Trends in Asymmetric Michael Reactions Catalysed by Tripeptides in Combination with an Achiral Additive in Different Solvents

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The potential of tripeptides **3**, **6** and **12** as chiral catalysts for asymmetric Michael addition reactions in the presence of an achiral additive has been tested in different solvents ($CHCl_3$, acetone, DMF, DMSO and the room-temperature ionic liquid [bmim]PF_6). The dependence of yields and enantiomeric excesses on the solvent used has been demonstrated. The experiments show that the combination of additive and peptides provides a catalytic system that appears to be better than the sum of its parts.

Introduction

Undoubtedly, the more elegant and economically most attractive way to introduce chirality into a molecule is through the use of a catalytic amount of a chiral controller to induce the chiral transformation. The fact that enzymes perform a variety of highly stereoselective reactions at highly organized chiral binding sites has resulted in particular interest in asymmetric synthesis through noncovalent binding of reactants in the chiral interiors of simple chemical inclusion aggregates and compounds.[1,2]

Aspiring to imitate enzymatic efficiencies, chemists have delved into Nature's toolbox, transforming amino acids into auxiliaries, catalysts, and ligands.[3] Even proline itself (and simple derivatives thereof) has been used as an organocatalyst for the direct asymmetric aldol reaction,[4] the Robinson annulation[5] and the Diels−Alder[6] and Michael reactions.[7]

Several decades after the first use of an amino acid as a catalyst, amino acids and simple peptides have become an important subset of asymmetric catalysts.[3]

Short peptides have recently been found to be excellent asymmetric catalysts for a number of organic transformations, including the enantioselective Strecker reaction[8] and the aldol reaction.[9a] Their ability to perform a variety of transformations is complemented by their ready availability, stability and ease of handling. In the majority of examples, both the amine and the acid functionalities in peptides are altered or eliminated.[3]

Unmodified peptides have been used as catalysts much less frequently, and so we decided to test the unprotected peptides H−Asp−Phe−Arg−OH (**3**) and H−Asp−Pro−Arg−OH (**6**) (known as active ingredients of anticholesteremic[10] and antiallergic agents[11]) as catalysts for asymmetric Michael addition reactions.

In this contribution, we also demonstrate the potential of the 4-*trans*-aminoproline-based tripeptide **12** (recently described[12] as an ingredient of a poor DNA binding agent) as a chiral catalyst for asymmetric C−C bond formation reactions.

Results and Discussion

Tripeptides **3** and **6** were prepared in good to high yields by known classical methods[13] as summarized in Scheme 1.

Compound **7**, obtained from the readily available (*S*)-(−)-*trans*-4-hydroxyproline by methods described in the literature,[14] was used as starting material for the synthesis of tripeptide **12**[12,15] (Scheme 2).

1,4-Addition of 2-nitropropane (**14**) to cyclohex-2-en-1-one (**13**) in the presence of *trans*-2,5-dimethylpiperazine as an additive[7] (Scheme 3) was chosen by us as a known, simple model reaction for testing peptides **3**, **6** and **12** as possible chiral catalysts.

We examined the influence of peptides and *trans*-2,5-dimethylpiperazine on the reaction in different solvents: $CHCl_3$, acetone, DMF, DMSO and the ionic liquid [bmim]PF_6. The achieved results are summarized in Table 1.

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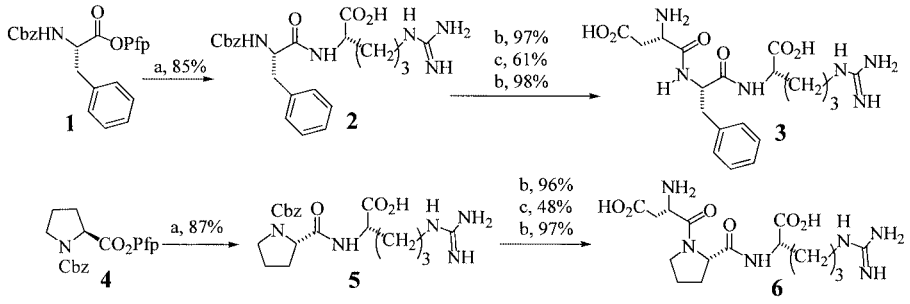
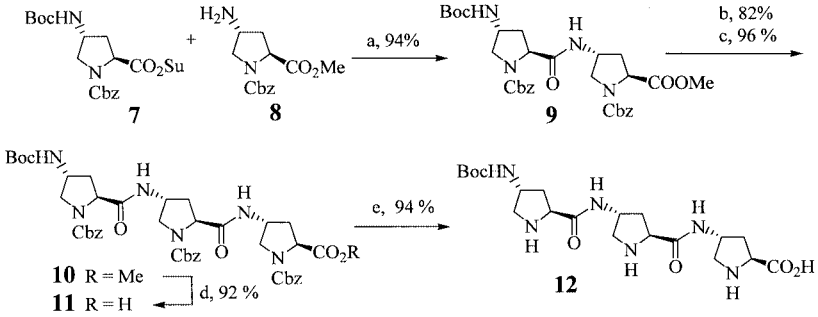
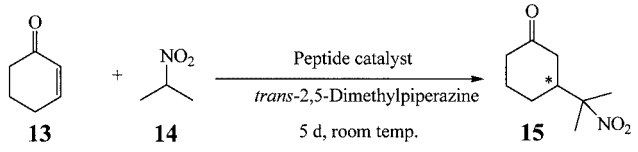


