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Cyclic Peptide–Polymer Complexes and Their Self-Assembly

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Abstract: The efficient synthesis of novel chiral cyclic peptides cyclo[NHCHX-CH=CHCH$_2$CO(NHCH$_2$-CH=CHCH$_2$CO)$_2$] designed to develop hydrogen-bonding interactions with suitable polymers is described. Complexation of a carboxylic acid derivatized cyclic peptide **2** (X = CH$_2$OCOCH$_2$CH$_2$CO$_2$H) capable of self-assembling as "endless" tubes, with poly(vinyl alcohol) (PVA) led to a vast weak-interaction network, in which the cyclopeptide developed extensive hydrogen-bonding interactions with the hydroxyl groups of PVA through not only the carboxylic acid, but also its ester carbonyl and amide groups. In aqueous solution, the peptide/PVA complexes self-assemble into long-grain ricelike aggregates compatible with the stacking of cyclic peptides through intercycle hydrogen bonds. Upon casting on silicon wafer, the anisotropic aggregates can coalesce to form filaments tens of micrometers long. The study demonstrates that complexing functionalized cyclic peptides with polymers through hydrogen bonding is a useful approach for using polymers to mediate the self-assembly and self-organization of cyclic peptides.

Keywords: cyclopeptides · nanotubes · polymer engineering · supramolecular chemistry · synthetic methods

Introduction

Rationally designed cyclic peptides with flat conformations can self-assemble into tubular nanostructures mainly stabilized by intercycle hydrogen bonding. With the numerous possibilities of functionalizing both the interior and exterior of the cyclic peptide nanotubes, they are believed to hold promise for many applications.[1–4] Generally, when the assembly of cyclic peptides occurs in solution from the molecularly dissolved state, which can be triggered either by cooling the solution or adding a nonsolvent, they can stack into nanotubes that, in turn, organize into a compact crystal structure. In a recent study,[5] our groups found that the medium in which the self-assembly of cyclic peptides takes place may have a profound effect on the final structure. Instead of forming a compact crystal in organic solvents, a macrolactam, namely, cyclo(NHCH$_2$CH=CHCH$_2$CO)$_3$ (*E* olefin), dissolved in a nematic liquid crystal (LC) formed micrometer-sized hexagonal hollow tubes on slow cooling of the mixture. The intermolecular interaction between the cyclic peptide and the medium, that is, the liquid crystal in this case, is no doubt a decisive factor that caused the dramatic change in the self-assembled structure of the peptide. It is clear that practical applications of tubular nanostructures of cyclic peptides not only rely on their functionalities, but also on their control and manipulation at a macroscopic scale.

Bringing together tubular nanostructures of cyclic peptides and polymers may give rise to interesting materials.[6] Among the possible features, the processability of polymers can be exploited for the manipulation of peptide nanotubes. For instance, if nanotubes are formed in a polymer matrix, stretching of the polymer film may align them. It can also be foreseen that acting as an interacting medium, the polymer can affect the self-assembly process of cyclic peptides in different ways. In the study by Biesalski and Couet,[7] cyclic peptides functionalized with initiator groups were first self-

