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Structural Insight into the Aggregation of L-Prolyl Dipeptides and its Effect on Organocatalytic Performance

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NMR and organocatalytic studies of four dipeptides derived from L-proline are described. Results indicate that important conformational changes around the catalytic L-proline moiety are observed for free dipeptides upon changing the adjacent amino acid. Also, an aggregation process is detected as the concentration increases. Self-association of the dipeptides has been fitted to a cooperative binding model. All the compounds have been assayed as catalysts for the conjugated addition of cyclohexanone to trans-β-nitrostyrene in toluene. In agreement with the structural studies, noticeable changes in the catalytic performance are detected upon changing catalyst concentration, being the catalyst activated by self-aggregation.

Introduction

Organocatalysis has become a major topic of research in organic chemistry during the last decades. The extensive work in this area has been reviewed very often and examples can be found of organocatalysts for quite a variety of reactions and media. An outstanding family of organocatalysts is that derived from peptides and in particular from L-proline. This kind of catalysts have been reported successfully in asymmetric catalysis for a wide range of synthetically reactions. Focusing on the reaction studied in the present work, L-proline derivatives have been used extensively as catalysts for conjugate addition reactions. Seminal work by List and coworkers reported first the use of L-proline and then of L-prolyl-peptides to catalyse efficiently the conjugate addition of cyclohexanone to β-nitrostyrene. Wennemers and coworkers have studied in detail peptide catalysed conjugated additions to nitroalkanes with excellent efficiency in terms of conversion and enantioselectivity. Advances in this line of research comprise the achievement of highly efficient 1,4-addition of aldehydes to nitroolefins using a continuous flow system containing solid-supported peptidic catalysts. Not long ago, a detailed mechanistic study including the determination of the stereoselectivity-determining step for this type of reaction has been described.

Although catalyst aggregation is sometimes neglected, there is a growing interest in addressing this behaviour and how it affects to catalytic performance. Aggregation effects have been analysed especially for thiourea catalysts and smart strategies have been developed to avoid this undesired assembled process. Non-linear effects in organocatalysis have been in some cases correlated with catalyst self-aggregation. Recently, the aggregation of catalytic L-proline containing block copolymers was studied. Previous work reported in our group shows how the aggregation of bolaamphiphilic organogelators containing L-proline catalytic moieties into fibrilar gel networks modified the enantioselectivity of the reaction when compared to solution. Also in that work the dipeptide soluble catalyst ProValPr (see Figure 1) was preliminary studied.

Here we report a detailed study of compound ProValPr and three other dipeptide analogues as catalysts, focusing on their structural analysis. Special attention is paid to the conformational preferences of these analogues and to their self-association in solution. Additionally, the efficiency of these molecules as catalysts in the conjugated addition of cyclohexanone to β-nitrostyrene is evaluated.
The present study aims to highlight the complexity of L-prolyl catalysts associated to conformational mobility and self-aggregation rather than to develop new catalysts with high enantioselectivity, which have been reported elsewhere as cited above.

Results and Discussion

Four different L-proline derivative dipeptides were studied containing respectively L-alanine, L-phenylalanine, L-valine and L-isoleucine. All of them were capped at C-terminus as propylamides (Figure 1). The self-assembly of the dipeptide catalysts in toluene was studied from a thermodynamic and structural point of view using NMR experiments. The compounds were fully soluble in this solvent in all the concentration range studied. Firstly, self-association of the molecules was monitored by NMR in D8-toluene following the chemical shift of the amide signals. It can be seen in Figure 2 that both amide resonances of ProIlePr were shifted downfield as the concentration increases, revealing intermolecular association by means of hydrogen bonding. A similar behaviour was observed for the other compounds.

![Figure 2](image)

Figure 2. Partial $^1$H NMR spectra of ProIlePr and ProAlaPr in D8-toluene at different concentrations.

