.32 (m, 2H); 2.73-2.55 (m, signal doubling because of rotamers, 2H); 2.47 (m, 1H); 2.37 (m, 1H); 2.25 (m, 1H); 2.04 (m, 3H); 1.92 (m, 4H). ^{13}C (75.5 MHz, CD_3OD) δ: 175.7, 172.3, 172.1, 171.4, 171.0, 167.7, 62.2, 60.8, 53.1, 53.0, 48.9, 47.6, 36.6, 36.1, 31.2, 31.0, 30.8, 28.1, 26.7, 26.6, 25.4, 25.0. (ES-MS) for 494.2, $C_{22}H_{30}N_4O_9$, (MH^+) was found: 495.2; High resolution mass (EI-MS) for 494.2013, $C_{22}H_{30}N_4O_9$, 494.2003 was found (δ = 2.0 ppm). FT-IR (solid) ν_{max}: 2988.5, 2976.5, 1734.1, 1687.1, 1669.4, 1616.4, 1612.5, 1423.6, 1412.5, 1294.6, 1200.5, 1198.2, 1167.5; mp: 230-232°C.



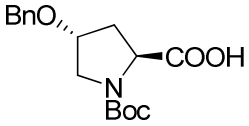
104

Synthesis of H-Pro-▼-Pro-Pro-OH (104): **102** (320 mg, 0.9 mmol) was dissolved in DCM (10 mL) and EDC·HCl (180 mg, 0.95 mmol, 1.05 equiv.) was added. After 30 min **103** (365 mg, 1.08 mmol, 1.2 equiv.) was added and the reaction was stirred overnight. Then DCM (10 mL) and water (10 mL) were added and the pH was adjusted to 4 by addition of small amounts of $KHSO_4$ solution (1M). The organic phase was extracted and then washed with $NaHCO_3$ solution (sat.) (10 mL) and brine (10 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give Boc-Pro-▼-Pro-Pro-OBn (460 mg, 0.72 mmol, 80%). Deprotection of this intermediate to furnish the final peptide has been carried out as described above for **78** (Boc-deprotection) and **88** (debenzylation), leading to **104** (275 mg, 0.61 mmol, 85%).

H-Pro-▼-Pro-Pro-OH (104), ^{1}H-NMR (300 MHz; CD_3OD) δ: 4.46-3.94 (m, signal doubling because of rotamers, 3H); 3.72 (s, 3H); 3.66 (m, 2H); 3.58 (m, 2H), 3.49 (m, 2H); 3.37 (m, 1H); 2.67 (m, 2H); 2.27 (m, 3H); 2.04 (m, 9H). ^{13}C (75.5 MHz, CD_3OD) δ: 172.4, 172.1, 171.4, 168.7, 166.1, 71.4, 61.8, 53.0, 50.5, 47.4, 46.3, 36.0, 31.6, 31.3, 30.7, 30.3, 29.2, 28.8, 26.2, 25.2, 24.2, (ES-MS) for 450.2, $C_{22}H_{30}N_4O_9$, (MH^+) was found: 451.2; High resolution mass (EI-MS) for 450.2114, $C_{21}H_{30}N_4O_7$, 450.2108 was found (δ = 1.3 ppm). FT-IR (film) ν_{max}: 2983.5, 2960.4, 1720.1, 1687.1, 1618.4, 1422.6, 1411.0, 1294.6, 1200.5, 1198.2, 1167.5; oil.



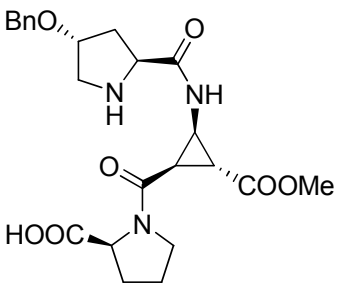
148



152

Synthesis of H-Hyp(OBn)-OH (148) and Boc-Hyp(OBn)-OH (152): **149** (1.9 g, 8.2 mmol) is dissolved in 15 mL of DMF. Ag_2O is added to this solution at 0°C (4.75 g, 20.5 mmol, 2.5 equiv.) and the reaction is left stirring for 24 hours. After this period, the inorganic precipitates are filtrated and water (30 mL) and Et_2O (20 mL) are added to the resulting solution. The organic layer is washed with 15 mL of water, dried over Na_2SO_4 and evaporated to yield a oil that is purified through column chromatography on silica gel (1:1 Hexanes/Et_2OAc) affording 1.85 g of **150** (4.4 mmol, 55%) along with 1.1 g of the mono-benzylated product Boc-Hyp(OH)-OBn (3.4 mmol, 43%). **150** (200 mg, 0.5 mmol) is Boc-deprotected following the same procedure used for the deprotection of **78**, obtaining **151** (140 mg, 0.45 mmol, 90%). **151** (140 mg, 0.45 mmol) was dissolved in MeOH and Pd/C (10 m%, 14 mg) was added. Stirring under H_2 atmosphere was continued for 30 min. After this period the Pd/C residue was filtered through celite and the collected solvent was evaporated to provide **148** (100 mg, quantitative). The remaining amount of **150** (1.65 g, 3.9 mmol) was dissolved in EtOAC and Pd/C (10 m%, 170 mg) was added. Stirring under H_2 atmosphere was continued for 1 hour. After this period the Pd/C residue was filtered through celite and the collected solvent was evaporated to provide **152** (1.19 g, 3.7 mmol, 95%).

H-Hyp(OBn)-OH (148), ^{1}H-NMR (300 MHz; CD_3OD) δ: 7.45-7.15 (m, 5H); 4.52 (s, 2H); 4.27 (brs 1H); 4.03 (m, 2H); 2.51 (m, 1H); 2.00 (m, 1H); 1.28 (m, 2H). ^{13}C (75.5 MHz, CD_3OD) δ: 177.5, 139.3, 130.3, 129.5, 129.1, 129.0, 128.9, 72.0, 61.7, 52.2, 37.0, 23.4. (ES-MS) for 221.1, $C_{12}H_{16}NO_3$, (MH^+) was found: 221.9; High resolution mass (EI-MS) for 221.1130, $C_{12}H_{16}NO_3$, 221.1130 was found (δ = 0). FT-IR (solid) ν_{max}: 2946.3, 2773.4, 1620.7, 1566.6, 1449.7, 1313.5, 1185.3, 1189.4; mp: 190-191°C.

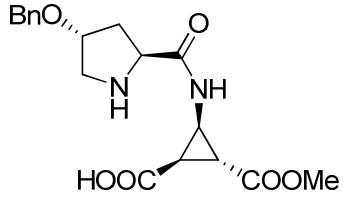


153

Synthesis of H-Hyp(OBn)-▲-Pro-OH (153): Racemic building block ***rac*-76** (2.0 g, 5.7 mmol, 1.0 equiv.) was dissolved in a saturated solution of HCl in EtOAC (c≈3 mol/L), 20 mL, at 0°C. After stirring for 2 hours the acid was removed under reduced pressure, leaving a white/pinkish solid as a residue. To this deprotected building block ***rac*-76a** was added a solution of **152** (1.3 g, 5.7 mmol, 1.0 equiv.) and EDC·HCl (805 g, 6.0 mmol, 1.05 equiv.) in DCM (25 mL) that had been stirred beforehand for 30 min. Using a dropping funnel TEA (0.87 mL, 6.8 mmol, 1.2 equiv.) in DCM (20 mL) was added over a period of 1.5 h. The mixture was stirred overnight. Then the reaction was quenched with water (20 mL) and the solution acidified to pH 3 using $KHSO_4$ solution (1 M), then extracted with DCM. The organic phase was extracted successively with $NaHCO_3$ solution and brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. Purification by chromatography on silica gel (3:1 hexanes/EtOAc) yielded 2.36 g (4.3 mmol, 75%) of the mixture of diastereomers **154**. The Boc-deprotection of this mixture was carried out as reported in the case of **78**, to yield H-Hyp(OBn)-▲/▼-OBn (**154a**, 1.94 g, 4.21 mmol, 98%). The two diastereomers **160** and **155** were separated through column chromatography on silica gel (DCM/MeOH 30:1) to give pure **160** (810 mg, 42% R_f=0.40) and pure **155** (620 mg, 32%, R_f=0.35) along with the recovered mixture **154a** (480 mg, 25%). **155** (500 mg, 1.1 mmol) was dissolved in DCM (10 mL) and then Boc_2O (360 mg, 1.65 mmol, 1.5 equiv.) was added. TEA (0.17 mL, 1.2 mmol, 1.1 equiv.) in DCM (10 mL) was then added dropwise into this mixture over a period of 1 h, and stirring continued for 10 h. The reaction was quenched with water and $KHSO_4$ solution (1 M) was used to acidify the solution, which was then extracted with DCM. The combined organic phases were extracted with brine, dried over Na_2SO_4, evaporated under reduced pressure to give Boc-Hyp(OBn)-▲-OBn (545 mg, 1.0 mmol, 90%). This intermediate (545 mg, 1.0 mmol) was dissolved in EtOAC

and Pd/C (10 m%, 55 mg) was added. Stirring under H_2 atmosphere was continued for 1 hour. After this period the Pd/C residue was filtered through celite and the collected solvent was evaporated to provide **157** (455 mg, 0.99 mmol, 99%). Dipeptide **157** (200 mg, 0.43 mmol) was then dissolved in DCM (10 mL) and EDC·HCl (90 mg, 0.45 mmol, 1.05 equiv.) was added to the solution. After stirring for 30 min NH(HCl)-Pro-OBn (160 mg, 0.65 mmol, 1.5 equiv.) followed by TEA (0.09 mL, 0.65 mmol, 1.5 equiv.) were added and the mixture was stirred for 36 h. DCM (10 mL) and water (10 mL) were added and the pH of the aqueous phase was adjusted to 4 using a $KHSO_4$ solution (1M). The organic phase was washed with $NaHCO_3$ solution (sat.) (10 mL) and brine (10 mL). Then it was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give **158** (140 mg, 0.22 mmol, 50%). **158** (140 mg, 0.22 mmol) is Boc-deprotected following the same procedure used for the deprotection of **78**, obtaining **159** (105 mg, 0.2 mmol, 90%). **159** (105 mg, 0.2 mmol) was dissolved in 5 mL of a 2:1 EtOAc/MeOH mixture and Pd/C (10 m%, 10 mg) was added. Stirring under H_2 atmosphere was continued for 2 hours. After this period the Pd/C residue was filtered through celite and the collected solvent was evaporated to provide **153** (92 mg, quantitative).

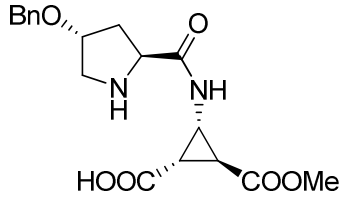
H-Hyp(OBn)-▲-Pro-OH (153), ^{1}H-NMR (300 MHz; CD_3OD) δ: 7.42-7.18 (m, 5H); 4.73-4.45 (m, 3H); 4.44-4.01 (m, 2H); 4.00-3.82 (m, 1H); 3.81-3.65 (m, 4H); 3.64-3.28 (m, 3H); 2.80-2.41 (m, 3H); 2.40-2.75 (m, 5H). ^{13}C (75.5 MHz, CD_3OD) δ (major conformer): 172.5, 171.2, 167.6, 166.5, 139.0, 129.54, 129.51, 129.02, 129.01, 128.9, 79.0, 71.9, 59.9, 52.9, 36.6, 36.4, 36.2, 31.2, 29.1, 26.9, 25.7, 23.9. (ES-MS) for 459.1, $C_{23}H_{29}N_3O_7$, (MH^+) was found: 460.0; High resolution mass (EI-MS) for 459.2006, $C_{23}H_{29}N_3O_7$, 459.2005 was found (δ = 0.2 ppm). FT-IR (solid) ν_{max}: 2958.3, 2744.4, 1622.7, 1556.6, 1445.7, 1303.5, 1195.3, 1169.4; solid, mp: decomposition at ca. 200°C.



161

Synthesis of H-Hyp(OBn)-▲-OH (161): Debenzylation of **155** was carried out by dissolving it (50 mg, 0.11 mmol) in a 2:1 EtOAc/MeOH mixture and adding Pd/C (10 m%, 5 mg). Stirring under H_2 atmosphere was continued for one hour. After this period the Pd/C residue was filtered through celite and the collected solvent was evaporated to provide **161** (40 mg, quantitative).

H-Hyp(OBn)-▲-OH (161), ^{1}H-NMR (300 MHz; CD_3OD) δ: 7.43-7.11 (m, 5H); 4.70-4.29 (m, 4H); 3.85-3.50 (m, 4H); 3.44 (m, 1H); 3.31 (m, 1H); 2.56 (m, 1H); 2.30 (m, 2H); 2.04 (m, 1H). ^{13}C (75.5 MHz, CD_3OD) δ (major conformer): 173.8, 172.4, 170.3, 138.9, 129.3, 129.2, 129.1, 78.4, 72.0, 60.3, 52.6, 52.2, 36.2, 36.0, 30.0, 28.1. (ES-MS) for 362.1, $C_{18}H_{22}N_2O_6$, (MH^+) was found: 363.0; High resolution mass (PI-LSIMS) for 362.1556, $C_{18}H_{22}N_2O_6$, 362.1560 was found (δ = 1.2 ppm). FT-IR (solid) ν_{max}: 2956.3, 2798.4, 1667.7, 1598.6, 1466.7, 1345.5, 1198.3, 1107.4; solid, mp: 220-221°C.



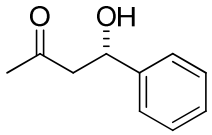
162

Synthesis of H-Hyp(OBn)-▼-OH (162): Debenzylation of **160** was carried out by dissolving it (50 mg, 0.11 mmol) in a 2:1 EtOAc/MeOH mixture and adding Pd/C (10 m%, 5 mg). Stirring under H_2 atmosphere was continued for one hour. After this period the Pd/C residue was filtered through celite and the collected solvent was evaporated to provide **162** (40 mg, quantitative).

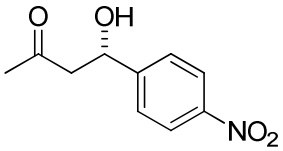
H-Hyp(OBn)-▼-OH (162), ^{1}H-NMR (300 MHz; CD_3OD) δ: 7.43-7.11 (m, 5H); 4.72-4.31 (m, 4H); 3.80-3.55 (m, 4H); 3.47 (m, 1H); 3.29 (m, 1H); 2.64 (m, 1H); 2.31 (m, 2H); 2.07 (m, 1H). ^{13}C (75.5 MHz, CD_3OD) δ (major conformer): 173.9, 172.7, 170.6, 139.1, 129.5, 129.0, 129.0, 78.7, 72.2, 60.1, 52.9, 52.4, 36.7, 36.3, 30.3, 28.2. (ES-MS) for 362.1, $C_{18}H_{22}N_2O_6$, (MH^+) was found: 363.0; High resolution mass (PI-LSIMS) for 362.1556, $C_{18}H_{22}N_2O_6$,

362.1566 was found (δ = 2.7 ppm). FT-IR (solid) ν_{max}: 2956.3, 2798.4, 1667.7, 1598.6, 1466.7, 1345.5, 1198.3, 1107.4; solid, mp: 223-225°C.

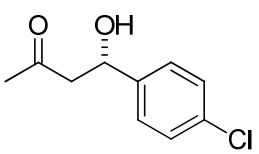
E.4 Catalysis products



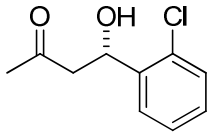
4-Hydroxy-4-phenyl-butan-2-one (52), $[\alpha]^{20}_D$ = -51.3 (*c* = 1.0, $CHCl_3$) for (*S*)-enantiomer, 79% *ee*. ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 7.27-7.38 (m, 5H); 5.15 (m, 1H); 3.32 (d, *J* = 3.0 Hz, 1H); 2.87 (m, 2H); 2.21 (s, 3H). ^{13}C (75.5 MHz, $CDCl_3$) δ: 209.2, 142.7, 128.6, 127.7, 125.7, 69.9, 52.0, 30.8. Enantiomeric excess determined by chiral GC as reported in the general information, at 120°C, t_R is 9.77 min for (*S*)-enantiomer, 10.12 min for (*R*)-enantiomer.



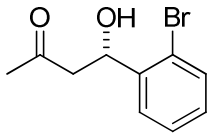
4-Hydroxy-4-(4'-nitrophenyl)-butan-2-one (60), $[\alpha]^{20}_D$ = -51.4 (*c* = 0.5, $CHCl_3$) for (*S*)-enantiomer, 88% *ee*. ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 8.15 (d, *J* = 6.8 Hz, 2H); 7.46 (d, *J* = 6.8 Hz, 2H); 5.18 (m, 1H); 3.65 (d, *J* = 3.2 Hz, 1H); 2.89 (m, 2H); 2.18 (s, 3H). ^{13}C (75.5 MHz, $CDCl_3$) δ: 208.6, 150.2, 147.4, 129.1, 126.7, 124.5, 123.9, 69.2, 51.9, 31.2. Enantiomeric excess determined by chiral GC as reported in the general information, at 165°C, t_R is 13.65 min for (*S*)-enantiomer, 14.04 min for (*R*)-enantiomer.



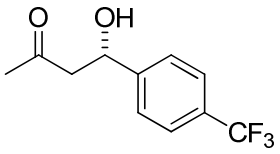
4-Hydroxy-4-(4'-chlorophenyl)-butan-2-one (108), $[\alpha]^{22}_D$ = -67.8 (*c* = 1.0, $CHCl_3$), for (*S*)-enantiomer, 84% *ee*. ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 7.27-7.21 (m, 4H); 5.08 (m, 1H); 3.31 (brd, 1H); 2.78 (m, 2H); 2.15 (s, 3H). ^{13}C (75.5 MHz, $CDCl_3$) δ: 219.0, 141.2, 133.4, 128.7, 127.0, 69.2, 51.8, 30.8. Enantiomeric excess determined by chiral GC as reported in the general information, at 140°C, t_R is 10.80 min for (*S*)-enantiomer, 11.15 min for (*R*)-enantiomer.



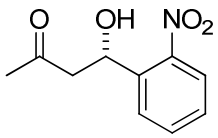
4-Hydroxy-4-(2'-chlorophenyl)-butan-2-one (109), $[\alpha]^{20}_D$ = -101.3 (*c* = 1.2, $CHCl_3$) for (*S*)-enantiomer, 80% *ee*. ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 7.61 (m, 1H); 7.44-7.18 (m, 3H); 5.50 (m, 1H); 3.61 (brs, 1H); 3.01-2.63 (m, 2H); 2.22 (s, 3H). ^{13}C (75.5 MHz, $CDCl_3$) δ: 209.3, 140.1, 131.1, 129.3, 128.6, 127.3, 127.1, 66.6, 50.0, 30.6. Enantiomeric excess determined by chiral GC as reported in the general information, at 120°C, t_R is 17.86 min for (*S*)-enantiomer, 18.38 min for (*R*)-enantiomer.



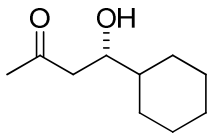
4-Hydroxy-4-(2'-bromophenyl)-butan-2-one (110), $[\alpha]^{22}_D$ = -77.8 (*c* = 1.5, $CHCl_3$), for (*S*)-enantiomer, 82% *ee*. ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 7.61-7.41 (m, 4H); 5.44 (m, 1H); 3.56 (brs, 1H); 2.99 (m, 1H); 2.65 (m, 1H); 2.22 (s, 3H). ^{13}C (75.5 MHz, $CDCl_3$) δ: 204.2, 141.6, 137.6, 129.0, 127.9, 127.3, 121.2, 68.8, 50.1, 30.6. Enantiomeric excess determined by chiral GC as reported in the general information, at 130°C, t_R is 16.64 min for (*S*)-enantiomer, 17.12 min for (*R*)-enantiomer.



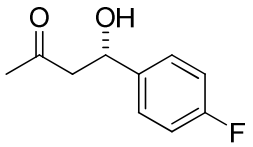
4-Hydroxy-4-(4'-trifluoromethyl)-butan-2-one (111), $[\alpha]^{22}_{D}$ = -47.8 (*c* = 1.5, $CHCl_3$), for (*S*)-enantiomer, 87% *ee*. ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 7.60 (d, *J* = 8.0 Hz, 2H); 7.48 (d, *J* = 8.0 Hz, 2H); 5.20 (m, 1H); 3.62 (brs, 1H); 2.84 (d, *J* = 6.3 Hz, 1H); 2.18 (s, 3H). ^{13}C (75.5 MHz, $CDCl_3$) δ: 208.8, 146.7, 129.6, 125.9, 125.5, 122.3, 69.2, 52.0, 30.7. Enantiomeric excess determined by chiral GC as reported in the general information, at 120°C, t_R is 9.04 min for (*S*)-enantiomer, 9.43 min for (*R*)-enantiomer.



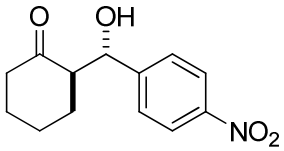
4-Hydroxy-4-(2'-nitrophenyl)-butan-2-one (112), $[\alpha]^{20}_{D}$ = +91.2 (*c* = 1.0, $CHCl_3$) for (*R*)-enantiomer, 91% *ee*. ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 7.93 (m, 2H); 7.67 (m, 1H); 7.44 (m, 1H); 5.68 (dd, *J* = 1.8; *J* = 9.7, 1H); 3.73 (brs, 1H); 3.18 (d, *J* = 1.8, 1H); 2.72 (m, 1H); 2.24 (s, 3H). ^{13}C (75.5 MHz, $CDCl_3$) δ: 208.9, 147.1, 138.4, 133.9, 128.3, 128.2, 124.5, 65.6, 51.1, 30.5. Enantiomeric excess determined by chiral GC as reported in the general information, at 160°C, t_R is 8.15 min for (*S*)-enantiomer, 10.55 min for (*R*)-enantiomer.



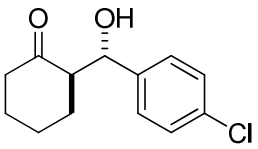
4-Hydroxy-4-(cyclohexyl)-butan-2-one (113), $[\alpha]^{22}_{D}$ = -35.3 (*c* = 2.0, $CHCl_3$), for (*S*)-enantiomer 82% *ee.* ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 3.82 (m, 1H); 2.89 (brs, 1H); 2.53 (m, 2H); 2.18 (s, 3H); 1.75-1.60 (m, 5H); 1.25-0.95 (m, 6H). ^{13}C (75.5 MHz, $CDCl_3$) δ: 210.7, 71.9, 47.5, 42.8, 30.7, 29.0, 28.1, 26.4, 26.2, 25.9. Enantiomeric excess determined by chiral GC as reported in the general information, at 100°C, t_R is 32.31 min for (*S*)-enantiomer, 33.11 min for (*R*)-enantiomer.



4-Hydroxy-4-(4'-fluoro)-butan-2-one (114), $[\alpha]^{22}_{D}$ = +55.6 (*c* = 1.5, $CHCl_3$), for (*R*)-enantiomer, 72% *ee*. ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 7.30 (m, 2H); 7.03 (m, 2H); 5.13 (m, 1H); 3.30 (brs, 1H); 2.83 (m, 1H); 2.21 (s, 3H). ^{13}C (75.5 MHz, $CDCl_3$) δ: 209.1, 163.9, 138.4, 127.4, 115.5, 69.2, 51.9, 30.8. Enantiomeric excess determined by chiral GC as reported in the general information, at 130°C, t_R is 6.71 min for (*S*)-enantiomer, 7.03 min for (*R*)-enantiomer.

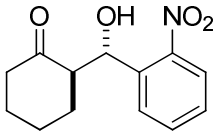


2-(Hydroxy(4-nitrophenyl)methyl)cyclohexanone (61), $[\alpha]^{22}_{D}$ = +11.8 (*c* = 1.0, $CHCl_3$), for *anti*/*syn* = 6:1 and 95% *ee* (*anti*). ^{1}H-NMR (300 MHz; $CDCl_3$) δ (*anti)*: 8.12 (d, *J* = 8.7 Hz, 2H); 7.34 (d, *J* = 8.7 Hz, 2H); 4.82 (d, 1H); 4.05 (brs, 1H); 2.53 (m, 1H); 2.38 (m, 2H); 2.01 (m, 1H); 1.68-1.23 (m, 5H). ^{13}C (75.5 MHz, $CDCl_3$) δ: 214.7, 148.5, 147.5, 127.9, 123.5, 73.9, 57.2, 42.7, 30.7, 27.6, 24.7. Diastereomeric ratio and enantiomeric excess determined by chiral GC as reported in the general information, at 170°C, t_R is 39.84 and 40.61 min (major) for the *anti* diastereomer, 44.59 and 46.10 for the *syn* diastereomer.

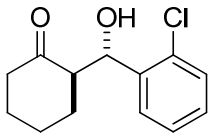


2-(Hydroxy(4-chlorophenyl)methyl)cyclohexanone (119), $[\alpha]^{22}_{D}$ = +20.4 (*c* = 1.0, $CHCl_3$), for *anti*/*syn* = 9:1 and 91% *ee* (*anti*). ^{1}H-NMR (300 MHz; $CDCl_3$) δ (*anti)*: 7.30 (d, *J* = 8.4 Hz, 2H); 7.27 (d, *J* = 8.4 Hz, 2H); 4.76 (d, 1H); 3.97 (brs, 1H); 2.60-2.44 (m, 2H); 2.35 (m, 1H); 2.10 (m, 1H); 1.82-1.45 (m, 4H); 1.29 (m, 1H). ^{13}C (75.5 MHz, $CDCl_3$) δ: 215.3, 139.5, 133.6, 128.6, 128.4, 74.2, 57.4, 42.7, 30.7, 27.7, 24.7. Diastereomeric ratio and enantiomeric

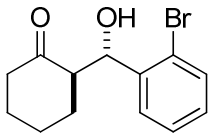
excess determined by chiral GC as reported in the general information, at 145°C, t_R is 36.71 and 37.47 min (major) for the *anti* diastereomer, 39.78 and 41.89 for the *syn* diastereomer.



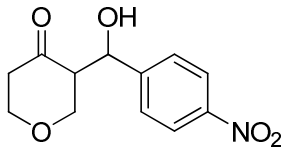
2-(Hydroxy(2-nitro)methyl)cyclohexanone (120), $[\alpha]^{22}_D$ = +24.9 (*c* = 0.8, $CHCl_3$), for *anti*/*syn* = 30:1 and 98% *ee* (*anti*). ^{1}H-NMR (300 MHz; $CDCl_3$) δ (*anti)*: 7.80 (m, 2H); 7.64 (m, 1H); 7.43 (m, 1H); 5.45 (d, *J* = 7.0 Hz, 1H); 4.19 (brs, 1H); 2.75 (m, 1H); 2.35 (m, 2H); 2.11 (m, 1H); 1.87-1.55 (m, 5H). ^{13}C (75.5 MHz, $CDCl_3$) δ: 215.0, 148.7, 136.4, 133.3., 128.9, 128.1, 124.0, 69.6, 57.1, 42.8, 30.8, 27.8, 25.1. Diastereomeric ratio and enantiomeric excess determined by chiral HPLC on a CHIRALCEL OD-H column (95:5 Hexane/i-PrOH, 1 ml/min) t_R is 18.07 and 21.59 min (major) for the *anti* diastereomer, 11.57 and 13.59 min for the *syn* diastereomer.



