Stereoselectivity of Proline / Cyclobutane Amino Acid-Containing Peptide Organocatalysts for Asymmetric Aldol Additions: a Rationale

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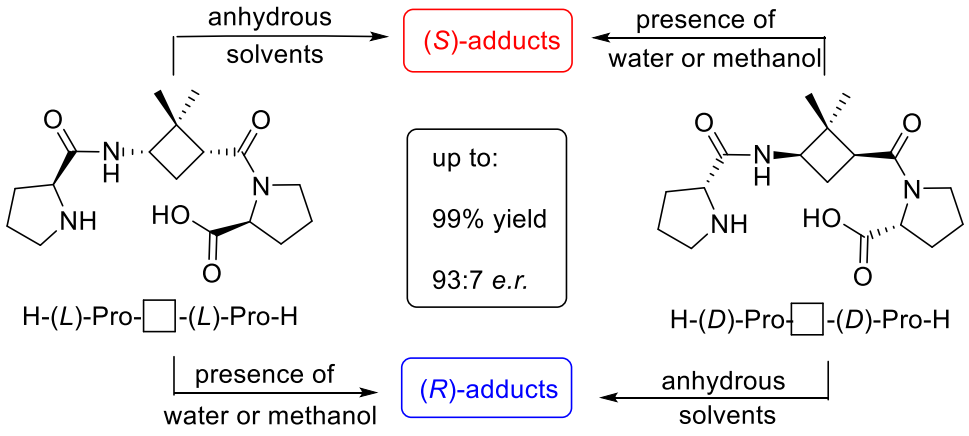
Abstract

Several α,β,α- or α,γ,α-tripeptides, consisting of a central cyclobutane β- or γ-amino acid being flanked by two (*D*)- or (*L*)-proline residues, have been synthesized and tested as organocatalysts in asymmetric aldol additions. High yields and enantioselectivities have been achieved with α,γ,α-tripeptides, being superior to the peptides containing a cyclobutane β-amino acid residue. This can probably be due to their high rigidity, which hinders the peptide catalysts to adopt the proper active conformation. This reasoning correlates with the major conformation of the peptides in the ground state, as suggested by ^{1}H NMR and computational calculations. The configuration of the aldol products is controlled by the proline chirality, and consequently, the *R/S* configuration of aldol products can be tuned by the use of either commercially available (*D*)- or (*L*)-proline enantiomers. The enantioselectivity in the aldol reactions is reversed if the reactions are carried out in the presence of water or other protic solvents such as methanol. Spectroscopic and theoretical investigations revealed that this effect is not the consequence of conformational changes in the catalyst but rather caused by the participation of a water molecule in the rate determining transition state, in such a way that the preferential nucleophilic attack is oriented to the opposite enantiotopic aldehyde face.

TOC



INTRODUCTION

The use of short peptides as catalysts in asymmetric reactions started flourishing in the last two decades.[1] Some earlier reports had been published by Inoue et al. on the use of a cyclic dipeptide as catalyst for stereoselective hydrocyannations of aldehydes,[2] by Juliá and Colonna on the epoxidation of chalcones by polypeptides,[3] and by Ueoka et al. on the enantioselective hydrolysis of esters using a tripeptide.[4] Nevertheless, it was not until some years later that this area of research got pushed forward, for example with the finding of Miller and coworkers[5] of a tripeptide containing a non-natural amino acid that catalyzed the kinetic resolution of alcohols by acetylation.

The enormous variety of possible combinations of natural and non-natural amino acids allows the preparation of chiral catalysts for several different applications. A privileged peptide conformation with adequate orientation of its functional groups is crucial for to high reactivity and selectivity in organocatalyzed reactions. For enzymes, this is achieved through long amino acid sequences that fold into highly ordered structures, thus creating a defined environment in which a reaction can be catalyzed. Short peptides are typically unordered and adopt many conformations, making it defying to have the various parts of the peptide participate in catalytic processes in a cooperative manner. Consequently, the rational design of a peptide catalyst for a given reaction remains a great challenge.

Short peptide catalysts have been explored for a great number of reactions, and especially C-C bond-forming methods such as Morita-Baylis-Hillman reactions, nitroalkane addition, Friedel-Crafts alkylations, Michael and aldol additions have been of special value for organic synthesis.[1]

The aldol reaction is one of the most preeminent methodologies for the stereoselective formation of C-C bonds. Nature uses this reaction in fundamental steps of the metabolism, for example in gluconeogenesis and glycolysis, which involve the reversible conversion of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate into fructose-1,6-bisphosphate.

In animals and higher plants, the process is catalyzed by type I aldolase enzymes that have also been used as biocatalysts for other transformations which include the synthesis of naturally occurring compounds as well as non-natural analogues.[6] In an effort to mimic the action of these aldolases, several organocatalysts have been developed to perform this essential C-C bond-forming reaction.[7] The organocatalyzed aldol addition has also been studied from a theoretical point of view by means of computational calculations, which have helped to visualize and to understand the mechanisms of these processes.[8]

Since the seminal work of Eder, Sauer and Weichert[9] and of Hajos and Parrish,[10] followed later by that of List et *al.*[11] on the use of (*L*)-Proline, (*L*)-Pro, as catalyst for the asymmetric aldol reaction, the inclusion of this amino acid in short peptides was reported by the groups of Reymond,[12] List[13] and Gong,[14] respectively. An excellent catalyst was reported by Wennemers and coworkers[15] with the H-Pro-Pro-Asp-NH_2 tripeptide, which was found through combinatorial screening of a large library of peptides.[16] Reiser et al. described the synthesis of short α,β-peptides containing conformationally restricted *cis*-β-cyclopropane amino acid (*cis*-β-CPAA) residues in combination with (*L*)-Pro. With these peptide catalysts, high yields and stereoselectivities in inter- and intramolecular aldol reactions even in aqueous solvents were achieved.[17] This was attributed to the remarkable stabilization of secondary-structure motifs in the peptides in which *cis*-β-CPAA is incorporated.[18] Since then, some other Pro-containing short peptides have been described as catalysts for the aldol reaction with comparable results of those described in the earlier reports.[19] The spatial arrangement of the functional groups in the peptide catalysts has been demonstrated to be a key factor for its adequate mode of action,[17,20] although a detailed study combining catalysis, spectroscopy and theory to explain the stereoselectivity observed with the catalysts has not been reported.

Unnatural *cis*-cyclobutane β-amino acids (*cis*-β-CBAA) have been synthesized in our laboratory in an enantioselective manner. The rigidity of their scaffold and their relative and absolute stereochemistry have proven to be beneficial for the stabilization of the well-defined

secondary structures and to play an important role for the properties of various homo or hybrid peptides[21] and other derivatives in which they have been incorporated.[22] In general, the *cis*-β-CBAA moiety, as part of a peptide, can give rise to two types of hydrogen-bonded arrangements (Figure 1), either an intra-residue 6-membered ring or an inter-residue 8-membered ring.[21a,b]

On the other hand, *cis*-cyclobutane γ-amino acids (*cis*-γ-CBAA) can easily be prepared from (–)-verbenone, a commercially available compound from the chiral pool, following enantiodivergent synthetic routes.[23,24] They have been incorporated in short peptides and have been studied as foldamers.[23,25] In general, the structures adopted by these *cis*-γ-CBAA-containing peptides are extended, and intra-residue 7-membered hydrogen-bonded rings between the carbonyl group substituent of the cyclobutane ring and the amino group are observed (Figure 1).

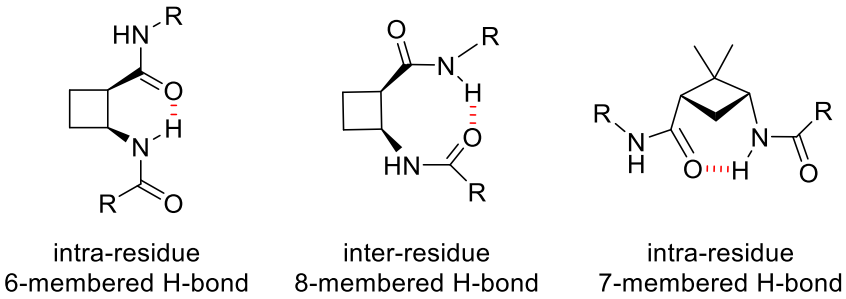


Figure 1. Intra- and inter-residue hydrogen bonds in peptides that incorporate either *cis*-β-CBAA or *cis*-γ-CBAA.

In this work, we report the synthesis of α,β,α– and α,γ,α-tripeptides prepared by the combination of the two enantiomers of rigid *cis*-β–CBAA and cis-γ-CBAA, respectively, with two residues of (*L*)-Pro (catalysts **1**-**4**) or (*D*)-Pro (catalysts *ent*-**3** and *ent*-**4**) (Chart 1) and their evaluation as chiral organocatalysts in asymmetric aldol reactions either in organic or in homogeneous aqueous solution. The effect of the β- or γ-substitution on the cyclobutane

residue along with its absolute configuration as well as the influence of proline chirality have been investigated and are discussed herein. A rationalization of the stereoinduction observed is provided by a combination of NMR and CD conformational analysis together with a computational study in anhydrous medium and in the presence of water.

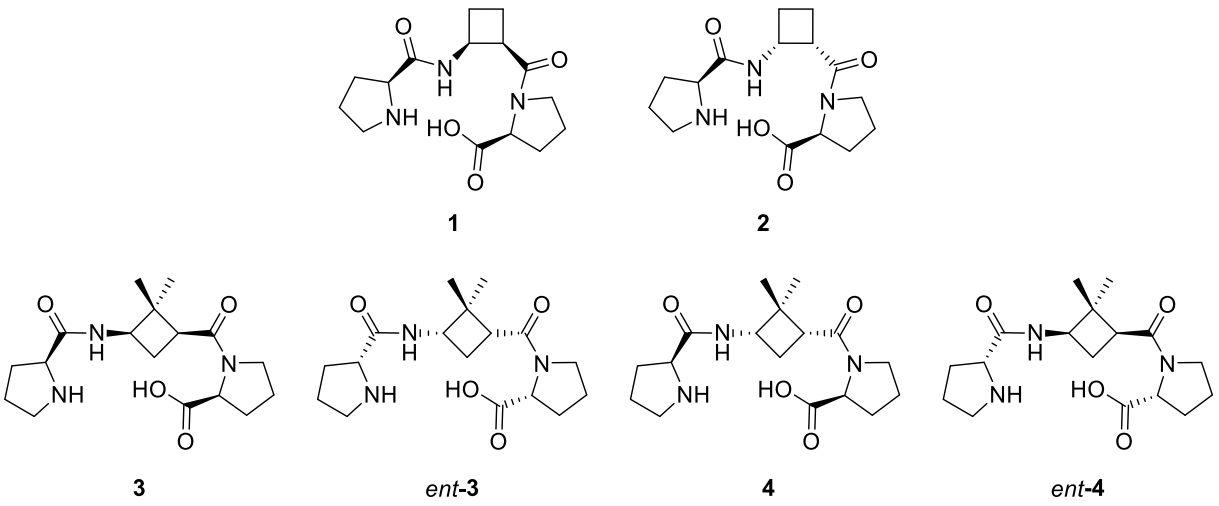
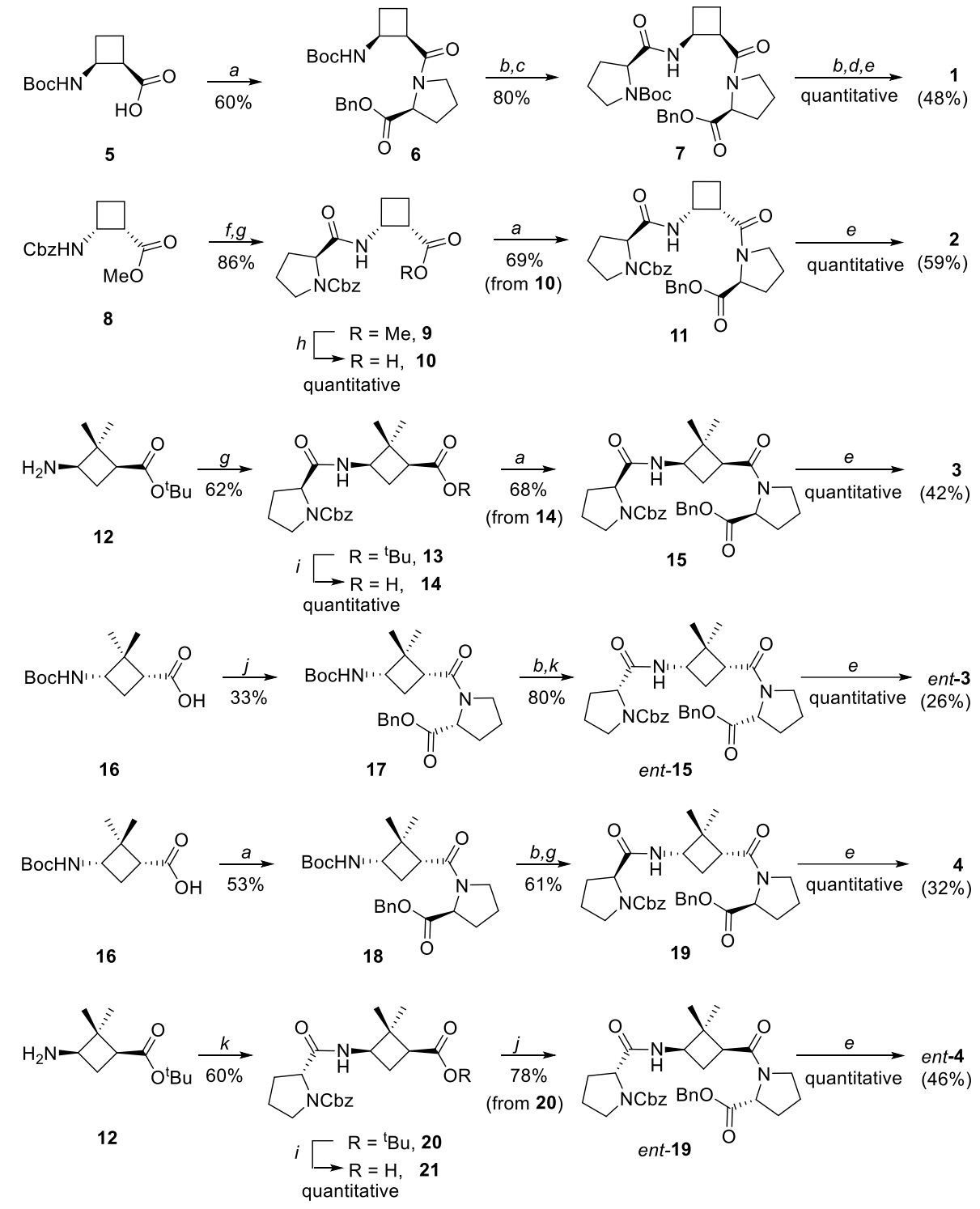