Scheme 1. (a) H−Arg−OH/DMF; (b) H_2, Pd/C, MeOH; (c) Z−Asp(OBz)−OPfp/DMF



Scheme 2. (a) EtOAc; (b) TFA, CH_2Cl_2; (c) **7**, TEA/CH_2Cl_2; (d) LiOH, MeOH/H_2O; (e) H_2, Pd/C, MeOH



Scheme 3

In all cases, tripeptides were screened under the same conditions (15 mol % peptide; room temperature).

The emerging results illustrate puzzlingly complex behaviour. Combinations of peptides **3**, **6** and **12** with *trans*-2,5-dimethylpiperazine in $CHCl_3$ provided **15** in 18%, 71% and 77% *ee*, respectively (Entries 1, 6, 11). The observed asymmetric induction in $CHCl_3$ is apparently due to a collaboration between tripeptides and *trans*-2,5-dimethylpiperazine, since neither conversion occurred with the peptide catalysts in the absence of *trans*-2,5-dimethylpiperazine, nor with the additive alone in the absence of the peptides (Entry 16).

In $CHCl_3$, while the tripeptides **3** and **6** in the presence of the additive gave the product **15** in similarly low yields (around 10%; Entries 1, 6), tripeptide **12** produced **15** in 80% yield (Entry 11). This may be explainable in terms of differences in their mechanisms of catalysis, but perhaps also by the low solubilities of **3** and **6** in $CHCl_3$.

The good yield and enantiomeric excess observed in the presence of **12** is an indication that aminocatalysis[9] does operate in this case. The tripeptides **3** and **6** most probably induce the enantioselectivity in $CHCl_3$ through hydrogen-bond formation with the substrate.

Table 1. Conjugate additions of 2-nitropropane to cyclohex-2-en-1-one catalysed by peptides **3**, **6** and **12** (15 mol %) in the presence of *trans*-2,5-dimethylpiperazine (100 mol %)

Solvent[a]	Entry	Peptide **3** Yield [%][b]	*ee* [%][c]	Entry	Peptide **6** Yield [%][b]	*ee* [%][c]	Entry	Peptide **12** Yield [%][b]	*ee* [%][c]	Entry	− Yield [%][b]
$CHCl_3$	1	< 10	18	6	< 10	71	11	80	77	16	−
Acetone	2	n.r.	−	7	n.r.	−	12	43	80	17	−
DMF	3	16	28	8	< 10	17	13	> 99	63	18	5
DMSO	4	53	29	9	73	23	14	85	7	19	39.5
[bmim]PF_6	5	44	5	10	35	< 5	15	> 95	51	20	25

[a] $CHCl_3$ (μ = 1.15 D, ε = 4.9), acetone (μ = 2.69 D, ε = 20.7), DMF (μ = 3.86 D, ε = 36.7), DMSO (μ = 4.3 D, ε = 48.7), [bmim]PF_6 (μ = ions, ε = cond.)[16] [b] Isolated yields after column chromatography. [c] % *ee* measured by ^{13}C NMR of the corresponding ketal with (2*R*,3*R*)-2,3-butanediol.

Peptides **3** and **6** are even less soluble in acetone than in $CHCl_3$, which probably explains the absence of any conversion of the substrate (Entries 2, 7), and the additive alone is also inactive here (Entry 17), similarly to the situation in $CHCl_3$. Peptide **12** affords 80% *ee* in acetone (Entry 12). The significant drop in yield (43%) for **12** could be due to competition between the two carbonyl compounds (acetone and cyclohex-2-en-1-one) for iminium ion formation.

The results in DMF again resemble those in $CHCl_3$, with the sole exception of the reduced enantiomeric excess achieved with **6** (Entries 3, 8, 13).

In the still more polar DMSO, better yields overall (53%, 73%, 85%; Entries 4, 9, 14, respectively), but − except in the case of **3** − lower enantioselectivities (29%, 23%, 7% *ee*, respectively) were attained in the presence of peptides **3**, **6** and **12** and *trans*-2,5-dimethylpiperazine, relative to the results in chloroform. Higher conversion rates in DMSO might be the result of better solvation and stabilisation of the nucleophile. In addition, the solvatating power measured by the dipole moments (μ) and/or dielectric constants (ε) of the solvent molecules (see Table 1), increases in the same direction. Solvent polarity has an adverse effect on the complexation of substrate with the peptide and consequently on the enantiomeric excess: entropy favours hydrogen bonding in nonpolar solvents while better solvation in polar media lets the solvent molecules get in the way. Polarity helps with the yields while the enantioselectivity drops sharply.