Data corresponding to the shift of amide resonances upon increasing concentration could be fitted to a supramolecular polymerization model with a dimerization constant, $K_2$, and equivalent successive aggregation constants, $K_n$ (see Experimental Section and Supporting Information). In all the cases moderate cooperativity was observed being $K_2 > K_n$ (Table 1). The association constants $K_2$ were similar for the four dipeptides but higher values of $K_n$ were obtained for ProValPr and ProIlePr. However these differences do not affect very significantly to the proportion of aggregated species in the range of concentrations studied for catalysis. As exemplified in Figure 3, species distribution diagram reveals that upon going from ca. 1 mM to 5 mM a very important linear increase of dimeric species takes place. Also exponential growth of oligomeric species is observed.

Molecular mechanics calculations (AMBER* force field) for the free catalysts predict in all the cases two energetically close folded conformations ($\Delta E$ ca. 7 kJ mol$^{-1}$) near the global minimum. In both cases the conformers contain a H-bond between propylamide NH and CO of the proline moiety, in accordance with the experimental results described below (see anti and syn conformers in Scheme 1; consult Supporting Information for pictures of the molecular models; details of computations in Experimental Section).

![Figure 3](image)

Figure 3. Species distribution diagram for the aggregation of ProIlePr in toluene.

![Scheme 1](image)

Scheme 1. Conformational and aggregation equilibria for the studied peptides.

Similar conformations can be obtained with semiempirical AM1 calculations (not shown). The main difference among the
found conformations is the dihedral angle N-C-C=O of the proline unit, giving place to syn and anti conformations which present respectively dihedral angles below and above 90°. Additionally anti conformation presents an intramolecular hydrogen bond between L-proline amine and the NH of the peptidic linkage as described previously (see Scheme 1).12, 14

The existence of this strong intramolecular hydrogen bond can be demonstrated by comparison of the NMR spectra recorded in different solvents. As shown in Figure 4, in difference with NH-a signal, chemical shift of NH-b, which is adjacent to proline ring, is insensitive to solvent polarity, indicating its involvement in intramolecular H-bonding. In this way its interaction with solvent molecules is precluded.

On the other hand, syn conformation presents an intramolecular hydrogen bond between L-proline amine and the carbonyl CO unit of the peptide linkage.

Structural studies using NMR were carried out to evaluate the presence of these conformations in solution. A whole set of evidences were collected which pointed to the majority of folded anti conformations in diluted solutions. Firstly, for all the compounds, VT-NMR experiments (c = 1 mM, almost no aggregation) revealed a significant variation of both amide signals with temperature indicating their participation in intramolecular H-bonding (see data for ProValPr in Figure 5). This fact fits with the presence of anti conformations which have two intramolecular H-bonds formed by amide NHs, as depicted in Scheme 1.

Secondly, as shown in the Figure 6, the splitting of the resonances of geminal protons in position 3 of the proline ring also might point to the presence of anti disposition. This type of conformation provokes the spatial proximity of C3 protons to the carbonyl, experiencing to a different degree its shielding/deshielding effect. Although this splitting is common in L-proline rings, its magnitude seems to be related to the particular conformation present, as shown below.

Finally, NOE detected between the NH of the propylamide chain and the proton of the chiral carbon of proline (Scheme 1 and Supporting Information, Figure S5) also fits with anti conformations which present a shorter distance between these protons in the models (3.8 and 4.5 Å for anti and syn dispositions respectively). It has to be noted that although NMR data indicate that ProValPr and ProIlePr present almost exclusively anti conformation, ProAlaPr and ProPhePr derivatives present a detectable amount of molecules in syn conformation. As seen in Figure 6, peptidic NH signals of ProAlaPr and ProPhePr present significantly lower chemical shift values than ProValPr and ProIlePr (7.85, 7.92, 8.04 and 8.02 ppm respectively).

These data point to the weakening of the intramolecular H-bond between the nitrogen atom of proline and the hydrogen atom of the peptide bond that results from syn type conformation. Furthermore, the geminal protons at C3 position of the proline ring in ProAlaPr and ProPhePr, although splitted, present a
reduced difference in chemical shift when compared to ProValPr and ProIlePr (ca. 0.07 ppm for the former peptides and ca. 0.15 ppm for the latter compounds) pointing again to the coexistence of fast exchanging syn and anti conformations for the alanine and phenylalanine derivatives (see Scheme 1). The higher steric demand of Val and Ile side chains (secondary carbon atom attached to chiral centre), compared to Ala and Phe (methyl and primary carbon atom attached to the chiral centre respectively) can explain the observed differences. In the case of anti conformations steric interactions between Val or Ile side chains and the L3-proline ring would arise as indicated by molecular models. (see Supporting Information, Figure S10, for schematic Newman projection).