Chart 1. New tripeptides prepared from *cis*-β–CBAA and cis-γ-CBAA in combination with (*L*)-Pro (**1**-**4**) and (*D*)-Pro (*ent*-**3** and *ent*-**4**).

RESULTS AND DISCUSSION

Synthesis of the organocatalysts. β-Amino acid **5**[21a] and **8**[21a] or γ-amino acid **12**[23] and **16**[24] derivatives served as the precursors for the synthesis of peptides **1**-**4**, *ent*-**3** and *ent*-**4** (Scheme 1). Their coupling with (*L*)- or (*D*)-Pro derivatives **I**-**V**, followed by selective deprotection of the resulting dipeptides, subsequent coupling with an additional Pro residue and final deprotection of the *N*- and *C*-termini, afforded the desired catalysts **1**-**4**, *ent*-**3** and *ent*-**4** in 26-59 % overall yields.



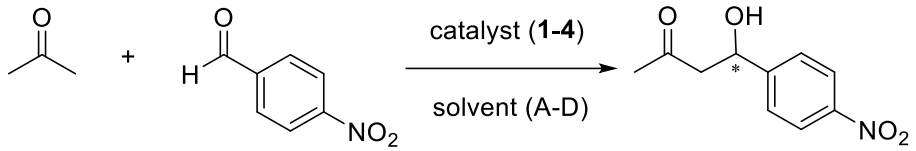
Scheme 1. Synthesis of catalysts **1-4,** *ent*-**3** and *ent*-**4**. Reagents: (*a*): PyBOP, DIPEA, **I**, CH_2Cl_2; (*b*): 2 M HCl in THF; (*c*): PyBOP, DIPEA, **II,** CH_2Cl_2; (*d*): $NaHCO_3$; (*e*): H_2, $Pd(OH)_2/C$, CH_3OH; (*f*): H_2, $Pd(OH)_2/C$, HCl, CH_3OH; (*g*): PyBOP, DIPEA, **III,** CH_2Cl_2; (*h*): 1) 0.25 M NaOH, 2) HCl; (*i*): H_3PO_4; (*j*): PyBOP, DIPEA, **IV,** CH_2Cl_2; (*k*): PyBOP, DIPEA, **V,** CH_2Cl_2.

Organocatalyzed reactions. Peptides **1-4** were initially tested as organocatalysts in the aldol reaction between acetone and *p*-nitrobenzaldehyde (Table 1).

All peptides catalyzed the title reaction with good to excellent yield. However, the *cis*-γ-CBAA containing peptides **3** and **4** showed considerable higher selectivities in non protic solvents than the *cis*-β-CBAA-containing compounds **1** and **2**. We attribute this fact to a more suitable conformation of peptides **3** and **4** in which the *N*- and *C*-terminus are able to interact with the substrate to promote the aldol reaction in agreement with the Houk-List model[26] (See below and the Supporting Information for structures of the major conformations of peptides **2** and **4**). In line with this argument, peptides **1** and **2** show approximately the same - moderate - selectivities, both in aqueous as well as in non-aqueous environment, while the selectivity for **3** and **4** is strongly influenced by the solvent: a significant erosion of enantioselectivity is seen in aqueous solvents (compare entries 12 and 17; entries 19 and 26 in Table 1) as well as a loss in activity, especially for catalyst **4** (compare entries 19 and 26 in Table 1). Peptide **4** proved to be the best catalyst, providing **24a** in an almost quantitative yield and good stereoselectivity up to 93:7 (entries 21 and 23 in Table 1). These results compare very well with the results obtained with *cis*-β-CPAA containing peptides as catalysts[17] and other small peptides[15] for this particular reaction. In general, an increase in the mol% of catalyst involves a slightly faster reaction, as does an increase in the aldehyde concentration.

In acetone, the major enantiomer of the formed aldol product **24a** showed *S* configuration in nearly all cases using peptides **1-4**, independently of the chirality of the corresponding β- or γ-CBAA. An exception is found for catalyst **2**, where the *R* isomer is major in some cases (entry 5), although the enantioselectivity is too poor to consider the asymmetric induction as significant. On the contrary, as expected, the *R* enantiomer was the major product obtained when the benchmark reaction was catalyzed by peptides *ent*-**3** and *ent*-**4** in anhydrous acetone (entries 35 and 38, respectively, in Table 1). These results suggest

Table 1. Selected aldol reactions between acetone and *p*-nitrobenzaldehyde catalyzed by **1-4**.



22 **23a** **24a**

Entry	Catalyst	Solvent[a]	mol%	T (ºC)	Time	Yield[b]	e.r.[c]	Abs. Config.[d]
1		A	5	r.t.	5	90	68:32	*S*
2	**1**	A	20	r.t.	4	95	69:31	*S*
3		B	5	r.t.	48	80	73:27	*R*
4		C	5	r.t.	48	90	69:31	*R*
5		A	5	r.t.	24	66	54:46	*R*
6		A	5	-20	48	17	50:50	-
7	**2**	A[e]	5	r.t.	24	86	52:48	*S*
8		A	20	r.t.	24	96	50:50	-
9		A	20	-20	48	56	53:47	*R*
10		B	5	r.t.	24	64	62:38	*R*
11		B	20	r.t.	18	99	63:37	*R*
12		A	5	r.t.	24	77	83:17	*S*
13		A[e]	5	-20	24	39	89:11	*S*
14	**3**	A	5	r.t.	24	54	81:19	*S*
15		A	20	r.t.	4	96	82:18	*S*
16		A	20	-20	24	69	90:10	*S*
17		B	5	r.t.	24	97	60:40	*R*
18		B	20	r.t.	18	99	60:40	*R*
19		A	5	r.t.	2	97	88:12	*S*
20		A	5	0	3	99	90:10	*S*
21		A	5	-20	15	99	93:7	*S*
22		A[e]	5	r.t	0.75	99	86:14	*S*
23		A[e]	5	0	2.5	99	93:7	*S*
24		A	10	r.t.	1	95	87:13	*S*
25		A	20	r.t.	1	92	87:13	*S*
26	**4**	B	5	r.t.	63	97	68:32	*R*
27		B[e]	5	r.t.	24	85	71:29	*R*
28		B	10	r.t.	63	97	67:33	*R*
29		B	15	r.t.	63	99	68:32	*R*
30		B	20	r.t.	24	99	69:31	*R*
31		C	5	r.t.	48	48	68:32	*R*
32		C[e]	5	r.t.	48	67	68:32	*R*
33		C	20	r.t.	24	80	69:31	*R*
34		E	5	r.t.	5	95	61:39	*S*
35		A	5	0	24	78	88:12	*R*
36	*ent*-**3**	A[e]	5	-20	24	41	90:10	*R*
37		B	5	r.t.	24	97	60:40	*S*
38		A[e]	5	0	2.5	95	93:7	*R*
39		B	5	r.t.	63	95	68:32	*S*
40	*ent*-**4**	C	5	r.t.	63	40	70:30	*S*
41		D	5	r.t.	63	<10	68:32	*S*
42		E	5	r.t.	5	95	60:40	*R*

43		F	5	r.t.	24	<10	73:27	*S*
44		G	5	r.t.	63	<10	77:23	*S*
45		H	5	r.t.	63	<10	74:26	*S*

[a] All reactions were carried out using 0.2 mmol of **23a** and the according volume of the corresponding solvent (2 mL for 0.1 M in aldehyde reactions and 0.7 mL for 0.3 M in aldehyde reactions). Solvent: A: anhydrous acetone; B: acetone/water 10:1 (v/v); C: acetone/water 3:1 (v/v); D: acetone/water 1:1 (v/v); E: acetone/methanol 10:1 (v/v); F: acetone/methanol 3:1 (v/v); G: anhydrous methanol; H: methanol/water 10:1 (v/v). [b] Yield of the isolated product. [c] e.r. determined by chiral stationary phase HPLC analysis. [d] Absolute configuration of the major enantiomer. [e] Experiments with a 0.3 M concentration of aldehyde.

that Pro residues, which are located at the two ends of the peptide catalysts, are responsible for the stereochemical information that controls the stereoselectivity of the process. Nevertheless, the chirality of the γ-CBAA had a significant effect on the rate of the reaction: comparing **3** and **4**, the latter catalyzed the aldol reaction more than 10 times faster.

Remarkably, the observed selectivity was reversed in the presence of water, being the *R* enantiomer the major one when catalysts **1, 3** and **4** were used (entries 3, 17 and 26) while the *S* enantiomer prevailed with peptides *ent*-**3** and *ent*-**4** as catalyst (entries 37 and 39). In the case of catalyst **2**, the major isomer observed when the aldol reaction was performed in the presence of water (entry 10) was again of *R* configuration but in this case the enantioselectivity switch was not as clear as in the other cases because the result in anhydrous acetone was essentially racemic. Although this switch in enantioselectivity was not observed in reactions catalyzed by (*L*)-Pro and β-CPAA-containing peptides,[17] it has been reported in other cases concerning proline derived catalysts.[19, 27] Additionally, a decrease in the rate of formation of the aldol product is observed when water is added in the case of catalyst **4** and *ent*-**4**, an observation that is in agreement with some data described in the bibliography.[28] Moreover, an increase in the quantity of water used in combination with acetone, from 10:1 v/v to 1:1 v/v leads to similar levels of enantioselectivity (compare entries 26 and 31, and 39, 40 and 41, respectively).

Interestingly, when an acetone/methanol 10:1 v/v mixture was tested with catalyst **4** (entry 34) and with catalyst *ent*-**4** (entry 42), the reaction was only slightly slower than the

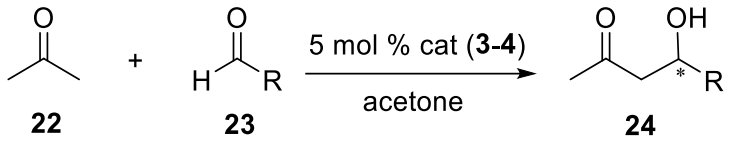
reaction carried out in anhydrous acetone but a significant drop in the enantioselectivity was observed. Nevertheless, the respective prevalent enantiomers were the same than those obtained in acetone. On the contrary, when the ratio of methanol was increased to 25%, the raction became very slow and the switch in enantioselectivity was observed, being the *S* enantiomer of **24a** the major one in the reaction catalyzed by *ent*-**4** (entry 43). A slight increase in the selectivity for the *S* enantiomer was obtained when anhydrous methanol was employed as solvent (entry 44) and similar levels of enantioselectivity were observed when a methanol/water 10:1 v/v mixture was used (entry 45).

These results suggest that the presence of a protic solvent slows down the reaction rate and provokes a switch in the enantioselectivity of the reactions, being more efficient in the case of water than methanol.

In all cases, the catalyst can be recovered (90 %) during work up of the reaction.

With catalysts **3** and **4** in acetone identified as promising catalysts for aldol reactions, their scope with other aldehydes was explored (Table 2). As expected, results with catalyst **3** were better for *p*-nitrobenzaldehyde than for benzaldehyde in terms of yield due to the activation exerted by the electron withdrawing nitro group in the *para* position, but the enantioselectivity levels were the same (entries 1 and 2). Catalyst **4** showed high activity and good enantioselectivity for electron deficient or neutral aromatic aldehydes **23** (entries 3-8, 10), while the electron rich *p*-anisaldehyde showed no conversion (entry 9). Steric hindrance of the substituents in the *ortho* position did not show any influence both on reactivity and stereoselectivity. The use of the more challenging cyclohexanecarbaldehyde led to the formation of the corresponding aldol product in 50% yield after 6 hours with an 82:18 enantiomeric ratio (entry 11), which is similar to the enantioselectivity levels obtained for the aromatic substrates.

