FULL PAPER   **WILEY**-VCH

Bifunctional Tripeptide Including a Phosphonic Acid as Brønsted Acid for Michael Addition: Mechanistic Insights

Margery Cortes-Clerget,[a] Jesús Jover,[b][c] Jade Dussart,[a] Emilie Kolodziej,[d] Maelle Monteil,[a] Evelyne Migianu-Griffoni,[a] Olivier Gager,[a] Julia Deschamp,*[a] Marc Lecouvey*[a]

Abstract: Enamine catalysis is a widespread activation mode in the field of organocatalysis and is often encountered in bifunctional organocatalysts. We previously described H-Pro-Pro-pAla-OMe as a bifunctional catalyst for Michael addition between aldehydes and aromatic nitroalkenes. As opposite selectivities were observed compared to its analogue H-Pro-Pro-Glu-NH₂ described by Wennemers, its activation mode was investigated through kinetic, linear effect studies, NMR analyses and structural modifications. It appeared that only one bifunctional catalyst was involved in the catalytic cycle, by activating aldehyde through an (E)-enamine and nitroalkene via an acidic interaction. A restrained tripeptide structure was optimal in terms of distance and rigidity for better selectivities and fast reaction rates. Transition state modeling unveiled the particular selectivity of this phosphonopeptide.

Introduction

The asymmetric characteristic of living organisms has long been a subject of interest especially in terms of stereospecificity and stereoselectivity. Inspiring by enzyme structures, organic chemists aim at developing more efficient, selective and sustainable catalysts. Despite the use of proline as catalyst has been known since the 70s, organocatalysis[1] has truly emerged as a powerful tool for asymmetric transformation since the independent works of Barbas, Lerner, List and MacMillan in 2000.[2] Nowadays, organocatalysis via covalent[3] or non-covalent[4] activations enables the selective formation of one or several stereogenic centers[5] at once. Moreover these methods have been successfully employed for the synthesis of biologically active compounds.[6] Thanks to their multifunctional structure, amino acids and peptides are especially efficient at multiactivating easily accessible or natural molecules.

[a]   Dr. M. Cortes-Clerget, Dr. O. Gager, Dr. M. Monteil, Dr. E. Migianu-Griffoni, J. Dussart, Dr. J. Deschamp and Prof. M. Lecouvey
        Université Paris 13, Sorbonne Paris Cité – Laboratoire CSPBAT – CNRS UMR 7244, 1 Rue de Chablis, 93000 Bobigny - France
        E-mail: marc.lecouvey@univ-paris13.fr
        E-mail: julia.deschamp@univ-paris13.fr
[b]   Dr. J. Jover, Institute of Chemical Research of Catalonia (ICIQ), The Barcelona Institute of Science and Technology, Avgda. Països Catalans, 16, 43007 Tarragona, Spain
[c]   Departament de Química Inorgànica i Orgànica, Secció de Química Inorgànica, Universitat de Barcelona, Martí i Franquès 1-11, 08028 Barcelona, Spain
[d]   E. Kolodziej, Université Paris Sud, ICMMO, UMR 8182, 15 Rue Georges Clemenceau 91405 Orsay Cedex

        Supporting information for this article is given via a link at the end of the document.

Combining either amino-, H-bonding or Lewis base catalysis, hydrophobic effect and in-solvent organization, they were early introduced to mimic enzyme behavior and selectively interact with substrates.[7] Beyond the structural peptide complexity, Miller has recently proposed combinatorial screenings to easily identify novel peptide-based catalysts to promote enantioselective processes.[8] Afterwards Wennemers has highlighted the tripeptides H-Pro-Pro-Asp-NH₂ and H-Pro-Pro-Glu-NH₂ as efficient catalysts for enantioselective aldol reaction and Michael addition, respectively (Scheme 1).[5d,9]
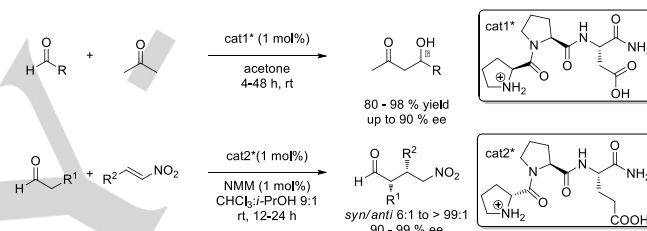
Scheme 1. Wennemers' catalysts effective on aldolisation and 1,4-addition reactions

Additionally, the same team showed that multiple modes of catalyst activation are required, where the absence of either the N-terminal secondary amine, or the carboxylic acid inhibited the reaction. They also provided evidence for an enamine rather than an enol mechanism by ESI-MS investigations.[10]

Our group have recently reported the first example of H-Pro-Pro-pAla-OMe[11] combining both aminocatalysis and phosphonic acid-activation for the diastereo- and enantioselective conjugate addition of aldehydes onto nitroalkenes.[12] We developed a straightforward synthesis of the modified amino-acids and their corresponding tripeptides by varying the absolute configuration of the three stereogenic centers and the lateral chain length of the amino-acid bearing the phosphonic acid moiety (Figure 1).
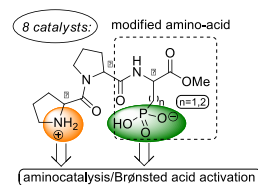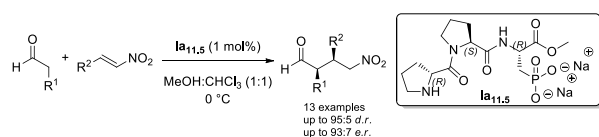

Figure 1. Novel bifunctional catalysts including a phosphonic acid moiety

Our preliminary studies focused on a Michael addition as a model reaction between aldehydes and nitroalkenes. Among our catalyst library, the tripeptide Ia₁₁.₅[13] enabled us to obtain the

corresponding γ-nitroaldehydes in excellent yields and with good to excellent stereoselectivities (up to 95:5 *d.r.*; 93:7 *e.r.*) without any external base or side product (Scheme 2).

Scheme 2. Michael addition between aldehydes and aromatic nitroalkenes catalyzed by **Ia₁₁.₅**

In order to propose a sustainable process, the catalyst recycling was performed. Thanks to their high water solubility, these catalysts were easily recovered by simple extractions and lyophilization. Several recycling tests were achieved without any significant loss of selectivities (10 times). Owing to the phosphonic acid group, our tripeptides have the advantage to be directly recyclable and thus, they do not require to be immobilized on a solid support.