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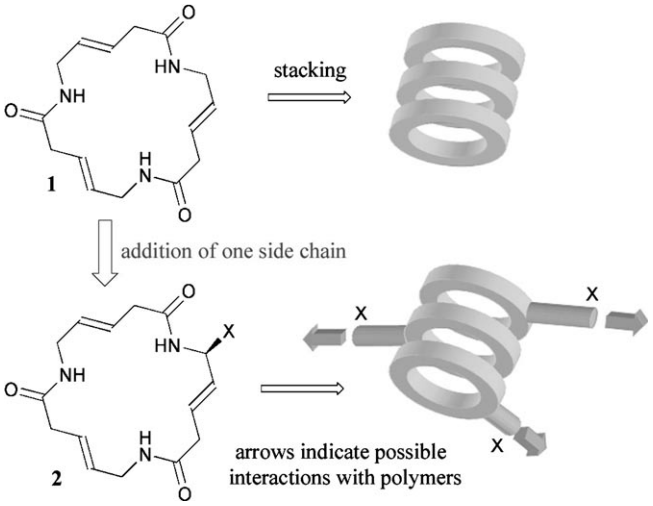
assembled into nanotubes; they were then used to initiate the polymerization of *N*-isopropylacrylamide with the initiation sites on their outer surface. Börner and co-workers employed a different approach:[8] a functionalized cyclic peptide was first attached to poly(*n*-butyl acrylate) through a coupling reaction to yield a peptide–polymer conjugate, which was followed by the hydrogen-bond-driven ring stacking assembly. In both cases, cyclic peptide nanotubes wrapped in a polymer were obtained. A particular interest of these approaches is the possibility of preventing the peptide nanotubes from organizing into a compact crystal, which may help the manipulation of such nano-objects. In another report,[9] a cyclic decapeptide gramicidin S was modified with a methacrylate group and the monomer was successfully polymerized by using atom-transfer radical polymerization; the β-sheet character of the peptide was retained in the resulting polymer.

Herein, we present a different approach of combining cyclic peptides and polymers. The basic idea, illustrated in Scheme 1, is to functionalize cyclic peptide **1** with a side

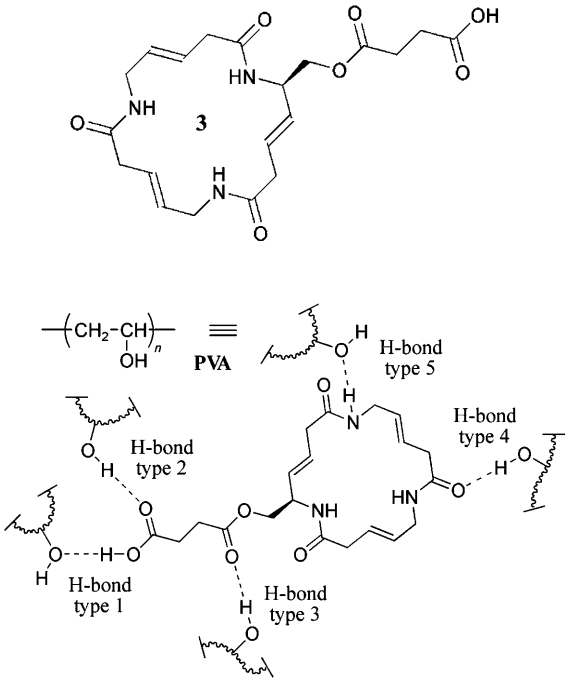


Scheme 1. Schematic illustration of the stacking of cyclic peptides functionalized with a side group capable of developing strong interactions with polymers.

group X that may develop strong interactions with polymers, while the resulting cyclopeptide **2** should still have the propensity to self-assemble into tubes like its parent **1**.

The type of interactions with polymers will be determined by the nature of the side chain X, which can end either with a group that forms hydrogen bonds with the polymer or with a polymerizable group that allows the cyclopeptide to be covalently linked to the polymer backbone.[9] We have made a carboxylic acid derivatized cyclic peptide **3** and used it to complex with poly(vinyl alcohol) (PVA).

The cyclic peptide can be noncovalently connected to the polymer through various types of hydrogen bonds formed between the acid, the ester carbonyl, and amide groups of **3** and the hydroxyl group of PVA (Scheme 2). In contrast to