Table 2. Aldol reactions between acetone and various aldehydes catalyzed by **3** and **4.**



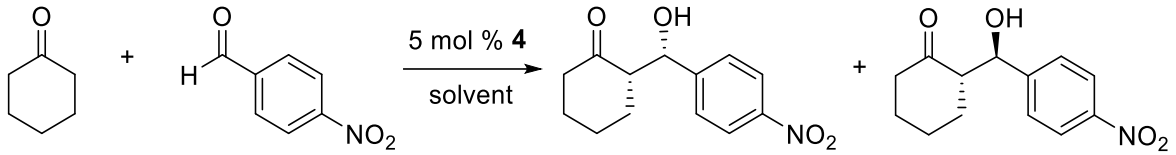
22 **23** **24**

Entry	Catalyst[a]	R	Time (h)	Yield (%)[b]	e.r.[c]	Abs. Config.[d]
1	**3**	*p*-NO_2-Ph	24	77	83:17	*S*
2		Ph	30	55	82:18	*S*
3	**4**	*p*-NO_2-Ph	2	97	88:12	*S*
4		*p*-Br-Ph	2	95	86:14	*S*
5		*p*-Cl-Ph	2	99	89:11	*S*
6		*p*-CF_3-Ph	2	90	85:15	*S*
7		*o*-NO_2-Ph	2	99	85:15	*S*
8		*o*-Br-Ph	2	95	85:15	*S*
9		*p*-MeO-Ph	72	1	-	-
10		Ph	3	90	87:13	*S*
11		*c*-C_6H_{11}	6	50	82:18	*S*

[a] Reactions were carried out at 20°C, using 0.2 mmol of **23** in 2 mL of anhydrous acetone. [b]Yield of the isolated product. [c] e.r. determined by chiral stationary phase HPLC analysis. [d] Absolute configuration of the major enantiomer.

To further explore the scope of the reaction with catalyst **4**, aldol reactions between *p*-nitrobenzaldehyde and cyclohexanone under two different sets of conditions were subsequently carried out (Table 3). In this case, not only enantioselectivity but also diastereoselectivity was considered.

Table 3. Aldol reactions between cyclohexanone and *p*-nitrobenzaldehyde catalyzed by **4.**



25 **23a** (*syn*)-**26** (*anti*)-**26**

Entry	Solvent	Time (h)	Yield (%)[c]	d.r.[d] (syn:anti)	e.r. syn[d,e]	e.r. anti[d,e]
1	MeOH[a]	24	0	-	-	
2	neat[b]	5	99	1:2	70:30	79:21

[a] The reaction was carried out at 20 ºC, using 0.2 mmol of **23a** and 0.2 mL of **25** in 2 mL of methanol. [b] 0.2 mmol of **23a** in 2 mL of **25**. [c] Yield of the isolated product. [d] d.r. and e.r. determined by chiral stationary phase HPLC analysis. [e] Absolute configuration of the major diastereomers: syn: (2*S*,1'*S*); anti: (2*R*,1'*S*)

In the case of using methanol as solvent, the reaction did not proceed at all, in contrast with the results reported with some other peptides.[27] Gratifyingly, the reaction proceeded in 99% yield after 5 hours when neat cyclohexanone was used. The diastereomeric ratio was 1:2 in favor of (*anti)*-**26**. The enantiomeric ratio was 70:30 for the syn isomer and 79:21 for the anti. The diastereomeric ratio is slightly lower than that reported for the β-CPAA containing catalysts[17] (3:1) under the same conditions but the enantiomeric ratios are of the same order.

In conclusion, tripeptide **4** performs very well in terms of catalyst loading and yields and enantioselectivities for the tested aldol reactions with a variety of differently substituted aldehydes with acetone and also with cyclohexanone.

Reaction mechanism. a) Computational calculations in acetone. In order to rationalize the findings obtained with catalyst **4**, which afforded the best results in all reactions explored, a detailed computational study of the reaction mechanism was performed. The simplified proposed mechanism is shown in Figure 2 (See the Supporting Information for

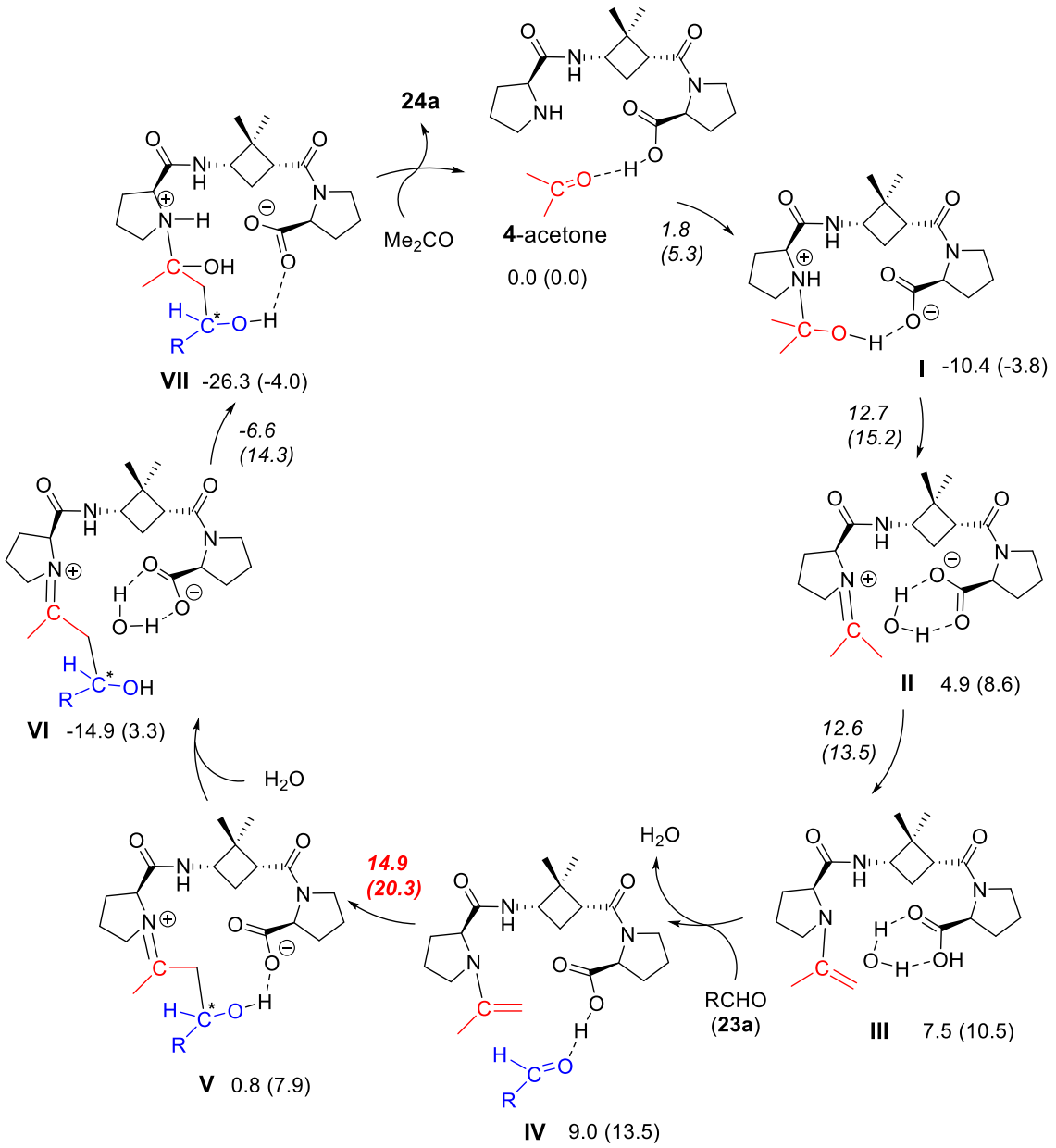


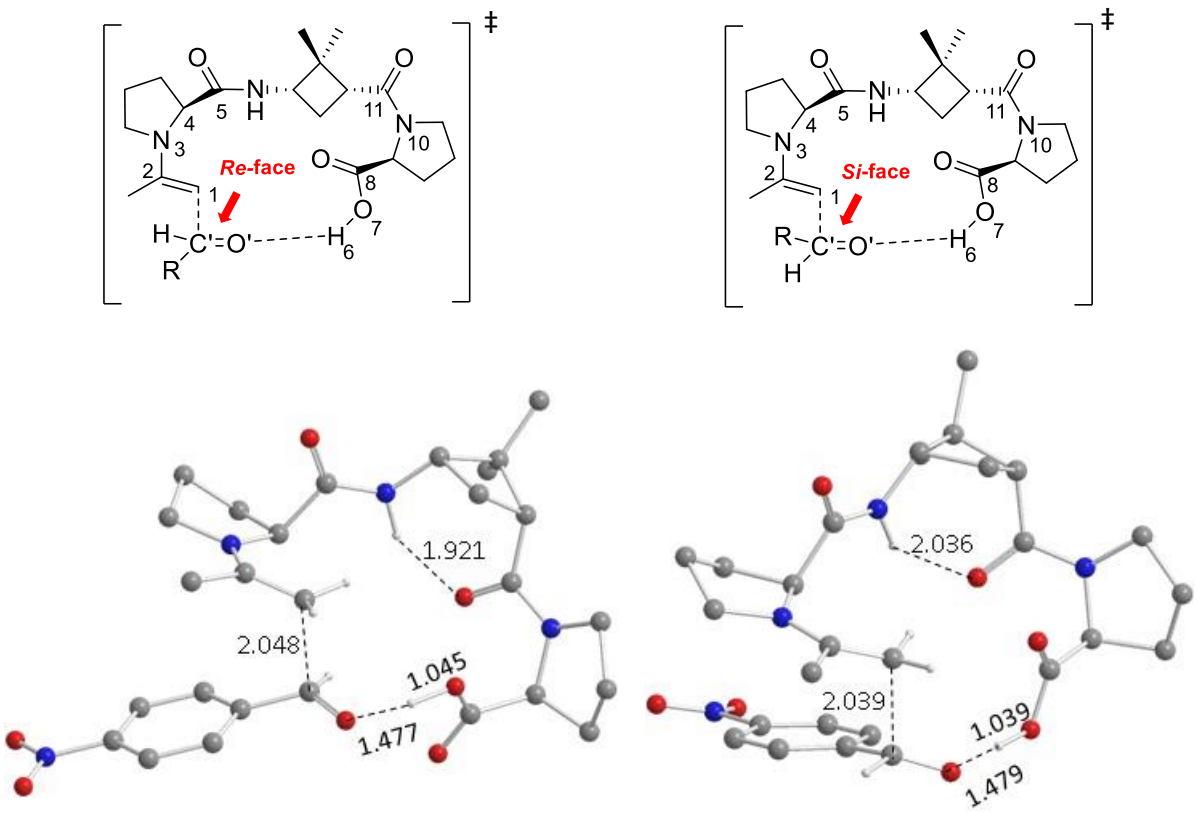
Figure 2. Proposed mechanism for the aldol reaction between acetone and *p*-nitrobenzaldehyde, **23a**, catalyzed by peptide **4**. For minima, relative energies are given in kcal mol^{-1} (and in parentheses Gibbs energies at 298.15 K and 1 mol L^{-1}). In italics are shown the energy and Gibbs energy values corresponding to transition states. From intermediate **IV**, the reported values correspond to the formation of the *S* enantiomer of **24a**. The energies corresponding to the rate determining transition state are marked in red.

a more detailed version). In a first stage, catalyst **4** reacts with acetone through the formation of an N-C bond via a series of intermediates involving the ammonium species **I**, an iminium,

II, and an enamine, **III**. Then, aldehyde **23a** is activated by the carboxylic acid group of enamine **III** and the new C-C bond is formed (**IV→V**). This step involves the rate determining transition state and also determines the enantioselectivity of the reaction. After that the addition of a water molecule helps liberate the aldol product **24a** and the recovery of the catalyst.

The enantioselectivity of the reaction is determined by the transition state of the reaction between the enamine intermediate and the aldehyde (**IV→V**).[29] For this reason, we have analyzed the diastereomeric transition states that lead to the formation of both enantiomers of the aldol product **24a**. Figure 3 shows a schematic representation and the calculated structures of these transition states. We can observe that the production of *R* enantiomers is due to the nucleophilic attack of the enamine to the *Re*-face of the aldehyde carbonyl group, while *S* enantiomer comes from the *Si*-attack. Table 4 presents the relative energies and Gibbs energies, selected geometry parameters and the charge transferred to the aldehyde for these transition states.

The results in Table 4 show a significant effect of the basis set on the energy barriers and Gibbs activation energies. However, the geometries of the transition sates only slightly change when they are located with the larger basis set. Most of the basis set effect is related to the fact that the stability of the enamine complex **III** is underestimated with the 6-31G(d) basis set (ΔE=7.5 kcal mol^{-1} as shown in Figure 2), whereas it is only 0.6 kcal mol^{-1} higher in energy than the **4**-acetone complex at the M06-2X/6-311+G(d,p) level.