Analysis of the aggregates by NMR was carried out at a concentration of 70 mM (ca. 80 % of aggregated species). It was found that in all the cases NOE correlations were obtained between protons of propyl chain moiety and protons of proline ring, pointing to the formation of antiparallel aggregates (see Scheme 1 and Supporting Information, Figure S6 and S7). Interestingly, 1H-NMR spectra of the aggregates formed by ProPhePr and ProAlaPr show a reduction of the splitting observed for the geminal protons at C3 in the proline ring when compared to spectra from diluted samples (Figure 7). However, for ProValPr and ProIlePr derivatives the mentioned splitting is maintained upon aggregation. This behaviour points to a conformational change around the L-proline moiety upon aggregation of ProPhePr and ProAlaPr which might be associated to a transition from anti to syn type conformations upon aggregation as suggested in Scheme 1.

Molecular mechanics calculations (AMBER* force field) permit to obtain energy minimized models for the dipeptide dimers studied. In these simulations several intermolecular H-bonds were found. In addition, the spatial proximity of the propyl and proline moieties in the model agrees with NMR NOE experiments. As an example, the structures obtained for ProllePr and ProAlaPr are shown in Figure 8.

In order to assess how the conformational preferences and aggregation behaviour affect the catalytic activity of the four dipeptides, the conjugate addition of cyclohexanone to trans-β-nitrostyrene was studied in toluene.

This reaction has been shown to be catalysed by proline moieties which forms an enamine-type intermediate by reaction with cyclohexanone (Scheme 2).

The reactions were carried out in the presence of an excess of cyclohexanone and therefore by means of simplicity the system was analysed in terms of pseudo first order kinetics (see equations 1-3; in equation (3) [alkene] = concentration at the end of the reaction time, [alkene] = initial concentration, k' = k
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Noticeable it was observed that upon increasing catalyst concentration from 1 to 5 mM, the kinetic constant of the reaction increased significantly for all the cases, being the catalyst almost inactive for diluted solutions. For example, a 15 fold and 10 fold increase are observed respectively for ProValPr and ProPhePr when the catalyst concentration grows from 1 to 5 mM. These results point to catalyst activation upon increasing concentration and agree with the fact that aggregates as those shown in Scheme 1 are much more active than non-aggregated species. A rationale for this behaviour is that upon aggregation the catalytic amino centre of proline ring is liberated from the intramolecular H-bonding which prevents its nucleophilic activity (see Scheme 1 and 2).

\[
\frac{d[\text{alkene}]}{dt} = k[\text{catalyst}][\text{ketone}][\text{alkene}] \quad (1);
\]

\[
\frac{d[\text{alkene}]}{dt} = k'[\text{alkene}] \quad (2); \quad \text{(pseudo first order kinetics)}
\]

\[
\ln \left( \frac{[\text{alkene}]}{[\text{alkene}]_0} \right) = -k't \quad (3); \quad \text{(integrated rate equation)}
\]

Conclusions

The structural studies carried out agree well with the experimentally observed catalytic activity of the different dipeptides. In first place, the catalyst activation observed upon increasing the concentration is clearly associated to the aggregation through hydrogen bonding. This process presumably activates the L-proline moiety as a result of the conformational changes associated to the aggregation. NNR studies point to the majoritarian presence of the so called anti conformations both for diluted and concentrated solutions of ProValPr and ProPhePr. In the case of ProPhePr and ProAlaPr a conformational change from anti to syn type conformation might be operating upon aggregation. Overall, the results support that organocatalysis can be rather sensitive to concentration and conformational effects associated to minor changes in the catalyst structure. Structurally simple compounds such as the reported dipeptides present a broad scope of species in solution arising both from aggregation and intrinsic conformational mobility.