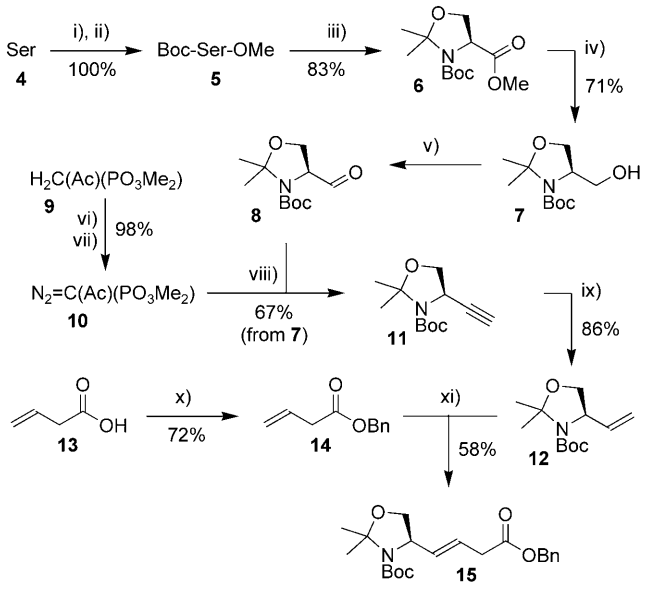
Scheme 2. Complexation through hydrogen bonding of the carboxylic acid derivatized cyclic peptide **3** with PVA.

the previous studies,[7,8] in which the stacking of peptide rings is the driving force to dictate the organization of polymer chains, in our cyclic peptide–polymer complexes it is easy to imagine that the polymer could actively alter the packing and organization of cyclic peptides. In addition to the preparation and characterization of the peptide–polymer complexes, their self-assembly behaviors in solution and in solution-cast films were investigated.

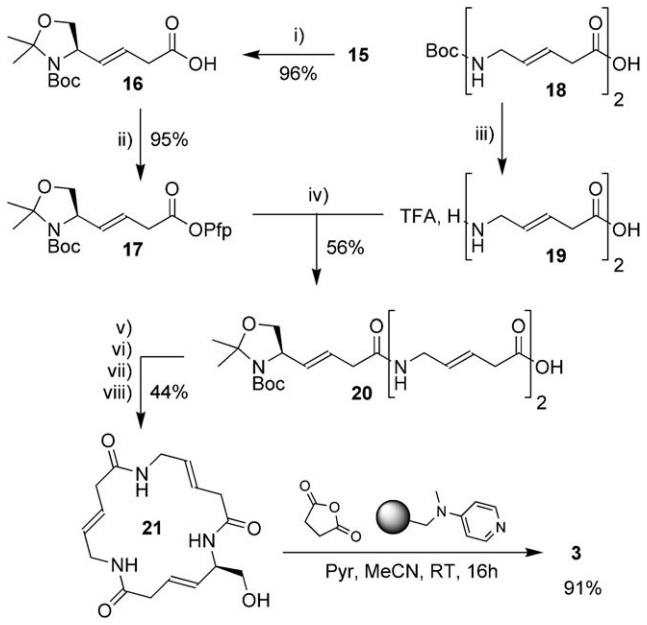
Results and Discussion

Synthesis: We have developed a versatile synthetic method to build chiral macrolactams **2**, in which X could be any side chain present in readily available natural α-amino acids. We wish to describe the synthesis of such a compound starting from serine (**4**). The resulting ring **2** ($X=CH_2OH$) displays a huge potential because it can develop weak hydrogen bonds with carefully chosen LCs or polymers. However, its main advantage comes from its easy derivatization to any kind of esters. We have selected ester **3** for its ease of preparation over other esters and because its acid functionality can prove very useful to hydrogen bond with amine-carrying polymers.[10] Compound **4** was sequentially protected at the amine and at the acid ends to yield ester **5** in a quantitative way (Scheme 3). Finally, the alcohol moiety and the carbamate nitrogen reacted with 2,2-dimethoxypropane (DMP) under acidic conditions to afford oxazolidine **6** in 83% yield.[11]

Ester **6** was transformed into the corresponding alcohol **7** with lithium aluminium hydride (71%). Oxidation of alco-