In our former work, the dependence of various parameters such as catalyst loading and protonated-state catalyst were thoroughly explored. Surprisingly, for a given spatial configuration catalyst, we obtained the opposite products when compared to Wennemers' catalyst (Scheme 3).
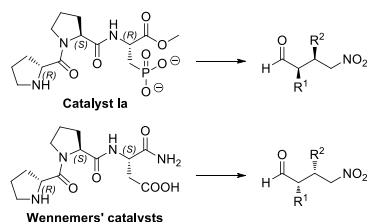

Scheme 3. Wennemers' catalyst vs. **Ia₁₁.₅**: opposite selectivities

As our research focused on the impact of a phosphonic acid compared to a carboxylic acid on both reactivity and selectivities, we have started some mechanistic investigations to understand our previous observations. Herein, we disclose our results in order to highlight the potential of our novel tripeptide-catalysts.

Results & Discussion

The first experiment was intended to elucidate which functional groups were required for asymmetric catalysis (Scheme 4). The reaction was carried out between propanal and *trans*-β-nitrostyrene in presence of the *N*-Boc-protected tripeptide **Boc-Ia** in the optimized conditions that we previously reported.[12] The ionization state of the phosphonic salt was pre-adjusted to pH = 7.5 with a sodium hydroxide solution during the catalyst preparation. As expected, no reaction occurred, demonstrating that the amine group is essential for activation. In the same

manner, the phosphonic acid was replaced by a phosphonate. Even with the terminal secondary amine-free proline, catalyst **Ia-(OMe)₂** was significantly poorer than **Ia₁₁.₅**. The reaction rate was very slow (conversion < 10 % after 48 h) and the selectivities dropped dramatically (*syn*:*anti* 66:34 and 32 % e.r.).
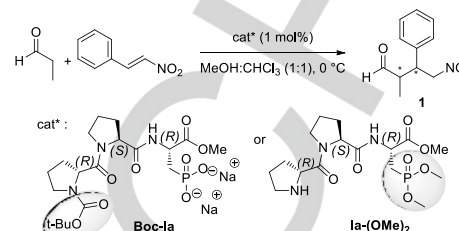
Scheme 4. Michael addition between propanal and *trans*-β-nitrostyrene catalyzed by tripeptides **Boc-Ia** or **Ia-(OMe)₂**

In view of these results, we conclude that both the secondary amine and the phosphonic acid groups are essential to keep good reactivity, diastereo- and enantioselectivities.

After that, 15 diastereoisomers of **Ia** were prepared to investigate the variations on both catalyst configurations and side chain length holding the phosphonic acid group.[14] The catalysts were screened in the presence of propanal and *trans*-β-nitrostyrene under the optimized conditions (Figure 2). The reactivity of the catalysts was excellent as total conversion was achieved within less than 6 hours for all the evaluated diastereoisomers. The selectivities varied accordingly to the catalytic pocket geometry. Changing the terminal proline absolute configuration can significantly reduce the enantiomeric (*e.g.* **IId → IIa** – row 2) and the diastereoisomeric ratios (*e.g.* **Id → Ic** – row 1). Moreover, opposite absolute configurations were obtained for product **1** (Figure 2 – red and green).

Increasing the side chain length (Figure 2 – columns) extended the time to completion and reduced the diastereoisomeric ratios indicating that higher flexibility implies lower selectivities. All these results showed the specific H-*R*-Pro-*S*-Pro-*R*-pAla-OMe structure was crucial for a good selectivity but also for a fast reaction in an intramolecular way. In our former works, we also noted the importance of the protonated-state of the catalyst as better selectivities were observed when the tripeptide catalyst was pre-adjusted at pH = 11.5.

Since the geometry of the catalytic pocket is crucial for a good stereoselectivity, we focused our interest on studying the importance of the central proline for selectivities (Table 1).

We started replacing the central proline by a less bulky and more flexible amino acid like glycine, obtaining catalyst **Ie** that was then evaluated in standard conditions (Table 1 – entry 2). The diastereoselectivity dropped ((65:35 vs. 91:9 d.r.) and a lower enantioselectivity was observed (23:77 vs. 93:7 e.r.) although the reaction time was extended (35 h vs. 20 h). The rigidity imposed by the proline residue seems to be crucial for an efficient diastereo-discrimination. As expected, the opposite enantiomer product **1** (2*S*,3*R*) was obtained as the enantiomer catalyst **Ie** was used (Table 1 – entries 2 vs. 1).
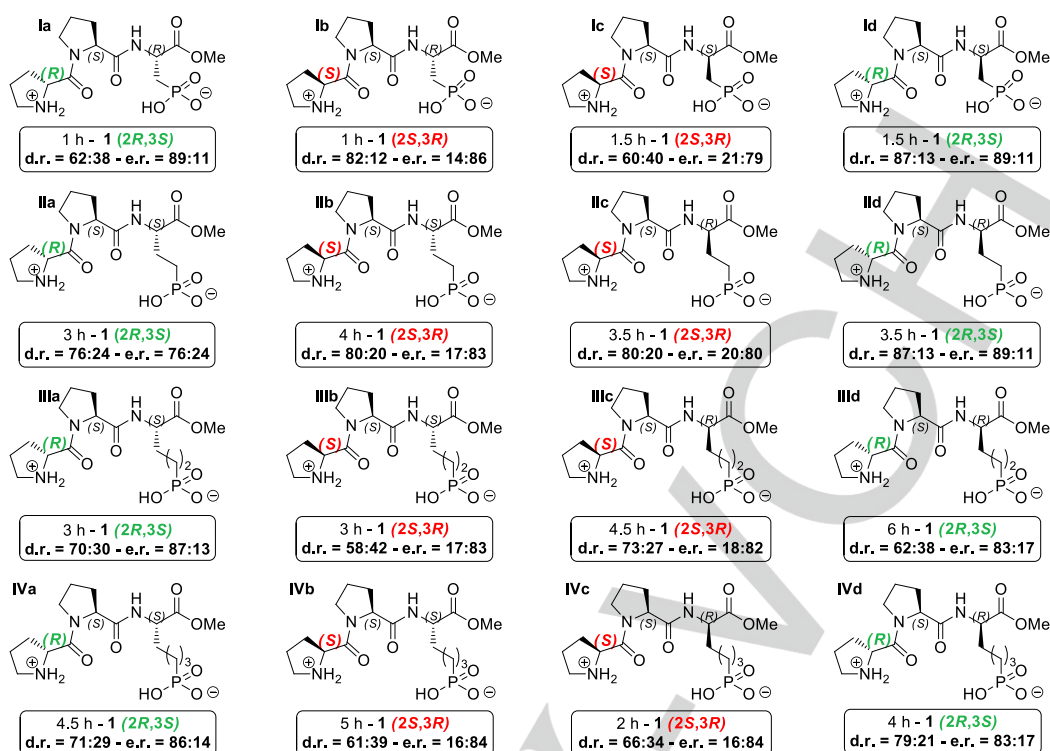
Ia — 1 h - **1** (2R,3S) — d.r. = 62:38 - e.r. = 89:11
Ib — 1 h - **1** (2S,3R) — d.r. = 82:12 - e.r. = 14:86
Ic — 1.5 h - **1** (2S,3R) — d.r. = 60:40 - e.r. = 21:79
Id — 1.5 h - **1** (2R,3S) — d.r. = 87:13 - e.r. = 89:11