No reaction took place in DMSO with peptides **3** and **12** in the absence of additive. Intriguingly, peptide **6** gave the product in 67% yield and with 8% *ee* under the same conditions.

Since the strongest base (guanidine group of arginine, $pK_a = 13.20$ in water) in the system with **3** and **6** is generally deactivated through formation of zwitterions, only the proline residue of peptide catalyst **6** appears to be basic enough to deprotonate the nitroalkane. [Second pK_a values of the amino acids making up the peptides: proline ($pK_a = 10.64$) is a better proton acceptor than phenylalanine ($pK_a = 9.46$); here we have employed the pK_a values of the individual amino acids in water as an approximation − it has been found that acid/base pairs have the same relative pK_a values in nonaqueous media as they do in water.[17]]

Surprisingly, even the presence of *trans*-2,5-dimethylpiperazine ($pK_a = 9.83$) alone results in the product in 5% yield in DMF (Entry 18) and in 39.5% yield in DMSO (Entry 19). Apparently, the substrate reacts with the nucleophile without being polarized at all. Alternatively, the protonated *trans*-2,5-dimethylpiperazine lives long enough in the more polar solvent to be able to transfer a proton to the oxo group of the enone, activating the β-position for the attack of the nucleophile. This results in competition between the peptide catalysts and the protonated additive for catalysing the reaction through direct interaction with the substrate, thereby lowering the enantiomeric excesses of the product by the ratio of the contribution of the achiral additive. Competition alone, however, cannot explain why the reaction in the presence of both peptide **6** and the additive gives a higher enantiomeric excess (23% *ee*, Entry 9) than obtained without additive (8% *ee*). There must also be a cooperative effect. One possible explanation for the cooperative effect might be the formation of a noncovalently bound complex of additive and peptide that interacts with the substrate through hydrogen bonds. The possibility for peptides to form noncovalent interactions with the additive seems particularly intriguing.

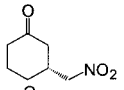
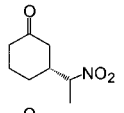
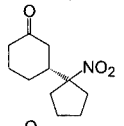
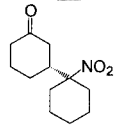
The lower enantioselectivities observed in DMSO with respect to $CHCl_3$ in the presence of a combination of peptides **6** or **12** and additive could thus be explained in terms of solvent polarity, while the individual results in DMSO arise from the balance of the competition and the cooperation effect (e.g., in case of **6**, the cooperation outweighs the competition, while in the case of **3**, the complex with the substrate might be a more stable one). The low enantiomeric excess with catalyst **12** in DMSO contradicts the assumption of the enamine mechanism here.

We next examined the room-temperature ionic liquid $[bmim]PF_6$ as an alternative solvent. The enantiomeric excesses of product obtained in the presence both of peptide (**3** or **6**, respectively) and of additive in the ionic liquid $[bmim]PF_6$ were further reduced to 5%, compared to the reaction in DMSO, accompanied by significant drops in yields (44% and 35%, respectively, Entries 5, 10). Notably, the Michael product is nearly racemic here, indicating the influence of peptides' **3** and **6** chirality as minimal in $[bmim]PF_6$ as solvent. In contrast, peptide **12** afforded the product in over 95% yield and with 51% *ee* in $[bmim]PF_6$ (Entry 15). Whereas tripeptide **6** alone gave the product in 37% yield and with 5% *ee*, no reaction took place in $[bmim]PF_6$ with peptides **3** and **12** in the absence of additive. Use of additive alone gave the Michael product in 25% yield.

In the highly polar ionic liquid we also encountered an additional phenomenon typical of the presence of ion clouds in solutions: screening. The screened nucleophile, shrouded by the cloud of cations, becomes less active (but more selective) than the nucleophile in the merely polar solvent DMSO. This might explain the stronger enantioselectivity observed with tripeptide **12** in $[bmim]PF_6$ (Entry 15) relative to DMSO (Entry 14) and the reduced activity of the additive when acting alone (Entry 20). That the enantiomeric excesses in the products formed in the presence of **3** and **6** decreased so dramatically in relation to the results in DMSO (or DMF, $CHCl_3$) could reflect the greater lability and reduced stability of hydrogen bond complexes that may form between Michael acceptor and the peptides and which could influence the outcome of the choice between enantiomeric forms. This is a further indication that **12** reacts by a different mechanism.

In the light of the more promising results that we achieved with tripeptide **12**, we next employed tripeptide **12** as catalyst of conjugate additions of various nitroalkanes, such as nitromethane, nitropropane, nitrocyclopentane and nitrocyclohexane, to cyclohex-2-en-1-one in $CHCl_3$ (defined as the best solvent for the peptide **12**). Moderate to good yields (up to 95%) and enantioselectivities ranging from 56% to 84% were obtained (Table 2).