R **(IV-V)** TS

S **(IV-V)** TS

Figure 3. Schematic representation and calculated structures of the diastereomeric rate determining transition states corresponding to the aldol reaction between acetone and *p*-nitrobenzaldehyde catalyzed by **4** in acetone solution at the M06-2X/6-31G(d) level of calculation. Noncritical hydrogen atoms have been omitted for clarity. Selected interatomic distances are in Å. Numbering of the atoms is arbitrary.

Table 4. Relative energies and Gibbs energies,[a] selected geometry parameters[b] and charge (Q) of the aldehyde fragment[c] for the the rate determining transition states of the reactions between acetone and *p*-nitrobenzaldehyde **23a** catalyzed by **4** in acetone solution using the M06-2X density functional with two different basis sets.

	Basis set	$\Delta E^{\ddagger}$		$\Delta G^{\ddagger}$	C1-C'	O'-H6	H6-O7	τ_1	τ_2	Q
R (**IV**-**V**) TS	6-31G(d)	16.0	(6.7)	21.3	2.048	1.477	1.045	–8.1	–35.7	–0.33
	6-311+G(d,p)	6.8		11.2	2.106	1.461	1.043	–6.3	–33.3	–0.32
S (**IV**-**V**) TS	6-31G(d)	14.9	(5.1)	20.3	2.039	1.479	1.039	–11.3	153.8	–0.35
	6-311+G(d,p)	5.3		10.4	2.089	1.494	1.028	–10.8	151.2	–0.36

[a] In kcal mol^{-1}, relative to **4**-acetone + **23a**. The values in parentheses have been computed with the 6-311+G(d,p) basis set from geometries optimized with the 6-31G(d) basis set. [b]Interatomic distances are in Å. C4-N3-C2-C1 (τ_1) and N10-C9-C8-O7 (τ_2) dihedral angles are in degrees. [c] From Natural Population Analysis, in a.u.

The difference of energy barriers and Gibbs activation energies shown in Table 4 are in good agreement with the experimentally observed diastereoselectivity (86:14 e.r.), with a predicted e.r. at 25 ºC of 90:10 (with the 6-31G(d) basis set) and 80:20 (with the 6-311+G(d,p) basis set).

In general, both diastereomeric transition states present the same conformation of the "Pro-enamine" moiety, with C4-N3-C2-C1 dihedral angles (τ_1) between −11º and −6º. Regarding the "Pro-carboxyl" moiety, the conformations of the carboxyl group (τ_2) are notably different for *R* and *S* transition states. Both structures present an intramolecular and intra-residue hydrogen bond in the enamine fragment involving the NH and CO groups directly bonded to the cyclobutane (Figure 3).

The reaction between the enamine and the aldehyde (**IV→V**) involves a nucleophilic attack to form the new C-C bond and a proton transfer from the carboxyl group of the enamine intermediate to the carbonyl oxygen of the aldehyde. The values of the C1-C' distances and of the charge transferred to the aldehyde fragment show that the nucleophilic attack is slightly more advanced for the *S* transition state than for the *R* transition state. Otherwise, the values of O'-H6 and H6-O7 distances show that *R* transition state presents a larger degree of proton transfer than the *S* transition state. Therefore, from all these data, we can conclude that the enamine derived from catalyst **4** allows the proper orientation of the aldehyde molecule in the *S* transition state to get an efficient degree of C1-C' bond formation (nucleophilic attack) and proton transfer to the carbonyl group.

We have also located the two diastereomeric rate determining transition states for the reaction catalyzed by **3** (see supporting information) and the results show that the difference in Gibbs energies is 0.7 kcal mol^{-1} at the M06-2X/6-31G(d) level, the formation of the *S* product being the most favorable one. This result is in excellent agreement with the experimental observation that **3** is less selective than **4** in the catalyzed aldol reaction.

b) Experimental studies. In order to gain more insight in how stereochemical features influence the reactivity and the stereochemical course of events, some additional studies were performed with catalyst **4**. Its preferential conformation was investigated by means of high resolution NMR experiments. First of all, catalyst **4** was studied in $(CD_3)_2CO$ solution. The analysis of the ^{1}H-NMR spectrum demonstrates the presence of at least two species in a ~60:40 proportion. The significant downfield shift of the signals corresponding to the methylene and methine protons neighbor to the secondary amine, along with a new peak appearing at 189 ppm in the ^{13}C NMR spectrum,[30] pointed towards the formation of iminium species **II** (Figure 2), which corresponds to the major component of the ~60:40 mixture. The minor species corresponds to catalyst **4** (See the Supporting Information for detailed NMR experiments). The HMBC spectrum of this sample showed cross peaks between the iminium

carbon and the protons neighbor to the nitrogen H2 and H5 (Figure 4). In order to corroborate these findings, a new sample of catalyst **4** was dissolved in CD_3CN and, subsequently, anhydrous non-deuterated acetone was added. A ^{1}H-NMR was recorded and two singlets at 2.35 and 2.55 ppm were observed. SELNOESY experiments revealed the spatial proximity of these methyl groups to the protons on the proline residue (See the Supporting Information for detailed NMR experiments). Moreover, an HMBC spectrum showed cross peaks between the two methyl signals with the iminium group carbon appearing at 189 ppm (Figure 4). These results suggest that the formation of the iminium species **II** is favored.

Having realized that the use of acetone did not allow the study of the catalyst itself, a conformational study was carried out in CD_3CN, as an equivalent non-protic polar solvent (See the Supporting Information). These experiments revealed the presence of two major conformers in a 93:7 ratio, as determined by integration of distinctive signals, and the major conformation was identified by NOE and ROE contacts as the *trans* rotamer (Figure 5). Moreover, trying to rationalize some of the obtained results in Table 1 and in view of some additional experiments to understand the effect of protic solvents in the catalysis (*vide infra*), the conformational study was also performed in CD_3OD (See the Supporting Information).

The results were very similar to those obtained for CD_3CN, but the ratio of conformers was 82:18 in this case. Careful NOE and ROE studies allowed the characterization of the minor rotamer too. These rotamers originate from the rotation around one of the amide bonds, as in the case of the similar β-CPAA-containing tripeptide.[17] The prevalence of the *trans* rotamer can be easily explained by the stabilization provided by inter-residue hydrogen-bonding.

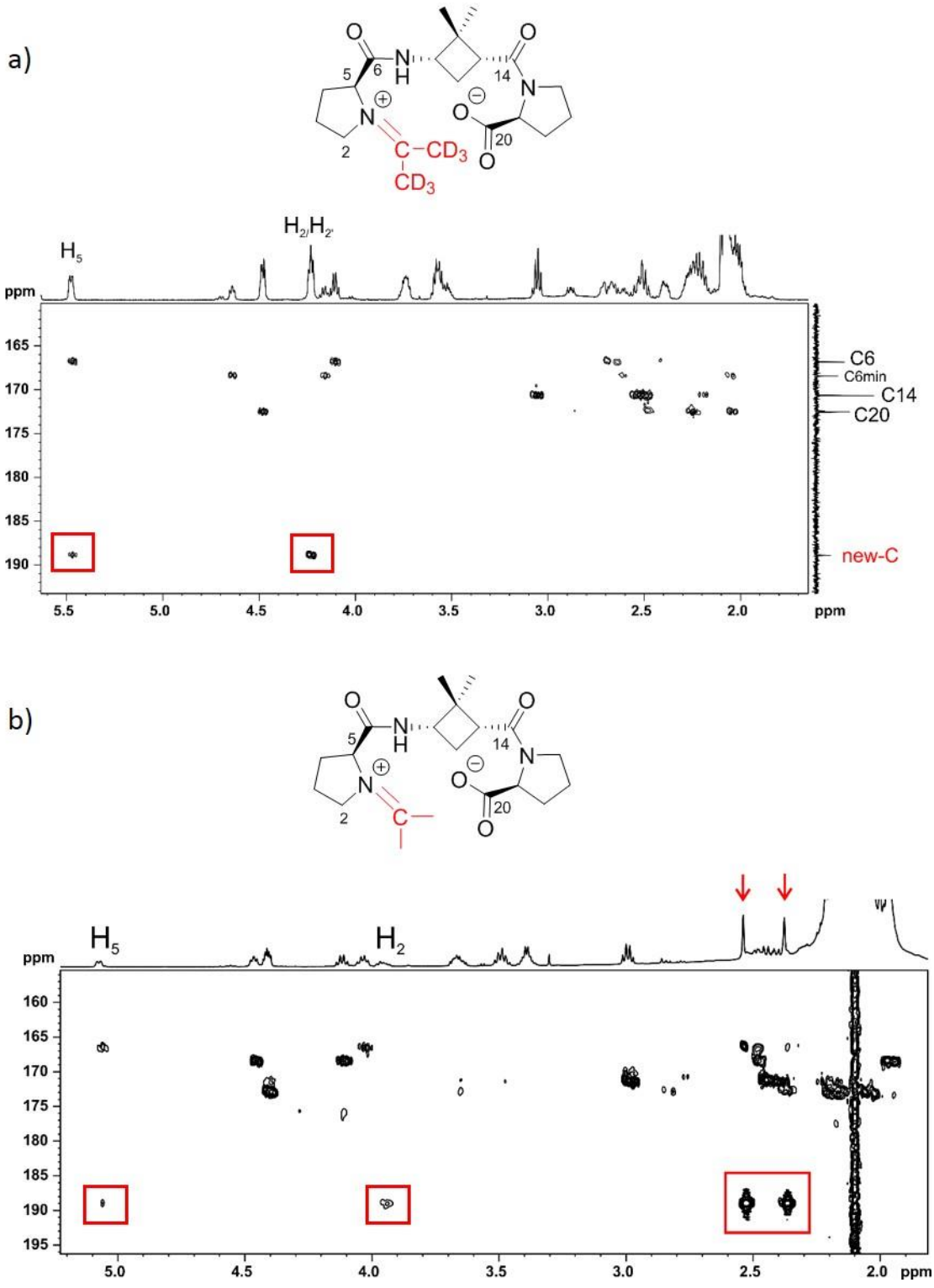


Figure 4. a) Expansion of the carbonyl region of the HMBC spectrum of the reaction product from catalyst **4** with $(CD_3)_2CO$ ($(CD_3)_2CO$, 600 MHz, 298 K). b) Expansion of the carbonyl region of the HMBC spectrum of the reaction product from catalyst **4** with $(CH_3)_2CO$ (CD_3CN, 600 MHz, 298 K).

It is noteworthy that this rotamer is retained in the active conformation of the catalyst as suggested by computational calculations (See the Supporting information).

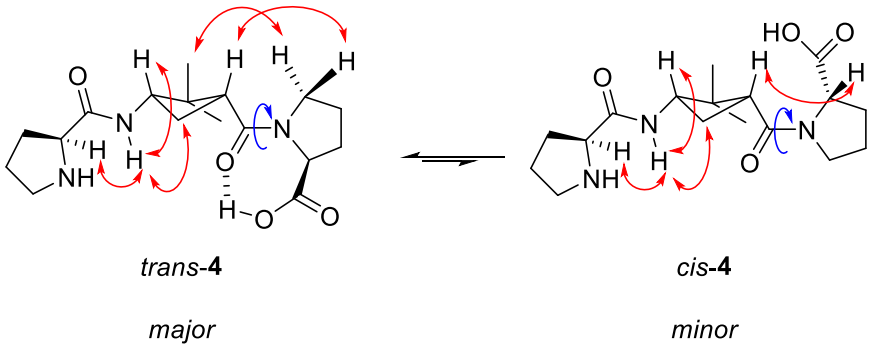


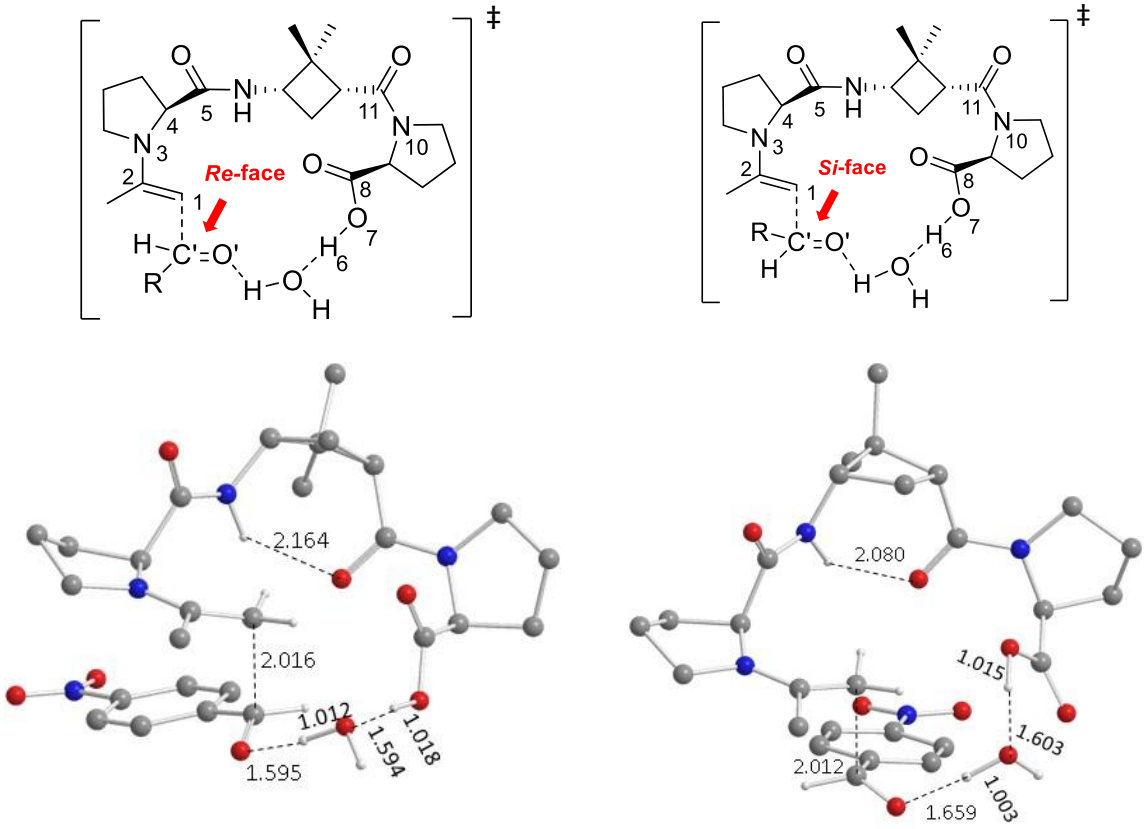
Figure 5. Equilibrium of the two main *cis/trans* rotamers of catalyst **4** in CD_3CN and in CD_3OD solution. The arrows in red correspond to the observed NOE and ROE contacts from NMR experiments. The arrows in blue correspond to the bond rotation responsible for the two conformations.