IIa — 3 h - **1** (2R,3S) — d.r. = 76:24 - e.r. = 76:24
IIb — 4 h - **1** (2S,3R) — d.r. = 80:20 - e.r. = 17:83
IIc — 3.5 h - **1** (2S,3R) — d.r. = 80:20 - e.r. = 20:80
IId — 3.5 h - **1** (2R,3S) — d.r. = 87:13 - e.r. = 89:11

IIIa — 3 h - **1** (2R,3S) — d.r. = 70:30 - e.r. = 87:13
IIIb — 3 h - **1** (2S,3R) — d.r. = 58:42 - e.r. = 17:83
IIIc — 4.5 h - **1** (2S,3R) — d.r. = 73:27 - e.r. = 18:82
IIId — 6 h - **1** (2R,3S) — d.r. = 62:38 - e.r. = 83:17

IVa — 4.5 h - **1** (2R,3S) — d.r. = 71:29 - e.r. = 86:14
IVb — 5 h - **1** (2S,3R) — d.r. = 61:39 - e.r. = 16:84
IVc — 2 h - **1** (2S,3R) — d.r. = 66:34 - e.r. = 16:84
IVd — 4 h - **1** (2R,3S) — d.r. = 79:21 - e.r. = 83:17

Figure 2. Influence of catalyst **I-IV** absolute configurations and lateral chain lengths on 1,4-conjugated addition of propanal onto *trans*-β-nitrostyrene

After that, the central proline was suppressed and dipeptides **If** and **Ig** were tested but, no total conversion was reached after a week (Table 1 - entries 3 and 4). As predicted by Wennemers,[9a] the catalytic pocket of these compound might be too small for a β-position attack and consequently an intermolecular reaction probably occurred with a drop of selectivities. However, it was noted that better selectivities were obtained in presence of **Ig** rather than **If**. The link between the two activating functions was then suppressed in order to investigate the synergic catalysis; it was done by replacing the tripeptide by amino-acid "cocktails". It was firstly noted that modified amino acid **AA₁** alone gave less than 1 % conversion after 5 days (Table 1 – entry 5). The primary amine appears not to be nucleophilic enough to form an enamine with propanal. Proline alone allowed the formation of the γ-nitroaldehyde **1** after a long reaction time with a low enantioselectivity (Table 2 – entry 6). A mixture of proline and amino acid **AA₁**, named **cocktail A** (1:1) and a mixture of H-Pro-OMe and *N*-Boc-protected **AA₁**, called **cocktail B** (1:1) were evaluated under the optimized conditions (Table 1 – entries 7 and 8). We observed that the reaction rates were 6 times slower for both mixtures. The diastereoisomeric ratio slightly decreased for **cocktail A** while remained the same for **cocktail B**. In both cases, the enantiomeric ratios were lower compared to those obtained with **Ia₁₁.₅**. Although these two cocktails have the same absolute configurations, interestingly, the opposite products were obtained. We suppose that this discrepancy arises from the difference between the transition states leading to the formation of the corresponding products (Figure 3).

Table 1. Influence of the catalyst structure.

Entry	Catalyst	t [h][a]	*syn:anti*[b]	e.r.[c]	Abs. conf.[d]
1	**Ia₁₁.₅**	20	91:9	93:7	2R,3S
2	**Ie**	35	65:35	23:77	2S,3R
3	**If**	(64)[e]	70:30	38:62	2S,3R
4	**Ig**	(76)[e]	85:15	28:72	2S,3R
5	**AA₁**	n.d.[f]	-	-	-
6	proline[g]	216	93:7	41:59	2S,3R
7	cocktail A	120	87:13	38:62	2S,3R
8	cocktail B	120	91:9	70:30	2R,3S

[a] Time to completion, estimated by GC analysis. [b] Determined by ^{1}H NMR spectroscopy on the crude material. [c] e.r. of the *syn*-adduct were determined by chiral-HPLC analysis. [d] Compared to optical rotation given in literature. [e] Expressed in conversion rate after a week. [f] Less than 1% of conversion after a week. [g] The reaction was conducted at pH = 11.5

In the case of **cocktail A,** an electrostatic interaction allows the self-assembly of the amino-acids while for **cocktail B** the steric bulkiness of the dangling *tert*-butyl group pulls away the two partners. The same enantioselectivity was obtained with **cocktail A** and catalyst **If** but the diastereoisomeric ratio is higher for the former (Table 1 – entries 3 vs 7). **Cocktail B** and catalyst **Ia$_{11.5}$** gave the same diastereoselectivity albeit a lower enantiomeric ratio was obtained with **cocktail B** (Table 1 – entries 1 vs 8).
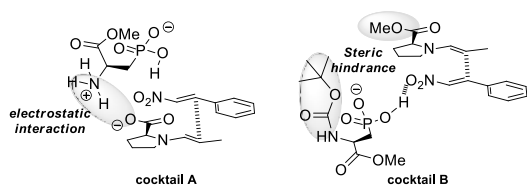
Figure 3. Postulated transition states involving **cocktail A** and **cocktail B**

After studying the importance of the catalyst structure, we decided to explore the catalyst activation mode. According to the literature,[15] the postulated mechanism should involve the formation of an *E*-enamine between the secondary amine group and the aldehyde (Scheme 5). Then, the nitroalkene activation through an acidic interaction should increase the electrophilicity of the β-carbon and allow the enamine attack. Thanks to the geometry of the catalytic pocket, the C-C bond could be created in a stereoselective way. The final hydrolysis should concomitantly permit the liberation of the expected γ-nitroaldehyde and regenerate the active catalyst.
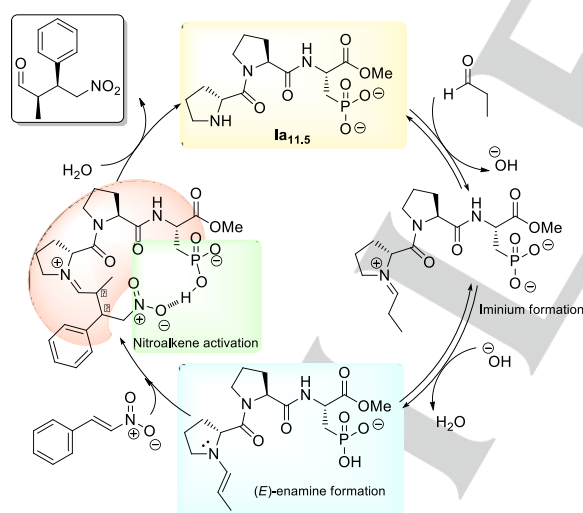
Scheme 5. Postulated catalytic cycle for the **Ia$_{11.5}$**-catalyzed 1,4-addition reaction