Table 2. Conjugate addition of nitroalkanes to cyclohex-2-en-1-one in $CHCl_3$ catalysed by peptide **12** (2 mol %) in the presence of *trans*-2,5-dimethylpiperazine (100 mol %)

Nitroalkane	Product	Yield (%)[a]	*ee* (%)[b]
CH_3NO_2		95	58
$CH_3CH_2NO_2$		83	LP: 56[c] MP: 65[d]
		71	84
		24	78

[a] Isolated yields after column chromatography. [b] % *ee* measured by ^{13}C NMR of corresponding ketal with (2*R*,3*R*)-butane-2,3-diol. [c] *ee* of less polar isomer (LP). [d] *ee* of more polar isomer (MP).

Comparison of the results for the nitroalkanes demonstrates the importance of steric effects for the reaction yields. During the attack of the nucleophile, the substrate is forming an iminium ion adduct with the peptide, impairing the approach of space-consuming nucleophiles. The large nucleophile reacts slowly with the activated enone. The highest yield (95%) was therefore observed for nitromethane, while nitrocyclohexane gave the lowest yield (24%) in this series. The same effects could explain the increased enantioselectivities in the cases of nitrocyclopentane (84% *ee*) and nitrocyclohexane (78% *ee*): the reactions run slowly, but more selectively, than with nitromethane (58% *ee*).

Besides these investigations, we also decided to carry out one reaction in the presence of additive and peptide **6** (2 mol %) in a mixture of $CHCl_3$ and DMSO (4:1), since we had observed better yields in DMSO but much lower enantioselectivities than had been obtained in $CHCl_3$ (Table 1, Entries 6 and 9). The reaction took place to give product **15** in 23% yield and with 69% *ee*, which represents approximately the same enantioselectivity and a 13% increase in yield relative to Entry 6.

Conclusion

At the outset, in analogy to Hanessian,[7] we established that the combination of a peptide catalyst and *trans*-2,5-dimethylpiperazine could provide an asymmetric co-catalysis of Michael reactions. We found that even *trans*-2,5-dimethylpiperazine alone can support the conversion into the product in polar solvents. Although the solvent influence on yields and enantioselectivities is obviously a rather complex phenomenon and has to be carefully analysed for each individual case, our results showed that solvent polarity is a double-edged sword in the case of the title reaction and the catalysts employed here: while the polarity helps to facilitate the reaction, it could also give rise to reduced enantiomeric excesses. The picture is further complicated because changes not only in the catalyst but also in the solvent could lead to different catalysis mechanisms or to competition between different mechanisms. The above experiments demonstrated that a combination of solvents could result in improved yields with roughly the same enantioselectivities.

We are continuing investigations in this area and are currently screening larger libraries of peptides on different C−C bond formation reactions.

Experimental Section

General: All solvents were purified by standard procedures and were distilled prior to use. Reagents obtained from commercial sources were used without further purification. TLC chromatography was performed on precoated aluminium silica gel SIL G/UV_{254} plates (Macherey−Nagel & Co.) or silica gel 60-F_{254} precoated glass plates (Merck). ^1H NMR spectra were recorded with Varian Unity 300 and Varian Inova 600 instruments. ESI mass spectra were measured with an LCQ Finnigan spectrometer. High-resolution mass spectra were recorded with a Bruker APEX IV 7T FT-ICR instrument. A Perkin−Elmer 241 polarimeter was used for optical rotation measurements.

***N*-Benzyloxycarbonyl-L-phenylalanyl-L-arginine (Z−Phe−Arg−OH, 2):** H−Arg−OH (3.63 g, 21 mmol) was added to a solution of Z−Phe−OPfp (**1**, 11.6 g, 25 mmol) in DMF (10 mL). The resulting mixture was stirred at room temperature for several hours until the H−Arg−OH had completely dissolved. After this period, the mixture was diluted with EtOAc (100 mL) and Et_2O (100 mL). The solvent mixture was decanted, and the residue was treated with *n*BuOH (75 mL) and H_2O (35 mL). The organic layer was separated and washed with H_2O (15 mL) and concentrated at reduced pressure. The addition of Et_2O (100 mL) to the residue resulted in the formation of a precipitate, which was filtered off, washed several times with Et_2O and dried to give the dipeptide **2** (8.17 g, 85%). ^1H NMR (600 MHz, [D_6]DMSO): δ = 9.11 (s, 1 H), 7.62−7.13 (m, 16 H, 4 NH, NH_2 and 10 Ar−H), 4.97−4.90 (m, 2 H), 4.23−4.19 (m, 1 H), 3.97−3.93 (m, 1 H), 3.08−3.06 (m, 3 H), 2.77−2.72 (m, 1 H), 1.73−1.45 (m, 4 H) ppm. ^{13}C NMR (150.8 MHz, [D_6]DMSO): δ = 175.1 (CO_2H), 170.2 (C=O), 157.4 (C=O), 155.7 (C=NH), 138.2 ($C_{quat.,arom}$), 136.9 ($C_{quat.,arom}$), 129.2 (CH), 129.0 (CH), 128.2 (CH), 128.1 (CH), 127.9 (CH), 127.7 (CH), 127.5 (CH), 127.2 (CH), 127.1 (CH), 126.1 (CH), 65.1 (CH_2, O*CH*$_2$Ph), 56.5 (CH), 53.6 (CH), 40.4 (CH_2), 37.3 (CH_2), 29.7 (CH_2), 25.2 (CH_2) ppm. ESI-MS (positive ion): *m*/*z* = 456.4 [M + H]$^+$, 478.3 [M + Na]$^+$. ESI-MS (negative ion): *m*/*z* = 454.3 [M − H]$^-$. HRMS (ESI): calcd. for $C_{23}H_{30}N_5O_5$ [M + H]$^+$ 456.22415; found 456.22384.