Experimental Section

General Considerations.

NMR spectra were recorded at 500 MHz, 300 MHz (\(^1\)H NMR) and 125 MHz, 75 MHz (\(^{13}\)C NMR) in different solvents at 30°C with the solvent signals as internal reference. Mass spectra were run in the electrospray (ESMS) mode.

Synthesis.

The catalysts were prepared with conventional peptide chemistry methodology. See Supporting Information for synthetic scheme. The compound ProValPr was synthetized as reported previously.\(^{15}\)

General procedure for the preparation of N-hydroxysuccinimide esters of amino acids. Synthesis of...
**General procedure to the synthesis of ZAIAPr:** To an ice-cooled solution of the corresponding N-Cbz-L-alanine (5.03 g, 22.5 mmol) and triethylamine (3.65 mL, 26.3 mmol) in THF (40 mL), a solution of ethyl chloroformate (2.05 mL, 25.7 mmol) in THF (10 mL) was added dropwise with vigorous stirring, a white precipitate was observed. After 30 min stirring in an ice-cold bath, a solution of propylamine (2.15 mL, 26.1 mmol) in THF (10 mL) was added. The mixture was stirred at 0 °C for 1 h and was left overnight at room temperature. The resulting white solid solution was filtered and the solvent was evaporated under vacuum. The resulting viscous solid was dissolved in dichloromethane (20 mL) and washed with HCl 0.1 M (3 x 20 mL), KOH 0.1 M (3 x 20 mL), NaHCO₃ (1 x 20 mL) and water (1 x 20 mL). The organic phase was dried with magnesium sulfate anhydrous and the solvent was evaporated under vacuum. The solid obtained was purified by column chromatography (silica gel, hexane/ethyl acetate; 1:1). A white solid compound was obtained. (Yield 35%). ¹³C NMR (300 MHz, DMSO-d₆) δ 175.0 (s, 1H), 174.5 (s, 1H); ¹H NMR (500 MHz, DMSO-d₆) δ 7.76 (s, 1H), 7.45 – 7.15 (m, 5H), 7.15 (m, 1H), 4.99 – 4.29 (m, 1H), 2.60 (dd, 2H), 1.09 (d, 3H), 1.34 (h, 1H); ²⁰⁷Pb NMR (165 MHz, DMSO-d₆) δ 207.2915; found, 207.1498 [M + H]⁺. ¹⁹F NMR (471 MHz, DMSO-d₆) δ –51.2 (s, 1F); ¹³C NMR (75 MHz, DMSO-d₆) δ 172.6, 136.0, 121.5, 84.0, 52.7, 40.1, 22.8, 11.7; (ESI-TOF, positive mode) m/z exp [M + H]⁺ calculated for C₁₄H₂₂N₂O₂Na⁺ 318.1370; found, 318.1370 [M + Na]⁺, (Δ = 0 ppm).

**Synthesis of the peptide of N-Bis(N-Cbz-L-aminocyl) amines.** Synthesis of ZPheOSu: The N-hydroxysuccinimide ester, ZPheOSu, (6.09 g, 15.3 mmol) was dissolved in DME (100 mL). The propylamine (1.0 g, 16.9 mmol) dissolved in DME (20 mL) was added dropwise and the resulting solution was stirred at room temperature for 18 hours and then was warned for 2 hours at 40-50°C. The solvent was evaporated under vacuum. The resulting solid was dissolved in dichloromethane (25 mL) and washed three times with HCl 0.1 M (3 x 25 mL) and water (3 x 25 mL). The organic phase was dried with magnesium sulfate anhydrous and the solvent was evaporated under vacuum. A white solid was obtained (Yield 93%). ¹³C NMR (75 MHz, DMSO-d₆) δ 176.0, 50.7, 40.4, 22.8, 22.1, 13.6, 15.3, 13.6; (ESI-TOF, positive mode) m/z exp [M + H]⁺ calculated for C₁₄H₃₃N₂O₂Na⁺ 341.1865; found, 341.1860 [M + Na]⁺, (Δ = 0 ppm).