In order to validate the involvement of only one molecule of catalyst, kinetic studies and non-linear effect experiments were conducted in presence of catalyst **Ia**. The 1,4-addition between propanal and *trans*-β-nitrostyrene was catalyzed at 1, 2.5, 5, 7.5

and 10 mol% of **Ia** at pH = 6.6 or 11.5. The partial order with regard to the catalyst was respectively 0.8 and 0.9 at pH = 6.6 and 11.5.[16] Both these values are close to 1, indicating that the rate of the reaction increases linearly with the catalyst loading.

Although Kagan and Agami had observed a slight non-linear effect on the proline-catalyzed Hajos-Parrish reaction.[17] List[18] contradicted these results by providing evidences for the involvement of only one pyrrolidine in the transition state of intra- and intermolecular aldol reaction.[19] To verify the linear relationship between the enantiomeric excess of the catalyst and the product, the reaction above was catalyzed by a mixture of **Ia** and its enantiomer in several ratios at 0 or 25 °C, with catalyst loadings of 1 or 10 mol% and pre-adjusted pH values of 6.6 or 11.5. A linear effect was observed regardless of the experimental conditions.[20] Both kinetic studies and non-linear effect experiments provide proofs that only one molecule of catalyst was involved in the catalytic process.

NMR experiments were performed to possibly identify some iminium/enamine intermediates. The catalyst **Ia** was mixed with an excess of propanal in a mixture of chloroform-d: methanol-d$_4$ (1:1). The signal of propanal (9.44 ppm) decreased and two new signals were detected at 6.26 ppm and 9.04 ppm which could correspond to enamine and iminium intermediates (Figure 4).[21]
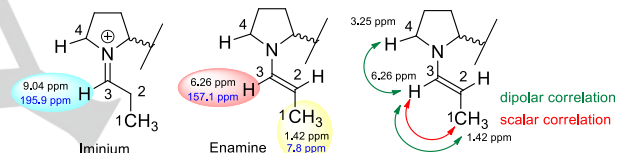

Figure 4. Identification of iminium and (*E*)-enamine by ^{1}H (in black) and ^{13}C NMR (in blue)

2D NMR studies were conducted to confirm the nature of the new products. COSY experiment highlighted the scalar correlation between the enamine proton (3) (6.26 ppm) and the CH$_3$ group (1) (1.42 ppm).
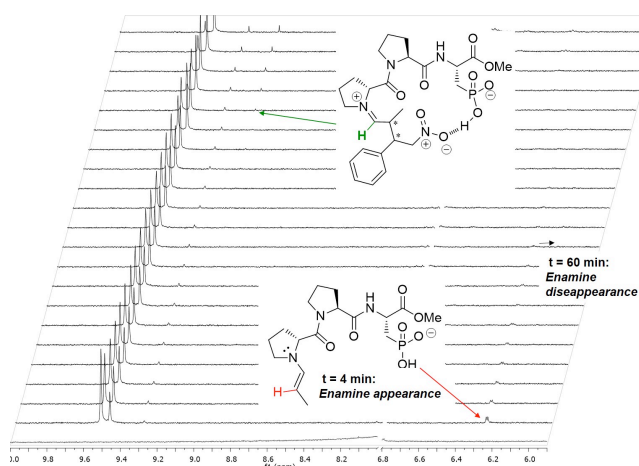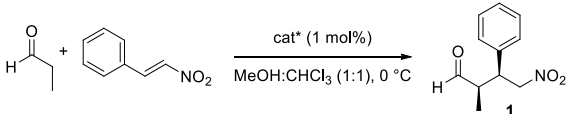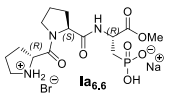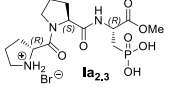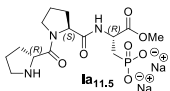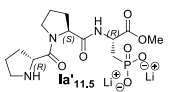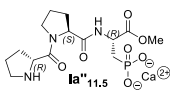

Figure 5. Enamine/iminium formation followed by ^{1}H NMR

Table 2. Influence of the pH and counter-cation nature in catalysis

Entry	Cat*	t [h][a]	d.r. [b] syn:anti	e.r.[c]
1	**Ia_{6.6}**	24	89:11	89:11
2	**Ia_{2.3}**	-	-	-
3	**Ia_{11.5}**	20	91:9	93:7
4	**Ia_{11.5}**[d]	168	90:10	90:10
5	**Ia'_{11.5}**	20	92:8	92:8
6	**Ia''_{11.5}**	192	94:6	94:6

[a] Time to completion, estimated by GC analysis. [b] Determined by 1H NMR spectroscopy of the crude material. [c] The *e.r.* of the *syn*-adduct were determined by chiral-phase HPLC analysis. [d] The reaction was carried out in presence of 2 equiv. of trimethylamine.

Moreover, NOESY showed a dipolar correlation with the α-proton (4) of the pyrrolidine (3.25 ppm) (Figure 4). These observations confirm the formation of an enamine with an *E*-configuration in agreement with the results reported by Blackmond, who observed an enamine proton at 6.1 ppm.[22] The addition of pre-activated molecular sieves in the NMR tube induced an increasing 6.26 ppm enamine signal at 9.44 ppm propanal signal expense. This observation seems to confirm the existing equilibrium at this step.

The reaction between propanal and *trans*-β-nitrostyrene was then carried out in presence of **Ia** and monitored by ¹H NMR (Figure 5). Enamine intermediate appeared at the first scan at 6.26 ppm. This signal rapidly decreased in favor of the formation of a novel signal which could correspond to a second iminium compound (9.2 ppm).