L-Aspartyl-L-phenylalanyl-L-arginine) H−Asp−Phe−Arg−OH, 3): A solution of **2** (2.69 g, 5.9 mmol) in MeOH (50 mL) was hydrogenated at room temperature in the presence of 10% Pd/C. After completion of the reaction, the palladium was removed by filtration, and the filtrate was concentrated to dryness under reduced pressure to give **H−Phe−Arg−OH** (1.85 g, 97%) as a white solid, which was used without further purification. ^1H NMR (600 MHz,

[D$_6$]DMSO): δ = 9.03 (s, 1 H), 8.0 (d, 1 H, NH), 7.75−7.08 (m, 11 H, 4 NH, NH$_2$ and 5 Ar−H), 3.89 (m, 1 H), 3.38 (m, 1 H), 3.09−2.98 (m, 3 H), 2.62 (m, 1 H), 1.67−1.38 (m, 4 H) ppm. ESI-MS (positive ion): *m*/*z* = 322.3 [M + H]$^+$. ESI-MS (negative ion): *m*/*z* = 320.5 [M − H]$^-$. HRMS (ESI): calcd. for $C_{15}H_{24}N_5O_3$ [M + H]$^+$ 322.18737; found 322.18751. The residue (1.85 g, 5.8 mmol) was taken up in DMF (5 mL) and treated with Z−Asp(OBzl)−OPfp (3.71 g, 7.08 mmol). After 15 h at ambient temperature, the reaction mixture was diluted with Et$_2$O (100 mL). The solvent was decanted and the residue was purified by silica gel chromatography (mobile phase: 5−20% MeOH/CHCl$_3$; TLC: *n*BuOH/AcOH/H$_2$O, 3:1:1) to afford **Z−Asp(OBzl)−Phe−Arg−OH** (2.3 g, 61%) as a colourless oil, which was dissolved in MeOH (45 mL). Palladium on charcoal (10% Pd/C) was added, and then the mixture was saturated with hydrogen gas. The precipitated product was dissolved by dropwise addition of 25% NH$_4$OH and water. The charcoal was separated from the reaction product by filtration. The solvent was co-evaporated with 2-propanol, and the product was isolated by precipitation from Et$_2$O and HPLC chromatography to give **3** (1.5 g, 98%) as a white solid. $[\alpha]_D^{20}$ = +12.5 (*c* = 0.24, 1 N HCl). ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 9.32 (br. s, 1 H), 8.68 (br. s, 1 H), 7.59 (d, 1 H), 7.22−7.15 (m, 5 H, Ar-H), 4.42 (br. s, 1 H), 3.89 (dd, *J* = 7.0, 11.7 Hz, 1 H), 3.58 (t, *J* = 6.4 Hz, 1 H), 3.12−3.00 (m, 4 H), 2.83 (dd, *J* = 9.8, 13.9 Hz, 1 H), 2.34 (dd, *J* = 6.7, 16.3 Hz, 1 H), 1.71−1.60 (m, 2 H), 1.46−1.44 (m, 2 H) ppm. ^{13}C NMR (75.4 MHz, [D$_6$]DMSO): δ = 174.3 (CO_2H), 173.9 (CO_2H), 170.9 (C=O), 169.6 (C=O), 157.5 (C=NH), 138.1 ($C_{quat.,arom}$), 129.1 (CH), 129.0 (CH), 128.1 (CH), 128.0 (CH), 126.1 (CH), 54.4 (CH), 53.3 (CH), 50.9 (CH), 40.4 (CH_2), 36.5 (CH_2), 29.1 (CH_2), 26.1 (CH_2), 24.5 (CH_2) ppm. ESI-MS (positive ion): *m*/*z* = 437.4 [M + H]$^+$, 459.4 [M + Na]$^+$. ESI-MS (negative ion): *m*/*z* = 435.5 [M − H]$^-$. HRMS (ESI): calcd. for $C_{19}H_{29}N_6O_6$ [M + H]$^+$ 437.21431; found 437.21454.