2-(Hydroxy(2-chlorophenyl)methyl)cyclohexanone (121), $[\alpha]^{22}_D$ = +19.4 (*c* = 2.7, $CHCl_3$), for *anti*/*syn* = 70:1 and 98% *ee* (*anti*). ^{1}H-NMR (300 MHz; $CDCl_3$) δ (*anti)*: 7.53 (m, 1H); 7.29 (m, 2H); 7.19 (m, 1H); 5.33 (dd, *J* = 3.7; *J* = 8.2, 1H); 4.05 (d, *J* = 3.7, 1H); 2.66 (m, 1H); 2.44 (m, 1H); 2.32 (m, 1H); 2.07 (m, 1H); 1.84-1.50 (m, 5H). ^{13}C (75.5 MHz, $CDCl_3$) δ: 215.2, 139.1, 132.9, 129.2, 128.8, 128.3, 127.3, 70.4, 57.6, 42.7, 30.4, 27.8, 24.9. Diastereomeric ratio and enantiomeric excess determined by chiral GC as reported in the general information, at 160°C, t_R is 13.57 and 14.98 min (major) for the *anti* diastereomer, 17.29 and 18.77 for the *syn* diastereomer.



2-(Hydroxy(2-bromophenyl)methyl)cyclohexanone (122), $[\alpha]^{22}_{D}$ = +14.0 (*c* = 2.5, $CHCl_3$), for *anti*/*syn* = 90:1 and 95% *ee* (*anti*). ^{1}H-NMR (300 MHz; $CDCl_3$) δ (*anti)*: 7.50 (m, 2H); 7.34 (m, 1H); 7.13 (m, 1H); 5.30 (m, *J* = 7.7, 1H); 3.85 (brs, 1H); 2.76 (m, 1H); 2.44 (m, 2H); 2.08 (m, 1H); 1.88-1.46 (m, 5H). ^{13}C (75.5 MHz, $CDCl_3$) δ: 215.3, 140.8, 132.5, 129.1, 128.8, 127.9, 123.4, 72.9, 57.7, 42.8, 30.6, 27.8, 25.0. Diastereomeric ratio and enantiomeric excess determined by chiral GC as reported in the general information, at 160°C, t_R is 18.36 and 20.24 min (major) for the *anti* diastereomer, 23.89 and 25.69 for the *syn* diastereomer.



3-(Hydroxy(4-nitrophenyl)methyl)-tetrahydropyran-4-one (123), $[\alpha]^{22}_{D}$ = +39.0 (*c* = 2.2, $CHCl_3$), for *anti*/*syn* = 1:2 and 87% *ee* (*syn*). ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 8.22 (d, *J* = 8.9, 2H); 7.51 (d, *J* = 8.9, 2H); 5.54 (s, 0.61H, *syn*); 4.98 (d, 0.39H, *anti*); 4.23 (m, 1H); 3.77 (m, 3H); 3.46 (m, 0.39H, *anti*); 2.97-2.85 (m, 1H + m, 0.61H, *syn*); 2.71 (m, 1H); 2.50 (m, 1H). ^{13}C (75.5 MHz, $CDCl_3$) δ (*syn*): 207.3, 147.0, 126.4, 125.3, 122.7, 67.9, 67.3, 66.5, 56.2, 42.1. ^{13}C (75.5 MHz, $CDCl_3$) δ (*anti*): 208.3, 146.3, 126.4, 125.3, 122.9, 70.3, 68.8, 67.3, 56.6, 41.9. Diastereomeric ratio and enantiomeric excess determined by chiral GC as reported in the general information, at 180°C, t_R is 26.18 and 26.57 min (major) for the *anti* diastereomer, 29.53 and 30.94 min (major) for the *syn* diastereomer

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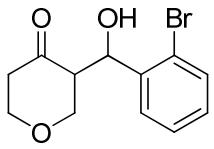
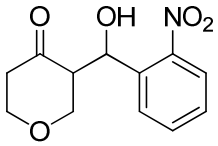
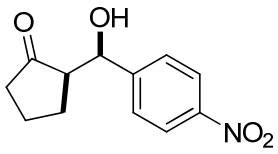


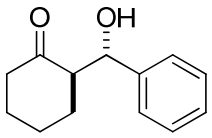
3-(Hydroxy(2-bromo)methyl)-tetrahydropyran-4-one (124), $[\alpha]^{22}_{D}$ = +12.4 (*c* = 0.7, $CHCl_3$), for *anti*/*syn* = 3:1 and 83% *ee* (*anti*). ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 7.55 (m, 2H); 7.37 (m, 1H); 7.16 (m, 1H); 5.73 (s, 0.25H, *syn*); 5.30 (d, *J* = 6.4 Hz, 0.75H, *anti*); 4.23 (m, 1H); 3.90-3.54 (m, 3H); 2.94 (m, 1H); 2.66 (m, 1H); 2.47 (m, 1H). ^{13}C (75.5 MHz, $CDCl_3$) δ (*anti*): 209.9, 139.8, 132.7, 129.5, 129.0, 128.4, 122.5, 70.4, 70.0, 68.4, 58.0, 43.2. Diastereomeric ratio and enantiomeric excess determined by chiral GC as reported in the general information, at 135°C, t_R is 51.30 and 52.49 min (major) for the *anti* diastereomer, 45.94 and 49.12 min (major) for the *syn* diastereomer.



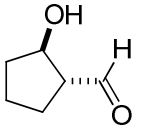
3-(Hydroxy(2-nitrophenyl)methyl)-tetrahydropyran-4-one (125), $[\alpha]^{22}_{D}$ = +36.5 (*c* = 0.7, $CHCl_3$), for *anti*/*syn* = 4:1 and 86% *ee* (*anti*). ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 7.93 (dd, *J* = 8.4 Hz, 0.8 Hz, 1H); 7.81 (t, *J* = 7.2 Hz, 1H); 7.68 (m, 1H); 7.46 (m, 1H); 6.03 (s, 0.2H, *syn*); 5.48 (d, *J* = 6.8 Hz, 0.8H, *anti*); 4.23 (m, 1H); 4.06 (brs, 1H); 3.83-3.79 (m, 2H); 3.10-3.04 (m, 1H); 2.72-2.64 (m, 1H); 2.54-2.49 (m, 1H). ^{13}C (75.5 MHz, $CDCl_3$) δ (*anti*): 209.6, 148.1, 136.1, 133.6, 128.8, 125.0, 70.5, 68.4, 68.2, 57.9, 43.2. Diastereomeric ratio and enantiomeric excess determined by chiral HPLC on a OD/ODH column (95:5 Heptane/i-PrOH, 0.5 ml/min). t_R is 84.64 (major) and 94.06 min (minor) for the *anti* diastereomer, 52.14 and 64.47 min (major) for the *syn* diastereomer.



2-(Hydroxy(4-nitrophenyl)methyl)cyclopentanone (126), ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 8.18 (d, *J* = 8.7 Hz, 2H); 7.51 (d, *J* = 8.7 Hz, 2H); 5.41 (d, 0.67H, *syn*); 4.83 (d, 0.34H, *anti*); 4.78 (brs, 0.34H, *anti*); 2.74 (brs, 0.67H, *syn*); 2.50-1.67 (m, 7H). ^{13}C (75.5 MHz, $CDCl_3$) δ (*syn)*: 219.6, 150.2, 127.3, 126.4, 123.7, 70.5, 56.2, 39.0, 22.4, 20.4. Diastereomeric ratio and enantiomeric excess determined by chiral GC as reported in the general information, at 165°C, t_R is 31.42 and 32.33 min (major) for the *syn* diastereomer, 36.33 and 39.16 min (major) for the *anti* diastereomer.

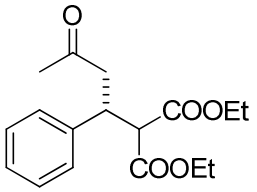


2-(Hydroxymethyl)cyclohexanone (163), $[\alpha]^{22}_{D}$ = +24.7 (*c* = 0.6, $CHCl_3$), for *anti*/*syn* = 9:1 and 93% *ee* (*anti*). ^{1}H-NMR (300 MHz; $CDCl_3$) δ (*anti)*: 7.31 (m, 5H); 4.78 (dd, *J* = 9.2; *J* = 2.4, 1H); 3.96 (d, *J* = 2.4, 1H); 2.52 (m, 1H); 2.47 (m, 1H); 2.35 (m, 1H); 2.06 (m, 1H); 1.77 (m, 1H); 1.71-1.46 (m, 3H); 1.27 (m, 1H). ^{13}C (75.5 MHz, $CDCl_3$) δ: 215.2, 140.7, 128.2, 127.6, 126.9, 74.5, 57.6, 42.7, 30.7, 27.8, 24.6. Diastereomeric ratio and enantiomeric excess determined by chiral GC as reported in the general information, at 130°C, t_R is 13.57 and 14.98 min (major) for the *anti* diastereomer, 17.29 and 18.77 for the *syn* diastereomer.

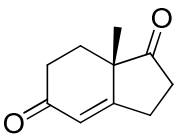


***trans*-2-hydroxycyclopentanecarboaldehyde (129),** $[\alpha]^{22}_{D}$ = +6.4 (*c* = 3.0, $CHCl_3$), for *anti*/*syn* = 7:1. To a suspension of silica gel (15 g) in 10 mL of DCM, 15 mL of a 0.65 M solution of $NaIO_4$ in water were added drop-wise. To this suspension, *trans*-cyclohexanediol (**136**, 2 g) was added and the reaction was monitored through TLC (5:1

Hexanes/EtOAc) until complete conversion of the starting material, in about 20 min. After the filtration of the inorganic precipitates, the organic layer is stirred over Na_2SO_4 and evaporated to provide adipaldehyde **128**. This aldehyde (70 mg, 0.6 mmol) is dissolved in chloroform and 13.8 of catalyst **104** (0.03 mmoles, 5 mol%) are added. After 5-10 min the complete conversion of the starting material is observed on TLC. The selectivity of the process is checked through chiral GC without isolating the reaction product (**129**); at 100°C, t_R is 10.43 and 10.53 min for the *anti* diastereomer, 12.42 and 13.03 min for the *syn* diastereomer. The organic layer is washed with water, stirred over Na_2SO_4 and evaporated obtaining the clean reaction product. (65 mg, 93%). **129** is not a stable product and is reduced to compound **137** using one equiv. of $NaBH_4$ in EtOH at 0°C, in 5-10 min. for **137**, ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 5.30 (brs, 2H); 3.60 (m, 2H); 3.45 (m, 1H); 1.92 (m, 1H); 1.66 (m, 3H); 1.22 (m, 3H).

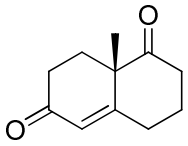


2-(3-oxo-1-phenylbuthyl) malonic acid diethyl ester (145), $[\alpha]^{22}_D$ = +6.1 (*c* = 0.5, $CHCl_3$). ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 7.28-7.18 (m, 5H); 4.18 (m, 2H); 3.96 (m, 1H); 3.94 (m, 2H); 3.69 (d, *J* = 9.8 Hz, 1H); 2.96 (m, 1H); 2.90 (m, 1H); 2.02 (s, 3H); 1.25 (dd, *J* = 7.2 Hz, 7.2 Hz, 3H); 1.01 (dd, *J* = 7.2 Hz, 7.2 Hz, 3H). (75.5 MHz, $CDCl_3$) δ: 206.1, 168.2, 167.7, 140.4, 128.5, 128.2, 127.2, 61.7, 61.3, 57.4, 47.4, 40.5, 30.3, 14.0, 13.8.



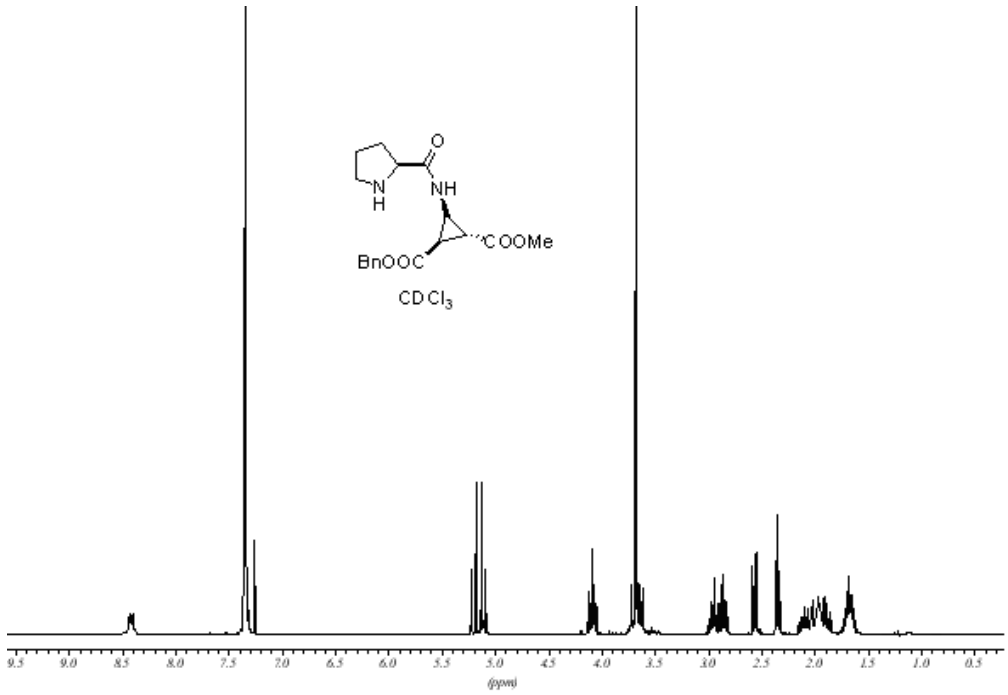
1H-Indene-1,5-(6H)-dione-2,3,7,7a-tetrahydro-7a-methyl (5), $[\alpha]^{22}_D$ = +244 (*c* = 0.5, $CHCl_3$), for (*S*)-enantiomer (83%) *ee*. ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 5.96 (s, 1H); 2.95 (m, 1H); 2.77 (m, 2H); 2.58-2.36 (m, 3H); 2.09 (m, 1H); 1.83 (m, 1H); 1.31 (s, 3H). ^{13}C (75.5 MHz, $CDCl_3$) δ: 216.5, 198.2, 169.7, 123.9, 48.7, 35.9, 32.9, 29.2, 26.8, 20.6. Enantiomeric

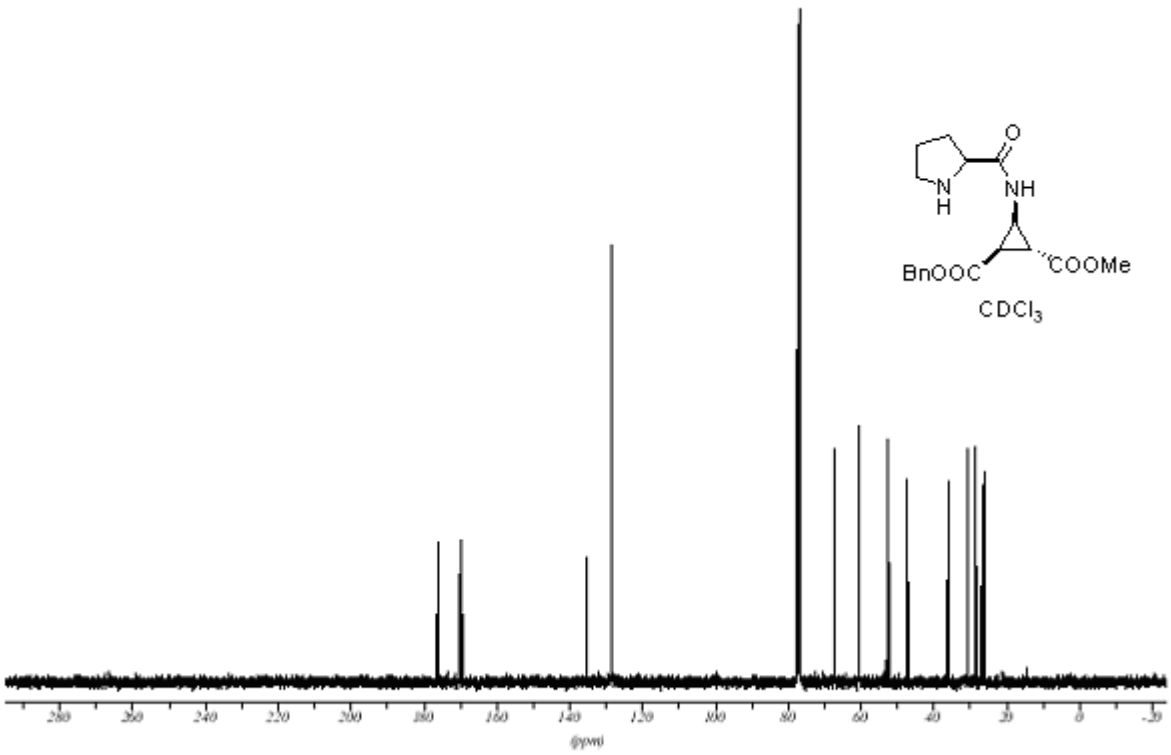
excess determined by chiral GC, at 155°C, t_R is 5.8 min for (*R*)-enantiomer, 6.2 min for (*S*)-enantiomer and 3.8 min for the starting material.



1,6-(2H,7H)-Naphtalenedione-3,4,8,8a-tetrahydro-8a-methyl (56), $[\alpha]^{22}_D$ = +107.5 (*c* = 2.0, $CHCl_3$), for (*S*)-enantiomer (92%) *ee*. ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 5.85 (s, 1H); 2.75-2.63 (m, 2H); 2.51-2.38 (m, 4H); 2.18-2.08 (m, 3H); 1.75-164 (m, 1H); 1.44 (s, 3H). ^{13}C (75.5 MHz, $CDCl_3$) δ: 210.2, 198.0, 165.7, 125.8, 50.5, 37.4, 33.7, 31.8, 29.7, 23.4, 21.0. Enantiomeric excess determined by chiral GC, at 140°C, t_R is 21.5 min for (*R*)-enantiomer, 22.5 min for (*S*)-enantiomer and 11.2 min for the starting material.

E.5 Copies of NMR spectra

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N
H
NH
BnOOC
COOMe
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180
160
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100
80
60
40
20
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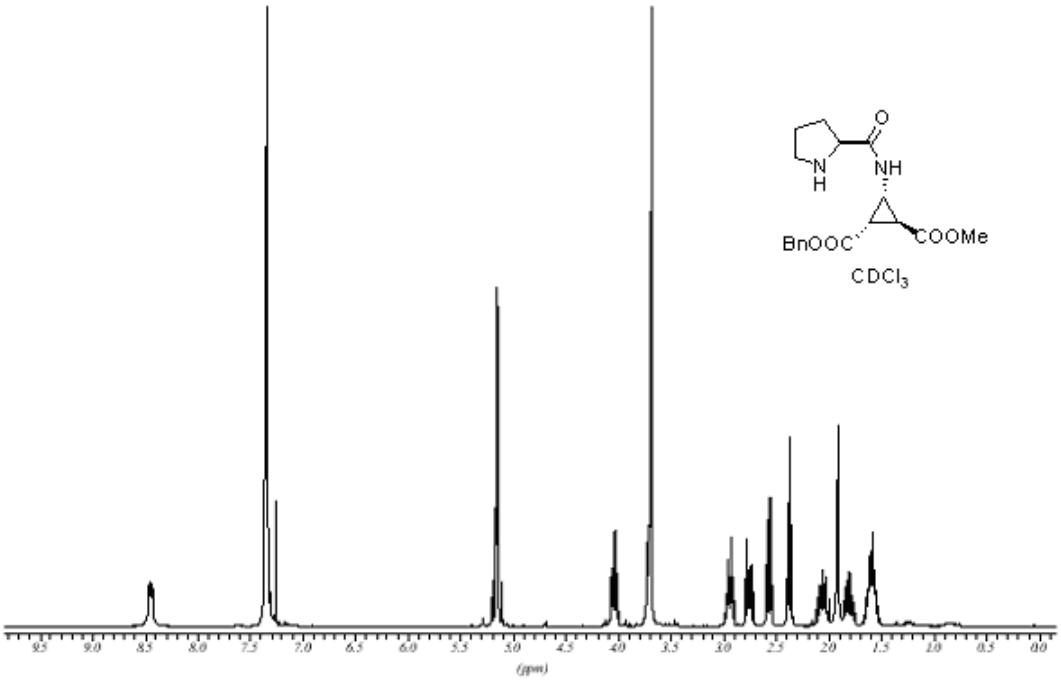
-20
(ppm)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COOMe
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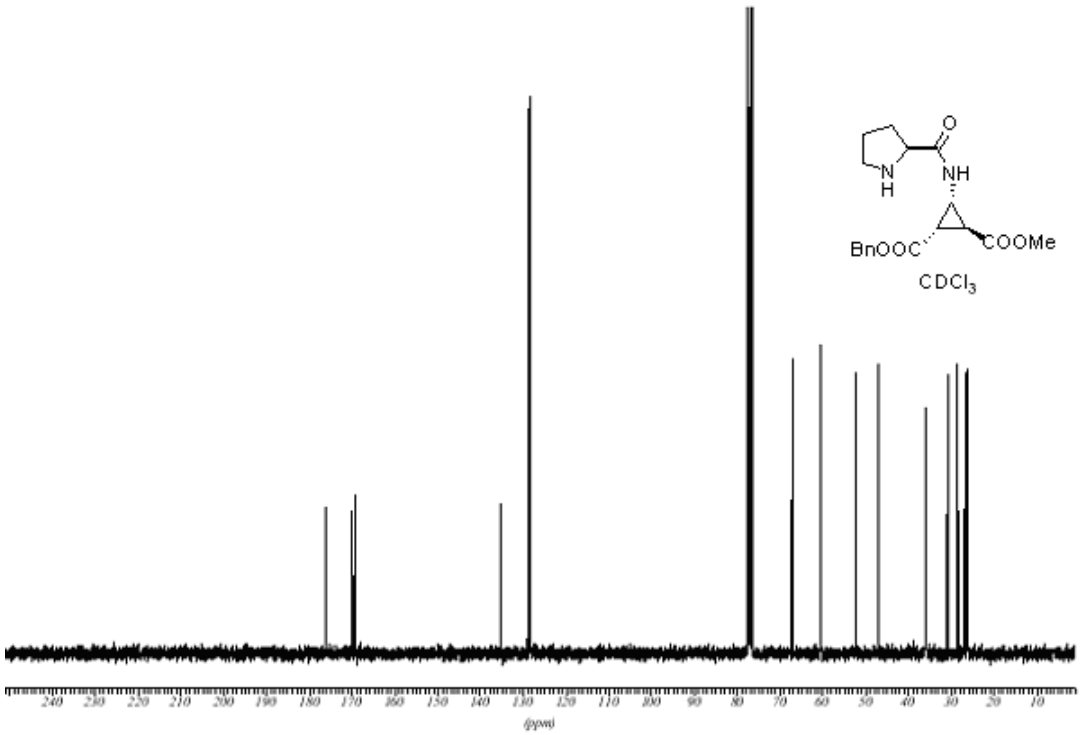
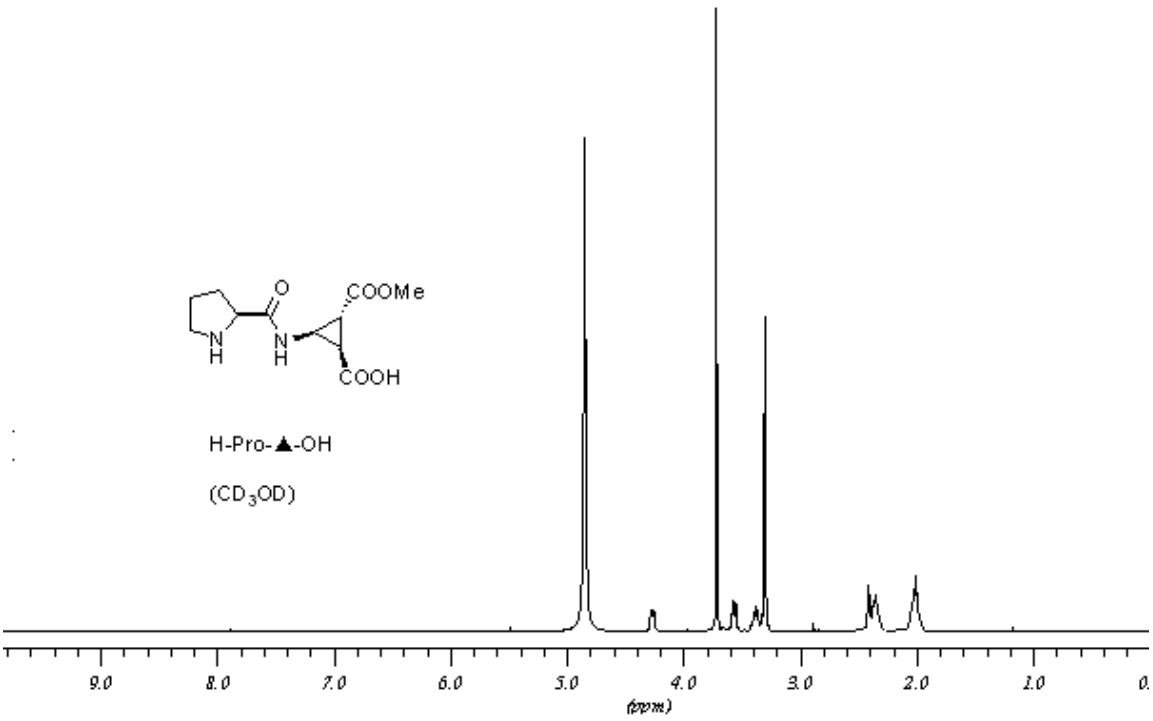
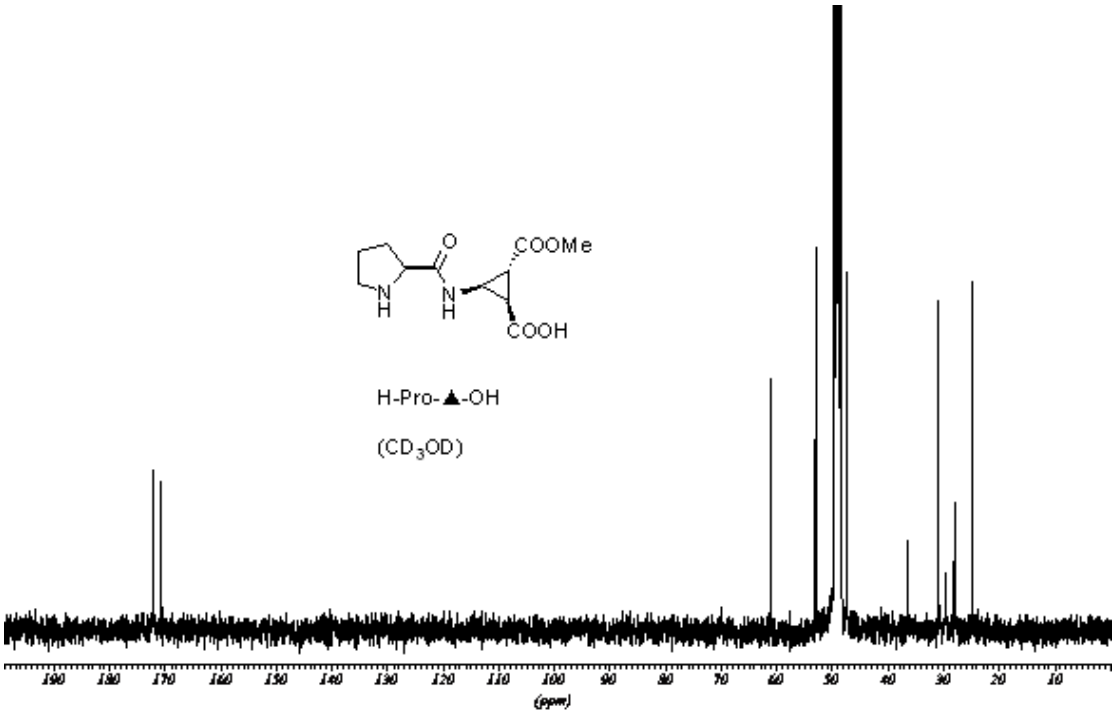
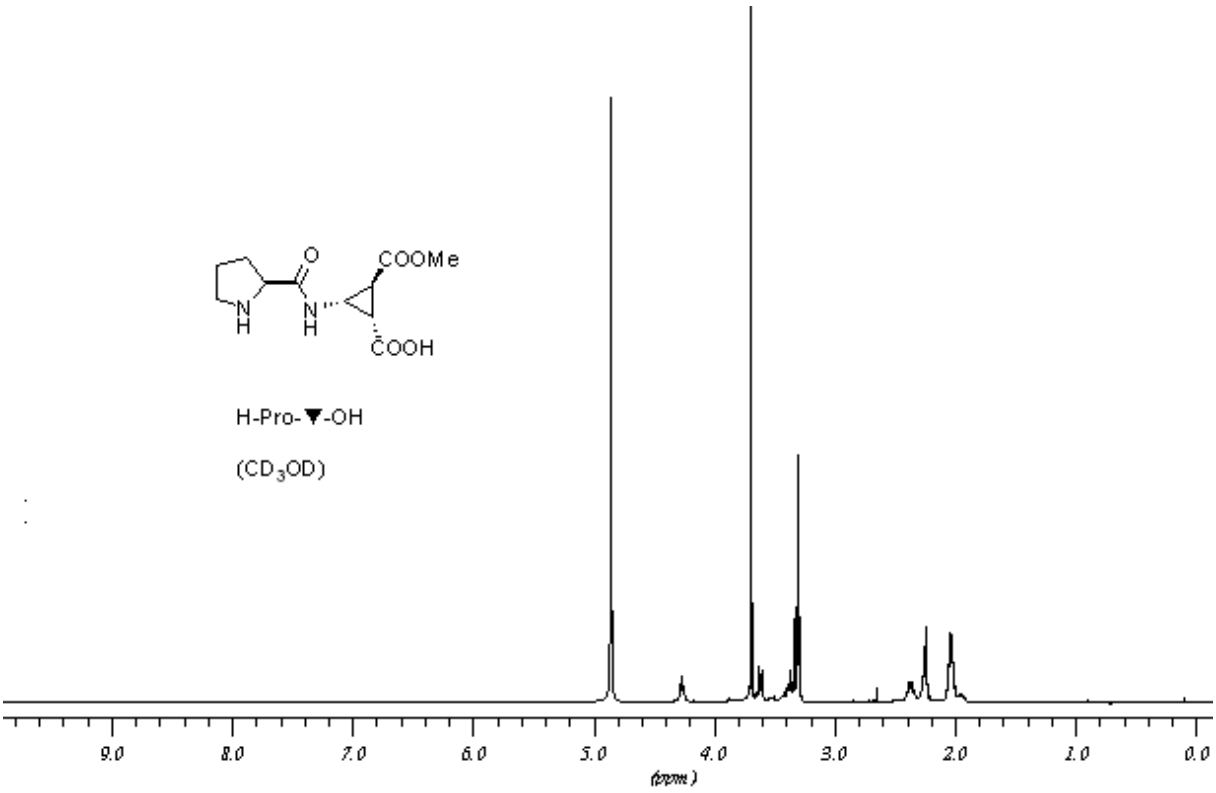
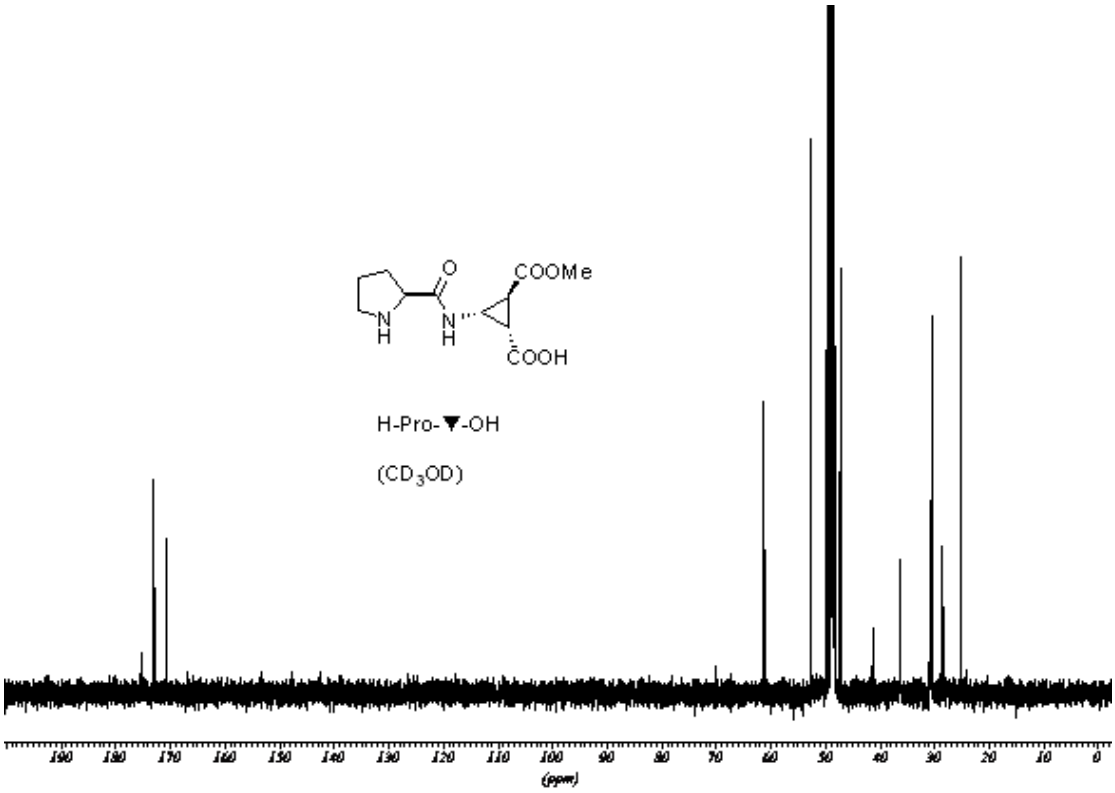
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)