Thereafter, kinetic studies were performed to determine the reaction partial orders with regard to the aldehyde and the nitroalkene in presence of **Ia,** initially pre-adjusted at pH = 6.6 and 11.5.[13] The partial order of propanal is 0.4 in presence of catalyst **Ia_{6.6}** and drops to 0.1 for catalyst **Ia_{11.5}**. Concerning *trans*-β-nitrostyrene, the partial order is 0.3 in presence of catalyst **Ia_{6.6}** and 0.2 for catalyst **Ia_{11.5}**.
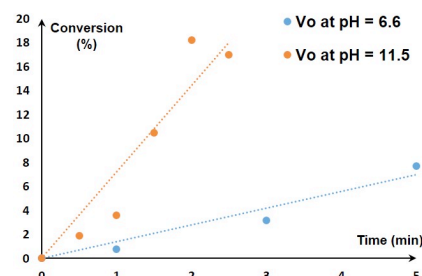
The protonated-state of the catalyst is strongly correlated with the partial orders of the aldehyde while its effect is limited on the nitroalkene partial order. Regardless of the pH pre-adjusted catalysts, enamine formation seems not to be the rate-limiting step. These kinetic studies corroborate our initial experimental observations about the postulated enamine formation.

Nevertheless, several questions remain concerning the influence of the peptide protonated-state on the catalytic process. We supposed that nitroalkene is activated by the phosphonic acid through an acidic interaction.

However, Ishihara showed that the calcium salt of the BINOL-phosphoric acid was as effective as the acidic form itself.[23] In order to identify the real activation mode of our tripeptide **Ia**, we studied the influence of its protonated-state (Table 2). The reaction still occurred with the all-deprotonated catalyst **Ia_{11.5}**, while no reaction was observed in presence of **Ia_{2.3}** (Table 2 – entries 2 and 3). Moreover, **Ia_{11.5}** was surprisingly a more reactive and selective catalyst than **Ia_{6.6}** and the reaction was completed after only 20 h. Nevertheless, when the reaction was conducted between propanal and *trans*-β-nitrostyrene in presence of **Ia_{11.5}**, the pH was monitored during the reaction. A drop of pH from 11.5 to 6.6 was early observed, which seems to confirm the presence of the acidic specie. We tested the same reaction in presence of **Ia_{11.5}** and 2 equivalents of triethylamine in order to keep the pH value around 8-9 (Table 2 – entry 4). Here, the reaction rate dramatically dropped and slightly lower selectivities were obtained. This observation provides the evidence that an acidic hydrogen on the phosphonic acid should be responsible for the nitroalkene activation, through a hydrogen bonding or ion-pairing.

To confirm the previous observations, the nature of the counter-cation was investigated by replacing the sodium salt by its lithium or calcium analogues (Table 2). The lithium salt **Ia'_{11.5}** gave the same results whereas the calcium salt **Ia''_{11.5}** showed poor reactivity (20 h vs. 192 h). It is not surprising that lithium which is quite similar to sodium, does not modify the catalytic pocket. On the other hand, calcium is bigger and divalent and probably disrupts the peptide conformation.

Based on our experiments, we propose the revised catalytic cycles in order to include the pH effect (Scheme 6). By comparing the initial rate for each protonated-state, it appears the reaction starts faster in the presence of **Ia_{11.5}** rather than **Ia_{6.6}** (Figure 6).

Figure 6. Comparison of initial rates depending on the catalyst protonated-state
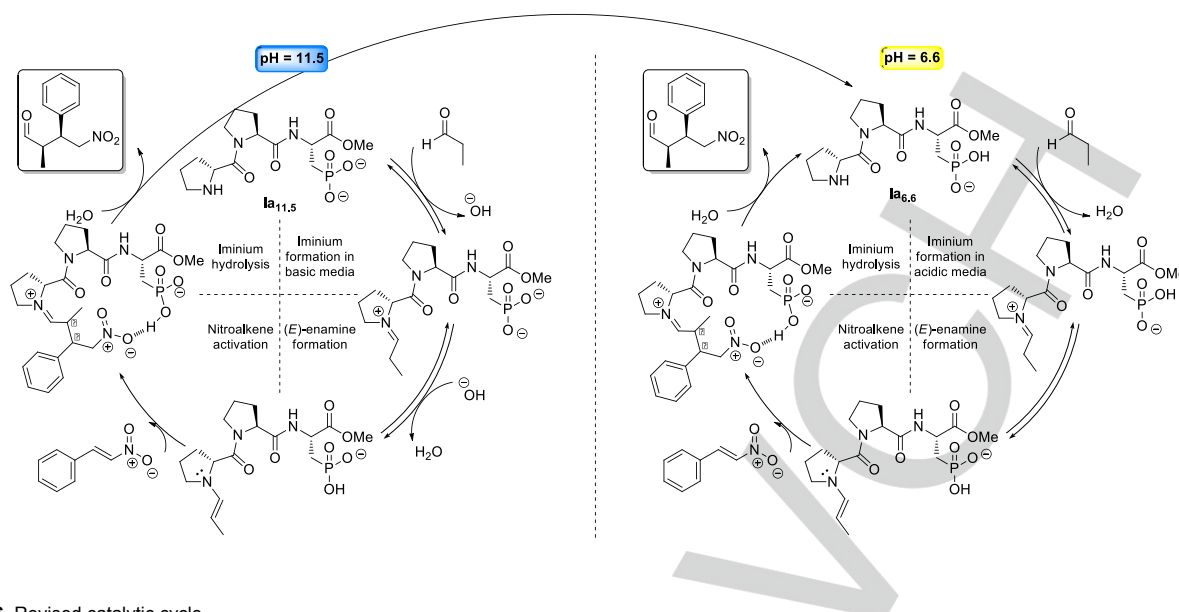
Scheme 6. Revised catalytic cycle

The catalyst **Ia₁₁.₅** already has the reactive amine on the terminal proline that could readily enable the formation of the iminium and then, enamine intermediates with propanal.

At this stage, the catalyst could be protonated allowing the nitroalkene H-bonding activation. The second iminium formation in presence of water could concomitantly release the expected γ-nitroaldehyde and regenerate the catalyst **Ia₆.₆** that permits the catalytic process to proceed.

Thereafter, the reaction was performed in presence of activated molecular sieves. The achieved conversion was never higher than 10 %. Besides, the 1,4-addition was carried out between propanal and *trans*-β-nitrostyrene in presence of **Ia₁₁.₅** and water, and no conversion was obtained. These results confirmed the harmful effect of water excess in the catalytic cycle, notably for the enamine formation. However the released water during the enamine formation is essential because it allows the second iminium hydrolysis. These observations support the formation of all the iminium/enamine intermediates.

Density functional theory (DFT) theoretical studies have been carried out in order to explain the observed enantioselectivity with peptide **Ia**.[20] The four transition states determining the final stereochemistry of the product *i.e. syn* (*2R, 3S*), *anti* (*2R, 3R*), *syn* (2S, 3R) and *anti* (2S, 3S), in the reaction between propanal and *trans*-β-nitrostyrene with peptide **Ia₆.₆** were computed. Their relative free energy barriers for the (*E*)-enamine attack onto *trans*-β-nitrostyrene are 4.5, 4.9, 9.4 and 10.8 kcal/mol for the above products, respectively (Scheme 7).