**Synthesis of the peptide of N-Bis(N-Cbz-L-aminocyl) amines.** Synthesis of ZPheOSu: A similar procedure to that described for ZPheOSu was used. Pure crystals were obtained (Yield 84%). ¹³C NMR (75 MHz, DMSO-d₆) δ 7.85 (s, 1H), 7.74 (s, 1H), 7.45 (d, J = 15.6 Hz, 1H), 7.38 – 7.10 (m, 10H), 4.99 – 4.29 (m, 1H), 2.60 (dd, 2H), 1.09 (d, 3H), 1.34 (h, 1H); ¹H NMR (500 MHz, DMSO-d₆) δ 7.82 (t, J = 5.5 Hz, 1H), 7.54 (d, J = 15.6 Hz, 1H), 7.38 – 7.10 (m, 10H), 4.99 – 4.89 (s, 2H), 4.21 (m, 1H), 3.10 – 2.86 (m, 3H), 2.77 (dd, J = 13.6, 10.1 Hz, 1H), 1.45 – 1.30 (m, 2H), 0.80 (t, J = 7.4 Hz, 3H); ¹¹B NMR (162 MHz, DMSO-d₆) δ 171.5, 156.2, 138.5, 129.6, 128.7, 128.4, 128.0, 127.8, 126.6, 65.6, 56.7, 40.7, 38.2, 22.7, 11.7; (ESI-TOF, positive mode) m/z exp [M + H]⁺ calculated for C₁₆H₂₅N₂O₂⁺ 341.1865; found, 341.1860 [M + Na]⁺, (Δ = 1.5 ppm).

**Synthesis of ZAlaPr:** A similar procedure to that described for ZPhePr was used starting from the N-hydroxysuccinimide ester of N-Cbz-isoleucine (ZIleOSu). A white solid was obtained (Yield 84%). ¹³C NMR (300 MHz, DMSO-d₆) δ 7.85 (s, 1H), 7.32 (m, 5H), 7.18 (d, J = 8.4 Hz, 1H), 5.01 (s, 2H), 3.80 (t, J = 8.1 Hz, 1H), 3.11 – 2.86 (m, 2H), 1.66 (s, 1H), 1.38 (dd, J = 14.3, 7.4 Hz, 3H), 1.03 (m, 1H), 0.95 – 0.66 (m, 9H); ¹H NMR (500 MHz, DMSO-d₆) δ 7.15 (m, 1H), 7.03 (m, 1H), 1.61 (s, 1H), 1.34 (h, J = 7.2 Hz, 2H), 0.77 (t, J = 7.4 Hz, 3H); ¹¹B NMR (162 MHz, DMSO-d₆) δ 174.5, 139.2, 129.7, 128.4, 126.4, 56.7, 41.7, 38.5, 22.8, 11.7; (ESI-TOF, positive mode) m/z exp [M + Na]⁺ calculated for C₁₅H₂₉N₂O₃Na⁺ 302.1836; found, 302.1840 [M + Na]⁺, (Δ = 0.3 ppm).

**Synthesis of ZLisPr:** A similar procedure to that described for ZAlaPr was used. A yellow oil was obtained (Yield 96%). ¹³C NMR (300 MHz, DMSO-d₆) δ 7.74 (s, 1H), 7.41 – 6.98 (m, 10H), 3.39 (m, 1H), 3.11 – 2.80 (m, 3H), 2.60 (dd, J = 13.3, 8.0 Hz, 1H), 1.61 (s, 1H), 1.34 (h, J = 7.2 Hz, 2H), 0.77 (t, J = 7.4 Hz, 3H); ¹¹B NMR (162 MHz, DMSO-d₆) δ 171.5, 156.2, 138.5, 129.6, 128.7, 128.4, 128.0, 127.8, 126.6, 65.6, 56.7, 40.7, 38.2, 22.7, 11.7; (ESI-TOF, positive mode) m/z exp [M + H]⁺ calculated for C₁₆H₂₅N₂O₂⁺ 302.1836; found, 302.1840 [M + Na]⁺, (Δ = 0.3 ppm).
(dd, J = 8.9, 3.6 Hz, 1H), 1.45 – 1.28 (m, 3H), 1.15 – 0.91 (m, 1H), 0.88 – 0.69 (m, 9H); 13C NMR (75 MHz, DMSO-d6) δ 174.9, 59.8, 40.5, 38.9, 24.2, 22.8, 16.2, 11.9, 11.8; (ESI-TOF, positive mode) m/z exp [M + H]+ calc'd for C16H22N2O2 273.1648; found, 273.1650 [M + H]+, (Δ = 1.2 ppm).