c) Reactions in protic solvents. As mentioned above, the use of acetone/water instead of dry acetone as solvent reverses the enantioselectivity of the reactions. There are also various examples in organocatalyzed reactions where the enantioswitching is controlled by the solvent polarity.[31] Wennemers et al. attributed this fact to a change in the conformational preference of the catalyst in the presence or absence of water as deduced by circular dichroism (CD) spectroscopy.[27] In our case, the CD spectra of catalyst **4** were recorded in pure methanol and pure water solutions, respectively, as well as in various mixtures of both solvents but no significant changes were observed. These experiments could not be carried out in acetone because it reacts quickly with catalyst **4** as explained above, and the use of acetonitrile as an equivalent polar non-protic solvent was precluded because of its absorbance in the CD spectra. In addition, a series of ^{1}H-NMR spectra were acquired in CD_3OD and with increasing quantities of D_2O but no relevant changes were observed either (See the

Supporting Information for details). Again, the use of acetone to study the conformational change of the catalyst was avoided due to their reactivity.

Trying to rationalize these results, for reactions with catalyst **4,** we have located transition states in which the proton transfer between the carboxyl group of the enamine and the carbonyl group of the aldehyde takes place through an intercalated water molecule (Figure 6). This water molecule is already present in intermediate **III** (see Figure 2) and its release to allow the coordination of the aldehyde molecule may become disfavored as the concentration of water increases.

The interaction with this water molecule stabilizes the *R* and *S* transition states by 13.7 and 7.2 kcal mol^{-1}, respectively, in such a way that the *R* transition state becomes lower in energy than the *S* transition state, in good agreement with the observed reversion of the enantioselectivity of the reaction. To discuss the competition between this water-mediated mechanism and the original mechanism shown in Figure 2, we should compute Gibbs energies of transition states with respect to a common reference. The values computed at the M06-2X/6-31G(d) level relative to **III** + **23a** are 10.8 (*R*) and 9.8 (*S*) kcal mol^{-1} for the direct proton transfer between the enamine and the aldehyde and 10.4 (*R*) and 13.9 (*S*) kcal mol^{-1} for the water-mediated proton transfer. These results show that for the formation of the *S* enantiomer the direct proton transfer is clearly the most favorable one, whereas for the *R* enantiomer both mechanisms may compete. In pure acetone, the water-aldehyde exchange in **III** should be efficient and the rate determining transition state does not involve the participation of the water molecule. However, as the concentration of water increases, the proton transfer through the water molecule may become operative, leading to the inversion of enantioselectivity.



R (**IV-V**) H_2O TS *S* (**IV-V**) H_2O TS

Figure 6. Schematic representation of the preferential nucleophilic attack and calculated structures of the transition states corresponding to the reactions of the enamine derived from catalyst **4** with *p*-nitrobenzaldehyde in the presence of a water molecule at the M06-2X/6-31G(d) level in acetone solution. Noncritical hydrogen atoms have been omitted for clarity. Selected interatomic distances are in Å. Numbering of the atoms is arbitrary.

As it has already been shown (see Table 1) the reaction in acetone-water mixtures is slower than in anhydrous acetone. Water may compete with acetone to interact with the catalyst. The complex **4**-H_2O is more stable than **4**-acetone (ΔG =7.9 kcal mol^{-1}), so that as the concentration of water increases the concentration of **4**-acetone may decrease and, therefore, the reaction rate too. A related competition could also be interpreted for the reactions in the presence of large amounts of other protic solvents such as methanol.

CONCLUSION

Six new hybrid tripeptides have been synthesized by alternate combination of two (*L*)- or (*D*)-Pro residues and the two enantiomers of a β- or a γ-CBAA, respectively. They have been tested as organocatalysts in the aldol reactions between several aldehydes and acetone or cyclohexanone under water-free conditions, and in acetone/water, in acetone/methanol and in water/methanol homogeneous solutions. Results show that β-CBAA-containing peptides are poor catalysts in view of reactivity and enantioselectivity, probably due to the severe conformational restrictions imposed by the high rigidity of the β-CBAA moiety. Better results have been obtained with γ-CBAA-containing derivatives, and the observed enantioselectivity has been rationalized by means of theoretical calculations, which point out that the active conformation in the transition state reproduces the major conformer observed by ^{1}H NMR spectroscopy in the ground state. The predominant absolute configuration of the adducts is controlled by the chirality of (*D*)- or (*L*)-Pro since, for a given Pro enantiomer, the same major aldol enantiomer is obtained independently of the CBAA chirality in each case. The *R* enantiomer always prevails with (*L*)-Pro-containing catalysts in anhydrous solvents as the result of the preferential approach of the reagent to the *Re*-face of the aldehyde, as predicted by computational calculations.

It is noteworthy that the enantioselectivity is reversed in the presence of water (and other protic solvents such as methanol). By using ^{1}H NMR and CD spectroscopies we have shown that conformational changes are not responsible for this reversion. Calculations suggest that a water molecule coordinates simultaneously with a carboxyl proton of the ending proline residue in the catalyst and with the oxygen carbonyl in the aldehyde. This coordination promotes a change in the geometry of the transition state and induces a preferential approach to the *Si*-face of the aldehyde. Also in protic solvents, the reaction rate decreases due to the competition between the aldehyde and the solvent to interact with the intermediate enamine.

The almost quantitative yields and good enantioselectivities achieved under easy reaction conditions, especially with catalyst **4**, jointly with the possibility to tune the adduct configuration by alternatively using easily available (*D*)- or (*L*)-Pro enantiomers, confers these peptide catalysts with interesting properties to be employed in aldol reaction and to be further explored in other chemical processes.

EXPERIMENTAL SECTION

General remarks. Melting points are uncorrected. Infrared analyses were performed with a FT-IR spectrophotometer equipped with an ATR component; NMR spectra were recorded at 250, 400 or 600 MHz for ^{1}H NMR and 62.5, 100 or 150 MHz for ^{13}C NMR; chemical shifts are given in (δ) parts per million (ppm) and coupling constants (*J*) in Hertz. HRMS analyses were carried out with an ESI-MS (QTOF) apparatus. Thin-layer chromatography was carried out on TLC aluminum sheets covered with silica gel. Column chromatography was performed using silica gel of 40−60 μm.

General procedure for the synthesis of dipeptides and tripeptides 6, 11, 15, 18: The adequate carboxylic acid (1 eq) and PyBOP (1.5 eq) were dissolved in CH_2Cl_2 (12 mL/mmol of acid) and DiPEA (4 eq) was added under nitrogen atmosphere. After stirring for 10 min, NH(HCl)-Pro-OBn (**I**) (1 eq) in CH_2Cl_2 (5 mL/ mmol of **I**) were added and the reaction was stirred at room temperature overnight. Then, the solution was washed with saturated $NaHCO_3$ (3 x) and brine. The organic phase was dried over $MgSO_4$, filtered and evaporated under reduced pressure to give crude compounds **6**, **11**, **15** and **18**, respectively. ***Compound 6*:** Synthesized from acid **5**[21a] (0.88 g, 4.1 mmol). The crude was purified by column chromatography (EtOAc/hexane, 1:1) to yield **6** (0.99 g, 2.5 mmol, 60%) as an oil. $[\alpha]_D^{20}$ -123 (*c* 1.2, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.33 (s, 9H), 1.88-2.66 (m, 8H), 3.43 (m, 3H), 4,45 (m, 2H), 5.16 (m, 2H), 5.75 (br d, 1H, *J* = 8.4 Hz), 7.27 (m, 5H). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 18.5, 25.1, 28.7, 29.5, 29.6, 44.4, 46.4, 47.1, 59.1, 67.1, 79.4, 128.4, 128.6,

128.8, 136.1, 155.3, 172.4. HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_{22}H_{30}N_2O_5Na$ 425.2052; Found 425.2047. ATR-IR ν_{max}: 3327, 2976, 1744, 1706, 1633, 1500, 1434 cm^{-1}. ***Compound 11*:** Synthesized from acid **10** (0.33 g, 0.95 mmol). The crude was purified by column chromatography (EtOAc/hexane, from 1:1 to 1:0) to afford **11** (0.35 g, 0.66 mmol, 69%) as a white solid. mp: 52-54 ºC (CH_3OH). $[\alpha]_D^{20}$ -30 (*c* 1.0, CH_3OH). ^1H NMR (CD_3OD, 400 MHz): δ 1.88-2.30 (m, 12H), 3.51-3.60 (m, 5H), 4.27 (m, 1H), 4.58 (m, 1H), 4.96 (m, 1H), 5.24 (m, 4H), 7.39 (m, 10H). ^{13}C NMR (CD_3OD, 100 MHz): δ 17.4, 22.3, 23.6, 24.4, 24.7, 26.6, 28.9, 29.0, 29.8, 30.7, 31.7, 43.3, 43.9, 45.2, 45.5, 46.5, 59.4, 60.5, 67.1, 127.7, 128.1, 128.5, 136.2, 137.23, 155.6, 173.3. HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_{30}H_{35}N_3O_6Na$ 556.2418; Found 556.2432. ATR-IR ν_{max}: 3315, 2951, 2880, 1685, 1629, 1524, 1421, 1355, 1173 cm^{-1}. ***Compound 15*:** Synthesized from acid **14** (0.36 g, 0.96 mmol). The crude was purified by column chromatography (EtOAc /hexane, from 1:1 to 1:0) to yield **15** (0.37 g, 0.66 mmol, 68%) as a white solid. mp: 91-92 ºC (CH_2Cl_2). $[\alpha]_D^{20}$ -40 (*c* 1.0, CH_3OH). ^1H NMR ($CDCl_3$, 400 MHz): δ 0.88 (s, 3H), 1.37 (s, 3H), 1.92-2.16 (m, 10H), 2.77 (t, 1H, *J* = 7.9 Hz), 3.54 (m, 4H), 4.06 (m, 1H), 4.33 (m, 1H), 4.55 (m, 1H), 5.16 (m, 4H), 7.34 (m, 10H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 17.5, 24.9, 25.5, 29.0, 30.0, 31.3, 43.4, 47.1, 50.2, 58.9, 60.8, 66.7, 67.3, 127.9, 128.1, 128.6, 135.7, 136.5, 171.1, 172.1. HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_{32}H_{39}N_3O_6Na$ 584.2731; Found 584.2737. ATR-IR ν_{max}: 2957, 1686, 1625, 1529, 1439, 1357 cm^{-1}. ***Compound 18*:** Synthesized from acid **16**[24] (1.00 g, 4.1 mmol). The crude was purified by column chromatography (EtOAc/hexane, 1:1) to afford **17** (0.94 g, 2.2 mmol, 53%) as a white solid. mp: 95-98 ºC (EtOAc). $[\alpha]_D^{20}$ -50 (*c* 0.8, CH_3OH). ^1H NMR ($CDCl_3$, 250 MHz): δ 0.88 (s, 3H), 1.36 (s, 3H), 1.43 (s, 9H), 1.95-2.25 (m, 6H), 2.78 (t, 1H, *J* = 8.0 Hz), 3.46 (m, 1H), 3.64 (m, 1H), 3.80 (m, 1H), 4.52 (d, 1H, *J* = 7.2 Hz), 5.11 (m, 2H), 7.33 (m, 5H). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 17.9, 25.2, 26.3, 28.8, 29.6, 30.3, 43.8, 45.8, 47.6, 51.9, 59.3, 67.2, 128.5, 128.9, 136.2, 155.9, 171.5, 172.6. HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd

for $C_{24}H_{34}N_2O_5Na$ 453.2360; Found 453.2368. ATR-IR ν_{max}: 3391, 2971, 2863, 1749, 1705, 1634, 1507 cm^{-1}.