Scheme 3. Synthesis of chiral δ-amino acid benzyl ester **12**. i) Boc_2O (Boc = *tert*-butyloxycarbonyl), K_2CO_3, dioxane, H_2O, RT, 16 h; ii) MeI, K_2CO_3, DMF, 0 °C, 16 h; iii) DMP, *p*-toluenesulfonic acid (PTSA), benzene (Bz), reflux, 30 min; iv) $LiAlH_4$, Et_2O, reflux, 2 h; v) trichloroisocyanuric acid (TCU), 2,2,6,6,-tetramethylpiperidine *N*-oxyl (TEMPO), CH_2Cl_2, 0 °C, 30 min; vi) NaH, Bz, THF, 0 °C, 1 h; vii) $TosN_3$ (Tos = tosyl), Bz, RT, 2 h; viii) K_2CO_3, MeOH, 0 °C, 1 h, RT, 16 h; ix) H_2, Lindlar catalyst (5%), quinoline, EtOH, RT, 16 h; x) benzyl chloroformate, pyridine, CH_2Cl_2, RT, 16 h; xi) Grubbs catalyst (2nd generation), CH_2Cl_2, reflux, 12 h.



Scheme 4. Synthesis of alcohol cyclopeptide **21** and its succinate derivative **3**. i) 0.5 M NaOH, MeOH, RT, 16 h; ii) PfpOH, DCC, EtOAc, RT, 3 h; iii) trifluoroacetic acid (TFA), CH_2Cl_2, RT, 1 h; iv) K_2CO_3, MeAc, H_2O, RT, 16 h; v) PfpOH, DIC, EtOAc, RT, 12 h; vi) AcOH, PTSA, Bz, H_2O, RT, 12 h; vii) TFA, CH_2Cl_2, RT, 40 min; viii) *N*-methyl morpholine (NMM), dioxane, 80 °C, 48 h.

hol **7** with TEMPO[12] produced aldehyde **8**,[11] which was never stored, but immediately used in subsequent reactions. Several attempts to prepare alkene **12**[13,14] by Wittig-type reactions on the aldehyde **8** were unreliable. However, compound **8** readily reacted with **10**[15] to give alkyne **11** with a two-step overall yield of 67%.[16] Compound **10** was prepared from **9** by nucleophilic attack of its anion onto tosyl azide (98%).[17] Alkyne **11** was then partially hydrogenated to the desired alkene **12** in 86% yield.[18] Therefore, amine side metathesis precursor **12** was made in seven steps from **4** and with an overall yield of 34% yield. Acid side metathesis precursor **14** was available from a single esterification reaction from acid **13** (72% yield).[19] Owing to the large difference of steric hindrance between the two metathesis precursors **12** and **14**, it was possible to favor the formation of the desired unsymmetrical alkene during their cross-coupling metathesis reaction.[20] Thus, compound **15** was formed in yield of 58% by using a 20% excess of **14** over **12**. Ester **15** was hydrolyzed with sodium hydroxide to give **16** (96%), which was subsequently transformed into its corresponding pentafluorophenyl (Pfp) ester **16** with dicyclohexylcarbodiimide (DCC) and PfpOH and with a yield of 95% (Scheme 4).[21] Dipeptide **18** used to prepare macrolactam **1**[22] was treated with TFA to afford the corresponding salt **19**. The free amine of **19** was left to react with the activated ester **17**. Trimeric linear peptide acid **20** was obtained with a yield of 56%.

Acid **20** was activated as its Pfp ester by means of diisopropylcarbodiimide (DIC) and PfpOH. The resulting urea byproduct was filtered off, but the product could not be purified further, since it was reacting with methanol, a solvent that would have been suitable to purify it by flash column chromatography. Nevertheless, its TLC purity was satisfactory. Many preliminary attempts to use TFA alone to cleave both the oxazolidine and the BOC group had shown that the oxazolidine ring remained intact in the absence of water. Therefore, the oxazolidine was opened up with acetic acid, PTSA and water, then; the Boc group was cleaved with TFA. The resulting TFA salt was treated with NMM as a base to free the amine and provoke its macrocyclization onto the intramolecularly activated Pfp ester. Medium dilution conditions were used for that step (5 mM), which led to the desired macrocycle **21** in a good yield of 44%. By treating alcohol **21** with succinic anhydride in the presence of DMAP, it was possible to synthesize the functionalized macrolactam **3**. However, DMAP proved to be difficult to remove from the final crude mixture. The problem was solved by using DMAP linked to polystyrene. Under these optimized conditions, the yield could be as high as 91%.