***N*-Benzyloxycarbonyl-L-prolyl-L-arginine (Z−Pro−Arg−OH, 5):** This dipeptide was prepared from Z−Pro−OPfp (**4**, 8.93 g, 21.5 mmol) and H−Arg−OH (3.15 g, 18.1 mmol) by the same procedure as described above for **2**, to give **5** (6.35 g, 87%) as a white solid. ^{1}H NMR (600 MHz, D$_2$O): δ = 7.44−7.31 (m, 5 H, Ar−H), 5.14−5.08 (m, 2 H), 4.38−4.29 (m, 1 H), 4.08−4.06 (m, 1 H), 3.56−3.49 (m, 2 H), 3.18−2.93 (m, 2 H), 2.33−2.28 (m, 1 H), 2.00−1.88 (m, 3 H), 1.70−1.32 (m, 4 H). ^{13}C NMR (150.8 MHz, D$_2$O): δ = 177.9 (CO_2H), 174.2 (C=O), 156.4 (C=O), 156.1 (C=NH), 136.14 ($C_{quat.,arom}$), 128.8 (CH), 128.7 (CH), 128.2 (CH), 127.6 (CH), 127.2 (CH), 67.4 (CH_2, O*C*H$_2$Ph), 60.4 (CH), 54.6 (CH), 47.5 (CH_2), 40.6 (CH_2), 31.1 (CH_2), 29.1 (CH_2), 24.4 (CH_2), 23.53 (CH_2) ppm. ESI-MS (positive ion): *m*/*z* = 406.4 [M + H]$^+$, 428.3 [M + Na]$^+$. ESI-MS (negative ion): *m*/*z* = 404.3 [M − H]$^-$. HRMS (ESI): calcd. for $C_{19}H_{28}N_5O_5$ [M + H]$^+$ 406.20850; found 406.20819.

L-Aspartyl-L-prolyl-L-arginine (H−Asp−Pro−Arg−OH, 6): The same procedure as described for the preparation of **3** was applied, with substitution of Z−Phe−Arg−OH for Z−Pro−Arg−OH (4.05 g, 10 mmol), to afford **H−Pro−Arg−OH** (2.6 g, 96%) as a white solid. ^{1}H NMR (600 MHz, D$_2$O): δ = 4.18 (dd, *J* = 7.9, 5.0 Hz, 1 H), 3.74 (dd, *J* = 8.4, 5.7 Hz, 1 H), 3.19 (t, 2 H), 2.93 (t, 2 H), 2.17−2.12 (m, 1 H), 1.89−1.82 (m, 1 H), 1.78−1.72 (m, 4 H), 1.60−1.55 (m, 2 H) ppm. ^{13}C NMR (150.8 MHz, D$_2$O): δ = 178.4 (CO_2H), 176.2 (C=O), 156.8 (C=NH), 60.3 (CH), 54.4 (CH), 46.5 (CH_2), 40.7 (CH_2), 30.4 (CH_2), 29.1 (CH_2), 25.3 (CH_2), 24.46 (CH_2) ppm. ESI-MS (positive ion): *m*/*z* = 272.3 [M + H]$^+$, 294.3 [M + Na]$^+$. ESI-MS (negative ion): *m*/*z* = 270.4 [M − H]$^-$. HRMS (ESI): calcd. for $C_{11}H_{22}N_5O_3$ [M + H]$^+$ 272.17172; found 272.17171.

Tripeptide **6** was prepared from H−Pro−Arg−OH (2.6 g, 9.6 mmol), by the same procedure as described for **3**, to give **6** (1.72 g) as a white solid. $[\alpha]_D^{20}$ = −58.8 (*c* = 0.165, 1 N HCl). ^{1}H NMR (600 MHz, D$_2$O): δ = 4.52 (m, 2 H), 4.17 (dd, *J* = 5.2, 8.1 Hz, 1 H), 3.75−3.72 (m, 1 H), 3.67−3.63 (m, 1 H), 3.23−3.19 (m, 2 H), 2.86 (dd, *J* = 3.5, 17.4 Hz, 1 H), 2.61 (dd, *J* = 10.4, 17.4 Hz, 1 H), 2.34−2.29 (m, 1 H), 2.10−1.97 (m, 3 H), 1.88−1.83 (m, 1 H), 1.76−1.70 (m, 1 H), 1.65−1.60 (m, 2 H) ppm. ^{13}C NMR (75.4 MHz, D$_2$O): δ = 178.2 (CO_2H), 175.8 (CO_2H), 172.9 (C=O), 168.1 (C=O), 156.8 (C=NH), 60.9 (CH), 54.8 (CH), 50.1 (CH), 47.9 (CH_2), 40.7 (CH_2), 35.7 (CH_2), 29.3 (CH_2), 28.9 (CH_2), 24.7 (CH_2), 24.5 (CH_2) ppm. ESI-MS (positive ion): *m*/*z* = 387.2 [M + H]$^+$. ESI-MS (negative ion): *m*/*z* = 385.3 [M − H]$^-$. HRMS (ESI): calcd. for $C_{15}H_{27}N_6O_6$ [[M + H]$^+$] 387.19866; found 387.19883.