***Compound 7*:** Protected dipeptide **6** (0.22 g, 0.55 mmol) was dissolved in CH_2Cl_2 (20 mL). 2 M HCl in THF (3.6 mL, 7.2 mmol, 13 eq) was added and the mixture was stirred overnight at room temperature. The evaporation of the solvent under reduced pressure affords the half-deprotected dipeptide. This product in CH_2Cl_2 (6 mL) was added to a solution of *N*-Boc-L-proline (**II**) (0.12 g, 0.55 mmol, 1 eq), PyBOP (0.43 g, 0.83 mmol, 1.5 eq) and DiPEA (0.38 mL, 2.2 mmol, 4 eq) in CH_2Cl_2 (12 mL), which was stirred beforehand for 10 min under nitrogen atmosphere. The reaction mixture was stirred overnight at room temperature. The solution was washed with saturated $NaHCO_3$ (3 x 10 mL) and brine (10 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated under reduced pressure. Purification by column chromatography (EtOAc/hexane, from 1:1 to 1:0) yields **7** (0.22 g, 0.44 mmol, 80%) as an oil. $[\alpha]_D^{20}$ -40 (*c* 1.6, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.39 (s, 9H), 1.77-2.24 (m, 12H), 3.39 (m, 5H), 3.98 (m, 1H), 4.17 (m, 1H), 4.51 (m, 1H), 4.72 (m,1H), 5.07 (m, 2H), 7,28 (m, 5H).^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 18.3, 24.2, 24.7, 28.2, 28.4, 28.9, 29.2, 30.0, 42.9, 43.9, 46.8, 47.2, 58.4, 60.5, 66.7, 80.2, 128.0, 128.4, 135.6, 155.2, 171.7, 172.4. HRMS (ESI) *m/z*: $[M]^+$ Calcd for $C_{27}H_{37}N_3O_6$ 499.2714; Found 499.2712. ATR-IR ν_{max}: 3327, 2976, 1744, 1705, 1633, 1500, 1434, 1163 cm^{-1}.

***Compound 9*:** Protected amino acid **8**[21a] (0.8 g, 3.0 mmol) was dissolved in CH_3OH (40 mL). Then, $Pd(OH)_2/C$ (80 mg, 10% weight) and 2 M HCl in THF (1.5 mL, 3 mmol, 1 eq) were added and the mixture was stirred under 7 atm of H_2 at room temperature overnight. After this period, the crude was filtered through Celite and washed with CH_3OH. The collected solvent was evaporated to provide the half-deprotected amino acid. This product in CH_2Cl_2 (20 mL) was added to a solution of *N*-Cbz-L-proline (**III**) (0.75 g, 3.0 mmol, 1 eq), PyBOP (2.34 g, 4.5 mmol, 1.5 eq) and DiPEA (2.1 mL, 12 mmol, 4 eq) in CH_2Cl_2 (25 mL), which was stirred

beforehand for 10 min under nitrogen atmosphere. The reaction mixture was stirred overnight at room temperature. The solution was washed with saturated $NaHCO_3$ (3 x 10 mL) and brine (10 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated under reduced pressure. Purification by column chromatography (EtOAc/hexane, from 1:1 to 1:0) yields **9** (0.94 g, 2.6 mmol, 86%) as a white solid. mp: 46-47 ºC (CH_2Cl_2). $[\alpha]_D^{20}$ +3 (*c* 1.0, CH_3OH). ^1H NMR ($CDCl_3$, 250 MHz): δ 1.88-2.22 (m, 8H), 3.35 (m, 1H), 3.52 (m, 2H), 3.65 (s, 3H), 4.31 (m, 1H), 4.67 (m, 1H), 5.20 (m, 2H), 7.33 (m, 5H). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 18.7, 19.1, 23.5, 24.4, 29.1, 31.0, 44.1, 44.5, 51.7, 60.6, 67.3, 127.9, 128.5, 136.4, 171.2, 174.4. HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_{19}H_{24}N_2O_5Na$ 383.1577; Found 383.1580. ATR-IR ν_{max}: 3401, 2956, 1671, 1526, 1421, 1357, 1204 cm^{-1}.

Compound 10**:** To an ice-cooled solution of protected dipeptide **9** (0.5 g, 1.4 mmol) in a 1:10 mixture of THF/water (60 mL), 0.25 M NaOH aqueous solution (14 mL, 3.5 mmol, 2.5 eq) was added and the resultant mixture was stirred for 4 h. Then, the mixture was washed with CH_2Cl_2 (20 mL) and 5% HCl aqueous solution was added to the aqueous phase to reach pH 2. The acid solution was extracted with EtOAc (3 x 20 mL) and the organic phases were dried over $MgSO_4$, filtered and evaporated under reduced pressure to give compound **10** (0.47 g, 1.4 mmol, 98%) as white solid without need for further purification. mp: 70-71 ºC (CH_3OH). $[\alpha]_D^{20}$ -18 (*c* 0.8, CH_3OH). ^1H NMR ($CDCl_3$, 360 MHz): δ 1.81-2.11 (m, 8H), 3.36 (m, 1H), 3.50 (m, 2H), 4.34 (m, 1H), 4.71 (m, 1H), 5.02 (m, 2H), 7.29 (m, 5H). ^{13}C NMR ($CDCl_3$, 90 MHz): δ 18.8, 28.4, 28.8, 29.5, 31.2, 43.9, 44.6, 47.0, 47.4, 60.9, 67.4, 127.8, 128.1, 128.5, 136.0, 155.8, 171.7, 176.5. HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_{18}H_{22}N_2O_5Na$ 369.1421; Found 369.1425. ATR-IR ν_{max}: 3306, 2952, 2881, 1701, 1655, 1524, 1409, 1353, 1177 cm^{-1}.

Compound 13**:** *N*-Cbz-L-proline (**III**) (1.13 g, 4.5 mmol, 1 eq) and PyBOP (3.51 g, 6.8 mmol, 1.5 eq) were dissolved in CH_2Cl_2 (30 mL) and DiPEA (3.2 mL, 18 mmol, 4 eq) was added under nitrogen atmosphere. After stirring for 10 min, amine **12**[23] (0.90 g, 4.5 mmol) and

CH_2Cl_2 (16 mL) were added. The reaction was stirred at room temperature overnight. Then, the solution was washed with saturated $NaHCO_3$ (3 x 20 mL) and brine (20 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (EtOAc/hexane, 2:3) to afford **13** (1.21 g, 2.8 mmol, 62%) as a white solid. mp: 47-48 ºC (CH_2Cl_2). $[\alpha]_D^{20}$ -4 (*c* 1.0, CH_3OH). ^1H NMR ($CDCl_3$, 400 MHz): δ 0.81 (s, 3H), 1.25 (s, 3H), 1.44 (s, 9H), 1.90-2.33 (m, 6H), 2.49 (dd, 1H, *J* = 9.7, 7.9 Hz), 3.50 (m, 2H), 3.99 (m, 1H), 4.32 (m, 1H), 5.16 (m, 2H), 7.35 (m, 5H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 16.8, 17.0, 24.5, 25.7, 28.2, 29.0, 43.9, 45.7, 48.6, 50.0, 60.3, 67.2, 80.5, 127.8, 128.5, 136.5, 161.1, 172.1. HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_{24}H_{34}N_2O_5Na$ 453.2360; Found 453.2369. ATR-IR ν_{max}: 3310, 2958, 1703, 1534, 1412, 1354 cm^{-1}.

***Compound 14*:** To a solution of dipeptide **13** (0.11 g, 0.26 mmol) in toluene (0.2 mL) was added aqueous phosphoric acid (0.25 mL, 3.8 mmol, 15 eq, 85% weight) dropwise and the resulting mixture was stirred for 6 h at room temperature. Water (5 mL) was added, and the mixture was extracted with EtOAc (3 x 10 mL). The organic phases were dried over $MgSO_4$, filtered and evaporated under reduced pressure to give the compound **14** (96 mg, 0.26 mmol, quantitative yield) as a white solid. mp: 65-66 ºC (CH_2Cl_2). $[\alpha]_D^{20}$ +4 (*c* 0.8, CH_3OH). ^1H NMR (CD_3OD, 400 MHz): δ 0.96 (s, 3H), 1.28 (s, 3H), 1.92 (m, 3H), 2.20 (m, 3H), 2.59 (m, 1H), 3.51 (m, 1H), 3.58 (m, 1H), 3.91 (m, 1H), 4.25 (m, 1H), 5.09 (m, 2H), 7.31 (m, 5H), 8.06 (m, 1H). ^{13}C NMR (CD_3OD, 100 MHz): δ 16.0, 23.1, 24.2, 26.2, 28.0, 31.5, 42.6, 45.7, 50.0, 59.8, 66.7, 127.6, 128.1, 136.5, 154.9, 173.7, 174.5. HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_{20}H_{26}N_2O_5Na$ 397.1734; Found 397.1735. ATR-IR ν_{max}: 3338, 2956, 1701, 1648, 1533, 1414, 1354 cm^{-1}.

***Compound 17*:** Compound **16**[24] (0.70 g, 2.90 mmol) and PyBOP (2.22 g, 4.25 mmol, 1.5 eq) were dissolved in CH_2Cl_2 (20 mL) and DiPEA (2 mL, 11.5 mmol, 4 eq) was added under

nitrogen atmosphere. After stirring for 10 min, NH(HCl)-(D)-Pro-OBn (**IV**) (0.71 g, 2.90 mmol, 1 eq) in CH_2Cl_2 (10 mL) were added and the reaction was stirred at room temperature overnight. Then, the solution was washed with saturated $NaHCO_3$ (3 x 20 mL) and brine (20 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated under reduced pressure to give crude compounds Purification by column chromatography (EtOAc/hexane, 1:1) afforded compound **17** (0.41 g, 0.95 mmol, 33%) as a white solid. mp: 43-45ºC (EtOAc). $[\alpha]_D^{20}$ -30 (*c* 1, CH_3OH). ^1H NMR ($CDCl_3$, 360 MHz): mixture of conformers observed δ 0.90 (s, 3H), 1.34 (s, 3H), 1.42 (s, 9H), 1.80-2.25 (m, 6H), 2.70 (t, 1H, *J* = 8.1 Hz), 3.46 (m, 1H), 3.54 (m, 1H), 3.80 (m, 1H), 4.56 (m, 1H), 4.90 (m, 1H), 5.14 (m, 2H), 7.33 (m, 5H). ^{13}C NMR ($CDCl_3$, 90 MHz): mixture of conformers observed δ 17.0, 24.9, 26.0, 28.4, 29.0, 29.9, 42.8, 46.3, 47.0, 51.2, 58.9, 66.7, 79.2 128.2, 128.5, 135.7, 155.5, 170.8, 172.2. HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_{24}H_{34}N_2O_5Na$ 453.2360; Found 453.2372. ATR-IR ν_{max}: 3325, 2953, 2875, 1742, 1702, 1629, 1522, 1427, 1158 cm^{-1}.

Compound ent-15*:* Protected dipeptide **17** (0.41 g, 0.95 mmol) was dissolved in CH_2Cl_2 (28 mL). 2 M HCl in THF (7 mL, 14 mmol, 15 eq) was added and the mixture was stirred at room temperature overnight. The evaporation of the solvent under reduced pressure affords the half-deprotected dipeptide. This product dissolved in CH_2Cl_2 (7 mL) was added to a solution of *N*-Cbz-D-proline (**V**) (0.25 g, 1.00 mmol, 1 eq), PyBOP (0.57 g, 1.1 mmol, 1.1 eq) and DiPEA (0.7 mL, 4 mmol, 4 eq) in CH_2Cl_2 (8 mL), which was stirred beforehand for 10 min under nitrogen atmosphere. The reaction mixture was stirred overnight at room temperature. The solution was washed with saturated $NaHCO_3$ (3 x 25 mL) and brine (25 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated under reduced pressure. Purification by column chromatography (EtOAc/hexane, from 3:1 to 1:0) afforded *ent*-**15** (0.46 g, 0.82 mmol, %) as a white solid. mp: 89-91 ºC (AcOEt). $[\alpha]_D^{20}$ +38 (*c* 1.0, CH_3OH). ^1H NMR ($CDCl_3$, 400 MHz): δ 0.88 (s, 3H), 1.37 (s, 3H), 1.92-2.16 (m, 10H), 2.77 (t, 1H, *J* = 7.9 Hz), 3.54 (m, 4H),

4.06 (m, 1H), 4.33 (m, 1H), 4.55 (m, 1H), 5.16 (m, 4H), 7.34 (m, 10H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 17.5, 24.9, 25.5, 29.0, 30.0, 31.3, 43.4, 47.1, 50.2, 58.9, 60.8, 66.7, 67.3, 127.9, 128.1, 128.6, 135.7, 136.5, 171.1, 172.1. HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_{32}H_{39}N_3O_6Na$ 584.2731; Found 584.2744. ATR-IR ν_{max}: 3311, 2956, 2878, 1686, 1620, 1529 cm^{-1}