Preparation and characterization of peptide–polymer complexes: The complexation of **3** was achieved by first dissolving both components in a common solvent to obtain a clear and homogeneous solution and then evaporating the solvent under reduced pressure and heating. Thus, PVA (M_w ~ 124000–186000 g mol^{-1}) was first dissolved in water heated to 90 °C (~0.5%), then the peptide was added into the hot

aqueous solution under stirring. After a clear solution was observed, it was cooled to room temperature. The samples used for the characterizations were directly cast from the solution. Most water was first evaporated slowly under ambient conditions, followed by drying under reduced pressure at 70 °C for 16 h.

In addition to the carboxylic acid group, the cyclic peptide has one ester carbonyl (on the spacer) and three amides groups (on the ring) that can also form hydrogen bonds with the hydroxyl groups of PVA (Scheme 2). The multiple hydrogen bonds should provide extensive intermolecular interactions between the two components. Another molecule of cyclic peptide could form hydrogen bonds with different hydroxyl groups either on the same or different polymer chains. The complexes of peptide/PVA were prepared by dissolving them in water (at 90 °C), followed by evaporation of the water, and drying. The compositions of the complexes were chosen to have peptide/PVA molar ratios of 1:1 and 1:3 (meaning one peptide ring for one or three PVA hydroxyl groups; Table 1).

Figure 1 compares the infrared spectra of peptides **1** and **3**, PVA, and the four complexes in the $\tilde{\nu}=1100$–$1900\ cm^{-1}$ region. The large spectral changes confirm the strong hydrogen-bonding interactions between peptide **3** and the polymer. PVA has no absorption in the region of 1600–$1800\ cm^{-1}$, whereas the peptide displays the carbonyl band around $1725\ cm^{-1}$ (contributed by the end acid group and the ester carbonyl group on the spacer) and the amide I band at $1635\ cm^{-1}$. For the **3**/PVA complexes, the ester carbonyl band exhibits a large shift to lower frequencies as reflected by the appearance of a new band at about $1695\ cm^{-1}$. This drastic change clearly indicates the formation of hydrogen bonds between the ester carbonyl groups of the peptide and the hydroxyl groups of PVA. The proportion of hydrogen bonds involving the carboxylic acid and the carbonyl group on the spacer cannot be assessed due to the band overlap. On the other hand, judging from the change of the amide I band, hydrogen bonding between the hydroxyl groups of PVA and the amide groups on the ring of the peptide looks much less certain. The apparent shift of the band to higher frequencies may be caused by the overlap with the new band of the ester carbonyl groups hydrogen bonded to PVA. Nevertheless, the significant spectral change in the region around $1200\ cm^{-1}$, where PVA virtually has no absorption, may provide more information on hydrogen bonding. The absorption band of the peptide at $\sim 1240\ cm^{-1}$ is assigned to the amide III band (mainly in-phase N–H bending coupled with C–N stretching)[23,24] and, like the amide I band, it is known to be sensitive in frequency and intensity to hydrogen bonding. It can be noticed that in the two complexes this band appears better resolved and shifted to lower frequencies. This result suggests the formation of some hydrogen bonds between the hydroxyl groups of PVA (as the hydrogen-bond acceptor) and the N–H of the amide groups of the peptide (as the hydrogen-bond donor) in their complexes. As a whole, the infrared spectroscopic measurements confirmed the massive hydrogen bonding between the two components in the complexes of **3**/PVA. Of course, the hydrogen bonding between the peptide and PVA is in competition and can then disrupt the hydrogen bonding between molecules of peptide and between the hydroxyl groups of PVA. In the latter case, we note that the characteristic band of the self-association of hydroxyl groups around $3300\ cm^{-1}$ overlaps with the hydrogen-bonded N–H stretching band of the peptide in the same region (spectra not shown).