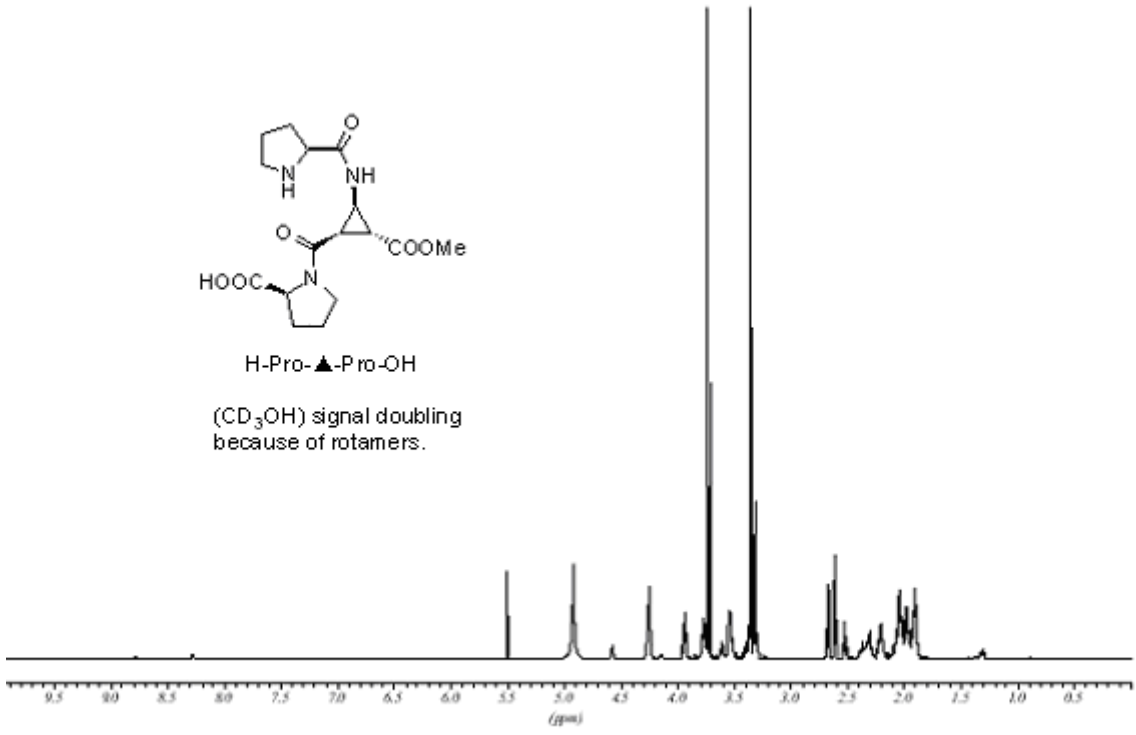

O
NH
N
H
BnOOC
COOMe
CDCl3
240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(ppm)

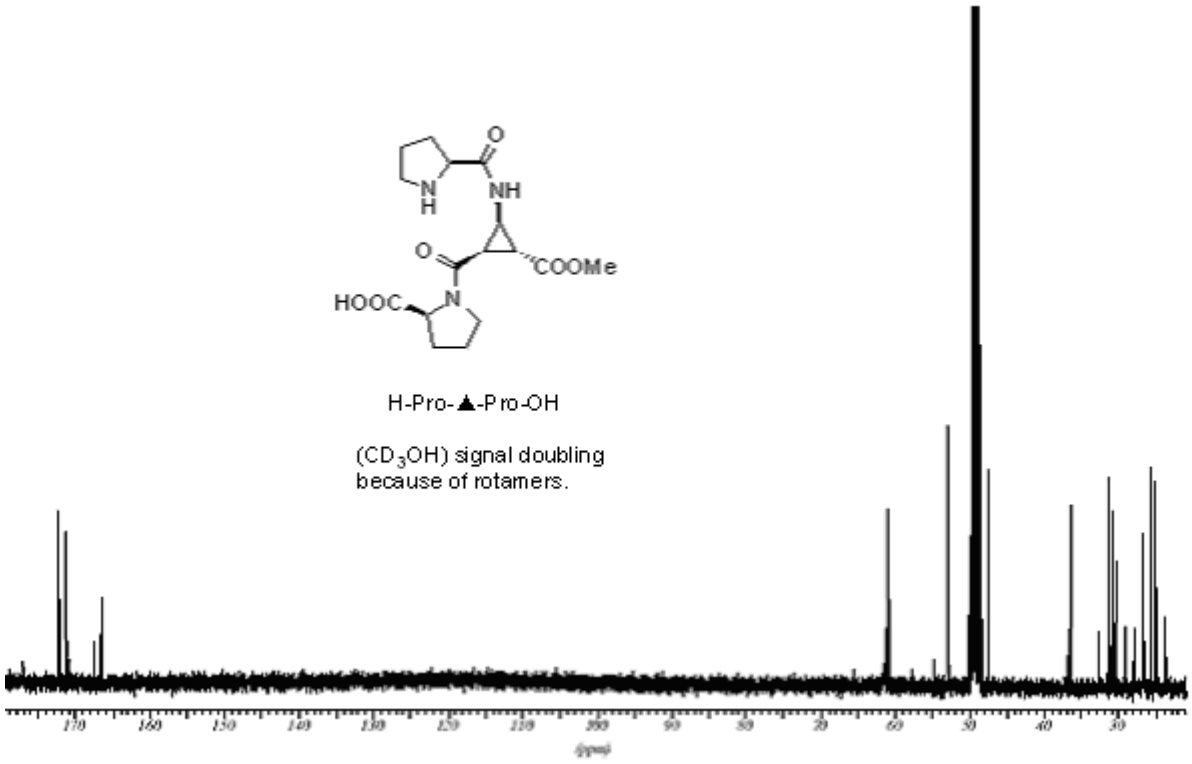

O
COOMe
N
H
N
H
COOH
H-Pro-▲-OH
(CD3OD)
9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.
(ppm)

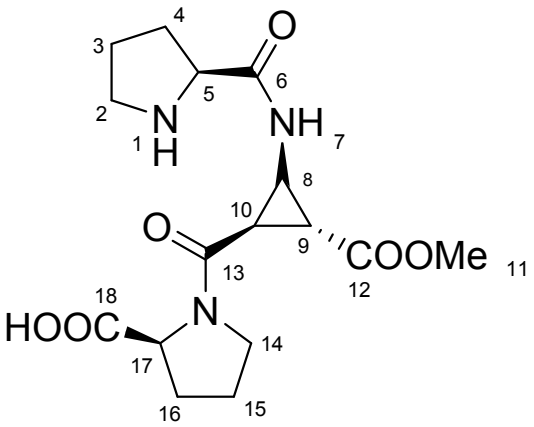

COOMe
COOH
H-Pro-▲-OH
(CD3OD)
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(ppm)

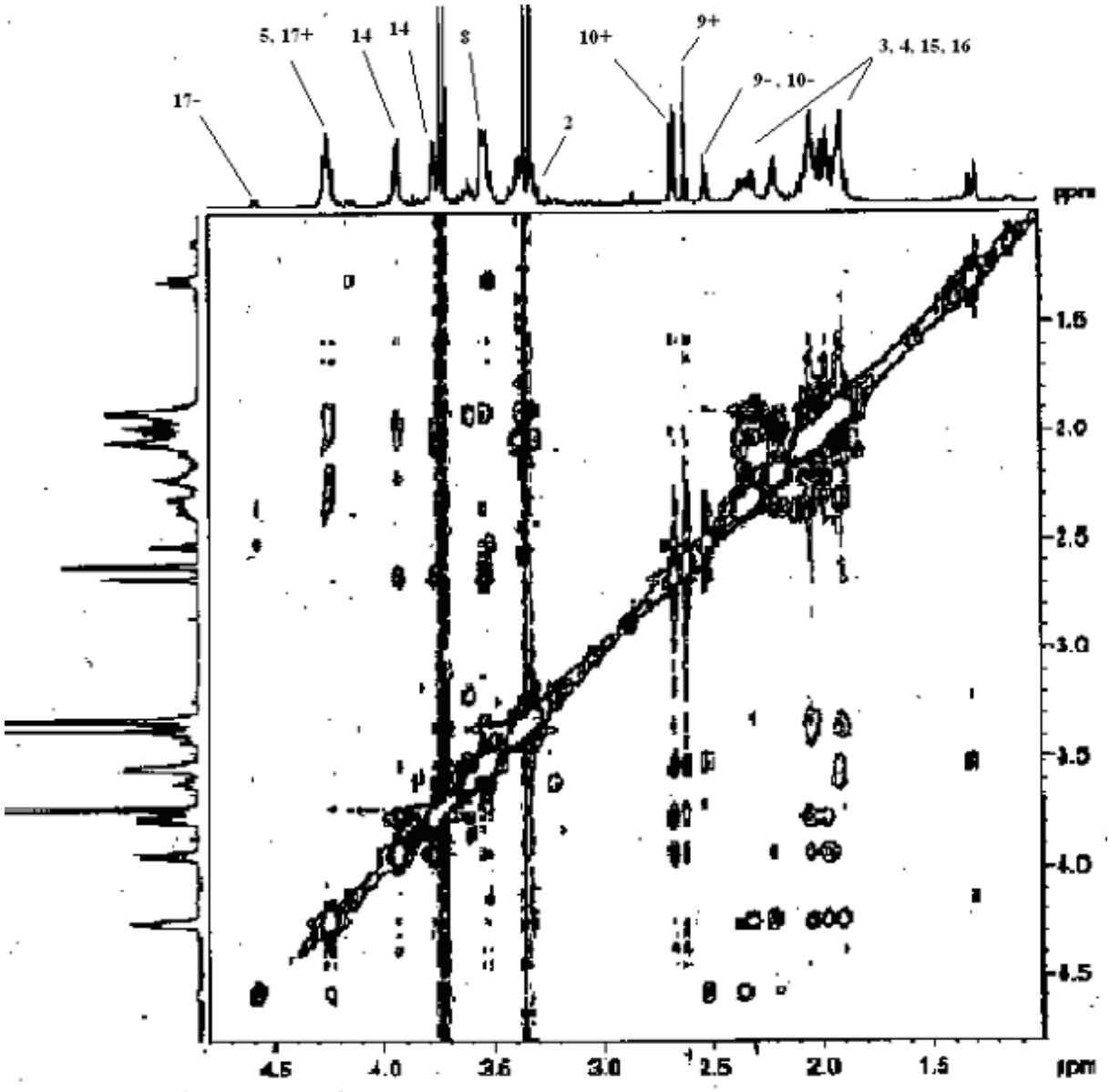

COOMe
COOH
H-Pro-▼-OH
(CD3OD)
9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0
(ppm)

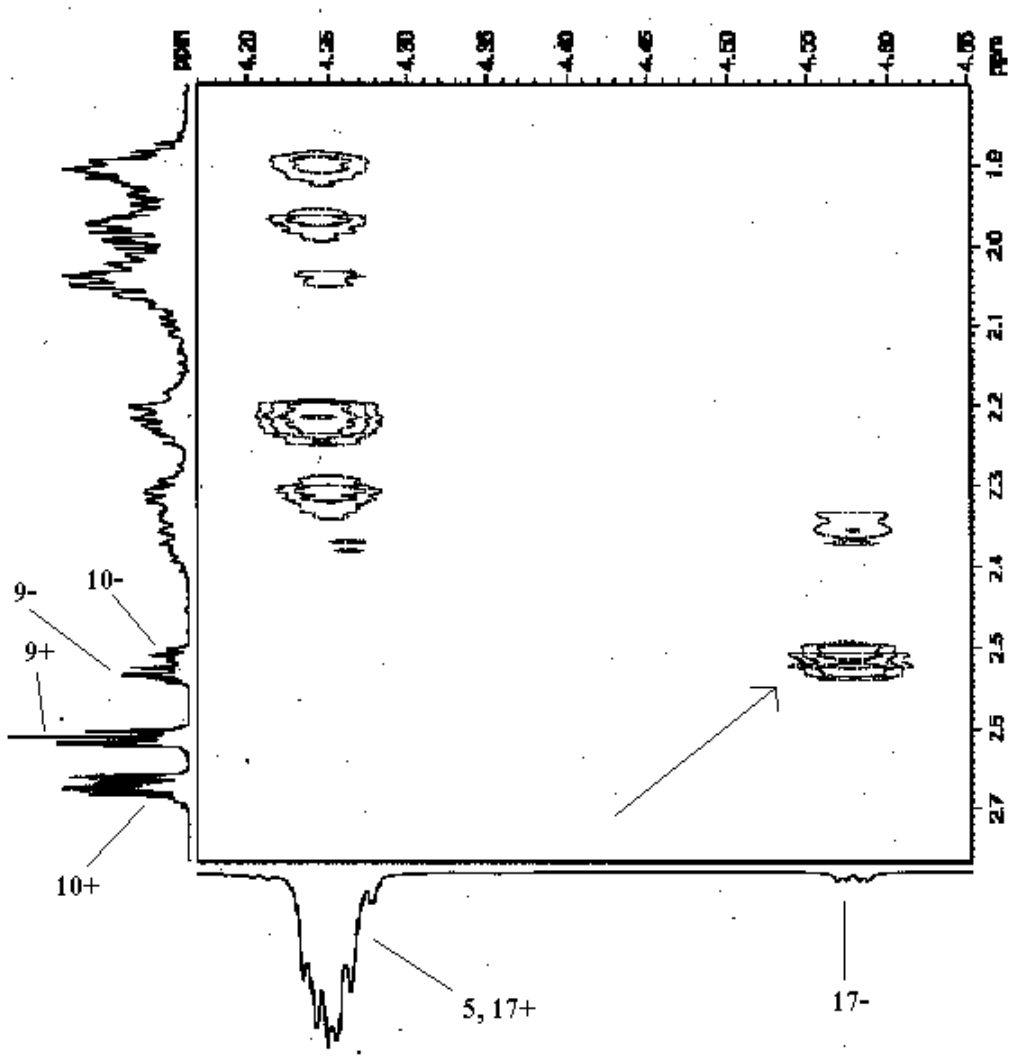

COOMe
COOH
H-Pro-▼-OH
(CD3OD)
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
(ppm)

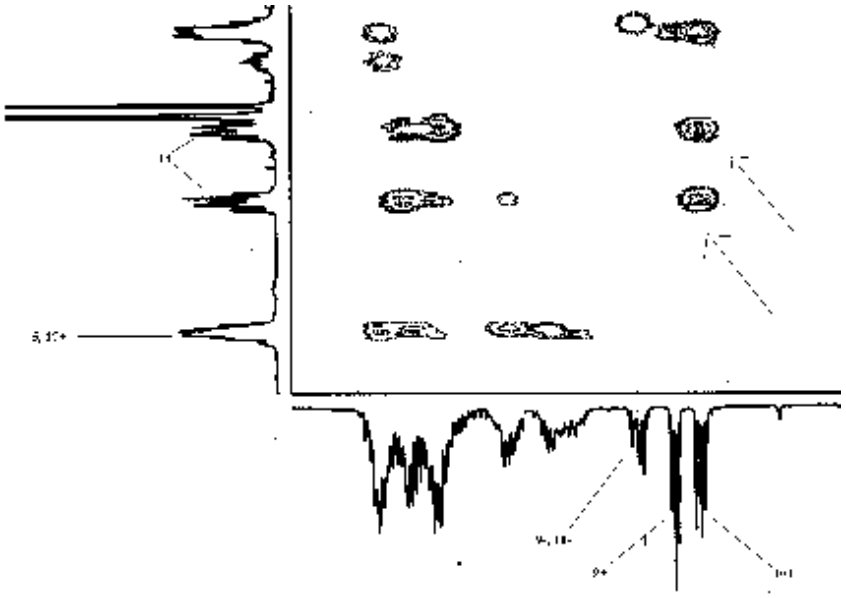

COOMe
HOOC
H-Pro-▲-Pro-OH
(CD3OH) signal doubling
because of rotamers.
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
(ppm)

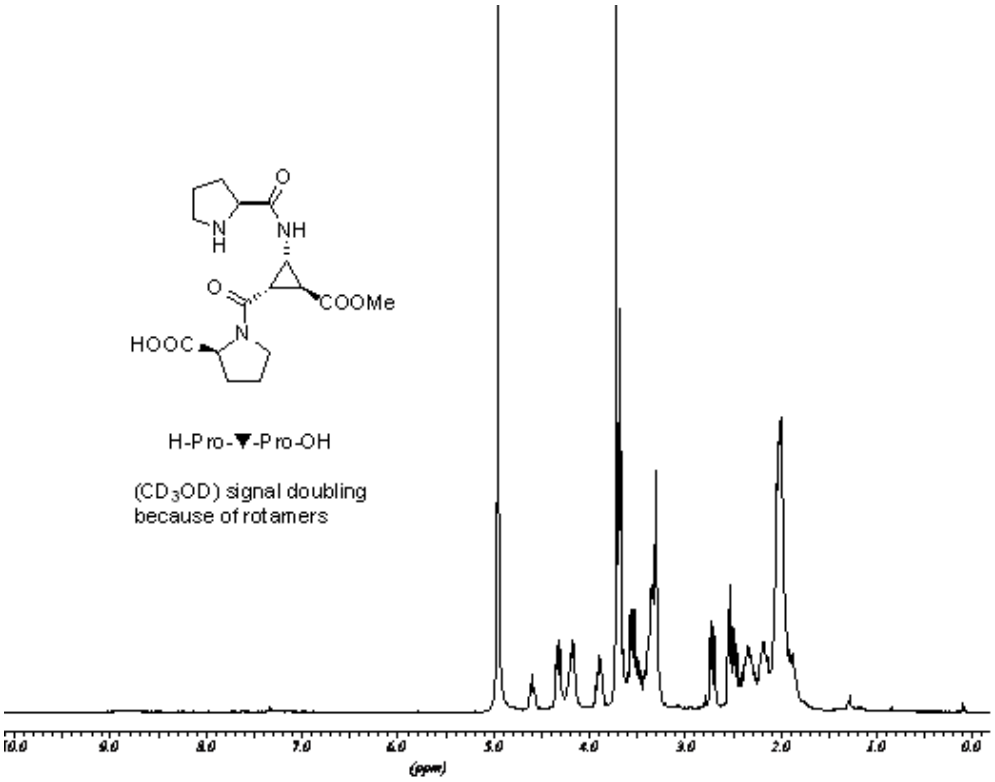

H-Pro-▲-Pro-OH
(CD3OH) signal doubling
because of rotamers.