General procedure for the preparation of N-Boc-protected compounds. Synthesis of BocProAlaPr: A solution of amino amide AlaPr (0.64 g, 4.8 mmole) in dry DME (10 mL) was added dropwise over a solution of Boc-L-Pro-OSu (1.87 g, 5.8 mmol) in dry DME (100 mL). The mixture was stirred at room temperature for 24 h and then at 40 °C for 5 h. The solvent was evaporated under vacuum and the resulting white solid was dissolved in dichloromethane (50 mL) and washed with NaHCO3 (3 x 15 mL). Afterwards, the organic layers were dried (Na2SO4) and the solvent was evaporated under vacuum to yield a white solid product. (Yield 60%). 1H NMR (300 MHz, DMSO-d6) δ 7.87 (s, 1H), 7.70 (d, J = 5.9 Hz, 1H), 4.55 (dd, J = 8.9, 3.7 Hz, 1H), 4.08 (dd, J = 8.2, 3.4 Hz, 1H), 3.34 (dd, J = 14.3, 7.4 Hz, 2H), 3.00 (dd, J = 12.8, 6.8 Hz, 2H), 2.38 (dd, J = 14.7, 7.3 Hz, 1H), 2.15 – 1.94 (m, 1H), 1.93 – 1.59 (m, 2H), 1.50 – 1.32 (m, 9H), 1.30 (s, 2H), 1.27 (dd, J = 7.0, 3Hz), 0.80 (t, J = 7.4 Hz, 3H); 13C NMR (75 MHz, DMSO-d6) δ 172.3, 153.7, 78.8, 59.7, 48.4, 46.9, 40.6, 31.3, 28.4, 25.7, 22.7, 19.1, 11.68, 11.7; (ESI-TOF, positive mode) m/z exp [M + H]+ calc'd for C16H22N2O2 328.2231; found, 328.2237 [M + H]+, (Δ = 0.3 ppm).

Synthesis of BocProPhePr: A similar procedure to that described for BocProAlaPr was used. A white solid product was obtained (Yield 95%). 1H NMR (300 MHz, DMSO-d6) δ 7.88 (s, 1H), 7.80 (d, J = 8.2 Hz, 1H), 7.22 (q, J = 8.7 Hz, 5H), 4.01 (dd, J = 8.7, 3.3 Hz, 1H), 3.41 (m, 1H), 3.24 – 2.89 (m, 6H), 2.00 (s, 1H), 1.64 (d, J = 12.8 Hz, 3H), 1.36 (dd, J = 14.1, 5.9 Hz, 6H), 1.22 (d, J = 11.9 Hz, 5H), 0.76 (t, J = 7.4 Hz, 3H); 13C NMR (75 MHz, DMSO-d6) δ 172.4, 171.1, 153.8, 138.2, 129.5, 128.4, 126.6, 78.8, 60.1, 54.3, 46.9, 38.4, 31.2, 28.3, 23.3, 22.6, 11.7; (ESI-TOF, positive mode) m/z exp [M + Na]+ calc'd for C23H20N4O4Na+ 426.2363; found, 426.2365 [M + Na]+, (Δ = 0.9 ppm).