Table 1. Composition information for mixtures of **1** and **3** and PVA.

Mixture	Molar ratio[a] (peptide/PVA)	PVA in mixture [wt %]
1/PVA	1:3	31
1/PVA	1:1	13.1
3/PVA	1:3	24
3/PVA	1:1	9.4

[a] One peptide molecule for one or three PVA OH groups.

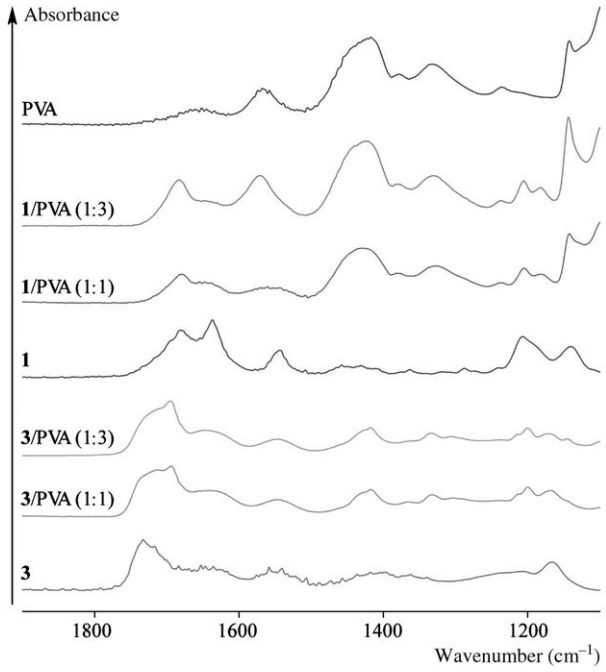


Figure 1. Infrared spectra of cyclic peptides **1** and **3**, PVA, and their complexes.

In the case of cyclopeptide **1**, no obvious changes can be seen in the IR spectra of the free peptide and its two PVA complexes. It may be inferred that **1** does not develop very strong noncovalent interactions with the PVA matrix. This provides additional proof that **3** is noncovalently linked to PVA mostly through its side-chain carboxyl groups.

The multisite, extensive hydrogen bonding in the **3**/PVA complexes results in materials that display no endo- or exotherms on the heating and cooling cycles of differential scanning calorimetry (DSC) indicating the absence of any ordered phases. No crystallization of PVA was observed due to the small amount of it complexed with the peptide matrix. The cyclic peptide hydrogen bonded to even a small amount of PVA chains gains much mechanical strength.

Figure 2 shows the photo of the peptide and its complex with 9.4 wt% of PVA side by side on a glass slide.

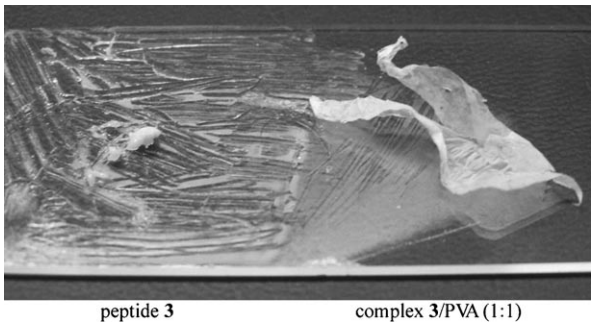


Figure 2. Photo showing **3** in an oily state at room temperature and the peelable film of the peptide complexed with 9.4 wt% PVA.