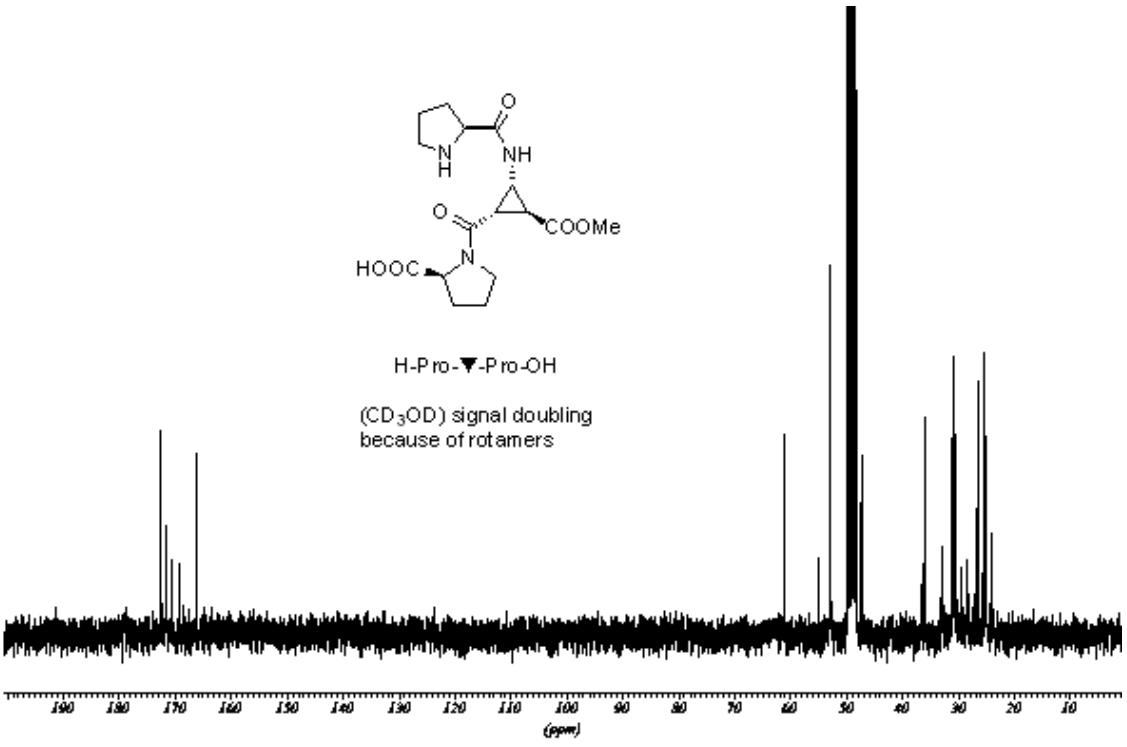

HOOC
COOMe
NH
O
N
H

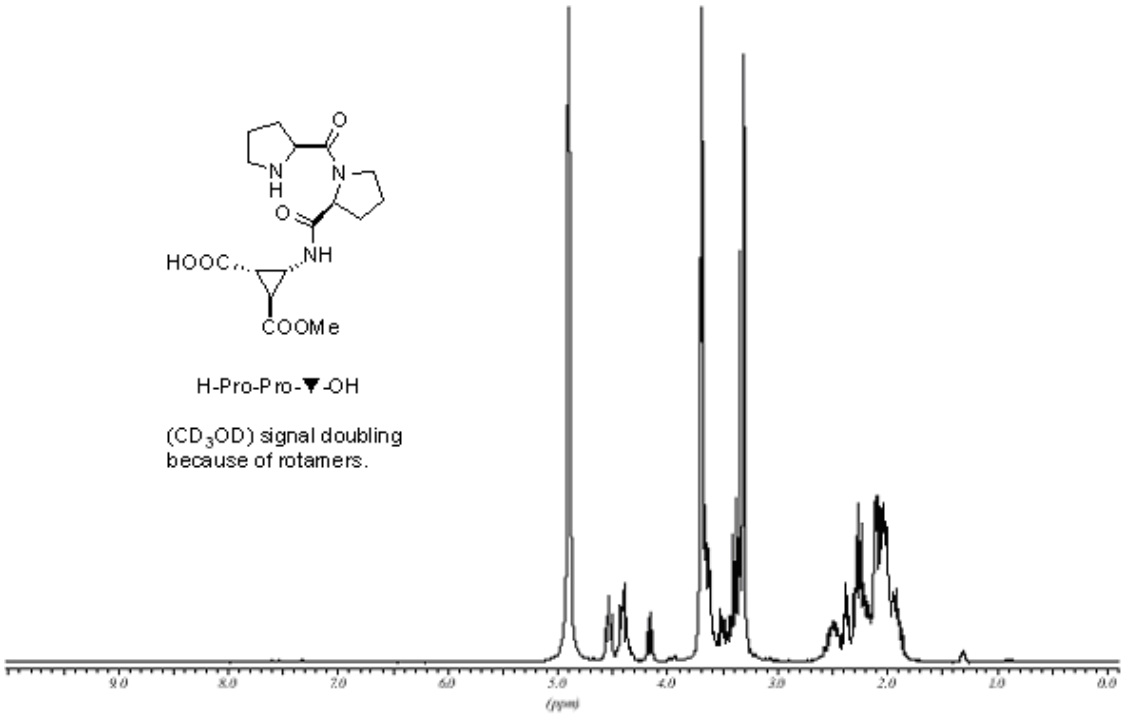

17-
5, 17+
14
14
8
2
10+
9+
9-, 10-
3, 4, 15, 16
ppm
1.5
2.0
2.5
3.0
3.5
4.0
4.5
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm

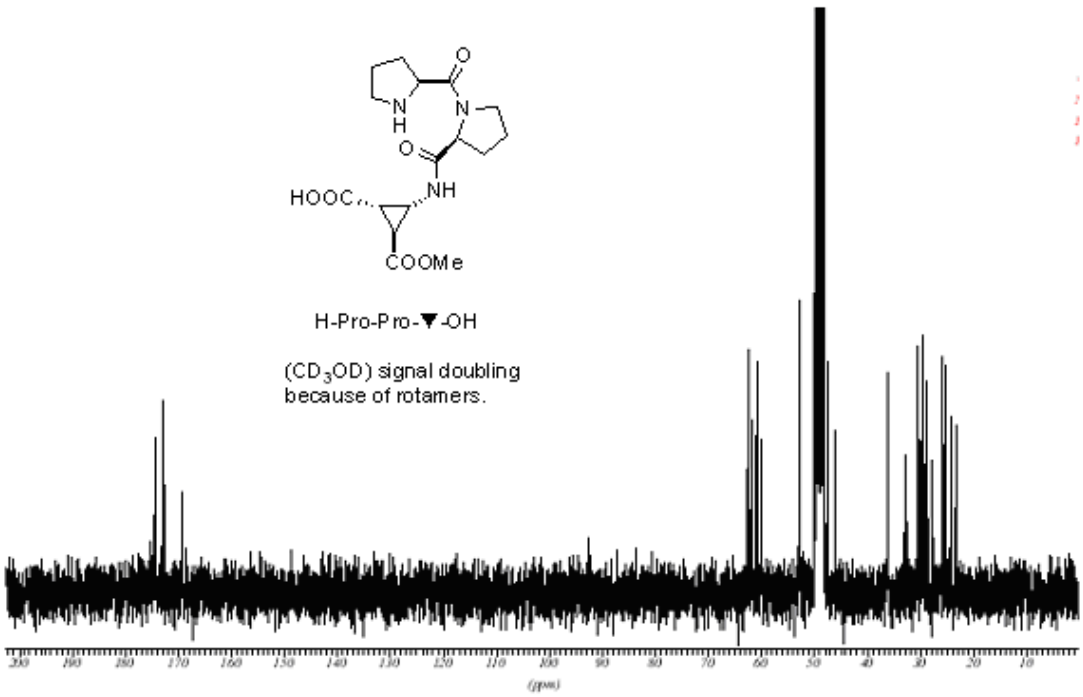

9-
10-
9+
10+
5, 17+
17-

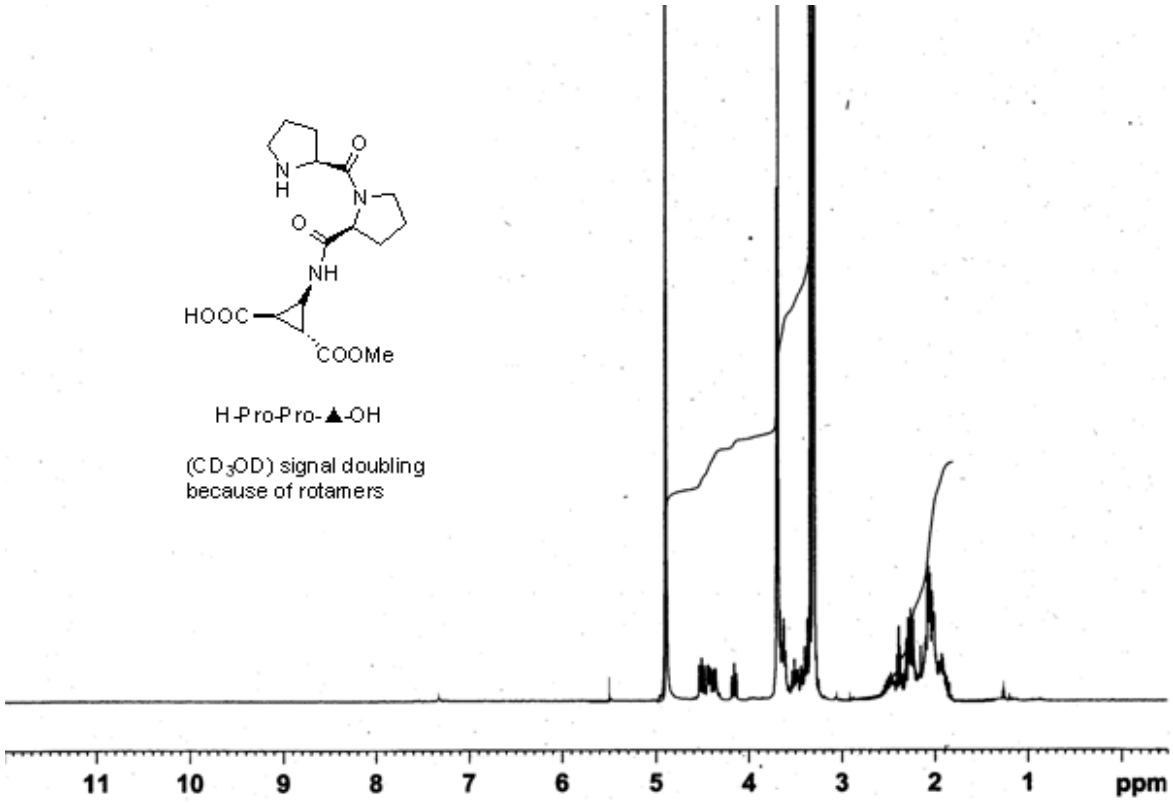


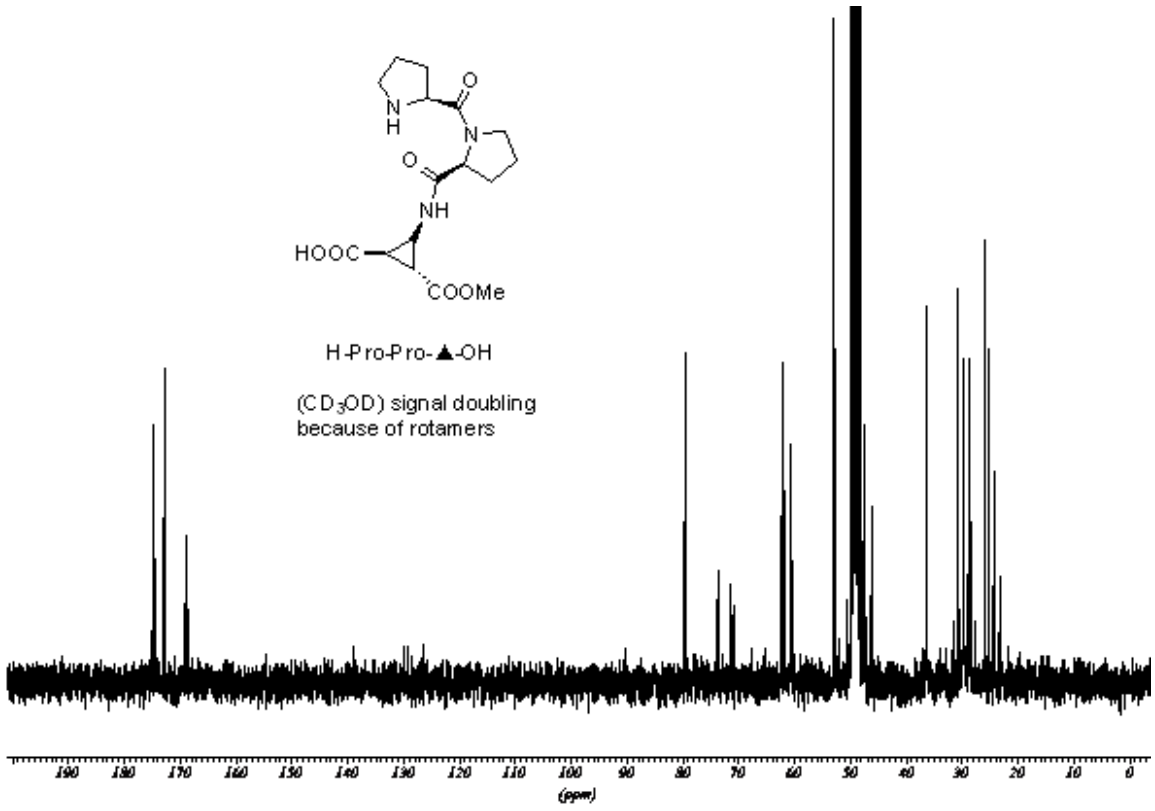

O
NH
N
H
O
COOMe
N
HOOC
H-Pro-▼-Pro-OH
(CD3OD) signal doubling
because of rotamers
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)

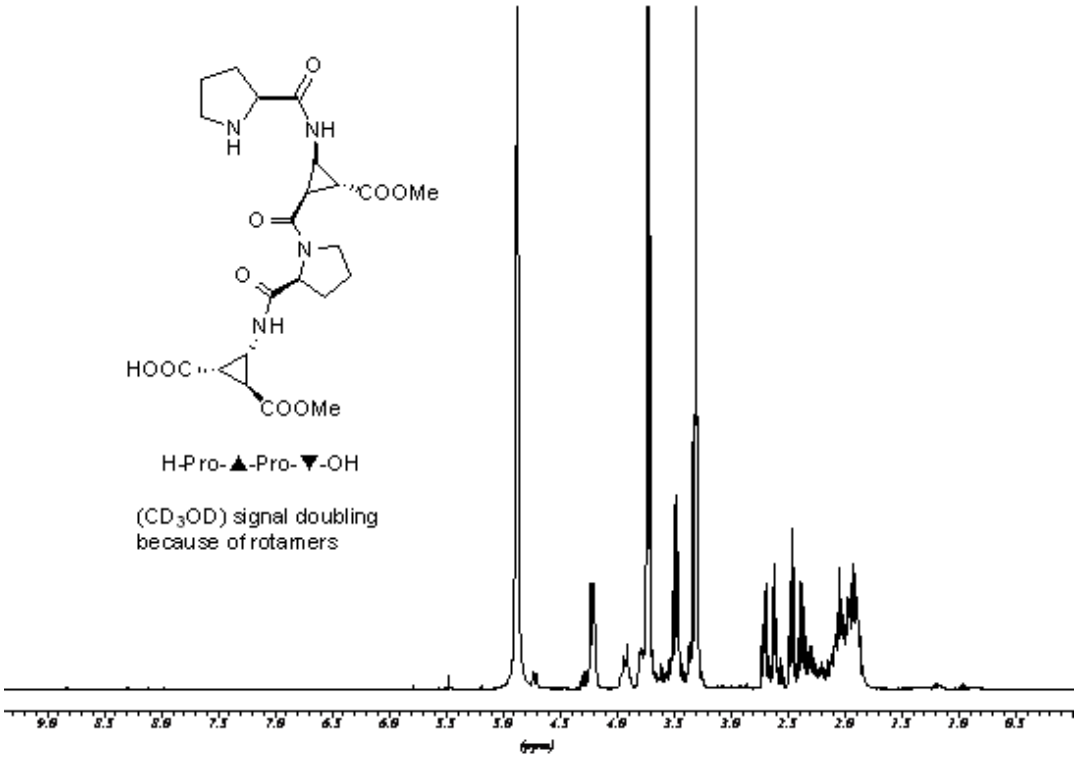

O
NH
N
H
O
COOMe
N
HOOC
H-Pro-▼-Pro-OH
(CD3OD) signal doubling
because of rotamers
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

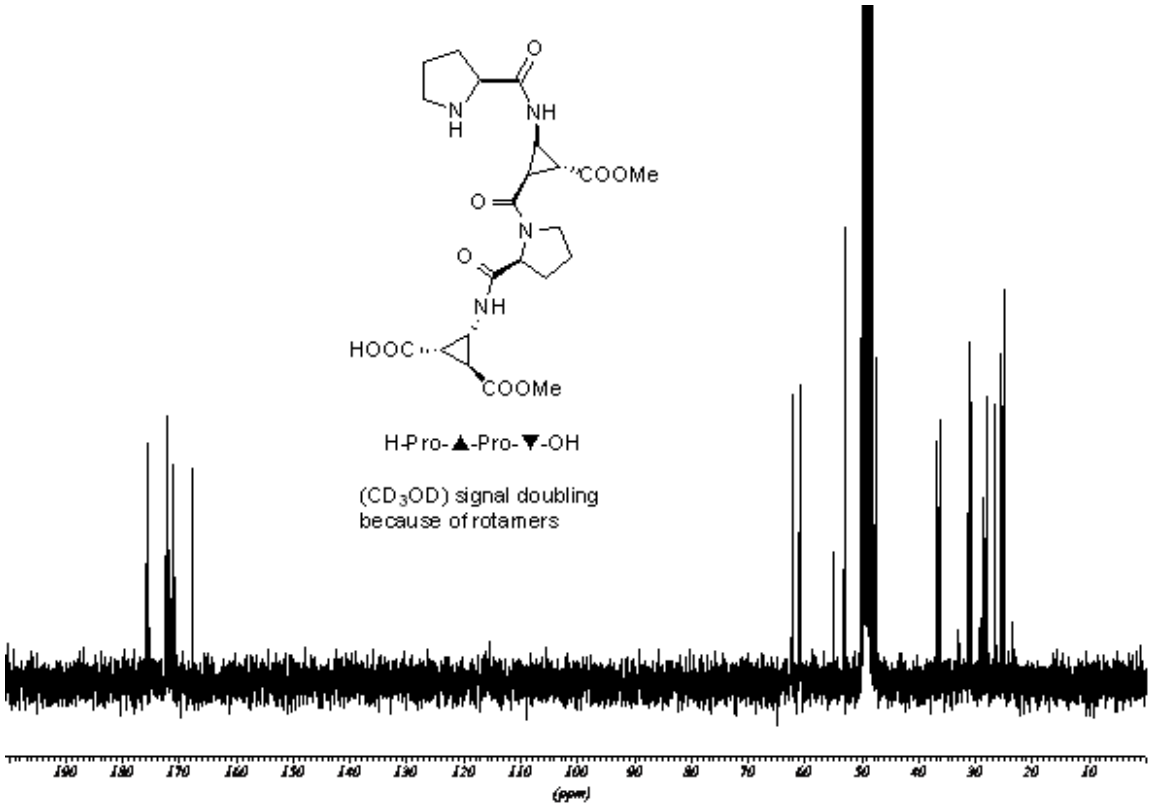

HOOC
NH
COOMe
H-Pro-Pro-▼-OH
(CD3OD) signal doubling
because of rotamers.
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)

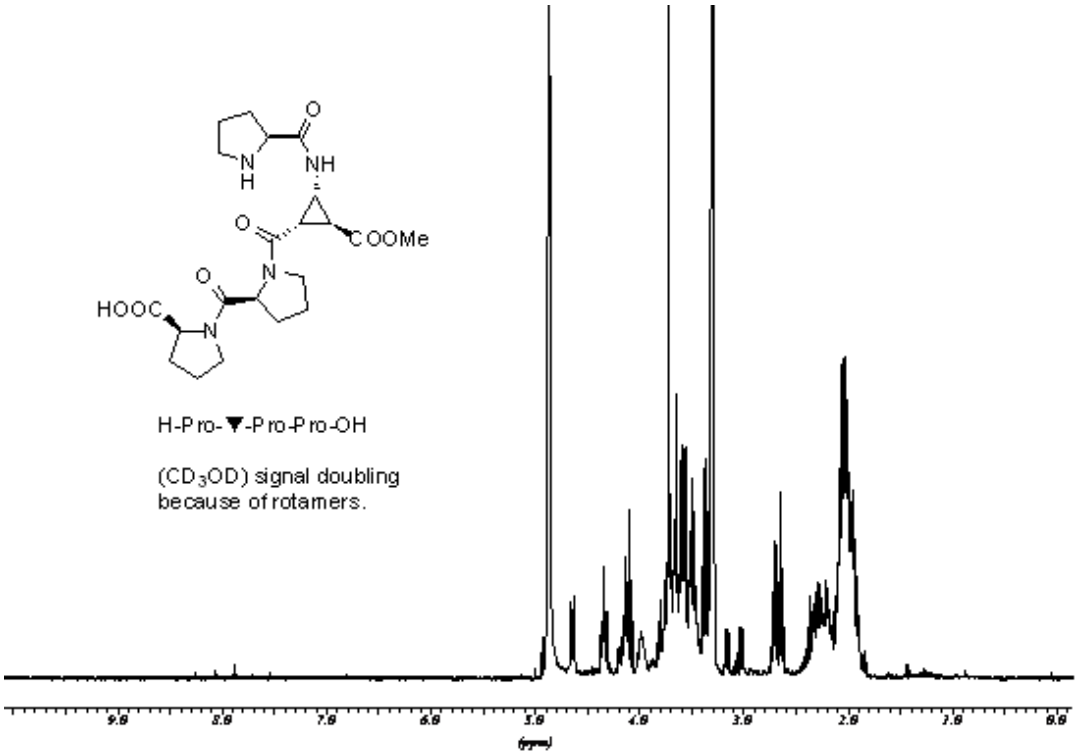

HOOC
NH
COOMe
H-Pro-Pro-▼-OH
(CD3OD) signal doubling
because of rotamers.
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

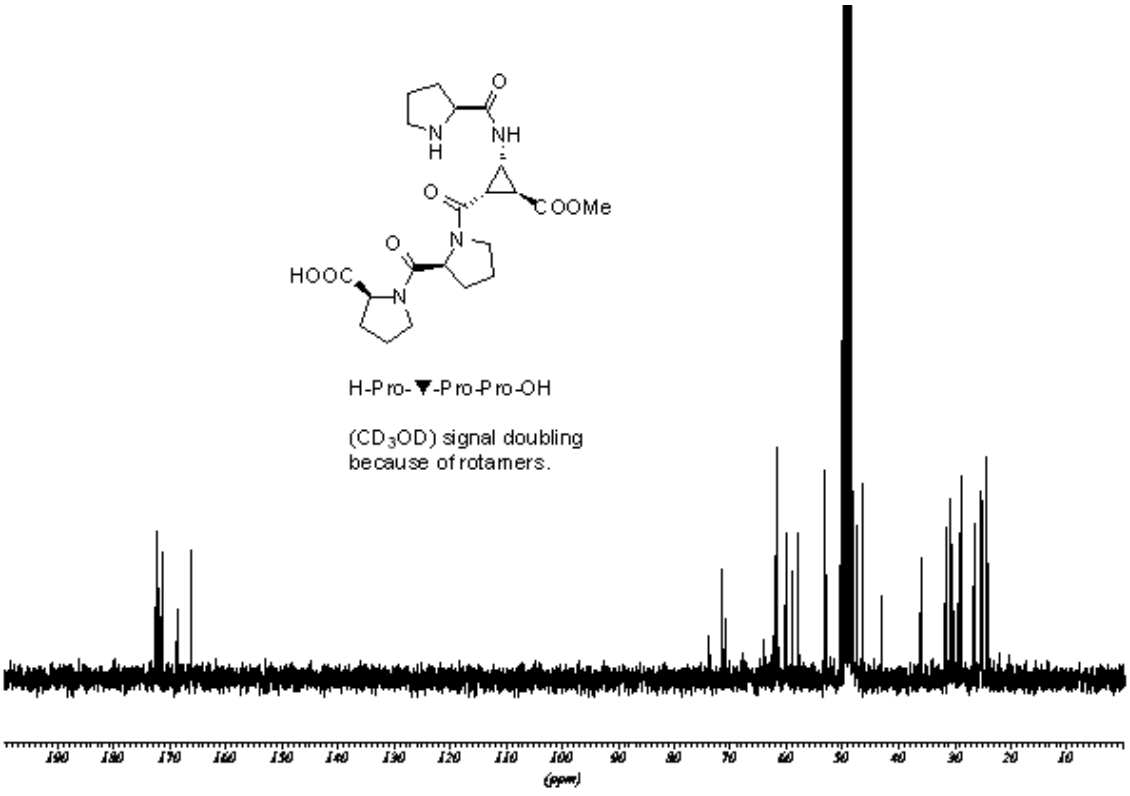

HOOC
COOMe
NH
H-Pro-Pro-▲-OH
(CD3OD) signal doubling
because of rotamers
11
10
9
8
7
6
5
4
3
2
1
ppm

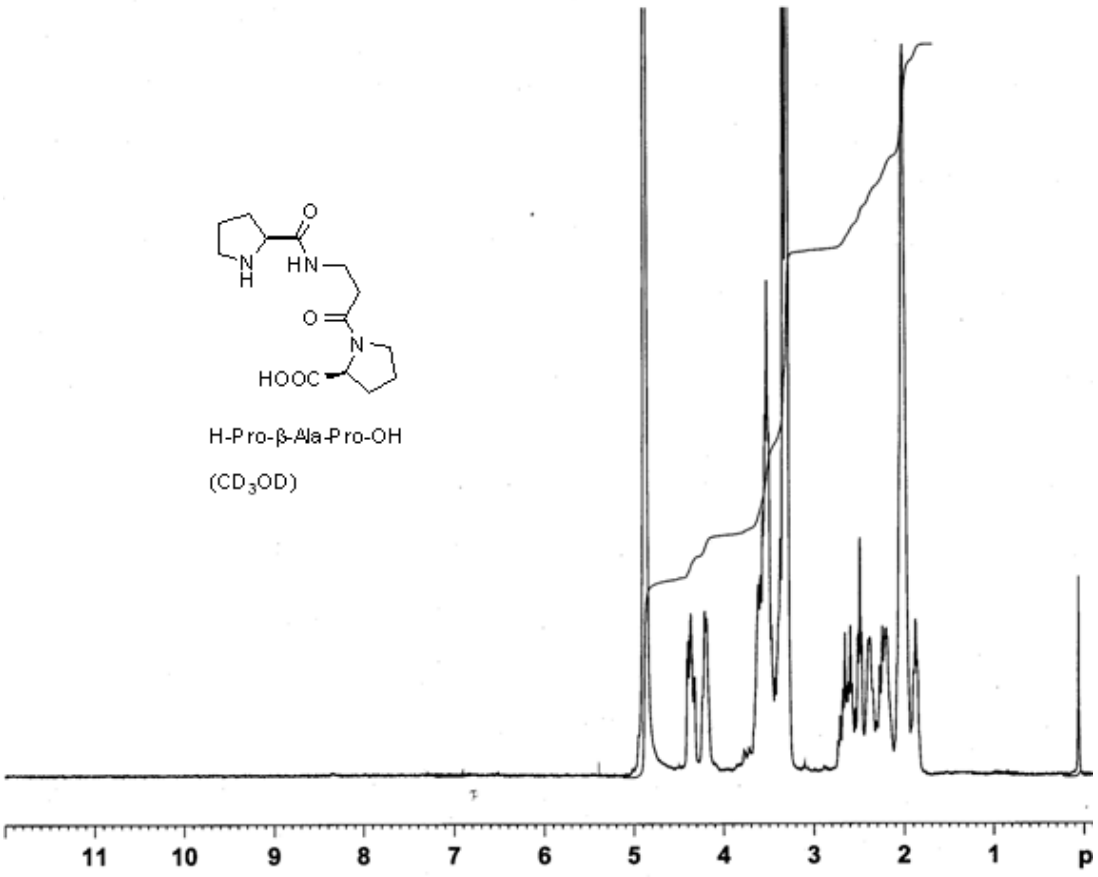

HOOC
COOMe
NH
H-Pro-Pro-▲-OH
(CD3OD) signal doubling
because of rotamers
(ppm)