Compound 19*:* Protected dipeptide **18** (0.65 g, 1.5 mmol) was dissolved in CH_2Cl_2 (40 mL). 2 M HCl in THF (10 mL, 20 mmol, 13 eq) was added and the mixture was stirred at room temperature overnight. The evaporation of the solvent under reduced pressure affords the half-deprotected dipeptide. This product dissolved in CH_2Cl_2 (16 mL) was added to a solution of *N*-Cbz-L-proline (**III**) (0.37 g, 1.5 mmol, 1 eq), PyBOP (1.19 g, 2.3 mmol, 1.5 eq) and DiPEA (1.0 mL, 6 mmol, 4 eq) in CH_2Cl_2 (33 mL), which was stirred beforehand for 10 min under nitrogen atmosphere. The reaction mixture was stirred overnight at room temperature. The solution was washed with saturated $NaHCO_3$ (3 x 25 mL) and brine (25 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated under reduced pressure. Purification by column chromatography (EtOAc/hexane, from 1:2 to 1:0) afforded **19** (0.51 g, 0.91 mmol, 61%) as a white solid. mp: 58-60 ºC (EtOAc). $[\alpha]_D^{20}$ -104 (*c* 1.1, CH_3OH). ^{1}H NMR ($CDCl_3$, 400 MHz): mixture of conformers observed δ 0.70 – 0.90 (m, 3H), 1.30-1.40 (m, 3H), 1.94-2.34 (m, 10H), 2.85 (m, 1H), 3.49-3.65 (m, 4H), 4.09 (m, 1H), 4.36 (m, 1H), 4.55 (m, 1H), 5.16 (m, 4H), 7.36 (m, 10H). ^{13}C NMR ($CDCl_3$, 100 MHz): mixture of conformers observed δ 17.8, 24.7, 25.5, 29.2, 29.8, 43.9, 47.1, 50.4, 58.8, 60.7, 66.6, 67.2, 128.0, 128.4, 135.7, 155.3, 155.8, 171.2, 172.2. HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_{32}H_{39}N_3O_6Na$ 584.2731; Found 584.2732. ATR-IR ν_{max}: 3312, 2952, 1702, 1677, 1636, 1529, 1415 cm^{-1}.

Compound 20**:** *N*-Cbz-D-proline (**V**) (0.715 g, 2.86 mmol, 1 eq) and PyBOP (2.23 g, 4.30 mmol, 1.5 eq) were dissolved in CH_2Cl_2 (20 mL) and DiPEA (1.4 mL, 8.04 mmol, 4 eq) was added under nitrogen atmosphere. After stirring for 10 min, amine **12**[23] (0.57 g, 2.86 mmol)

and CH_2Cl_2 (10 mL) were added. The reaction was stirred at room temperature overnight. Then, the solution was washed with saturated $NaHCO_3$ (3 x 20 mL) and brine (15 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (EtOAc/hexane, 2:3) to afford **20** (0.73 g, 1.72 mmol, 60%) as a white solid. mp: 83-85 ºC (EtOAc). $[\alpha]_D^{20}$ +37 (*c* 1.0, CH_3OH). ^1H NMR ($CDCl_3$, 360 MHz): mixture of conformers observed δ 0.90 (s, 3H), 1.26 (s, 3H), 1.45 (s, 9H), 1.91 (m, 4H), 2.15-2.47 (m, 2H), 2.50 (t, *J* = 9 Hz, 1H), 3.43-3.53 (m, 2H), 4.02 (m, 1H), 4.33 (m, 1H), 5.17 (m, 2H), 6.13 (m, NH), 6.97 (m, NH), 7.36 (m, 5H). ^{13}C NMR ($CDCl_3$, 90 MHz): mixture of conformers observed δ 16.8, 24.5, 26.1, 28.2, 28.9, 43.9, 45.9, 47.1, 49.9, 60.5, 67.4, 80.4, 128.2, 128.5, 136.4, 156.2, 171.4, 172.1. HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_{24}H_{34}N_2O_5Na$ 453.2360; Found 453.2371. ATR-IR ν_{max}: 3354, 2956, 1724, 1669, 1532, 1426 cm^{-1}.

***Compound 21*:** To a solution of dipeptide **20** (0.73 g, 1.70 mmol) in toluene (3.1 mL) was added aqueous phosphoric acid (1.67 mL, 28.9 mmol, 17 eq, 85% weight) dropwise and the resulting mixture was stirred for 6 h at room temperature. Water (15 mL) was added, and the mixture was extracted with DCM (3 x 20 mL). The organic phases were dried over $MgSO_4$, filtered and evaporated under reduced pressure to give compound **21** (0.63 g, 1.70 mmol, quantitative yield) as a white solid. mp: 63-64 ºC (CH_2Cl_2). $[\alpha]_D^{20}$ +68 (*c* 1.2, CH_3OH). ^1H NMR ($CDCl_3$, 250 MHz): mixture of conformers observed δ 0.73 (s, 3H), 1.30 (s, 3H), 1.89 (m, 2H), 2.18 (m, 3H), 2.66 (m, 2H), 3.53 (m, 2H), 4.20 (m, 1H), 4.45 (m, 1H), 4.98 (d, *J* = 12 Hz, 1H), 5.30 (d, *J* = 12 Hz, 1H), 7.34 (m, 5H). ^{13}C NMR ($CDCl_3$, 90 MHz): mixture of conformers observed δ 16.6, 23.4, 23.7, 29.0, 31.4, 42.6, 46.9, 47.5, 49.9, 61.1, 68.0, 128.0, 128.6, 135.3, 157.0, 171.9, 175.5. HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_{20}H_{26}N_2O_5Na$ 397.1734; Found 397.1730. ATR-IR ν_{max}: 3352, 2958, 1711, 1648, 1530, 1416 cm^{-1}.

Compound ent-19: Carboxylic acid **21** (0.57 g, 1.52 mmol) and PyBOP (0.87 g, 1.68 mmol, 1.1 eq) were dissolved in CH_2Cl_2 (10 mL) and DiPEA (1.2 mL, 6.76 mmol, 4 eq) was added under nitrogen atmosphere. After stirring for 10 min, NH(HCl)-(D)-Pro-OBn (**IV**) (0.41 g, 1.70 mmol, 1.1 eq) in CH_2Cl_2 (10 mL) were added and the reaction was stirred at room temperature overnight. Then, the solution was washed with saturated $NaHCO_3$ (3 x 15 mL) and brine (15 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated under reduced pressure to give crude compounds Purification by column chromatography (EtOAc/hexane, from 1:2 to 1:0) afforded *ent*-**19** (0.67 g, 1.18 mmol, 78%) as a white solid. mp: 60-61 ºC (EtOAc). $[\alpha]_D^{20}$ +99 (*c* 1.0, CH_3OH). ^1H NMR ($CDCl_3$, 360 MHz): mixture of conformers observed δ 0.70 – 0.90 (m, 3H), 1.30-1.40 (m, 3H), 1.94-2.34 (m, 10H), 2.85 (m, 1H), 3.49-3.65 (m, 4H), 4.09 (m, 1H), 4.36 (m, 1H), 4.55 (m, 1H), 5.16 (m, 4H), 7.36 (m, 10H). ^{13}C NMR ($CDCl_3$, 90 MHz): mixture of conformers observed δ 17.8, 24.7, 25.5, 29.2, 29.8, 43.9, 47.1, 50.4, 58.8, 60.7, 66.6, 67.2, 128.0, 128.4, 135.7, 155.3, 155.8, 171.2, 172.2. HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_{32}H_{39}N_3O_6Na$ 584.2731; Found 584.2748. ATR-IR ν_{max}: 3304, 2954, 1739, 1670, 1624, 1529 cm^{-1}.

***Catalyst 1*.** Protected tripeptide **7** (0.15 g, 0.3 mmol) was dissolved in CH_2Cl_2 (10 mL). 2 M HCl in THF (2 mL, 3.9 mmol, 13 eq) was added and the mixture was stirred overnight at room temperature. The solvent was evaporated under reduced pressure. The resulting crude was dissolved in water (4 mL) followed by the addition of a $NaHCO_3$ solution until pH = 9 was reached. This aqueous solution was extracted with CH_2Cl_2 (3 x 20 mL). The organic phases were dried over $MgSO_4$, filtered and evaporated under reduced pressure to give the half-protected tripeptide. This product was dissolved in CH_3OH (8 mL) and $Pd(OH)_2/C$ (12 mg, 10% weight) was added. The mixture was stirred under 7 atm of H_2 at room temperature overnight. After this period the crude was filtered through Celite and washed with CH_3OH. The collected solvent was evaporated to provide compound **1** (92 mg, 0.3 mmol, 99%) as a white solid. mp: 142-144 ºC (CH_3OH). $[\alpha]_D^{20}$ -107 (*c* 1.0, CH_3OH).^1H NMR (CD_3OD, 600

MHz): δ 1.83-2.17 (m, 9H, H3, H3', H4, H9, H10, H15, H15', H16, H16'), 2.38 (m, 3H, H4', H9', H10'), 3.27-3.36 (m, 2H, H2, H2'), 3.55 (m, 2H, H14, H14'), 3.67 (m, 1H, H11), 4.26 (m, 2H, H5, H17), 4.65 (m, 1H, H8). ^{13}C NMR (CD_3OD, 150 MHz): δ 18.4 (C10), 23.7 (C3), 24.3 (C15), 25.8 (C9), 29.1 (C16), 30.2 (C4), 43.6 (C11), 46.1 (C2), 46.4 (C8), 46.9 (C14), 59.0 (C17), 59.5 (C5), 167.8 (C6), 171.0 (C12), 174.5 (C18). HRMS (ESI) *m/z*: $[M]^+$ Calcd for $C_{15}H_{23}N_3O_4$ 309.1791; Found 309.1782. ATR-IR ν_{max}: 3321, 2967, 2872, 1747, 1628, 1543, 1387, 1376, 1155 cm^{-1}.

Procedure for the synthesis of catalysts 2-4, *ent*-3 and *ent*-4. Protected tripeptide (1 eq) was dissolved in CH_3OH (25 mL/mmol tripeptide) and $Pd(OH)_2/C$ (10% weight) was added. The mixture was stirred under 7 atm of H_2 at room temperature overnight. After this period, the crude was filtered through Celite and washed with CH_3OH. The collected solvent was evaporated to provide the catalyst. ***Catalyst 2:*** Synthesized from tripeptide **11** (0.24 g, 0.45 mmol). The evaporation of the solvent yields compound **2** (0.14 g, 0.45 mmol, quantitative yield) as a white solid without need for further purification. mp: 173-176 ºC (CH_3OH). $[\alpha]_D^{20}$ -33 (*c* 1.0, CH_3OH). ^{1}H NMR (CD_3OD, 400 MHz): δ 1.92-2.17 (m, 9H), 2.30-2.44 (m, 3H), 3.38 (m, 2H), 3.48 (m, 2H), 3.69 (m, 1H), 4.31 (m, 1H), 4.43 (m, 1H), 4.81 (m, 1H). ^{13}C NMR (CD_3OD, 100 MHz): δ 16.7, 23.7, 24.1, 27.9, 29.4, 29.7, 43.3, 45.8, 59.7, 61.2, 168.2, 170.3, 177.9. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{15}H_{24}N_3O_4$ 310.1761; Found 310.1760. ATR-IR ν_{max}: 3204, 2950, 1673, 1573, 1447, 1387 cm^{-1}. ***Catalyst 3***: Synthesized from tripeptide **15** (0.18 g, 0.32 mmol). The evaporation of the solvent yields compound **3** (0.11 g, 0.32 mmol, quantitative yield) as a white solid without need for further purification. mp: 154-156 ºC (CH_3OH). $[\alpha]_D^{20}$ -10 (*c* 0.5, CH_3OH). ^{1}H NMR (CD_3OD, 400 MHz): mixture of conformers observed δ 0.98 (s, 3H), 1.40 (s, 3H), 2.04 (m, 8H), 2.44 (m, 2H), 2.99 (m, 1H), 3.38 (m, 3H), 3.59 (m, 1H), 3.71 (m, 1H), 4.06 (m, 1H), 4.22 (m, 1H). ^{13}C NMR (CD_3OD, 100 MHz): δ 16.2, 16.5, 22.1, 23.6, 23.9, 24.4, 28.8, 29.8, 42.0, 45.9, 50.4, 59.6, 168.1. HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_{17}H_{28}N_3O_4Na$ 338.2074; Found 338.2085. ATR-IR