Whereas the peptide is in an oily soft state under ambient conditions, a thin film of the complex can be solution cast and peeled off from the glass surface after drying. This dramatic change in mechanical strength and integrity should be accounted for by the presence of the small amount of PVA chains that, by establishing an extensive hydrogen-bonding network with the cyclic peptides, act as a glue to bind the peptides together over large distances. Actually, the film of the **3**/PVA (24 wt%) complex could readily be stretched at an elevated temperature. Figure 3 shows the polarized infrared spectra for a film stretched to 100% deformation at

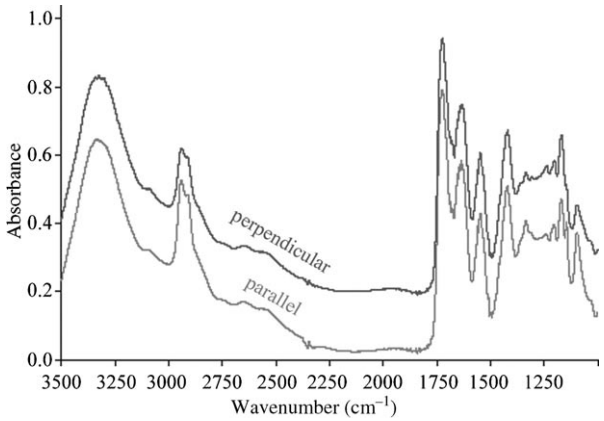


Figure 3. Polarized infrared spectra at room temperature for a film of peptide/PVA (24 wt%) stretched to 100% strain at 95°C.

95°C, followed by cooling to room temperature. The two spectra were recorded with the infrared beam polarized in a parallel and perpendicular arrangement to the stretching direction.

The absorption bands exhibiting a significant parallel dichroism, such as those around 2935 and 1100 cm^{-1}, come from PVA, indicating the alignment of the PVA chain along the stretching direction. Both the ester carbonyl band (1725 cm^{-1}) and the amide I band (1635 cm^{-1}) show only a very small parallel dichroism, indicating the absence of a high degree of orientation for peptide molecules in this complex. It can be imagined that if peptide nanotubes dispersed in a polymer matrix are aligned along the stretching direction, the amide I band should display high parallel dichroism because the transition moment of the C=O stretching mode is parallel to the long axis of the nanotube.

Dilute aqueous solutions of the **3**/PVA complexes were cast on silicon wafer for SEM observations. Presented in Figures 4 and 5 are the results obtained with peptide/PVA

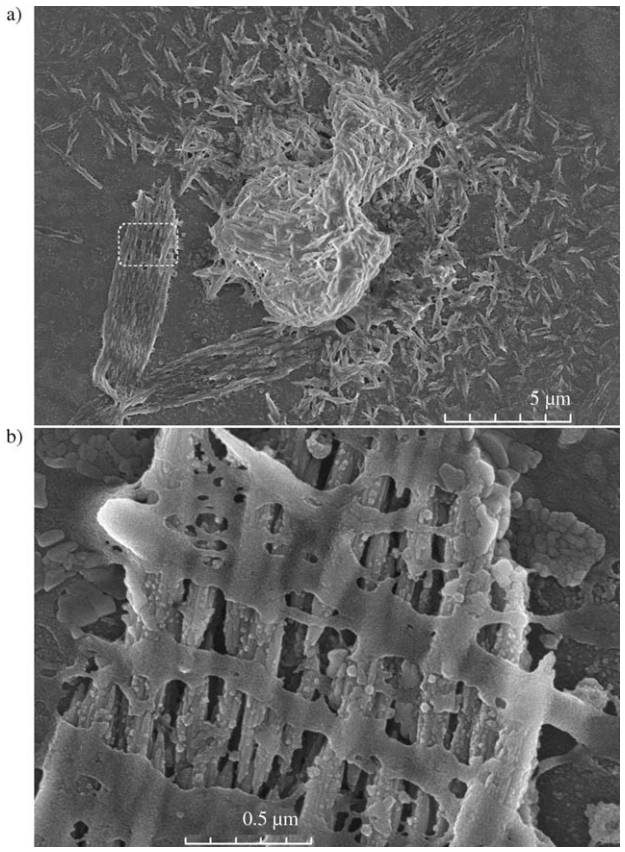


Figure 4. SEM images for films of peptide/PVA (24 wt%) on a silicon wafer cast from a 0.1 mg mL^{-1} solution of the complex. Image b shows the magnification of the marked region on image a.