Dipeptide 9: A solution of **7** (1.8 g, 3.9 mmol) in ethyl acetate (35 mL) was added to a stirred solution of amine **8** (1.19 g, 4.3 mmol) in ethyl acetate (20 mL). The reaction mixture was stirred at room temperature for 12 h and then washed with H$_2$SO$_4$ (2 N, 2 × 30 mL), H$_2$O, NaHCO$_3$ (5%, 2 × 30 mL), H$_2$O and brine and dried with Na$_2$SO$_4$. The solvent was removed under reduced pressure to give an oily residue, which was precipitated from diethyl ether (75 mL); filtration gave **9** as the white precipitate (2.3 g, 94%). ^{1}H NMR (300 MHz, CD$_3$OD): δ = 7.35−7.23 (m, 10 H), 5.19−5.03 (m, 4 H, 2 × PhC*H*$_2$), 4.48−4.14 (m, 4 H), 3.83 and 3.64 (m, 2 H), 3.72 and 3.58 (s, 3 H, *CH*$_3$O), 3.49−3.01 (m, 2 H), 2.28−2.09 (m, 4 H), 1.42 [s, 9 H, C(C*H*$_3$)$_3$] ppm. ESI-MS (positive ion): *m*/*z* = 647.3 [M + Na]$^+$. $C_{32}H_{40}N_4O_9$ (624.68): calcd. C 61.53, H 6.45; found C 61.35, H 6.52.

Tripeptide 10: Trifluoroacetic acid (10 mL) was added at 0 °C to a stirred solution of **9** (1 g, 1.6 mmol) in CH_2Cl_2 (20 mL). The stirring was continued at room temperature for 2 h, followed by concentration in vacuo. The oily residue was precipitated with dry diethyl ether (50 mL), filtered off and dried in vacuo. The crude product (843 mg, 1.32 mmol) was dissolved in CH_2Cl_2 (20 mL). TEA (0.187 mL, 1.32 mmol) and **7** (670 mg, 1.45 mmol) were then added, and the resultant mixture was stirred at room temperature overnight. The reaction mixture was then washed with H$_2$SO$_4$ (2 N, 2 × 30 mL), H$_2$O, NaHCO$_3$ (5%, 2 × 30 mL), H$_2$O and brine and dried with Na$_2$SO$_4$. The solvent was removed under reduced pressure to give **10** (1.084 g, 96%) as a white solid. ^{1}H NMR (600 MHz, CD$_3$OD): δ = 7.36−7.24 (m, 15 H), 5.15−4.89 (m, 6 H, 3 × PhC*H*$_2$), 4.42−4.16 (m, 6 H), 3.83 and 3.64 (m, 3 H), 3.78 and 3.58 (s, 3 H, *CH*$_3$O), 3.48−3.05 (m, 3 H), 2.24−2.02 (m, 6 H), 1.41 [s, 9 H, C(C*H*$_3$)$_3$] ppm. ESI-MS (positive ion): *m*/*z* = 893.5 [M + Na]$^+$, 1763.1 [2M + Na]$^+$. HRMS (ESI): calcd. for $C_{45}H_{54}N_6O_{12}$ [M + H]$^+$ 871.38725; found 871.38735.

Tripeptide 11: The above methyl ester **10** (2.12 g, 2.43 mmol) was saponified by stirring at room temperature with aqueous methanol (50%, 20 mL) containing lithium hydroxide monohydrate (112.2 g, 2.67 mmol). The product was isolated in the usual manner to give **11** (1.916 g, 92%) as a white solid. ^{1}H NMR (600 MHz, CD$_3$OD): δ = 7.36−7.24 (m, 15 H), 5.13−5.05 (m, 6 H, 3 × PhC*H*$_2$), 4.43−4.17 (m, 6 H), 3.83−3.75 (m, 3 H), 3.48−3.03 (m, 3 H), 2.30−2.09 (m, 6 H), 1.41 [s, 9 H, C(C*H*$_3$)$_3$] ppm. ESI-MS (positive ion): *m*/*z* = 879.6 [M + Na]$^+$, 1735.3 [2M + Na]$^+$. ESI-MS (negative ion): *m*/*z* = 855.5 [M − H]$^-$, 1711.5 [2 M − H]$^-$. HRMS (ESI): calcd. for $C_{44}H_{52}N_6O_{12}$ [M + H]$^+$ 857.37160; found 857.37180.

Tripeptide 12: Compound **11** (200 mg, 0.23 mmol) was hydrogenated in the presence of 10% Pd/C in methanol (5 mL). The product was isolated in the usual manner to give **12** (99.5 mg, 94%) as a

white solid. $[\alpha]_D^{20} = -7.8$ ($c = 0.32$, MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ = 4.37 (m, 1 H), 4.27 (m, 1 H), 4.17 (m, 1 H), 4.05 (m, 1 H), 3.95 (m, 2 H), 3.60 (m, 1 H), 3.28−3.20 (m, 3 H), 2.99−2.91 (m, 2 H), 2.39−2.32 (m, 2 H), 2.2−1.95 (m, 4 H), 1.43 [s, 9 H, C(CH_3)$_3$] ppm. ESI-MS (positive ion): *m*/*z* = 455.2 [M + H]$^+$, 909.1 [2 M + H]$^+$. ESI-MS (negative ion): *m*/*z* = 453.5 [M − H]$^-$. HRMS (ESI): calcd. for $C_{20}H_{34}N_6O_6$ [M + H]$^+$ 455.26126; found 455.26144.