These results clearly indicate that product syn (*2R, 3S*) is the most favored reaction outcome, in a perfect agreement with our NMR experiments. The free energy difference between the *syn* (*2R, 3S*) and *anti* (*2R, 3R*) diastereoisomers also agrees with the experimentally observed proportions, a difference of 0.4 kcal/mol at room temperature should roughly produce a selectivity ratio of 70:30, not very far of that observed

experimentally. Figure 7 shows the transition states leading to these products and includes some selected bond distances.

The C–C bond distances in these transition states are quite long: 2.41 and 2.24 Å for the *syn*- and *anti*-species respectively, indicating the early character of these structures. As may be observed, the presence of the sodium cation actively participates in the reaction by binding the nitro group of the substrate, the Na–O$_{nitro}$ distances are always shorter than 2.5 Å. As predicted in our experiments, the binding between *trans*-β-nitrostyrene and the peptide is reinforced by the hydrogen bond interaction established between the nitro and phosphoric groups, which show very short distances in both cases (ca. 1.85 Å).

The alternative *syn* (*2S, 3R*) and *anti* (*2S, 3S*) products are obtained in a much lower extent because they require the formation of a starting (*Z*)-enamine between **Ia** and propanal. This process is highly unlikely since this latter species is almost 10 kcal/mol higher than its (*E*)-counterpart.
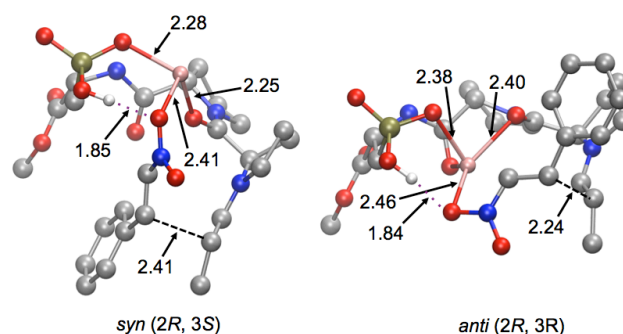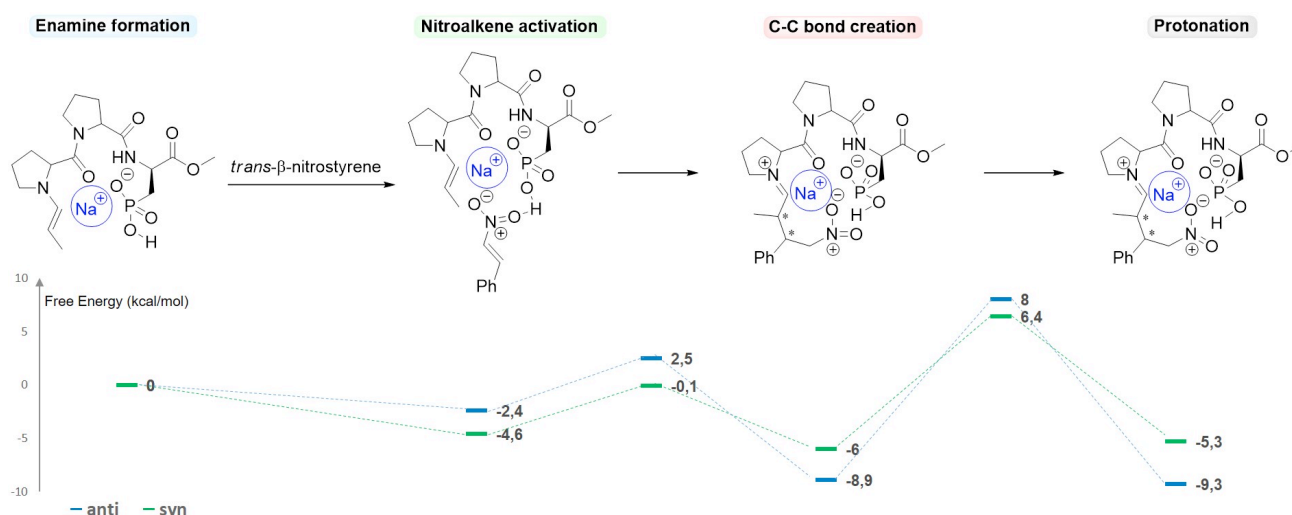

Figure 7. Transition states leading to the major products (distances in Å, color code: C = gray, N = blue, O = red, P = tan, Na = pink, H = white; for clarity most H-atoms have been omitted)

Scheme 7. Computed free energy profiles (kcal/mol) for the reaction of *trans*-β-nitrostyrene with the propanal (*E*)-enamine of peptide **Ia$_{6.6}$** leading to the syn (*2R, 3S*) and *anti* (2R,3R) products

The free energy profiles describing the reaction of *trans*-β-nitrostyrene with the propanal (*E*)-enamine of peptide **Ia$_{6.6}$** are shown in Scheme 7. As expected, the barrier for the formation of the *syn* (*2R, 3S*) product remains lower than that for the obtention of the *anti* (2R,3R) analogue: 12.4 and 16.9 kcal/mol, respectively. These calculations show also that the highest free energy barriers of the reaction are not those determining the final product configuration but the ones corresponding to the protonation after the new C–C bond formation.

The computed barriers are, however, too low to account for the observed reaction rate. This is probably due to a different reaction stage e.g. the (*E*)-enamine formation or the final product release, not directly related to the enantioselection process, has a higher free energy barrier in the overall reaction pathway.

Figure 8. Postulated transition states leading to the two enantiomers

The experimental and computed studies enable us to propose the following transition state in which the phosphonic acid

activation leads to the formation of the opposite enantiomers compared to those obtained by Wennemers (Figure 8).