ν_{max}: 2956, 1671, 1608, 1552, 1448 cm^{-1}. ***Catalyst ent-3***: Synthesized from tripeptide *ent*-**15** (0.35 g, 0.62 mmol). The evaporation of the solvent yields compound *ent*-**3** (0.21 g, 0.62 mmol, quantitative yield) as a white solid without need for further purification. All the physical constants and NMR data coincide with those for catalyst **3**, except the optical rotation, which is $[\alpha]_D^{20}$ +11 (*c* 1, CH_3OH). HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_{17}H_{28}N_3O_4Na$ 338.2074; Found 338.2079. ***Catalyst 4***: Synthesized from tripeptide **19** (0.25 g, 0.45 mmol). The evaporation of the solvent yields compound **4** (0.15 g, 0.45 mmol, quantitative yield) as a white solid without need for further purification. mp: 137-140 ºC (CH_3OH). $[\alpha]_D^{20}$ -110 (*c* 0.9, CH_3OH). ^1H NMR (CD_3OD, 600 MHz) major conformer: δ 0.92 (s, 3H, $CH_3$12), 1.40 (s, 3H, $CH_3$13), 1.96-2.09 (m, 6H, H3, H3', H4, H17, H17', H18), 2.21-2.26 (m, 2H, H11, H18'), 2.42 (m, 1H, H11'), 2.49 (m, 1H, H4'), 3.04 (t, 1H, J = 8.6 Hz, H10), 3.41 (m, 2H, H2, H2'), 3.54 (m, 1H, H16), 3.74 (m, 1H, H16'), 4.13 (t, 1H, J = 9.1 Hz, H8), 4.31 (m, 1H, H5), 4.38 (m, 1H, H19). minor conformer: δ 0.99 (s, 3H, $CH_3$12), 1.31 (s, 3H, $CH_3$13), 1.96-2.09 (m, 5H, H3, H3', H4, H17, H17'), 2.17-2.25 (m, 3H, H11, H18, H18'), 2.42 (m, 1H, H11'), 2.49 (m, 1H, H4'), 2.87 (t, 1H, *J* = 8.6 Hz, H10), 3.41 (m, 2H, H2, H2'), 3.61 (m, 1H, H16), 3.74 (m, 1H, H16'), 4.05 (t, 1H, *J* = 8.9 Hz, H8), 4.31 (m, 1H, H5), 4.50 (m, 1H, H19). ^{13}C NMR (CD_3OD, 150 MHz) major conformer: δ 16.5 ($CH_3$12), 23.8 (C3), 24.3 (C11, C17), 29.0 ($CH_3$13), 29.3(C18), 30.3 (C4), 42.7 (C10), 45.9 (C2, C9), 47.3 (C16), 50.0 (C8), 59.5 (C5), 60.2 (C19), 168.3 (C6), 170.9 (C14), 176.3 (C20). minor conformer: δ 16.1 (C12), 21.8 (C17), 23.7 (C3), 25.2 (C11), 28.5 (C13), 30.2 (C4), 31.3 (C18), 42.2 (C10), 46.1 (C2), 46.4 (C9), 47.3 (C16), 50.4 (C8), 60.2 (C5), 61.1 (C19), 168.1 (C6), 171.5 (C14), 176.5 (C20). HRMS (ESI) *m/z*: $[M]^+$ Calcd for $C_{17}H_{27}N_3O_4$ 337.2001; Found 337.2002. ATR-IR ν_{max}: 3383, 3067, 2956, 1721, 1671, 1609, 1562, 1447 cm^{-1}. ***Catalyst ent-4****:* Synthesized from tripeptide *ent*-**19** (0.46 g, 0.82 mmol). The evaporation of the solvent yields compound *ent*-**4** (0.28 g, 0.82 mmol, quantitative yield) as a white solid without need for further purification. All the physical constants and NMR data coincide with

those for catalyst **4**, except the optical rotation, which is $[\alpha]_D^{20}$ +106 (*c* 1.2, CH_3OH). HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_{17}H_{27}N_3O_4Na$ 360.1894; Found 360.1891.

General procedure for catalytic asymmetric aldol reaction. a. Water free conditions: 0.01 to 0.04 mmol (5 to 20%) of the selected catalyst were added to 0.2 mmol of aldehyde and dissolved in 0.75 to 2 mL (0.1 M to 0.3 M) of solvent (acetone, methanol or various acetone/methanol mixtures) under N_2 atmosphere at the desired temperature. The reaction was stirred for the time indicated. The solvent was evaporated and 5 mL of EtOAc and 5 mL of water were added. The organic phase was washed with 2 x 3 mL of water, dried over $MgSO_4$, filtered and evaporated under reduced pressure. Purification by column chromatography (EtOAc/hexane 1:3) yielded the desired aldol product. **b. Acetone/water mixture:** 0.01 to 0.04 mmol (5 to 20% catalyst) of the selected catalyst were added to 0.2 mmol of aldehyde and dissolved in 0.75 to 2 mL (0.1 M to 0.3 M) of acetone/water (10:1 or 3:1). The reaction mixture was stirred for the time indicated. The acetone was evaporated and 5 mL of EtOAc and 5 mL of water were added. The organic phase was washed with 2 x 3 mL of water, dried over $MgSO_4$, filtered and evaporated under reduced pressure. Purification by column chromatography (EtOAc/hexane 1:3) yields the desired aldol product.

Catalyst recovery: The combined water phases were washed with 3 x 5 mL of Et_2O. The lyophilization of the aqueous layers allowed the recovery of the catalyst (90%).

Reaction between *p*-nitrobenzaldehyde and cyclohexanone: Catalyst **4** (3.4 mg, 0.01 mmol, 0.05 eq) and *p*-nitrobenzaldehyde (30 mg, 0.2 mmol) were dissolved in 2 mL of cyclohexanone under N_2 atmosphere. The reaction was stirred for 5 h and the solvent was evaporated. 5 mL of EtOAc and 5 mL of water were added and the organic phase was washed with 2 x 3 mL of water, dried over $MgSO_4$, filtered and evaporated under reduced pressure. Purification by column chromatography (EtOAc/hexane 1:1) yielded the diastereomeric mixture of aldols.

Experimental data of the organocatalyzed reaction products: 4-Hydroxy-4-(4'-nitrophenyl)butan-2-one, 24a: ^{1}H NMR ($CDCl_3$, 360 MHz): δ 2.22 (s, 3H), 2.86 (m, 2H), 3.58 (d, 1H, *J* = 3.2 Hz), 5.26 (m, 1H), 7.54 (d, 2H, *J* = 8.4 Hz), 8.21 (d, 2H, *J* = 8.4 Hz). Enantiomeric ratio determined by chiral HPLC analysis. Chiralpak AS column, hexane/2-propanol 70:30, 0.5 mL/min; t_R = 25.37 min for (*R*)-enantiomer, t_R = 34.50 min for (*S*)-enantiomer. The absolute configuration was assigned by comparison with the values reported in literature.[17]

4-Hydroxy-4-phenylbutan-2-one, 24b: ^{1}H NMR ($CDCl_3$, 250 MHz): δ 2.25 (s, 3H), 2.87 (m, 2H), 4.40 (br s, 1H), 5.23 (m, 2H), 7.47 (m, 5H). Enantiomeric ratio determined by chiral HPLC analysis. Chiralpak AS column, hexane/2-propanol 90:10, 0.5 mL/min; t_R = 28.01 min for (*R*)-enantiomer, t_R = 35.76 min for (*S*)-enantiomer. The absolute configuration was assigned by comparison with the values reported in literature.[32]

4-(4'-Bromophenyl)-4-hydroxybutan-2-one, 24c: ^{1}H NMR ($CDCl_3$, 250 MHz): δ 2.22 (s, 3H), 2.82 (m, 2H), 5.13 (m, 1H), 7.27 (m, 2H), 7.48 (m, 2H). Enantiomeric ratio determined by chiral HPLC analysis. Chiralpak AS column, hexane/2-propanol 90:10, 0.5 mL/min; t_R = 30.57 min for (*R*)-enantiomer, t_R = 41.62 min for (*S*)-enantiomer. The absolute configuration was assigned by comparison with the values reported in literature.[32]

4-(4'-Chlorophenyl)-4-hydroxybutan-2-one, 24d: ^{1}H NMR ($CDCl_3$, 250 MHz): δ 2.22 (s, 3H), 2.81 (m, 2H), 5.13 (m, 1H), 7.30 (m, 1H). Enantiomeric ratio determined by chiral HPLC analysis. Chiralpak AS column, hexane/2-propanol 90:10, 0.5 mL/min; t_R = 27.64 min for (*R*)-enantiomer, t_R = 35.84 min for (*S*)-enantiomer. The absolute configuration was assigned by comparison with the values reported in literature.[32]

4-Hydroxy-4-(4'-(trifluoromethyl)phenyl)butan-2-one, 24e: ^{1}H NMR ($CDCl_3$, 250 MHz): δ 2.21 (s, 3H), 2.86 (m, 2H); 5.22 (m, 1H), 7.50 (m, 2H), 7.60 (m, 2H). Enantiomeric ratio determined by chiral HPLC analysis. Chiralpak AS column, hexane/2-propanol 92/8, 0.5

mL/min; t_R = 22.79 min for (*R*)-enantiomer, t_R = 28.85 min for (*S*)-enantiomer. The absolute configuration was assigned by comparison with the values reported in literature.[32]

4-Hydroxy-4-(2'-nitrophenyl)butan-2-one, 24f: ^{1}H NMR ($CDCl_3$, 250 MHz): δ 2.25 (s, 3H), 2.76 (m, 1H), 3.14 (m, 1H), 3.78 (br s, 1H), 5.69 (dd, 1H, *J* = 9.4, 2.2 Hz), 7.45 (m, 1H), 7.68 (m, 1H), 7.95 (m, 2H). Enantiomeric ratio determined by chiral HPLC analysis. Chiralpak AS column, hexane/2-propanol 70/30, 0.5 mL/min; t_R = 17.51 min for (*S*)-enantiomer, t_R = 21.78 min for (*R*)-enantiomer. The absolute configuration was assigned by comparison with the values reported in literature.[17, 33]

4-(2'-Bromophenyl)-4-hydroxybutan-2-one, 24g: ^{1}H NMR ($CDCl_3$, 250 MHz): δ 2.22 (s, 3H), 2.67 (m, 1H), 3.02 (m, 1H), 5.43 (m, 1H), 7.14 (t, 1H, *J* = 7.6 Hz), 7.35 (t, 1H, *J* = 7.6 Hz), 7.51 (d, 1H, *J* = 7.9 Hz), 7.61 (d, 1H, *J* = 7.7 Hz). Enantiomeric ratio determined by chiral HPLC analysis. Chiralpak AS column, hexane/2-propanol 90/10, 0.3 mL/min; t_R = 37.34 min for (*S*)-enantiomer, t_R = 43.54 min for (*R*)-enantiomer. The absolute configuration was assigned by comparison with the values reported in literature.[17, 34]

4-Cyclohexyl-4-hydroxybutan-2-one, 24i: ^{1}H NMR ($CDCl_3$, 250 MHz): δ 0.84–1.22 (m, 6H), 1.50-1.74 (m, 5H), 2.06 (s, 3H), 2.45 (m, 2H), 3.28 (br s, 1H), 3.69 (m, 1H). Enantiomeric ratio determined by chiral HPLC analysis. Chiralpak AS column, hexane/2-propanol 92/8, 0.5 mL/min; t_R = 16.75 min for (*R*)-enantiomer, t_R = 19.37 min for (*S*)-enantiomer. The absolute configuration was assigned by comparison with the values reported in literature.[32]

2-(Hydroxy(4'-nitrophenyl)methyl)cyclohexan-1-one, 26: ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.53–1.83 (m, 5H), 2.10 (m, 1H), 2.35-2.59 (m, 3H), 4.90 (d, 1H, *J* = 8.4 Hz), 7.51 (d, 2H, *J* = 8.7 Hz), 8.21 (d, 2H, *J* = 8.8 Hz). Enantiomeric and diastereomeric ratio determined by chiral HPLC analysis. Chiralpak AD-H column, hexane/2-propanol 88/12, 0.7 mL/min; t_R =

23.60 min for (2*R*, 1'*R*)-diastereomer, t_R = 25.79 min for (2*S*, 1'*S*)-diastereomer, t_R = 28.73 min for (2*S*,1'*R*)-diastereomer, and t_R = 37.65 min for (2*R*, 1'*S*)- diastereomer. The absolute configuration was assigned by comparison with the values reported in literature.[27]

Computational details:

The geometries of all structures have been optimized using the M06-2X[35] density functional method with the 6-31G(d) basis set in acetone solution. The solvent effects have been included using the CPCM method.[36] Harmonic vibrational frequencies were computed for all structures in solution to characterize them as energy minima or transitions states. The energies of selected structures were recalculated with the 6-311+G(d,p) basis set and some of them were also optimized with the largest basis set. Restricted conformational searches were carried out to obtain starting structures for the localization of the transition states using a mixed low mode/torsional sampling[37] with the OPLS-2005[38] force field implemented in the MacroModel[39] program (see the Supporting Information for details corresponding to the rate determining transition state). For each transition state displacements following the transition vector were done to locate the corresponding energy minima. Atomic charges were computed from Natural Population Analysis (NPA).[40] All density functional calculations were done using the Gaussian-09 program.[41]

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: xxxxxxx.

Conformational NMR studies for catalyst **4** in various solvents, Conformational NMR studies for catalyst **2** in CD_3OD, CD spectra for various solutions of catalyst **4**, HPLC chromatograms for the aldol products, NMR spectra of all intermediates and final products, detailed computed mechanism of the aldol reaction catalyzed by **4**, total energies and Gibbs energies in acetone of computed structures, Cartesian coordinates of computed structures, complete reference 42.

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