General Procedure for the Michael Reactions: 2-Nitropropane (0.63 mmol) was added to a stirred solution of 2-cyclohexen-1-one (0.5 mmol), *trans*-2,5-dimethylpiperazine (0.5 mmol) and peptide catalyst (15 mol %) in pre-dried solvent ($CHCl_3$, acetone, DMF, DMSO or [bmim]PF_6, 4 mL), and the reaction mixture was stirred at room temperature for 5 d. The reaction mixture in $CHCl_3$ was worked up as described in the literature.[7] When the reaction was carried out in acetone, DMF or DMSO, the solvent was evaporated, and the residue was dissolved in $CHCl_3$ and washed with diluted aqueous HCl (3%). The organic layer was dried with Na_2SO_4 and filtered, and the solvents were evaporated. In the case of [bmim]PF_6 as a solvent, in a deviation from the above workup, the product **15** was extracted from the reaction mixture with diethyl ether. The residues were purified by column chromatography on SiO_2 (hexane/ethyl acetate) to afford the desired product **15**. The enantiomeric excess of the product was measured by ^{13}C NMR of the corresponding ketal formed with (2*R*,3*R*)-butane-2,3-diol.[7] ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.48−2.34 (m, 3 H), 2.31−2.21 (m, 1 H), 2.19−2.08 (m, 2 H), 1.85−1.76 (m, 1 H), 1.71−1.53 (m, 1 H), 1.58 (s, 3 H), 1.57 (s, 3 H), 1.48−1.34 (m, 1 H) ppm. ^{13}C NMR (150.8 MHz, $CDCl_3$): δ = 208.9 (C=O), 90.6 ($C_{quat.}$), 46.5 (CH), 42.6 (CH_2), 40.7 (CH_2), 25.9 (CH_2), 24.3 (CH_2), 23.3 (CH_3), 22.5 (CH_3) ppm. ESI-MS (positive ion): *m*/*z* = 208.1 [M + Na]$^+$.

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[1] P. I. Dalko, L. Moisan, *Angew. Chem. Int. Ed.* **2001**, *40*, 3726−2042; *Angew. Chem.* **2001**, *113*, 3840−3864.
[2] T. Sugimoto, Y. Matsumura, S. Tanimoto, M. Okano, *J. Chem. Soc., Chem. Commun.* **1978**, 926−927.
[3] E. R. Jarvo, S. J. Miller, *Tetrahedron* **2002**, *58*, 2481−2495.
[4] B. List, R. A. Lerner, C. F. Barbas III, *J. Am. Chem. Soc.* **2000**, *122*, 2395−2396.
[5] Z. G. Hajos, D. R. Parrish, *J. Org. Chem.* **1974**, *39*, 1615−1621.
[6] K. A. Ahrendt, Ch. J. Borths, D. W. C. MacMillan, *J. Am. Chem. Soc.* **2000**, *122*, 4243−4244.
[7] S. Hanessian, V. Pham, *Org. Lett.* **2000**, *2*, 2975−2978.
[8] [8a] M. S. Sigman, E. N. Jacobsen, *J. Am. Chem. Soc.* **1998**, *120*, 4901−4902. [8b] M. S. Iyer, K. M. Gigstad, N. D. Namdev, M. Lipton, *J. Am. Chem. Soc.* **1996**, *118*, 4910−4911.
[9] [9a] H. J. Martin, B. List, *Synlett* **2003**, 1901−1902. [9b] B. List, P. Pojarliev, H. J. Martin, *Org. Lett.* **2001**, *3*, 2423−2425.
[10] M. Yoshikawa, *Antiamnesic and anticholesteremic peptides*, JP 2002080393, **2002**, 7 pp.
[11] J. C. Tolle, W. Z. Gifford, K. W. Funk, *Antiallergic pentapeptide synthesis*, US 5212287, **1993**, 9 pp.
[12] C. R. Woods, N. Faucher, B. Eschgfaller, K. W. Bair, D. L. Boger, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2647−2650.
[13] [13a] See Exp. Sect. for details. [13b]M. V. Ovchinnikov, Z. D. Bespalova, A. S. Molokoedov, I. V. Revenko, N. F. Sepetov, O. L. Isakova, M. I. Titov, *Collect. Czech. Chem. Commun.* **1989**, *54*, 772−783. [13c] V. I. Unkovskii, G. A. Vasil′eva, R. P. Evstigneeva, *J. Gen. Chem. USSR* **1986**, *56*, 1455−1460.
[14] M. Tamaki, G. Han, V. J. Hruby, *J. Org. Chem.* **2001**, *66*, 1038−1042.
[15] See Exp. Sect. for details.
[16] A. Berthod, S. Carda-Broch, *The Annals of the Marie Curie Fellowships*, vol. 3, http://www.mariecurie.org/annals/volume3/berthod.pdf
[17] [17a] J. F. Coetzee, G. R. Padmanabhan, *J. Am. Chem. Soc.* **1965**, *87*, 5005−5010. [17b] B. W. Clare, D. Cook, E. C. F. Ko, Y. C. Mac, A. J. Parker, *J. Am. Chem. Soc.* **1966**, *88*, 1911−1916.

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