Conclusions

This study shows that peptide H-*R*-Pro-*S*-Pro-*R*-pAla-OMe **Ia$_{11.5}$** is a catalyst with multifunctional activations. Covalent activation of carbonyl compounds through secondary amine and non-covalent activation by the phosphonic acid moiety allowed the 1,4-addition between aldehydes and nitroalkenes. NMR and modelisation studies confirmed the (*E*)-enamine formation while the presence of an acidic proton on the phosphonic acid seems to be crucial for the nitroalkene activation. The distance and the rigidity between both activating sites are also decisive for a good selectivity while replacing the central proline with a more flexible amino acid reduces the selectivities. Finally, the replacement of the carboxylic acid (a glutamic acid in Wennemer's catalyst) by a phosphonic acid has truly a striking impact in the reaction outcome, producing products with opposite configurations. These two groups have a strong influence in the transition state determining the final products, probably because the nitroalkene orientation is different in both catalytic pockets that differ in terms of nature and size.
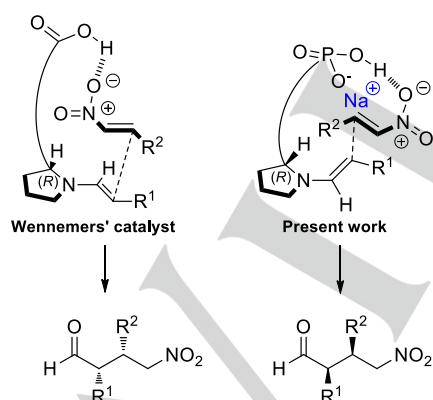
Experimental Section

General Procedure for the Asymmetric Michael Reaction

1 mol% of catalyst was dissolved in methanol and chloroform (1:1) (20 mL). *trans*-β-nitrostyrene (1.0 mmol, 1.0 equiv.) and propanal (3.0 mmol, 3.0 equiv.) were added and the reaction mixture (homogeneous solution) was stirred at 0°C. The reaction conversion was monitored by GC. After completion, the reaction mixture was concentrated under vacuum and the residue was

dissolved in ethyl acetate. This solution was washed twice with water (2 x 20 mL), dried over magnesium sulfate and concentrated to yield the desired product.

Acknowledgements

**Keywords:** organocatalysis • mechanism studies • Michael addition • DFT calculations • phosphonopeptide

[1] D. W. MacMillan, *Nature.* **2008**, *455*, 304-308.

[2] a) B. List, R. A. Lerner, C. F. Barbas, *J. Am. Chem. Soc.* **2000**, *122*, 2395-2396; b) K. A. Ahrendt, C. J. Borths, D. W. C. MacMillan, *J. Am. Chem. Soc.* **2000**, *122*, 4243-4244.

[3] For reviews of enamine and iminium catalysis, see: a) S. Mukherjee, J. W. Yang, S. Hoffmann, B. List, *Chem. Rev. (Washington, DC, U. S.)* **2007**, *107*, 5471-5569; b) A. Erkkilä, I. Majander, P. M. Pihko, *Chem. Rev.* **2007**, *107*, 5416−5470.

[4] For selected general reviews for non-covalent activation mode, see: a) J.-F. Brière, S. Oudeyer, V. Dalla, V. Levacher, *Chem. Soc. Rev.* **2012**, *41*, 1696; b) M. Mahlau, B. List. *Angew. Chem. Int. Ed.* **2013**, *52*, 518–533; c) K. Brak, E. N. Jacobsen. *Angew. Chem. Int. Ed.* **2013**, *52*, 534–561; d) A. Zamfir, S. Schenker, M. Freund, S. B. Tsogoeva, *Org. Biomol. Chem.* **2010**, *8*, 5262–5276; for selected reviews on thiourea as H-bond donating catalyst, see: e) Z. Zhang, P. R. Schreiner, *Chem. Soc. Rev.* **2009**, *38*, 1187–1198; f) M. S. Taylor, E. N. Jacobsen, *Angew. Chem. Int. Ed.* **2006**, *45*, 1520–1543; g) M. Terada, *Bull. Chem. Soc. Jpn.* **2010**, *83*, 101–119; h) S. Schenker, A. Zamfir, M. Freund, S.B. Tsogoeva, *Eur. J. Org. Chem.* **2011**, 2209–2222; i) D. Parmar, E. Sugiono, S. Raja, M. Rueping, *Chem. Rev.* **2014**, *114*, 9047–9153.

[5] a) B. List, *J. Am. Chem. Soc.* **2000**, *122*, 9336-9337; b) A. Ting, S. E. Schaus, *Eur. J. Org. Chem.* **2007**, 5797-5815; c) J. M. Verkade, L. J. van Hemert, P. J. Quaedflieg, F. P. Rutjes, *Chem. Soc. Rev.* **2008**, *37*, 29-41; d) M. Wiesner, J. D. Revell, H. Wennemers, *Angew. Chem., Int. Ed.* **2008**, *47*, 1871-1874; e) N. Halland, A. Braunton, S. Bachmann, M. Marigo, K. A. Jørgensen, *J. Am. Chem. Soc.* **2004**, *126*, 4790-4791; f) X. Fan, S. Sayalero, M. A. Pericàs, *Adv. Synth. Catal.* **2012**, *354*, 2971-2976; g) Y. Hayashi, S. Aratake, Y. Imai, K. Hibino, Q. Y. Chen, J. Yamaguchi, T. Uchimaru, *Chem. Asian J.* **2008**, *3*, 225-232; h) D. Enders, C. Grondal, M. R. Huttl, *Angew. Chem., Int. Ed.* **2007**, *46*, 1570-1581; i) R. Marcia de Figueiredo, M. Christmann, *Eur. J. Org. Chem.* **2007**, 2575-2600; j) K. S. Halskov, B. S. Donslund, S. Barfusser, K. A. Jorgensen, *Angew. Chem., Int. Ed.* **2014**, *53*, 4137-4141; k) J. McNulty, C. Zepeda-Velazquez, *Angew. Chem., Int. Ed.* **2014**, *53*, 8450-8454; l) T. Akiyama, J. Itoh, K. Yokota, K. Fuchibe, *Angew. Chem., Int. Ed.* **2004**, *43*, 1566-1568; m) D. Uraguchi, M. Terada, *J. Am. Chem. Soc.* **2004**, *126*, 5356-5357.

[6] For selected examples of bioactive molecule syntheses by organocatalysis, see: a) R. Marcia de Figueiredo, M. Christmann, *Eur. J. Org. Chem.* **2007**, 2575–2600; b) E. Marqués-López, R. P. Herrera, M. Christmann, *Nat. Prod. Rep.* **2010**, *27*, 1138–1167; c) S. B. Jones, B. Simmons, A. Mastracchio, D. W. C. MacMillan, *Nature* **2011**, *475*, 183–188; d) Z. Zhang, J. C. Antilla, *Angew. Chem. Int. Ed.* **2012**, *51*, 11778–11782; e) M. E. Abbasov, D. Romo, *Nat. Prod. Rep.* **2014**, *31*, 1318–1327. f) Y. Hayashi, S.Ogasawara, *Org. Lett.* **2016**, *18*, 3426-3429. g) M. Candy, T. Durand, J.-M. Galano, C. Oger, *Eur. J. Org. Chem.* **2016**, 5813-5816.