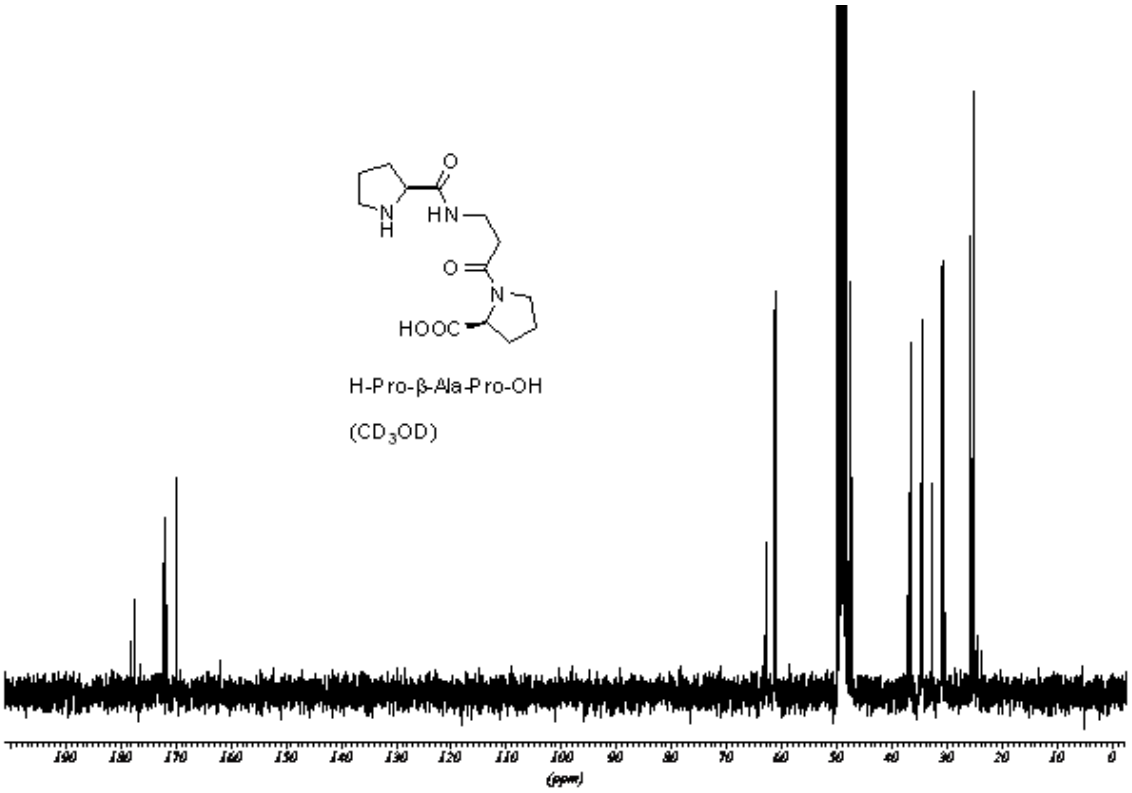

H-Pro-▲-Pro-▼-OH
(CD3OD) signal doubling
because of rotamers

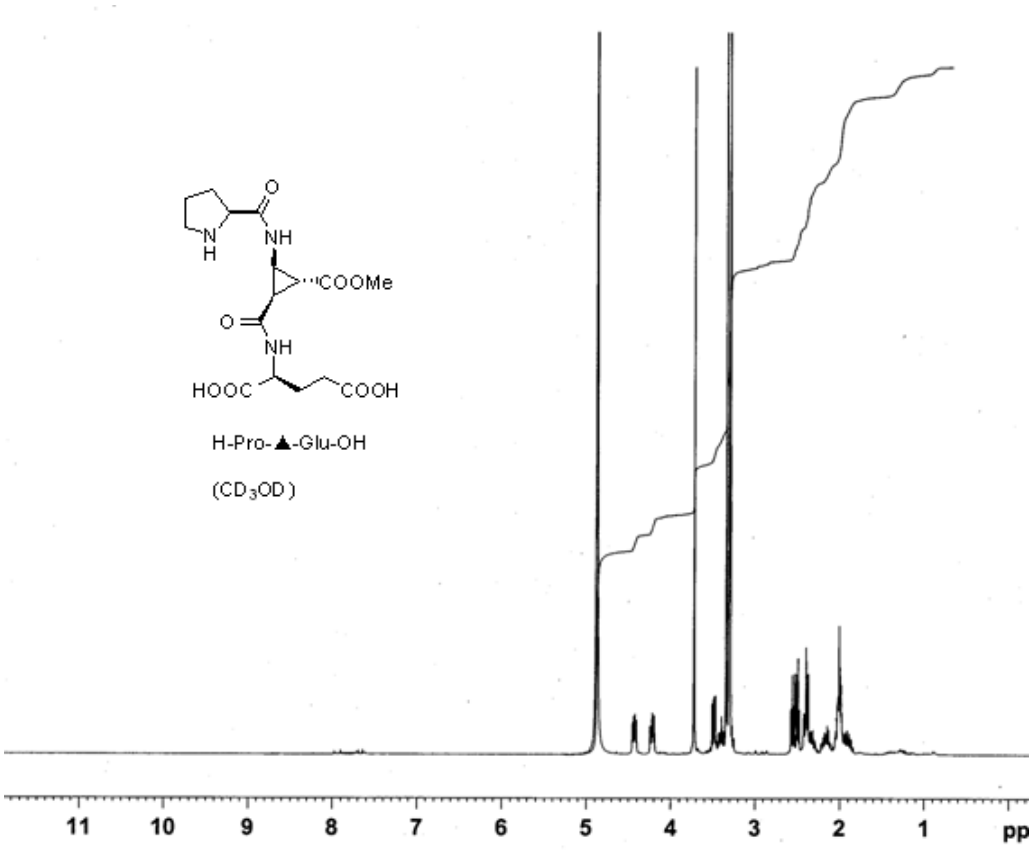

H-Pro-▲-Pro-▼-OH
(CD3OD) signal doubling
because of rotamers

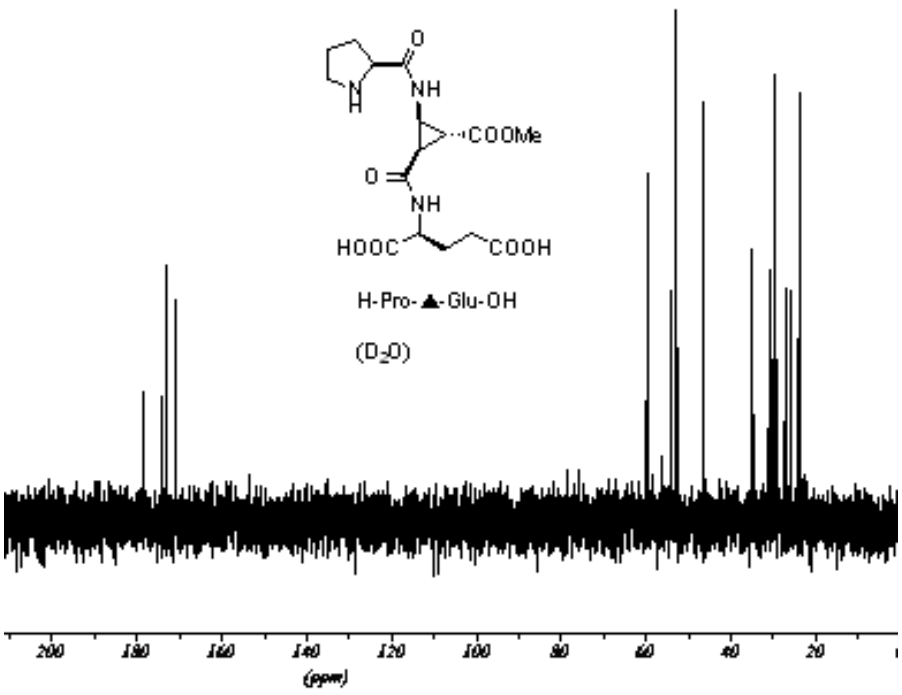

H-Pro-▼-Pro-Pro-OH
(CD3OD) signal doubling
because of rotamers.
(ppm)

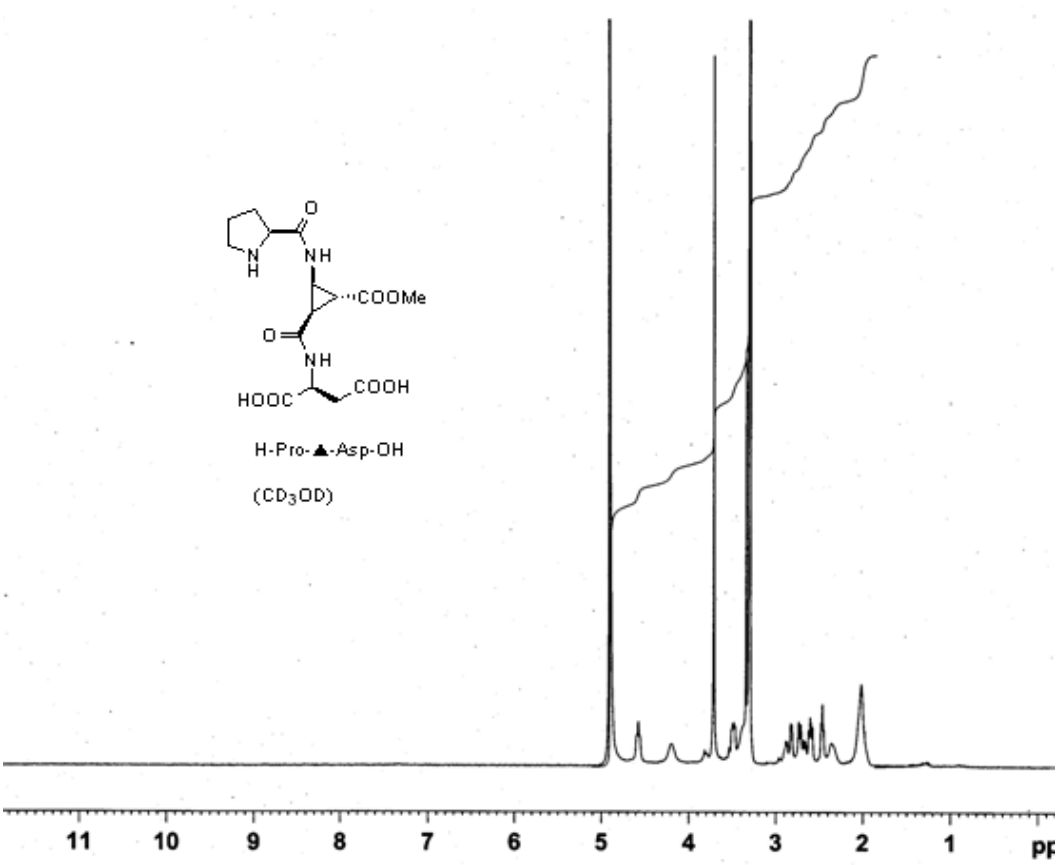

H-Pro-▼-Pro-Pro-OH
(CD3OD) signal doubling
because of rotamers.
(ppm)

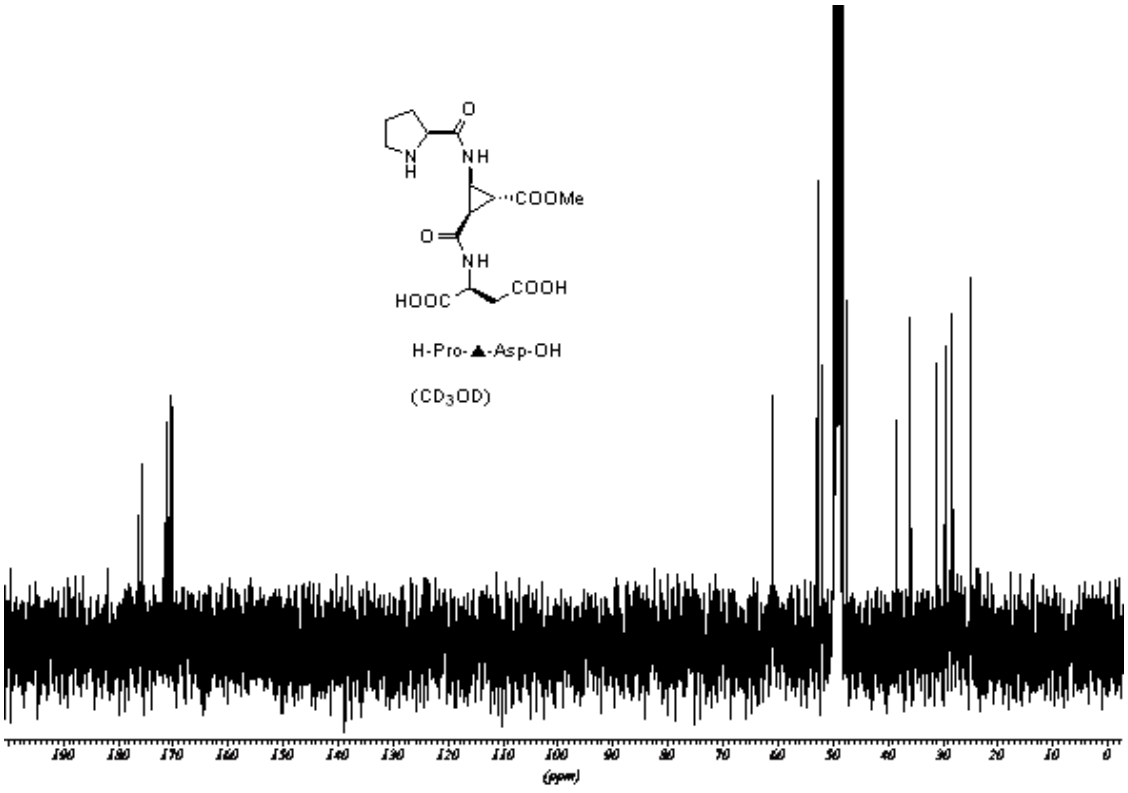

H-Pro-β-Ala-Pro-OH
(CD3OD)
11
10
9
8
7
6
5
4
3
2
1
p

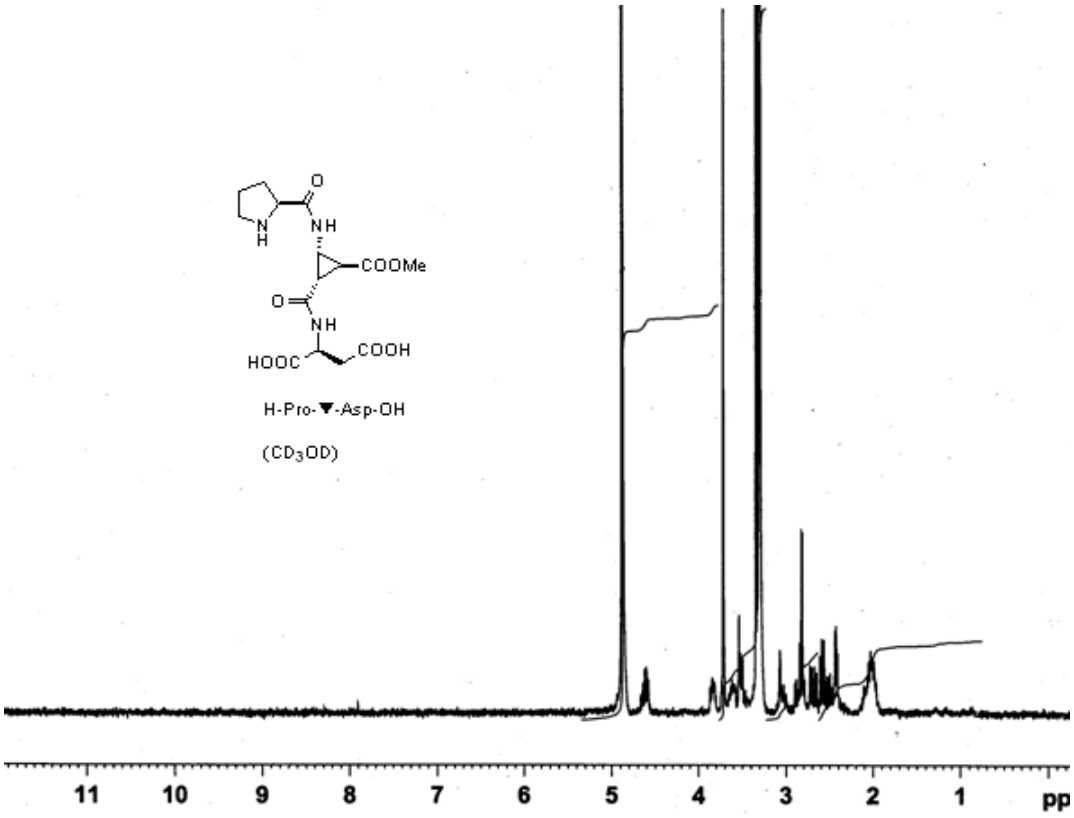

H-Pro-β-Ala-Pro-OH
(CD3OD)
(ppm)

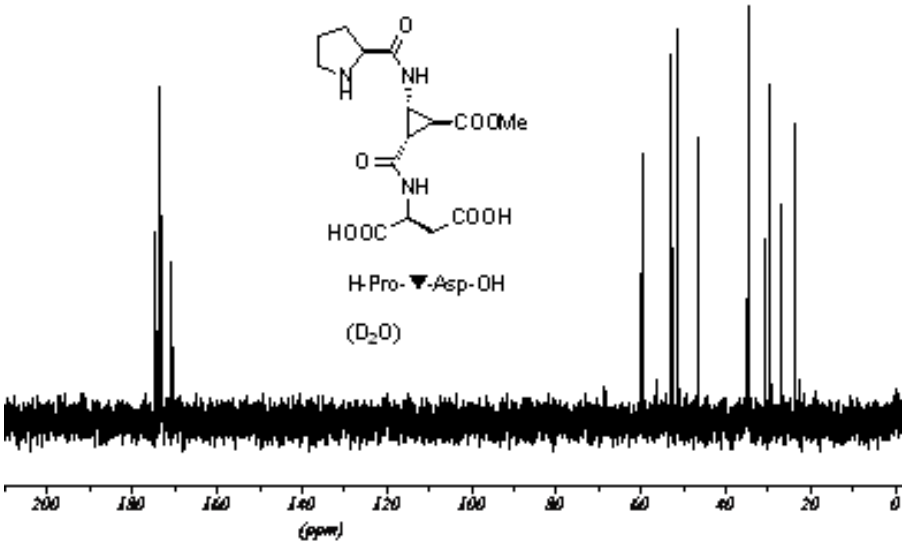

H-Pro-▲-Glu-OH
(CD3OD)
11
10
9
8
7
6
5
4
3
2
1
pp

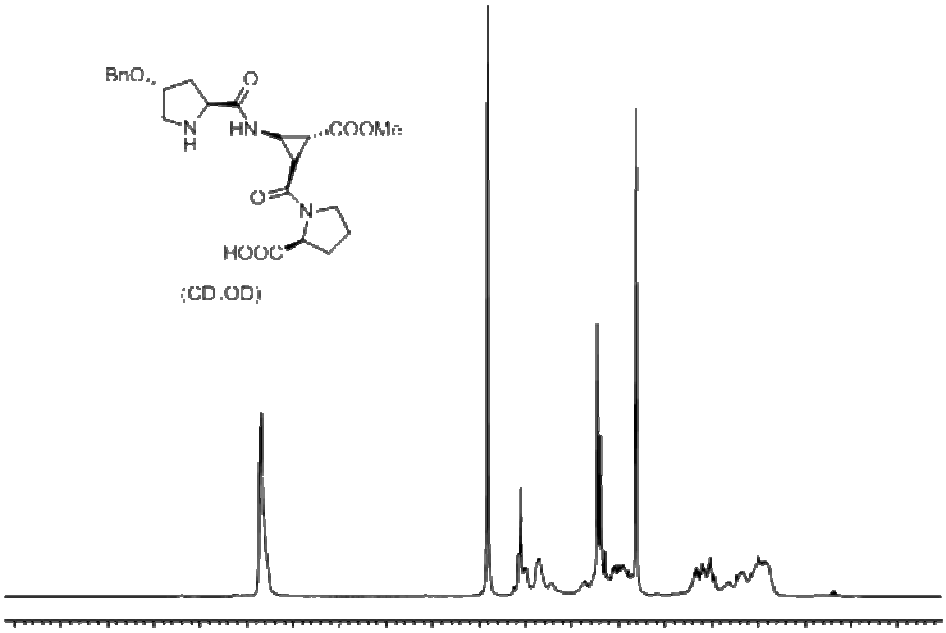

H-Pro-▲-Glu-OH
(D2O)
200
180
160
140
120
100
80
60
40
20
(ppm)

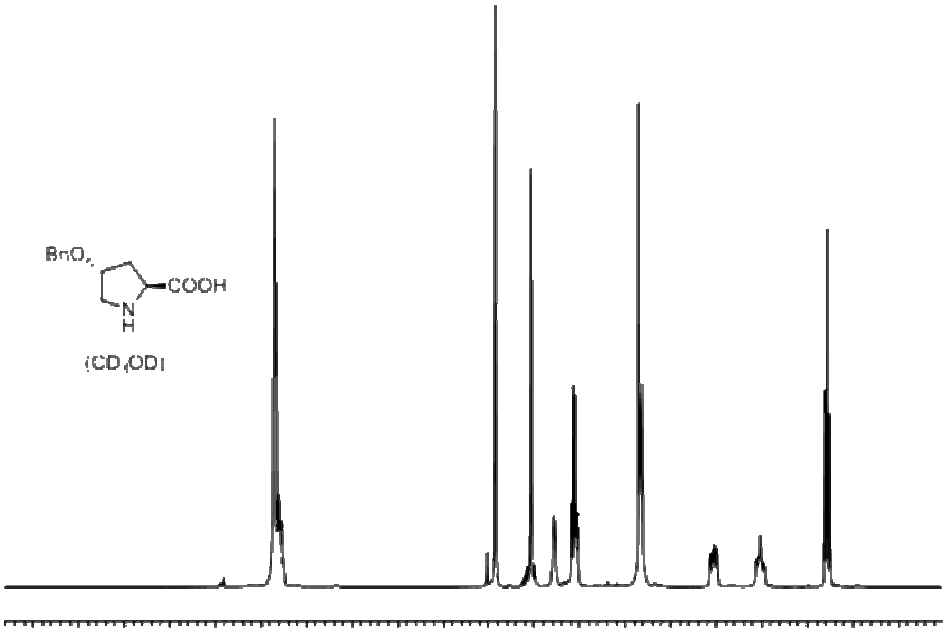

H-Pro-▲-Asp-OH
(CD3OD)
11
10
9
8
7
6
5
4
3
2
1

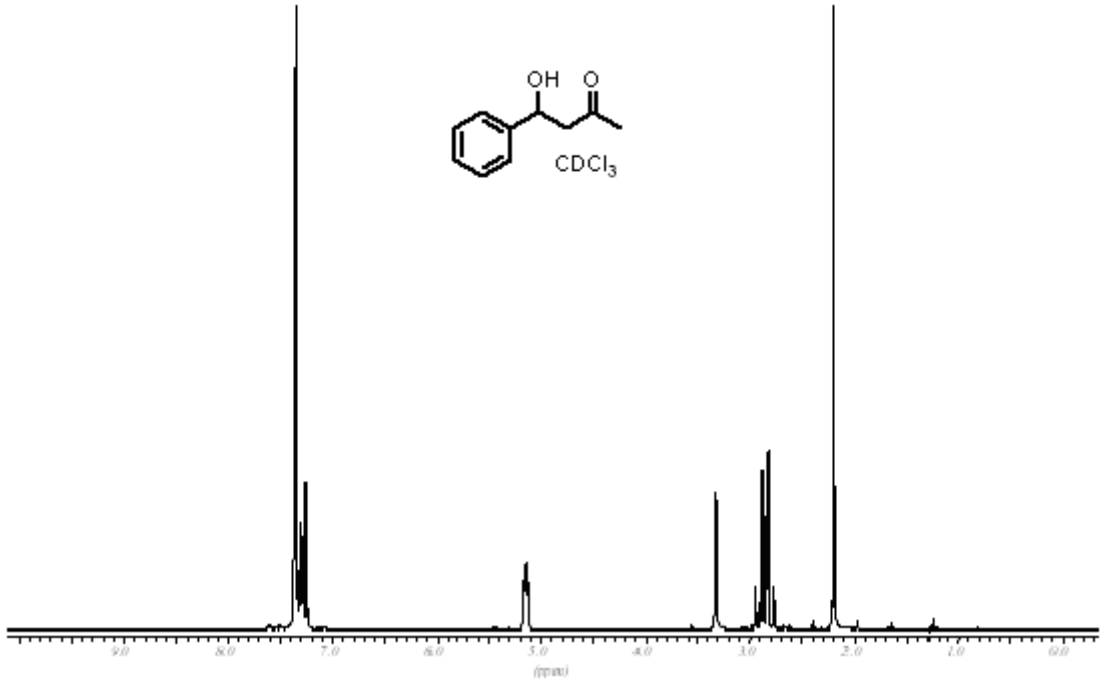

H-Pro-▲-Asp-OH
(CD3OD)
(ppm)