Synthesis of BocProPhePr: A similar procedure to that described for BocProAlaPr was used. A white solid product was obtained (Yield 95%). 1H NMR (300 MHz, DMSO-d6) δ 8.13 – 7.89 (m, 2H), 4.11 (dd, J = 25.7, 13.2, 7.8 Hz, 1H), 3.53 (dd, J = 9.0, 5.0 Hz, 1H), 3.14 – 2.91 (m, 2H), 2.91 – 2.84 (m, 1H), 2.72 (dt, J = 10.2, 6.4 Hz, 1H), 1.94 (dd, J = 16.3, 12.4, 7.5 Hz, 1H), 1.78 – 1.64 (m, 2H), 1.63 – 1.48 (m, 2H), 1.48 – 1.28 (m, 3H), 1.08 – 0.91 (m, 1H), 0.81 (dq, J = 11.8, 7.3 Hz, 9H); 13C NMR (300 MHz, dmsso) δ 174.2, 171.0, 60.6, 56.2, 47.1, 40.5, 37.8, 31.0, 26.3, 24.6, 22.6, 15.8, 11.8, 11.3; (ESI-TOF, positive mode) m/z exp [M + Na]+ calc'd for C14H23N3O2Na+ 270.2176; found, 270.2186 [M + Na]+, (Δ = 1.5 ppm).

General procedure for the 1,4-conjugated Michael addition reaction.2 Catalyst (0.033 mmol) was dissolved in a vial using the amount of toluene required to reach the targeted final concentration (for diluted systems, 1 mM and 2.5 mM, 0.006 mmol of catalyst were added). Then, trans-β-nitrostyrene (0.16 mmol) and cyclohexaneone (3.29 mmol) were added and the reaction was left at room temperature the required time. The reaction was quenched by addition of a 0.25 M aqueous acetic acid solution (2 mL) and toluene (1 mL). The aqueous layer was treated with toluene (2 x 2 mL). Then the solvent of the combined organic extracts was removed until dryness. The reaction crude was analyzed by 1H-NMR in CDCl3 in order to determine the yield and the diastereoselectivity (syn = 3.76 ppm; anti = 4.01 ppm) and was further purified by column chromatography (silica gel, mixture of hexane /ethyl acetate, 3:1) to isolate the pure product used to determine enantioselectivity.
Determination of the enantiomeric excess. The enantiomeric excess was determined by HPLC using a Chiral Pack IA column, hexane/IPA (v/v: 85/15), flow rate 1 mL/min, λ = 210 nm, t₁ = 7.76 min (2S, 1'R), t₂ = 8.76 min (2R, 1'S).

Kinetic studies. Kinetic constants were estimated from the reaction yield at a given final time. The concentration of cyclohexanone was approximated to be constant and the system analyzed as having a pseudo first order kinetics.

NMR studies. ¹H and ¹³C NMR spectra were recorded in a Varian Mercury 300 MHz or Varian Inova 500 MHz spectrometer at 30 °C. NOESY-1D experiments were recorded using a mixing time of 800 ms at 30 °C.

Determination of aggregation constants. The NMR chemical shift of the NH signal from the propylamide unit in D₆-toluene was used as a probe for the determination of the association constants. The samples were prepared dissolving the required amount of catalyst in toluene and were left overnight at r.t. A set of data corresponding to at least 10 different concentrations were acquired at 30 °C. The results could be fitted to a cooperative binding model using the equations reported previously in the literature (see Supporting Information). The equations were solved by non-linear regression with Solver (Microsoft Excel) to afford K₂ (dimerization constant, see equation (4)), K₃ (successive oligomerization constant, see equation (5)) and maximum chemical shift (δₑₘₓ).

A + A = A₂; K₂ (4)

Aₙ + A = Aₙ₊₁; Kₙ (5)

Molecular modeling studies. The models reported were obtained by molecular mechanics calculations performed with MacroModel using AMBER® as force field. Exhaustive Monte Carlo conformational search (1000 steps of torsional angles variation) was carried out for the isolated molecules. The structures described for the folded conformations in scheme 1 and Supporting Information correspond to energy minimized structures near the global minimum (within ca. 10 kJmol⁻¹). The models for the aggregates shown in Figure 8 were built manually from unfolded energy minimized conformers. Then the dimeric structures were energy minimized to the nearest local energy minimum. The feasibility of the molecular mechanics models was checked with AM1 semiempirical energy minimizations which afforded basically similar conformations within a similar energy range.

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Notes and references
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