[7] a) S. Inoue, *Adv. Polym. Sci.* **1976**, *21*, 78-106; b) J.-I. Oku, N. Ito, S. Inoue, *Makromol. Chem.* **1979**, *180*, 1089-1091; c) M. S. Iyer, K. M. Gigstad, N. D. Namdev, M. Lipton, *J. Am. Chem. Soc.* **1996**, *118*, 4910-4911; d) T. E. Horstmann, D. J. Guerin, S. J. Miller, *Angew. Chem., Int. Ed.* **2000**, *39*, 3635-3638; e) E. R. Jarvo, S. J. Miller, *Tetrahedron Lett.* **2002**, *58*, 2481-2495; f) A. Berkessel, *Curr. Opin. Chem. Biol.* **2003**, *7*, 409-419; g) S. J. Miller, *Acc. Chem. Res.* **2004**, *37*, 601-610; h) S. M. Mennen, J. T. Blank, M. B. Tran-Dube, J. E. Imbriglio, S. J. Miller, *Chem. Comm.* **2005**, 195-197. i) Y. Arakawa, M. Wiesner, H. Wennemers, *Adv. Synth. Catal.* **2011**, *353*, 1201-1206; j) J. Li, G. Yang, Y. Qin, X. Yang, Y. Cui, *Tetrahedron: Asymmetry* **2011**, *22*, 613-618; k) X. Fan, S. Sayalero, M. A. Pericàs, *Adv. Synth. Catal.* **2012**, *354*, 2971-2976; l) L. Tuchman-Shukron, S. J. Miller, M. Portnoy, *Chem. Eur. J.* **2012**, *18*, 2290-2296; m) K. S. Feu, A. F. de la Torre, S. Silva, M. A. F. de Moraes Junior, A. G. Corrêa, M. W. Paixão, *Green Chem.* **2014**, *16*, 3169-3174; n) C. Rodríguez-Escrich, M. A. Pericàs, *Eur. J. Org. Chem.* **2015**, 1173-1188.

[8] a) E.R. Jarvo, S. J. Miller. *Tetrahedron* **2002**, *58*, 2481-2495; b) S. J. Miller. *Chem. Res.* **2004**, *37*, 601-610; c) E. A. Colby Davie, S. M. Mennen, Y. Xu, S. J. Miller. *Chem. Rev.* **2007**, *107*, 5759-5812.

[9] a) P. Krattiger, R. Kovasy, J. D. Revell, S. Ivan, H. Wennemers, *Org. Lett.* **2005**, *7*, 1101-1103 b) M. Wiesner, H. Wennemers, *Synthesis*, **2010**, *9*, 1568-1571.

[10] a) M. Wiesner, M. Neuburger, H. Wennemers, *Chem. Eur. J.* **2009**, *15*, 10103-10109; b) F. Bächle, J. Duschmalé, C. Ebner, A. Pfaltz, H. Wennemers, *Angew. Chem. Int. Ed.* **2013**, *52*, 12619–12623.

[11] We define our modified amino-acid H-**AA₁**-OMe bearing a phosphonic acid as pAla for phosphonoalanine.

[12] M. Cortes-Clerget, O. Gager, M. Monteil, J.-L. Pirat, E. Migianu-Griffoni, J. Deschamp, M. Lecouvey, *Adv. Synth. Catal.* **2016**, *358*, 34–40.

[13] 11.5, 6.6 and 2.3 refer to the pH adjustment during the catalyst preparation. 11.5 refer to the all-deprotonated catalyst while 6.6 refer to the NH₂⁺/OH/O⁻ form and 2.3 refer to the all-protonated catalyst.

[14] The syntheses of **Ia-d** and **IIa-d** were reported in our previous work (ref 12). **IIIa-d** and **IVa-d** were synthesized starting from aspartic acid and glutamic acid respectively according to the same strategy than **Ia-d** and **IIa-d.** For more details, see supporting information.

[15] M. Wiesner, G. Upert, G. Angelici, H. Wennemers. *J. Am. Chem. Soc* **2010**, *132*, 6–7.

[16] Initial rates were determined by GC. For more details, see Supporting information.

[17] C. Puchot, O. Samuel, E. Duiiach, S. Zhao, C. Agami, H. B. Kagan, *J. Am. Chem. Soc.* **1986**, *108*, 2353-2357.

[18] L. Hoang, S. Bahmanyar, K. N. Houk, B. List, *J. Am. Chem. Soc.* **2003**, *125*, 16-17.

[19] Blackmond interpreted the first results by a solubility issues for more details see a) M. Klussmann, H. Iwamura, S. P. Mathew, D. H. Wells, Jr., U. Pandya, A. Armstrong, D. G. Blackmond, *Nature* **2006**, *441*, 621-623 b) M. Klussmann, S. P. Mathew, H. Iwamura, D. H. Wells, Jr., A. Armstrong, D. G. Blackmond, *Angew. Chem. Int. Ed.* **2006**, *45*, 7989-7992.

[20] For more details, see Supporting Information

[21] Preliminary Mass experiment were performed and the two new species were detected at m/z = 416 (enamine) and m/z = 417 (iminium intermediate). For more details see Supporting Information.

[22] J. E. Hein, J. Bures, Y. H. Lam, M. Hughes, K. N. Houk, A. Armstrong, D. G. Blackmond, *Org. Lett.* **2011**, *13*, 5644-5647.

[23] M. Hatano, K. Moriyama, T. Maki, K. Ishihara, *Angew. Chem. Int. Ed.* **2010**, *49*, 3823-3826.

Entry for the Table of Contents (Please choose one layout)

Layout 1:

FULL PAPER

One catalyst to rule them all. Mechanistic investigations were performed in order to unveil the activation modes and catalytic efficiency of the H-Pro-Pro-pAla-OMe catalyst in the 1,4-addition between aldehydes and nitroalkenes. This tripeptide behaves as a multiactivating catalyst, able to interact through a secondary amine group and non-covalent activations. A restrained tripeptide is optimal for better selectivities and fast reaction.
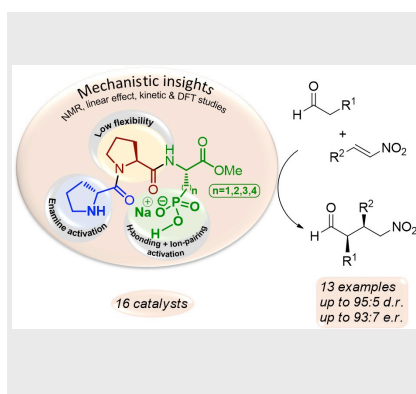
Margery Cortes-Clerget, Jesús Jover, Jade Dussart, Emilie Kolodziej, Maelle Monteil, Evelyne Migianu-Griffoni, Olivier Gager, Julia Deschamp,* Marc Lecouvey*

Page No. – Page No.

Bifunctional Tripeptide Including a Phosphonic Acid as Brønsted Acid for Michael Addition: Mechanistic Insights