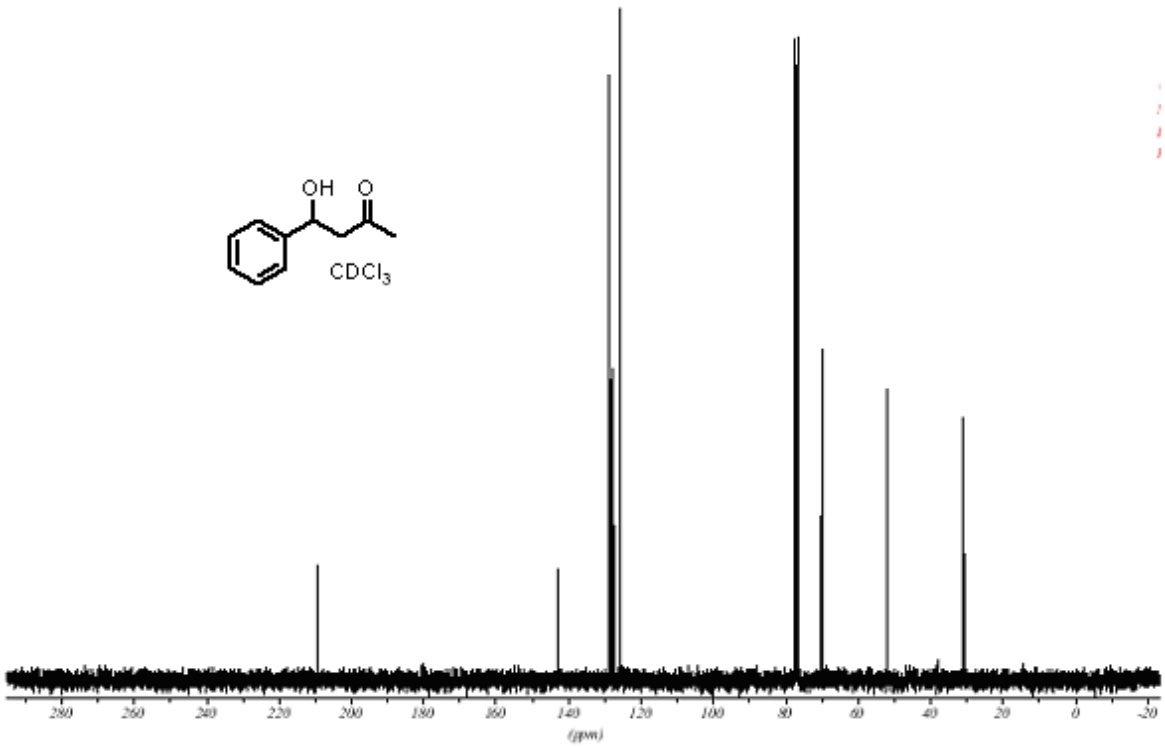

H-Pro-▼-Asp-OH
(CD3OD)
11
10
9
8
7
6
5
4
3
2
1
pp

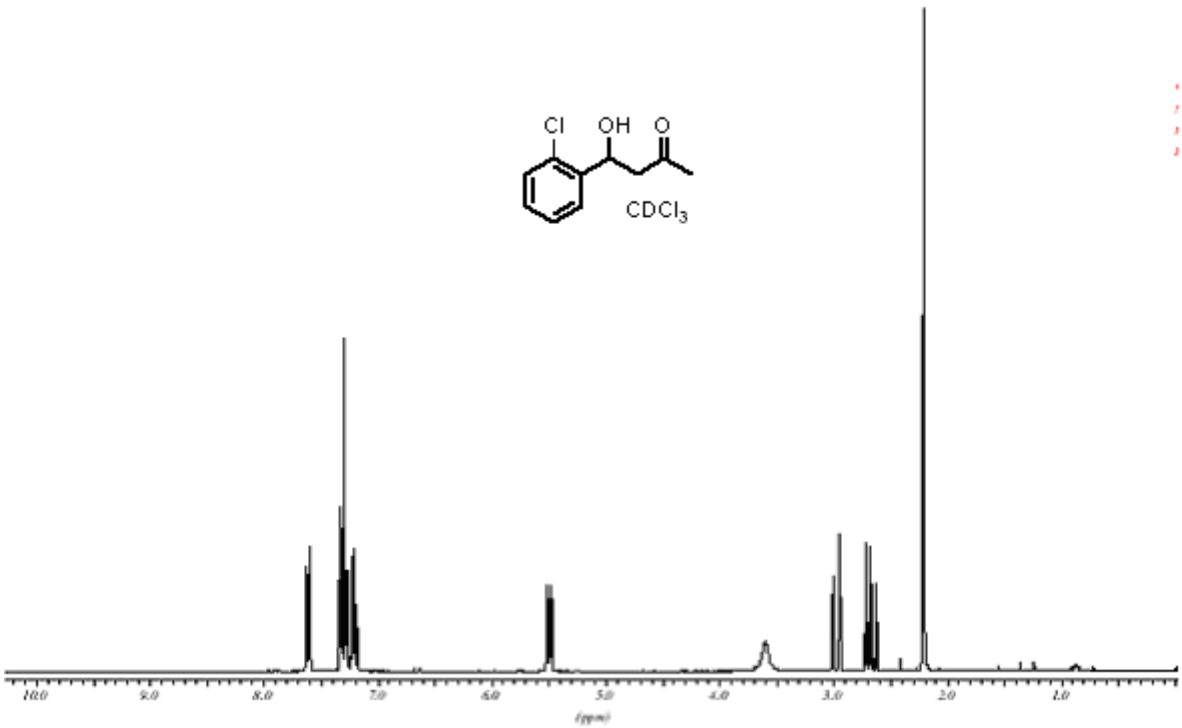

H-Pro-▼-Asp-OH
(D2O)
200
180
160
140
120
100
80
60
40
20
0
(ppm)

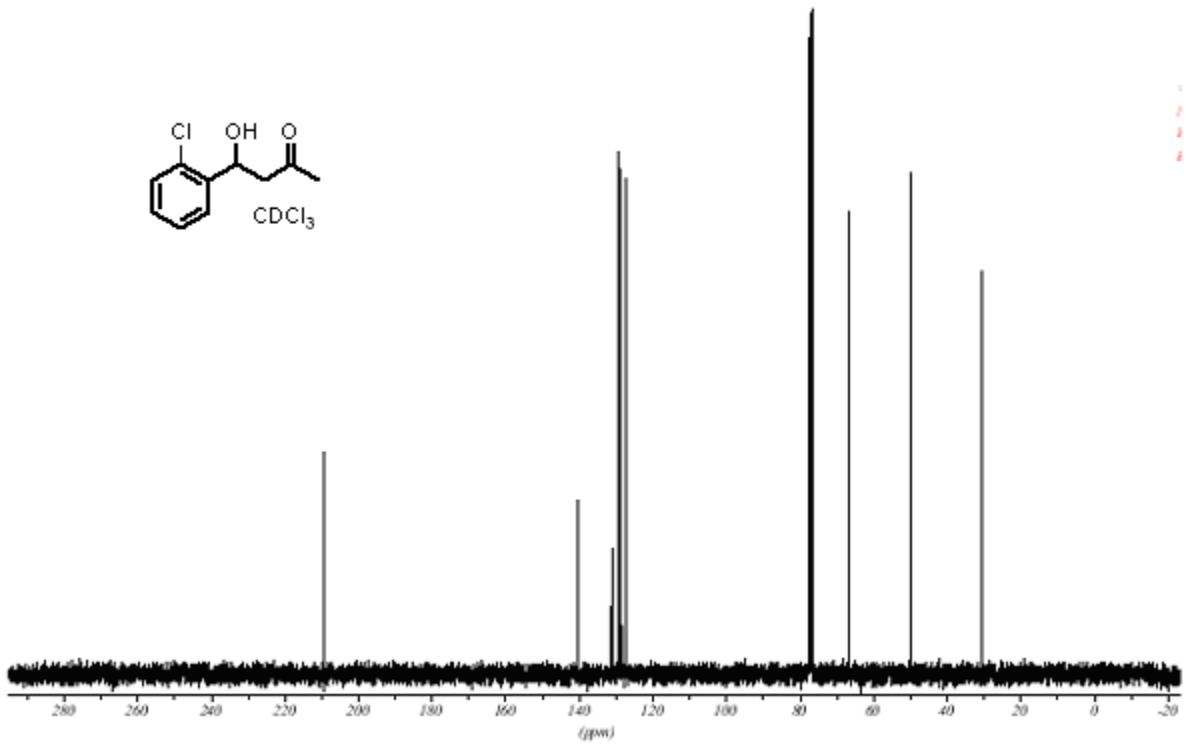

BnO
O
N
H
HN
COOMe
O
N
HOOC
(CD3OD)

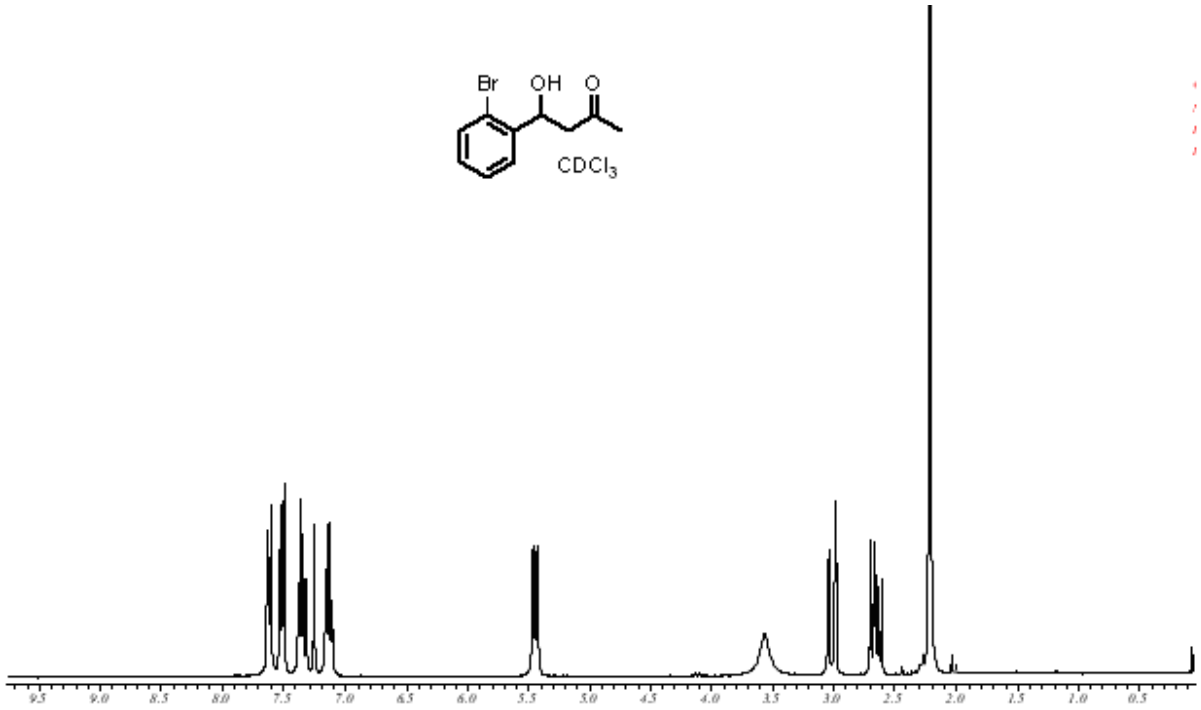

BnO
COOH
N
H
(CD3OD)

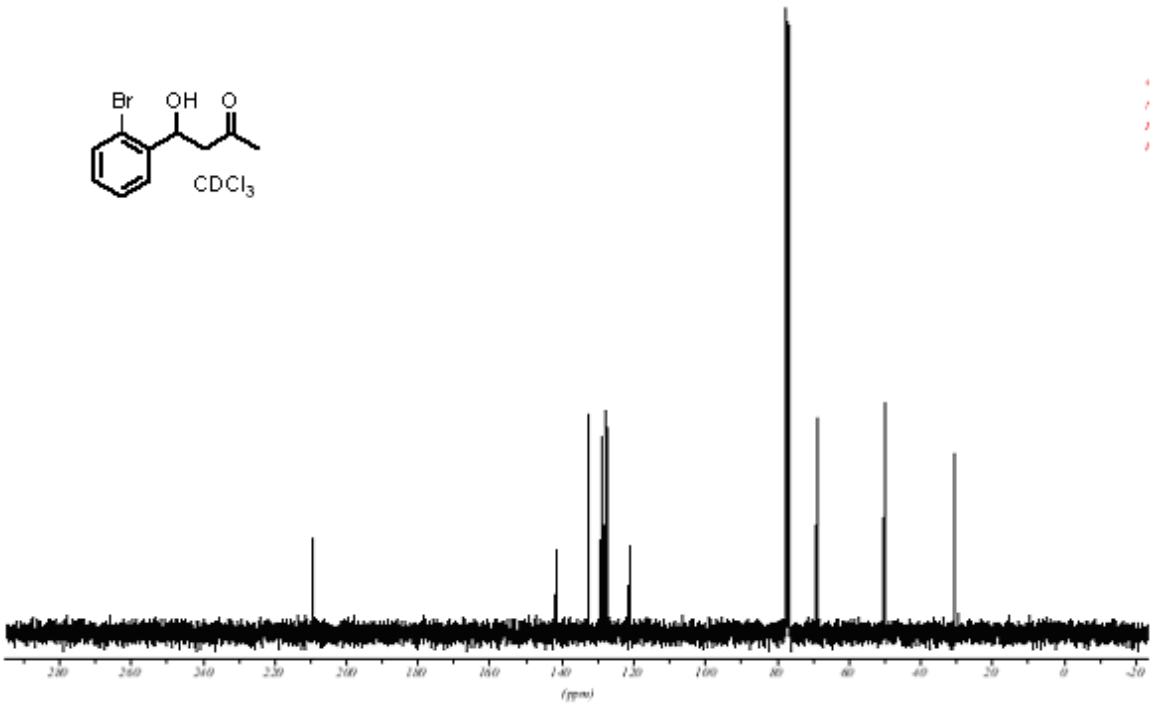

OH
O
CDCl3
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)

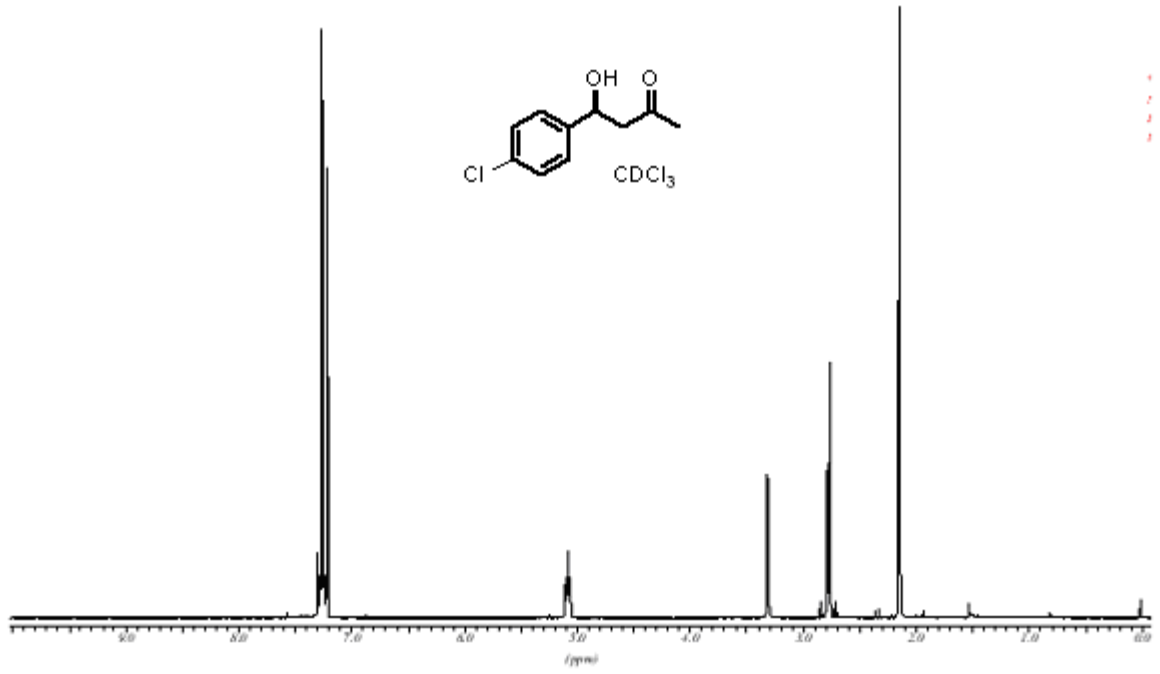

OH
O
CDCl3
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
-20
(ppm)

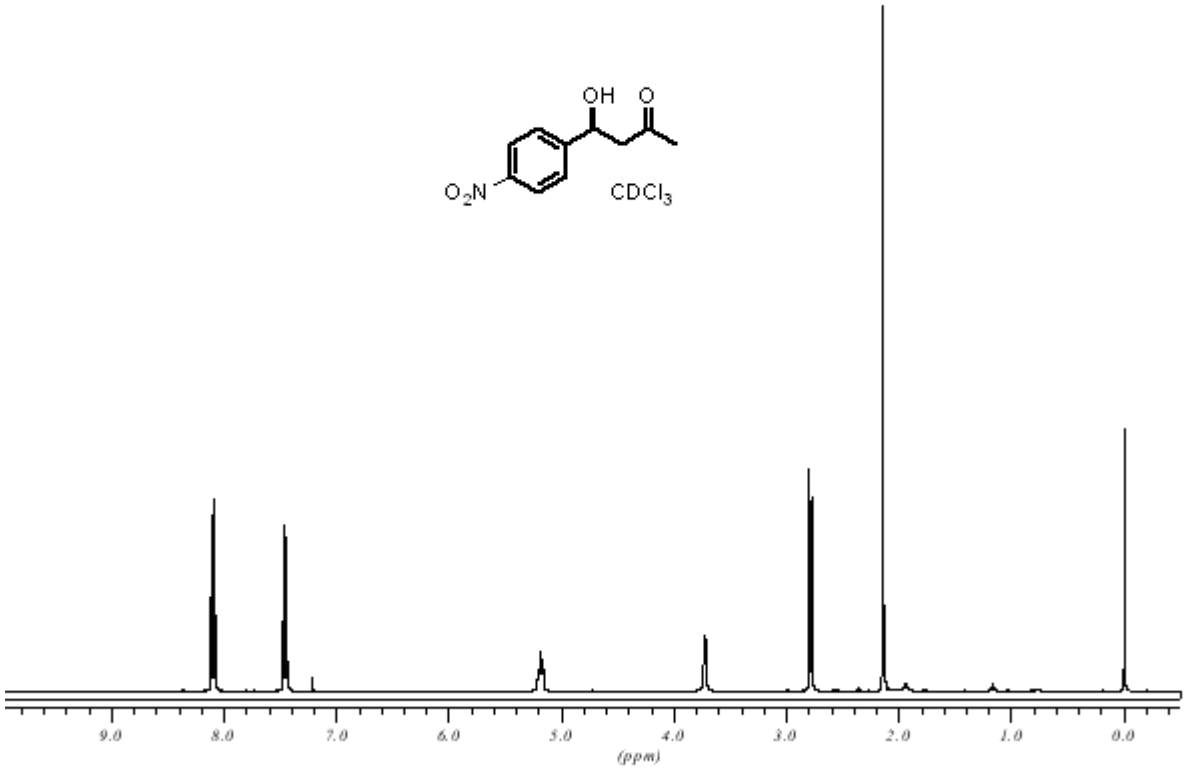

Cl
OH
O
CDCl3
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
(ppm)

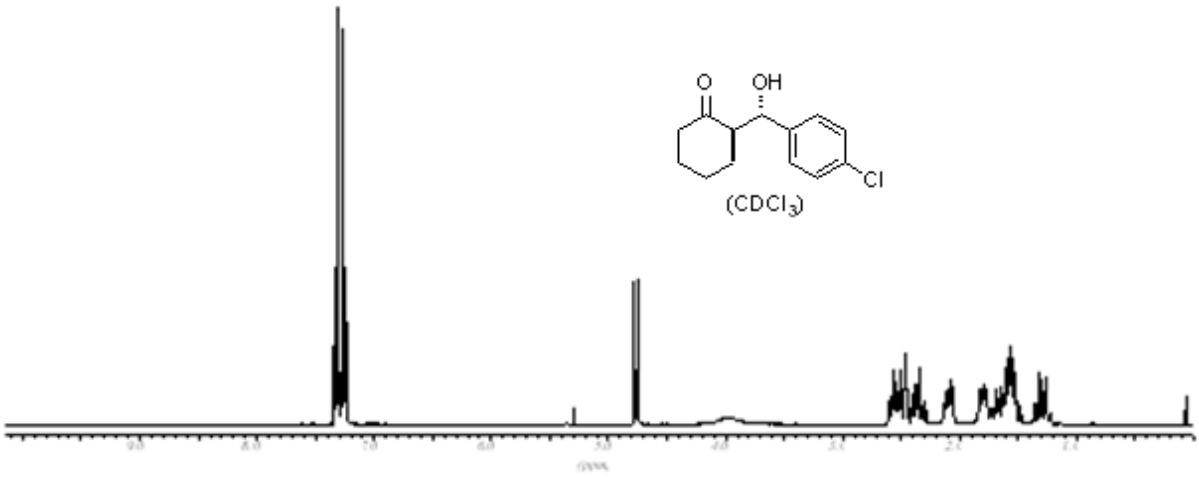

Cl
OH
O
CDCl3
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
-20
(ppm)

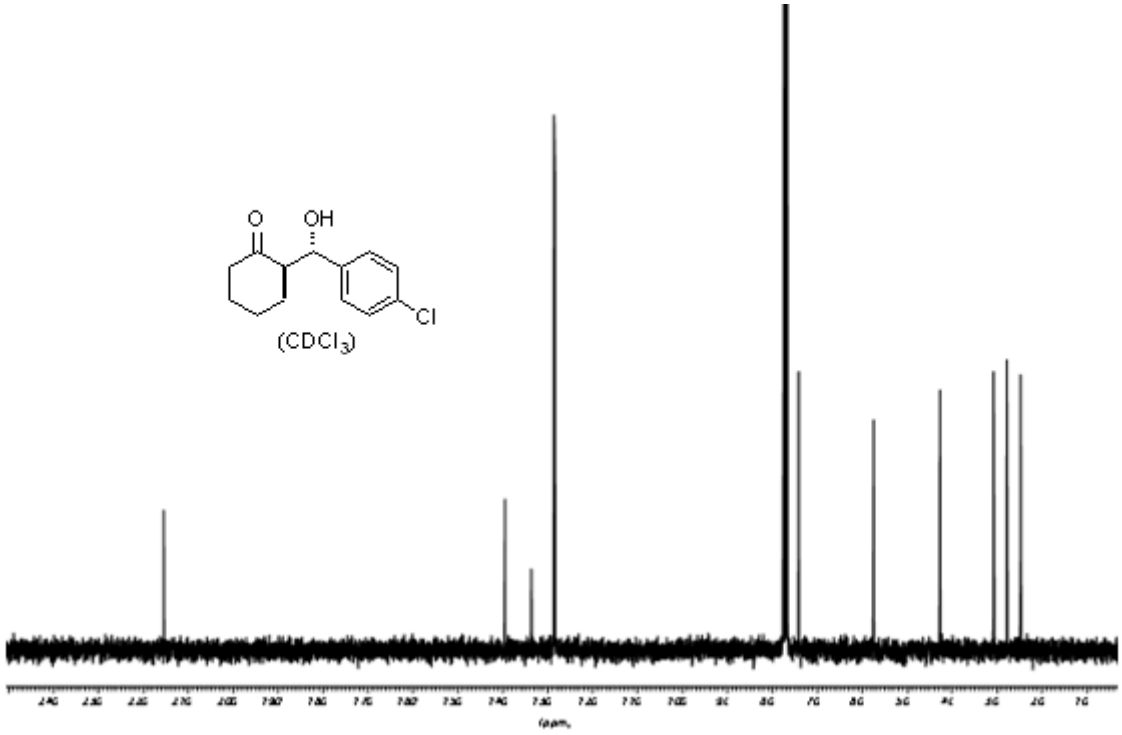

Br
OH
O
CDCl3

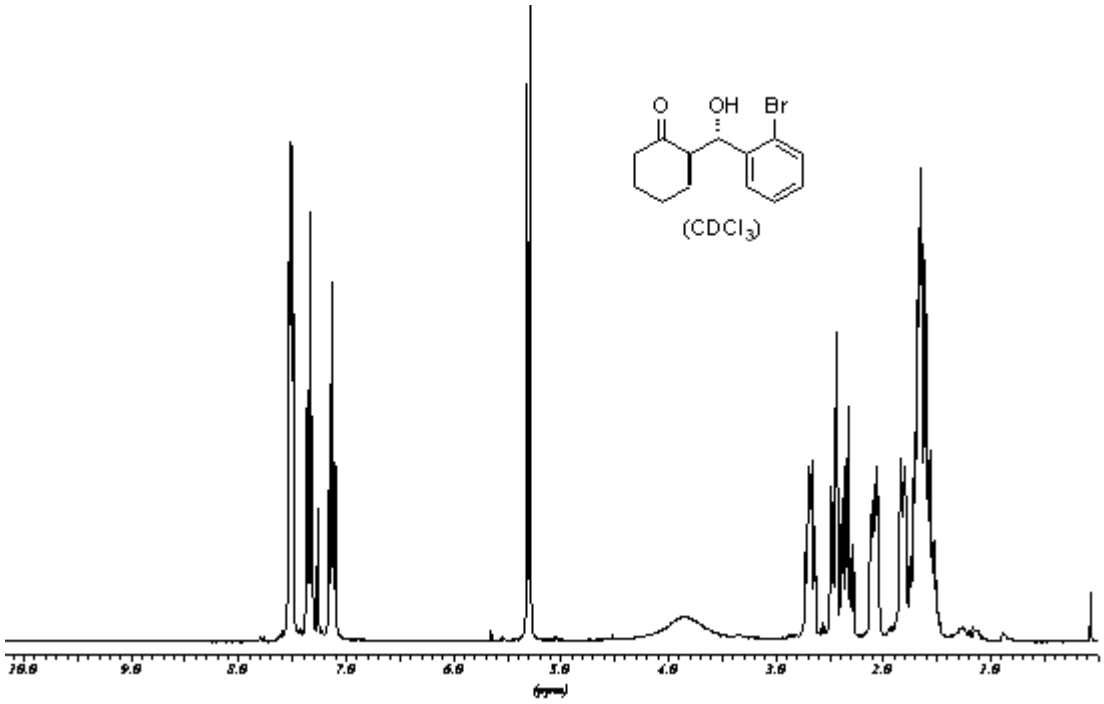

Br
OH
O
CDCl3

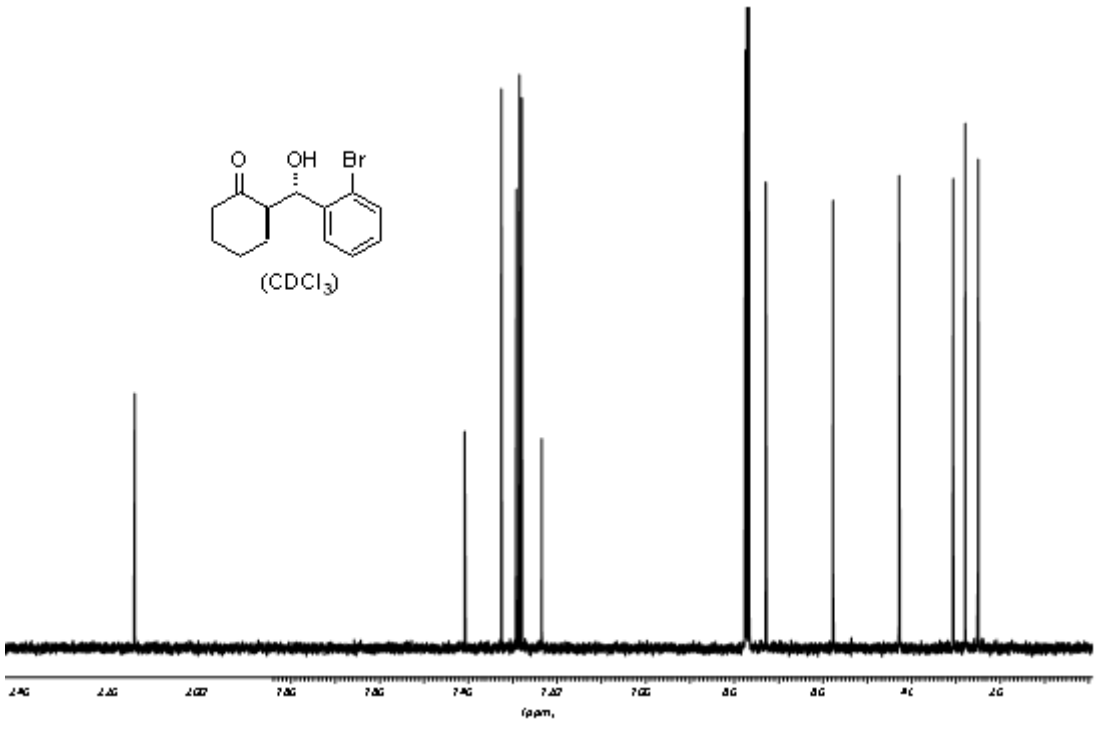

OH
O
Cl
CDCl3
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)

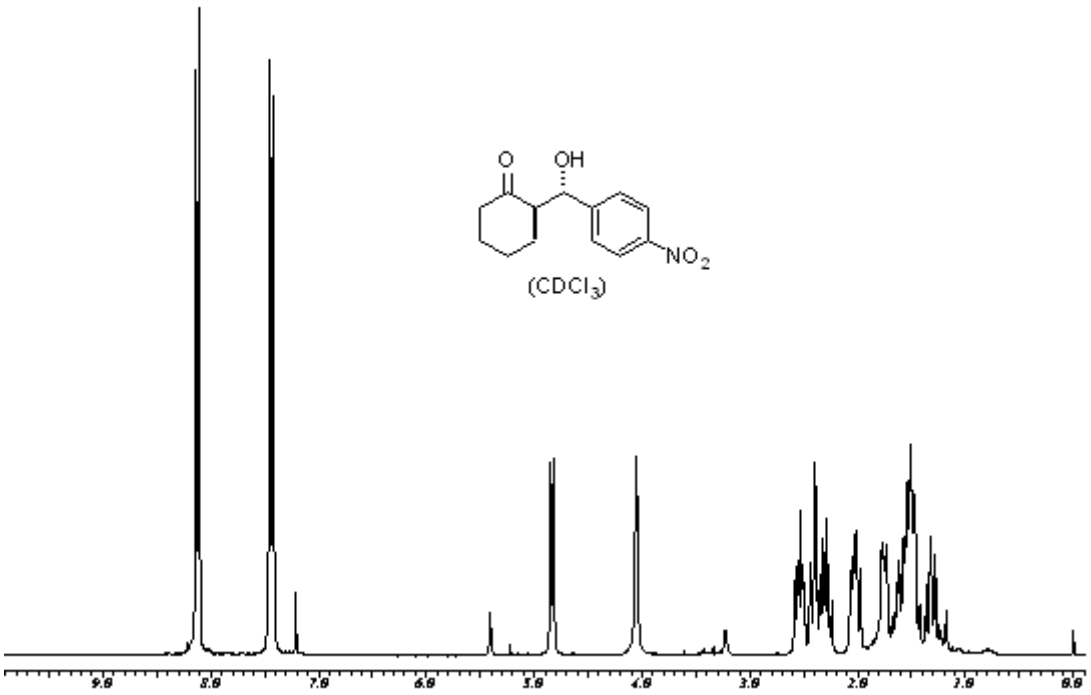

OH
O
O2N
CDCl3
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)

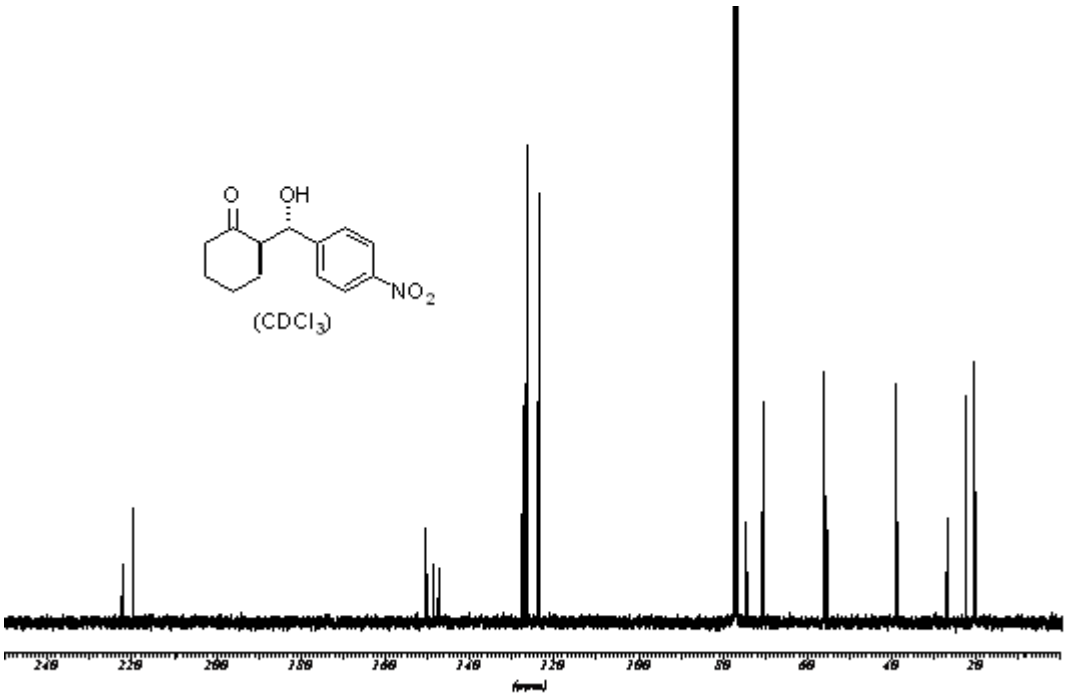

O
OH
Cl
(CDCl3)

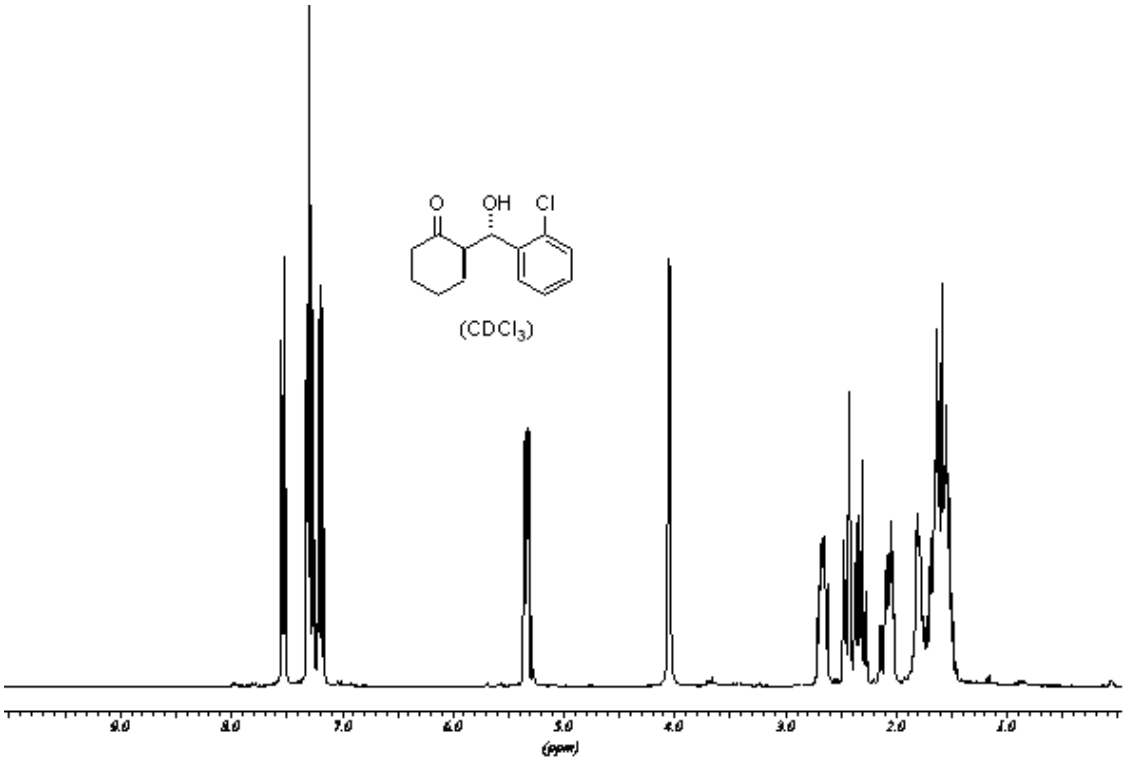

O
OH
Cl
(CDCl3)

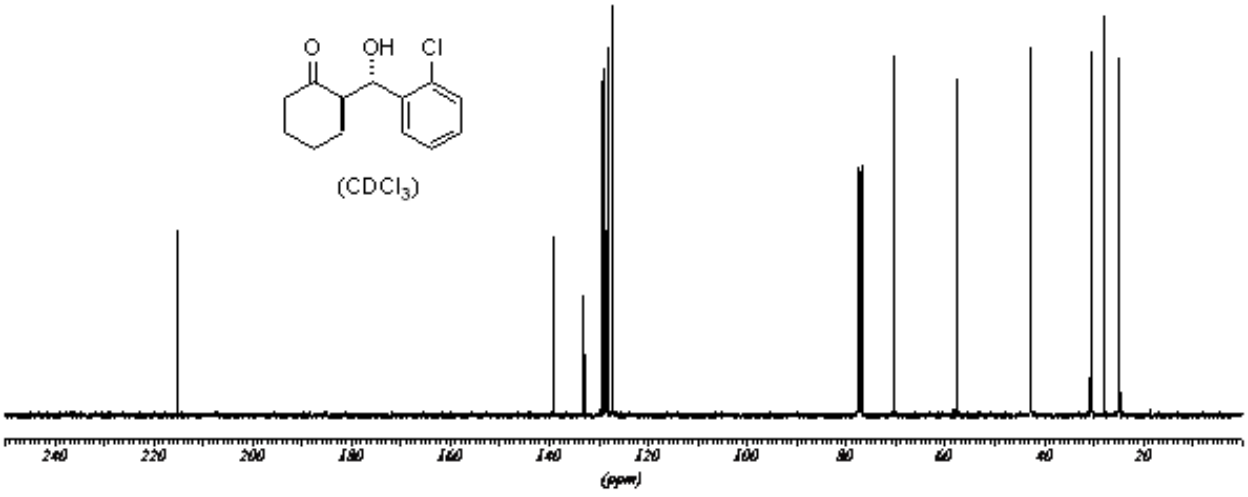

O
OH
Br
(CDCl3)

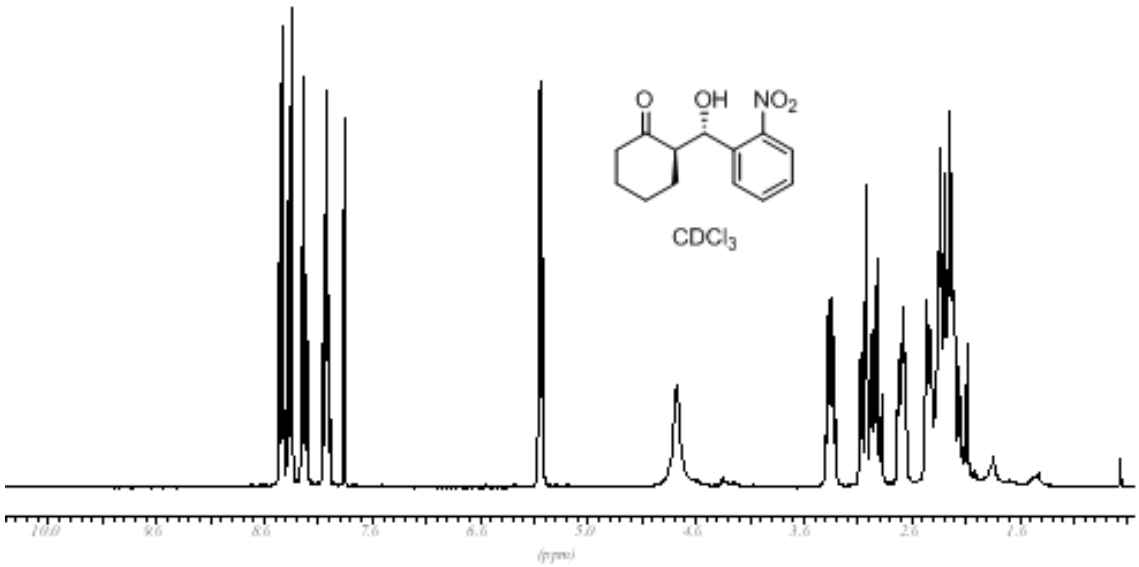

O
OH
Br
(CDCl3)

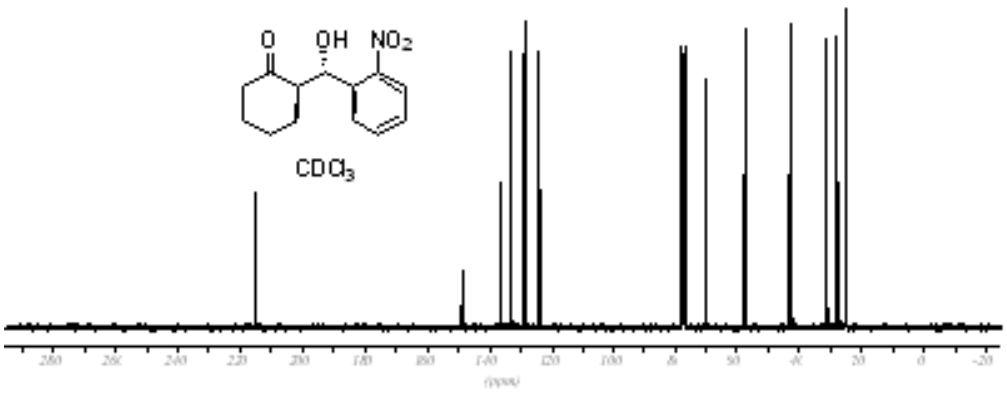

O
OH
NO2
(CDCl3)

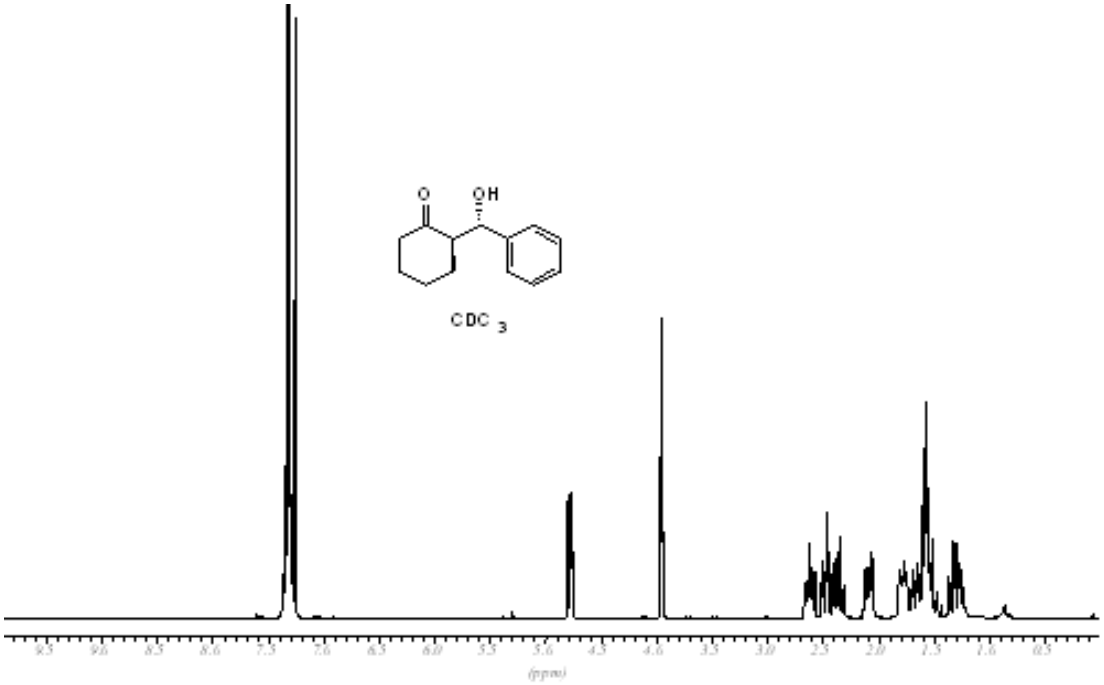

O
OH
NO2
(CDCl3)

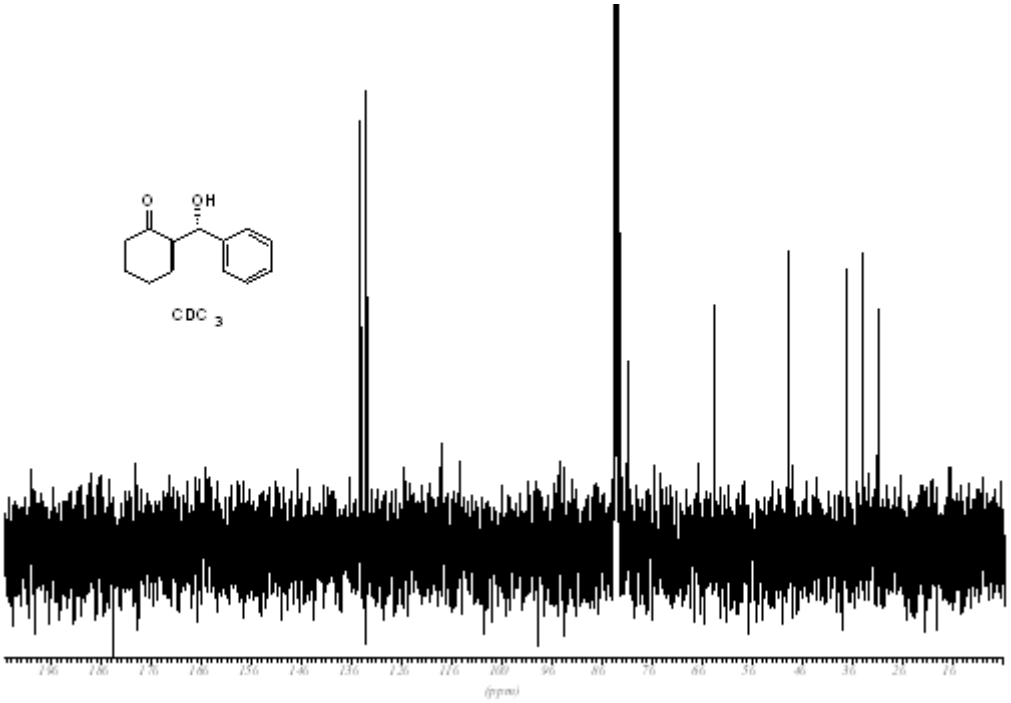

O
OH
Cl
(CDCl3)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
(ppm)

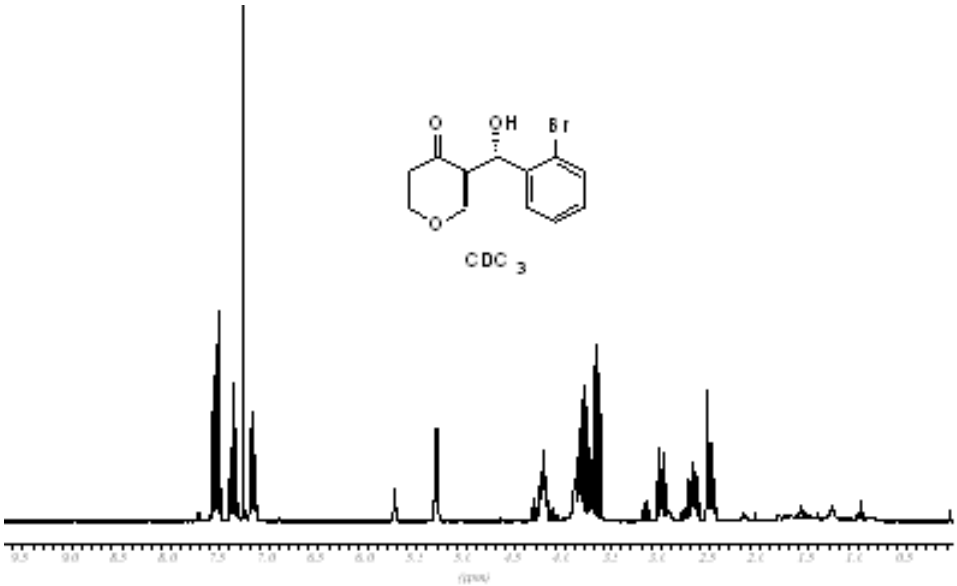

O
OH
Cl
(CDCl3)
240
220
200
180
160
140
120
100
80
60
40
20
(ppm)

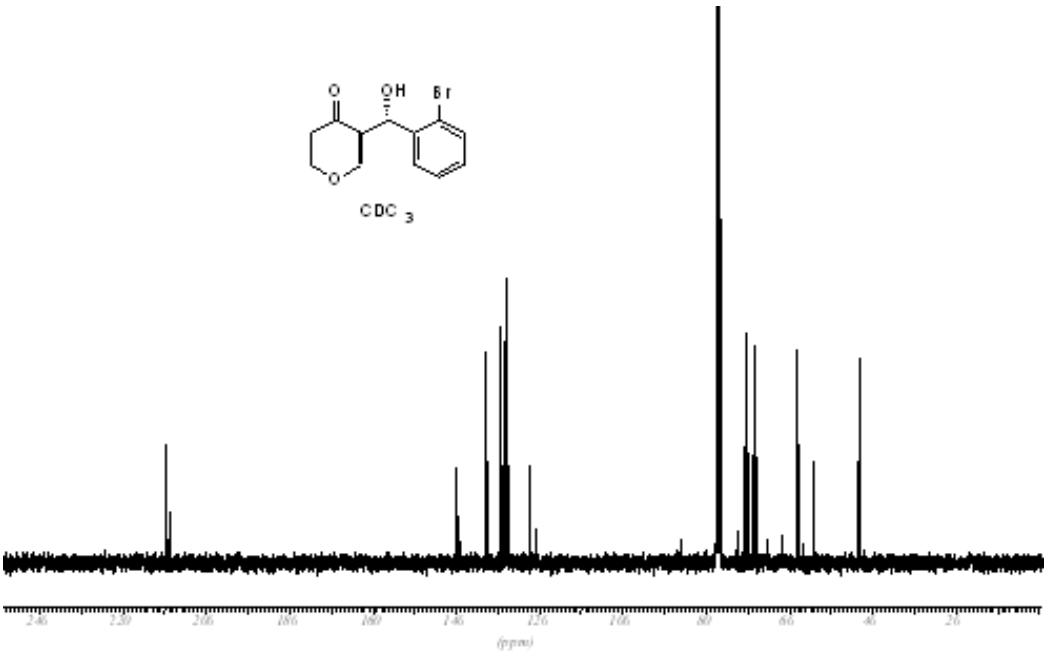

O
OH
NO2
CDCl3
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
(ppm)

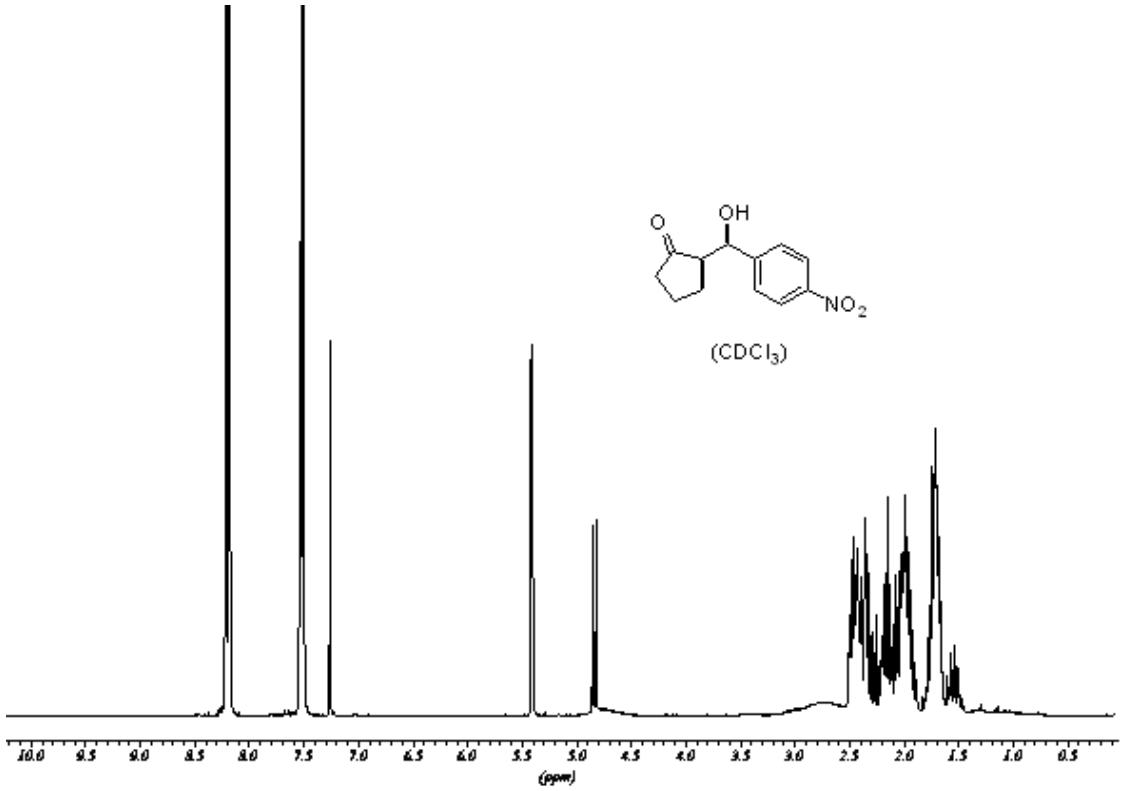

O
OH
NO2
CDCl3
(ppm)

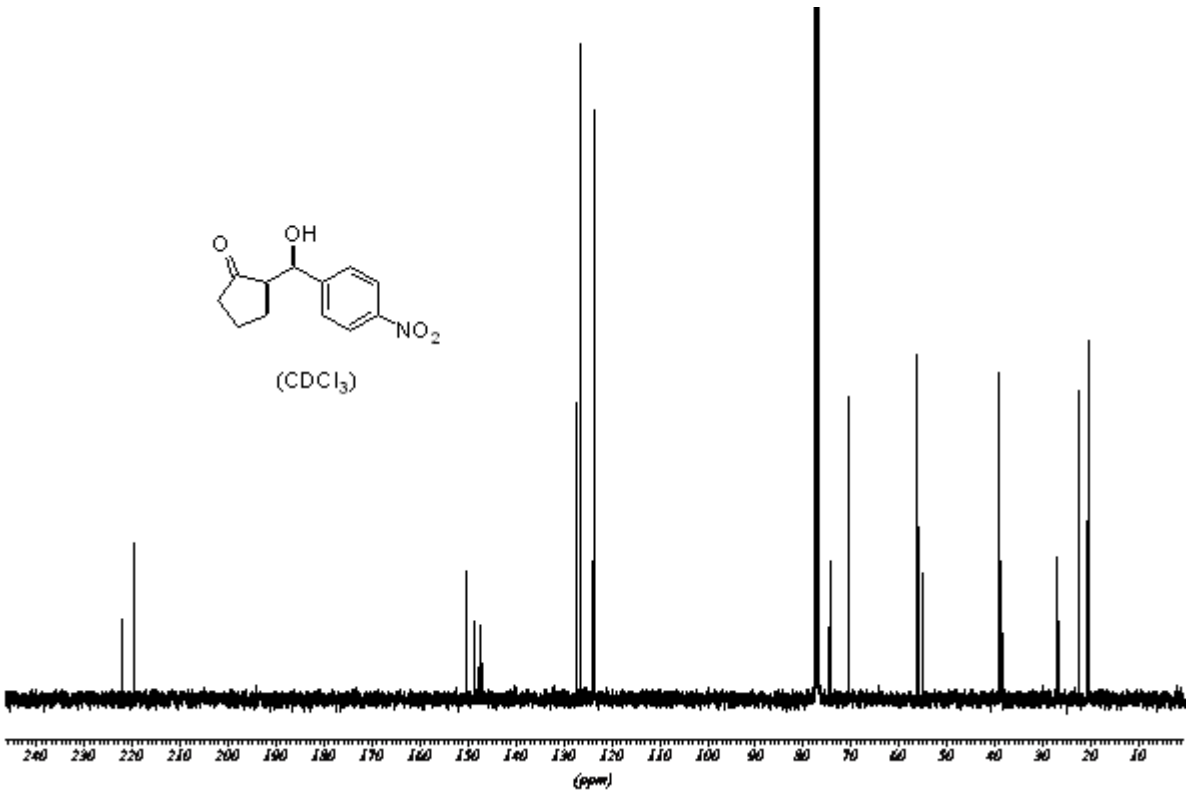

O
OH
CDC 3

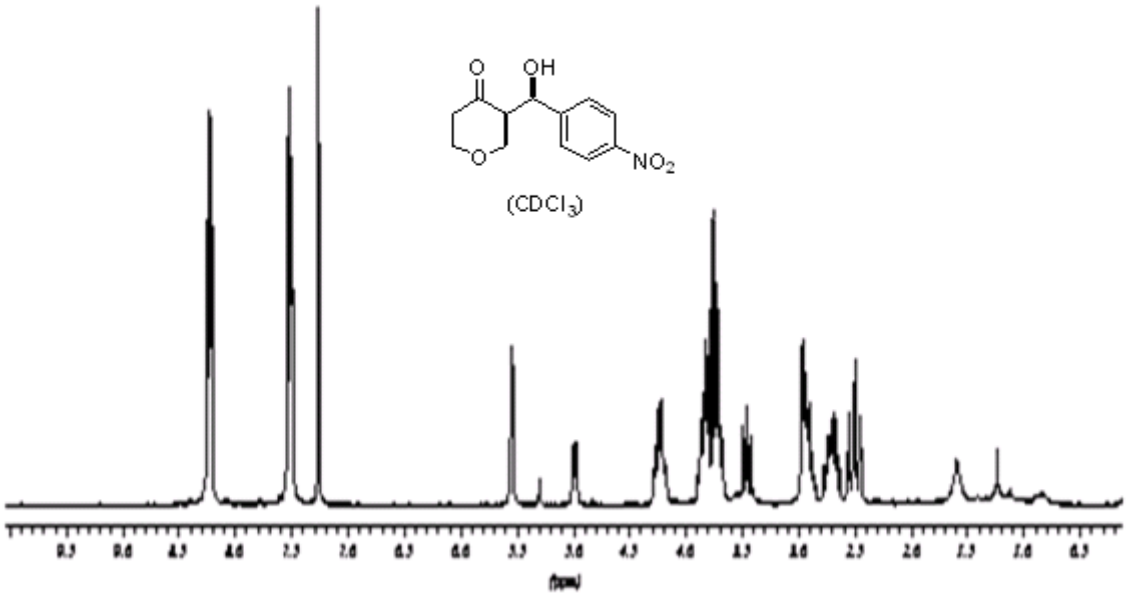

O
OH
CDC 3

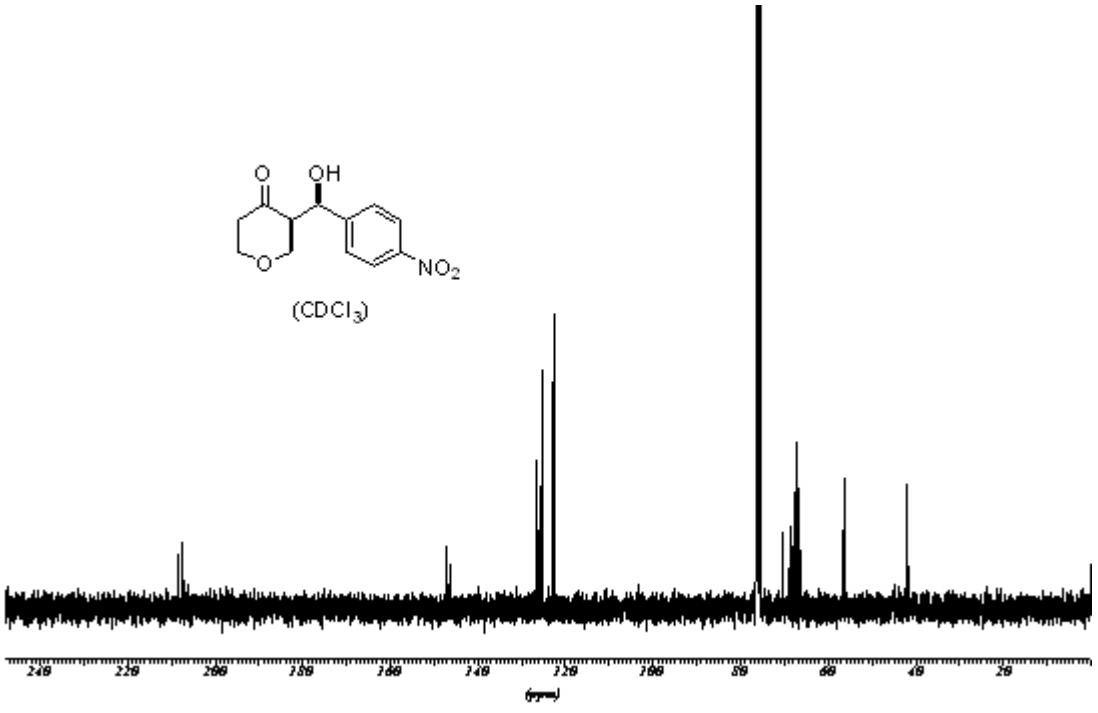

O
OH
Br
O
CDC 3

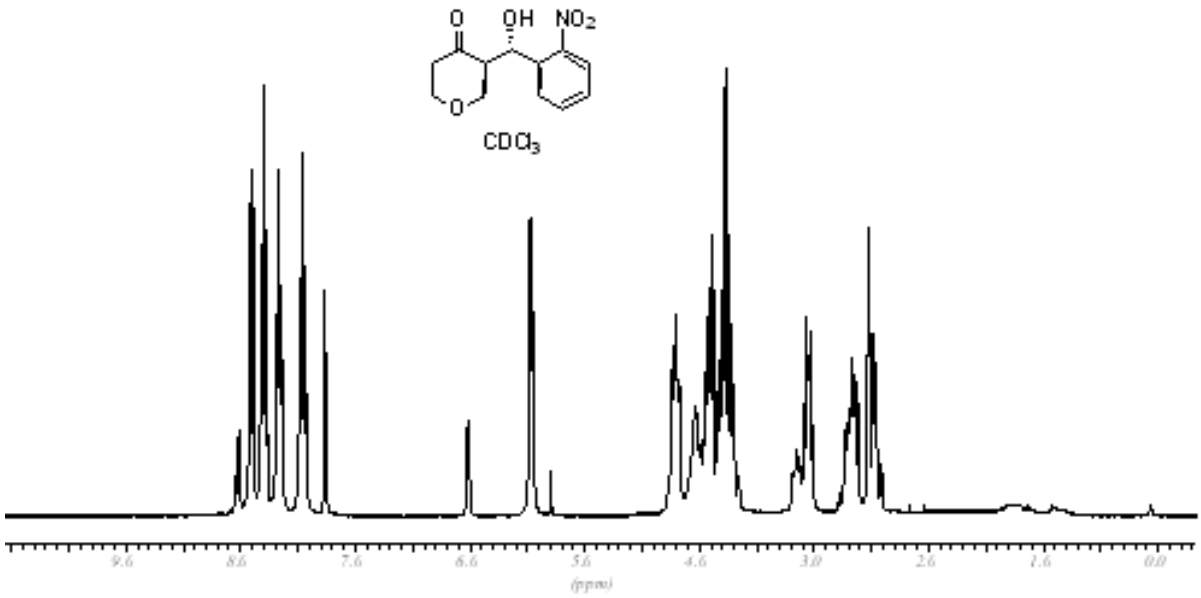

O
OH
Br
O
CDC 3

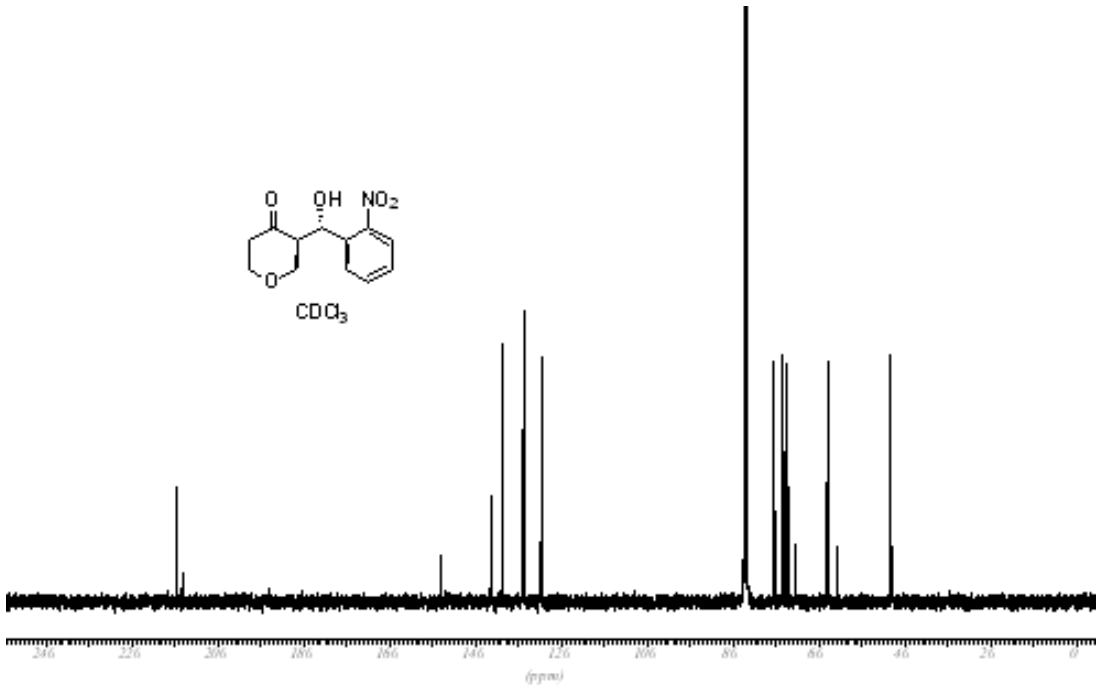

O
OH
NO2
(CDCl3)
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
(ppm)

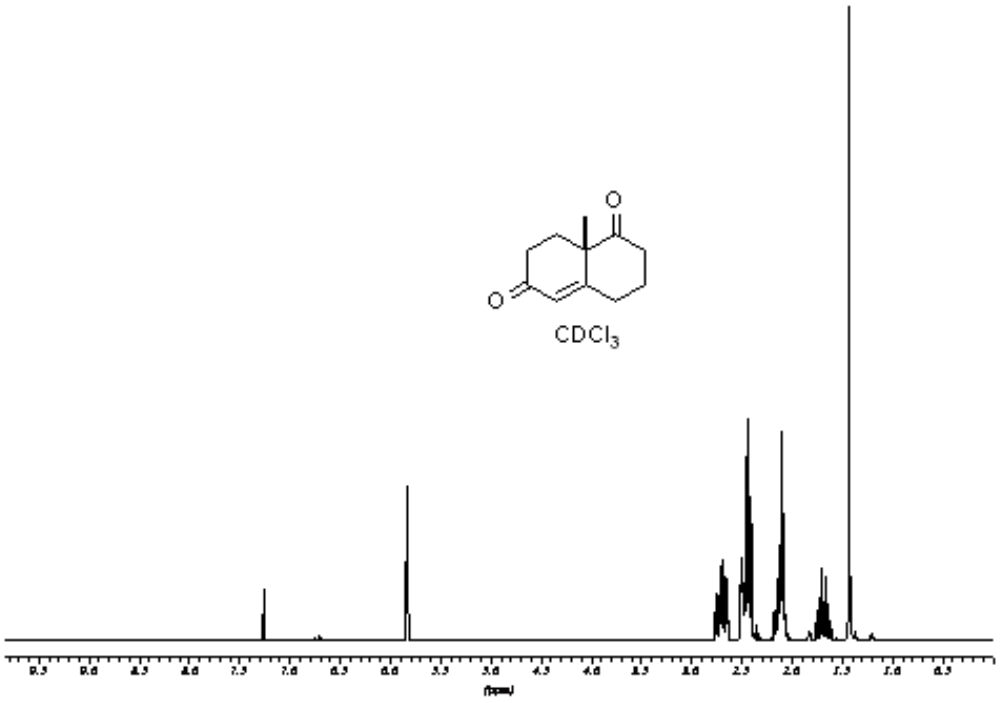

O
OH
NO2
(CDCl3)
240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(ppm)

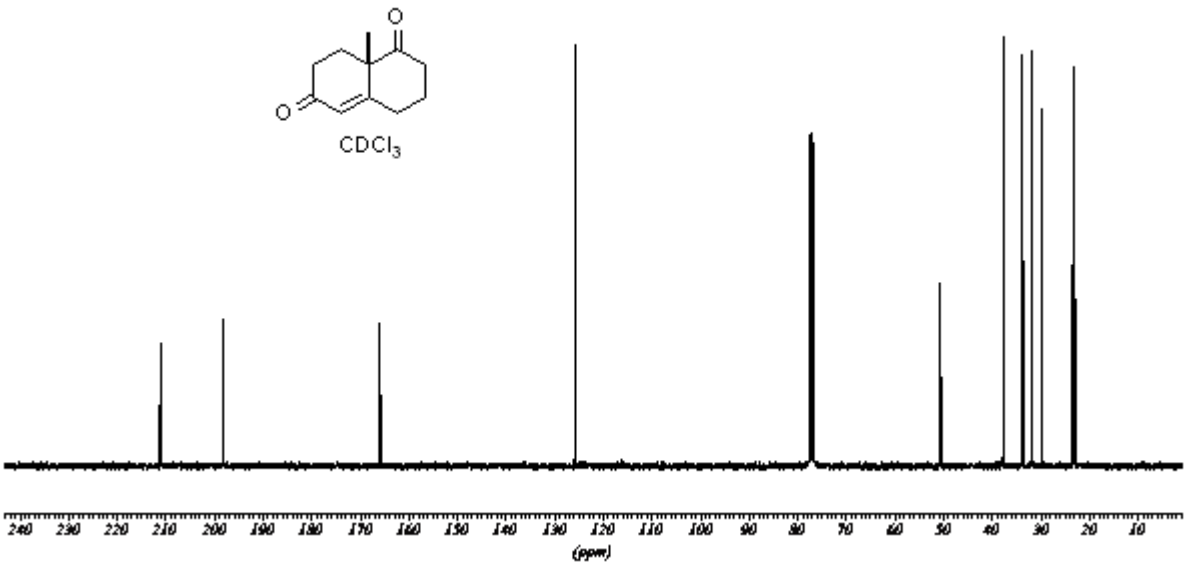

O
OH
NO2
(CDCl3)

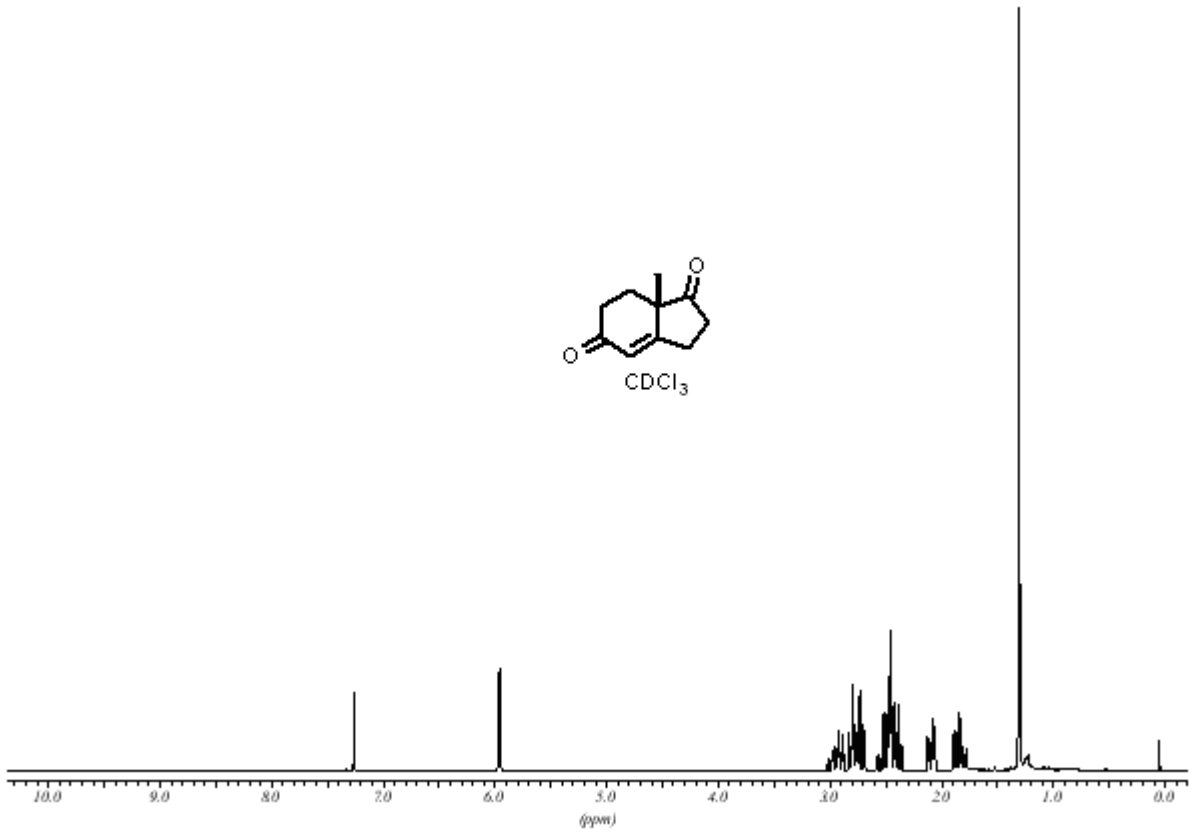

O
OH
NO2
(CDCl3)

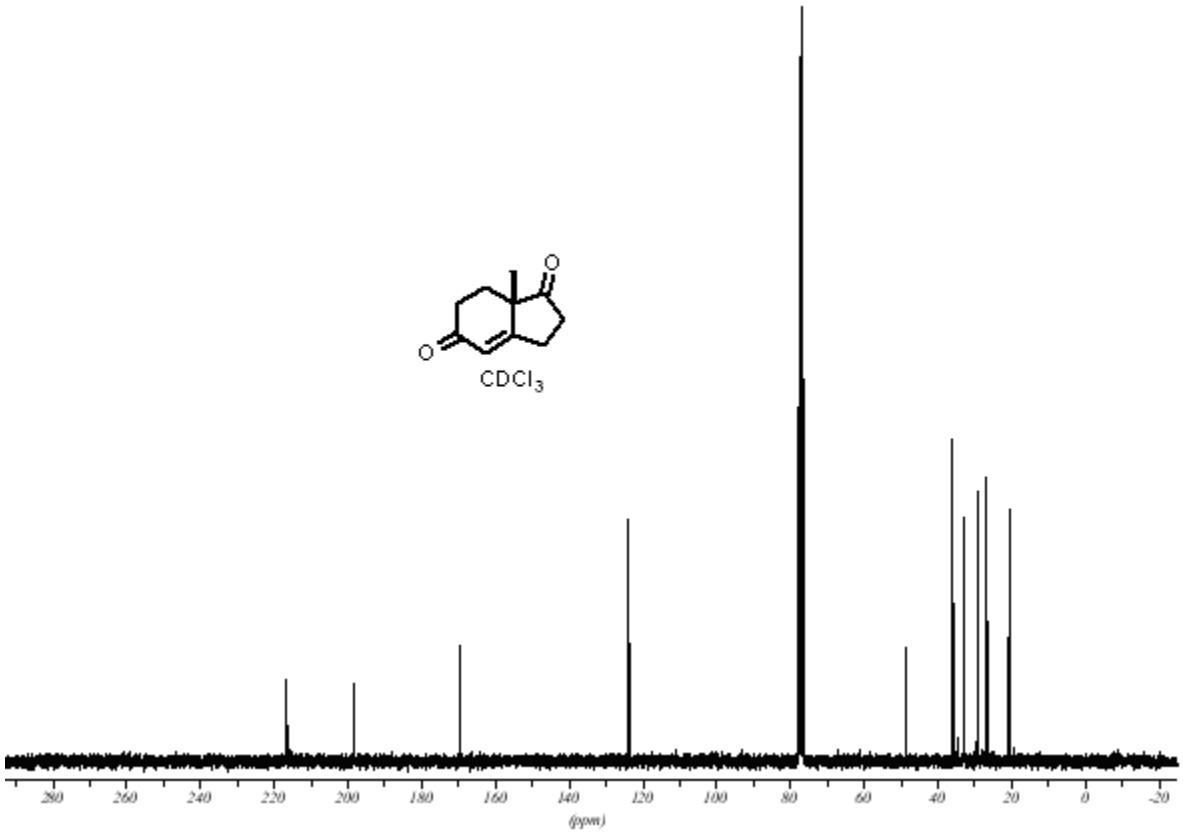

O
OH
NO2
O
CDCl3
9.6
8.6
7.6
6.6
5.6
4.6
3.0
2.6
1.6
0.0
(ppm)

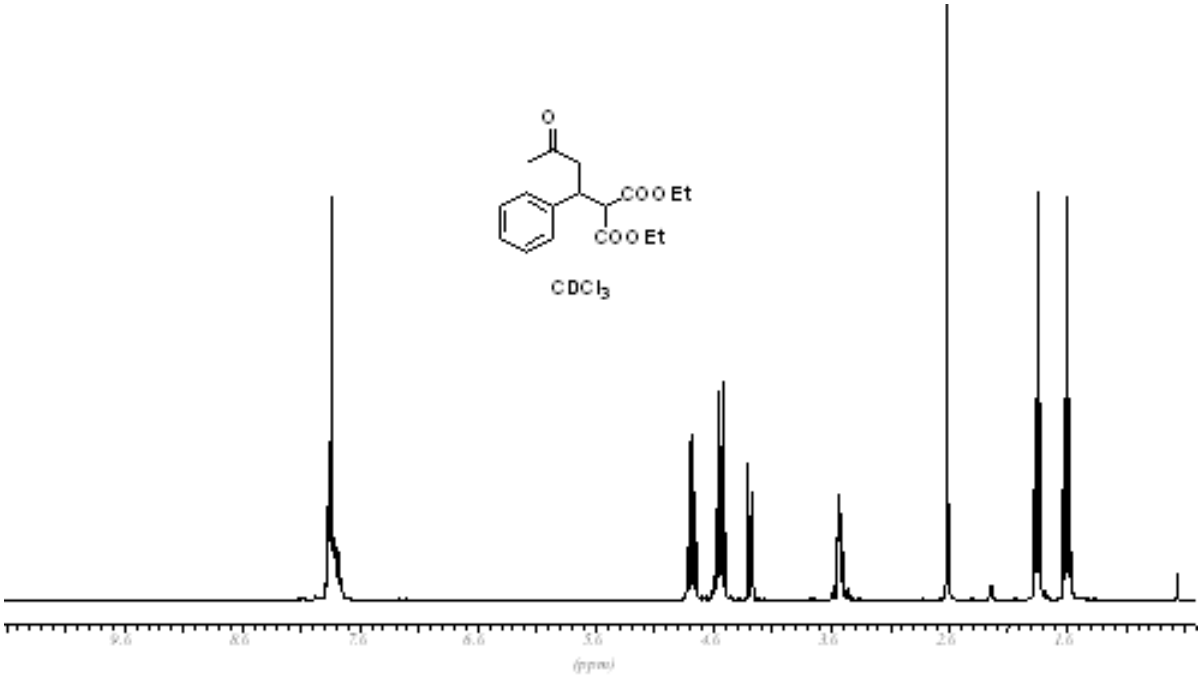

O
OH
NO2
O
CDCl3
246
226
206
186
166
146
126
106
86
66
46
26
0
(ppm)

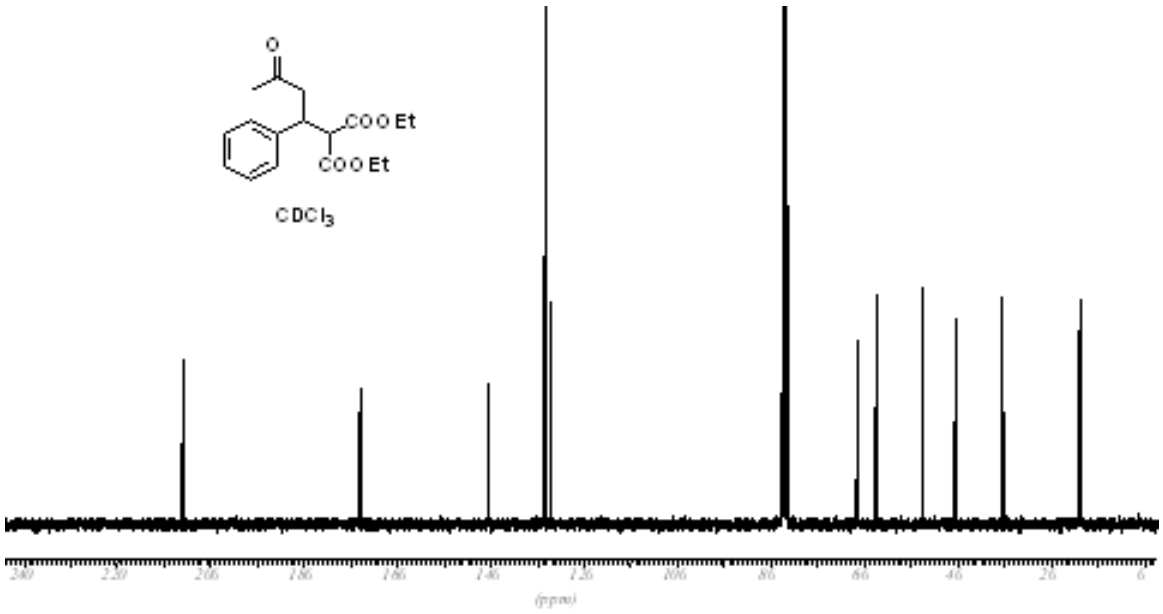

O
O
CDCl3


O
O
CDCl3
240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(ppm)


O
O
CDCl3
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)


O
O
CDCl3
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
-20
(ppm)


O
COO Et
COO Et
CDCl3


O
COO Et
COO Et
CDCl3

Ich erkläre hiermit an Eides statt, das ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe; die aus anderen Quellen direkt oder indirekt übernommenen Daten und Konzepte sind unter Angabe des Literaturzitats gekennzeichnet.

Regensburg, den