(9.4 wt%) (similar results were found with 24 wt% of PVA) at two different complex concentrations (0.1 and 0.5 mg mL^{-1}). The solutions were heated to 90°C for 30 min before being cast and dried at the same temperature for 2 h. At a concentration of 0.1 mg mL^{-1} (Figure 4), image a shows the formation of long-grain ricelike aggregates. The magnification of an area in a large agglomeration (Figure 4b) shows the packing of the long aggregates. It is clear that in solution the peptide/PVA complex self-assembles into the long-grain ricelike aggregates that agglomerate during the drying process.

When the complex concentration in solution was increased to 0.5 mg mL^{-1} (Figure 5), image a shows that the

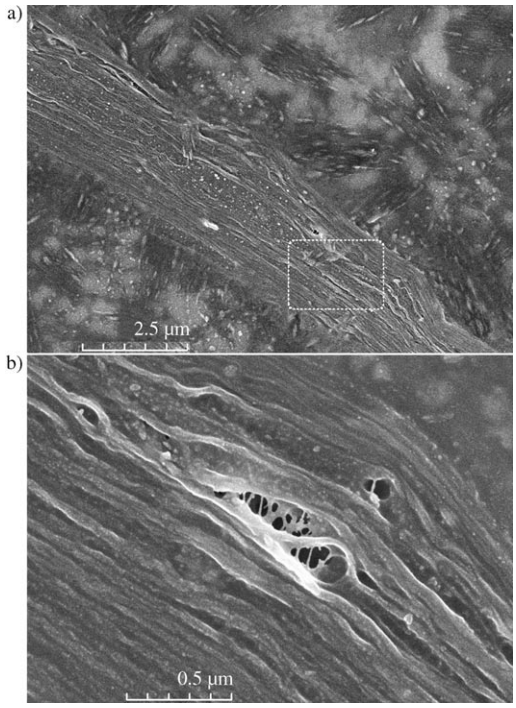


Figure 5. SEM images for films of peptide/PVA (24 wt%) on a silicon wafer cast from a 0.5 mg mL^{-1} solution of the complex. Image b shows the magnification of the marked region on image a.

primary long-grain ricelike aggregates are the same, but on the substrate surface they can now further assemble into filaments that can be as long as tens of micrometers. The coalescence of the primary aggregates to the long filaments can clearly be noticed in the interfacial region. Figure 5b gives a magnified area of the filaments, showing the coalescence/fusion of the primary aggregates.

Using SEM, we performed a number of control tests on samples prepared under the same aforementioned conditions. For pure cyclic peptide **3**, Figure 6a shows its crystallization on the substrate surface resulting in large dendritic and flowerlike patterns, which is completely different from the peptide/PVA complex. In another experiment, the unfunctionalized counterpart of the cyclic peptide[4] (compound **1** in Scheme 1) was mixed with PVA and investigated by SEM. In this case, the absence of the side group for the peptide means that PVA could only possibly have hydrogen bonding with the amide groups on the ring of **1**. Images b and c in Figure 6 compare the pure unfunctionalized cyclic peptide with its mixture containing 90 wt% PVA (both cast from 0.1 mg mL^{-1} aqueous solution). It is seen that the absence of the side group, that is, the absence of hydrogen bonding between the carboxylic acid/ester carbonyl group and the hydroxyl groups of PVA, led to drastically different results. Indeed, no complexation between the unfunctionalized peptide and PVA could be observed, since the peptide blended with PVA crystallized basically the same way as in the pure form, with the peptide crystals wrapped in the PVA film (similar results were obtained with less PVA). This experiment confirmed the crucial role of hydrogen

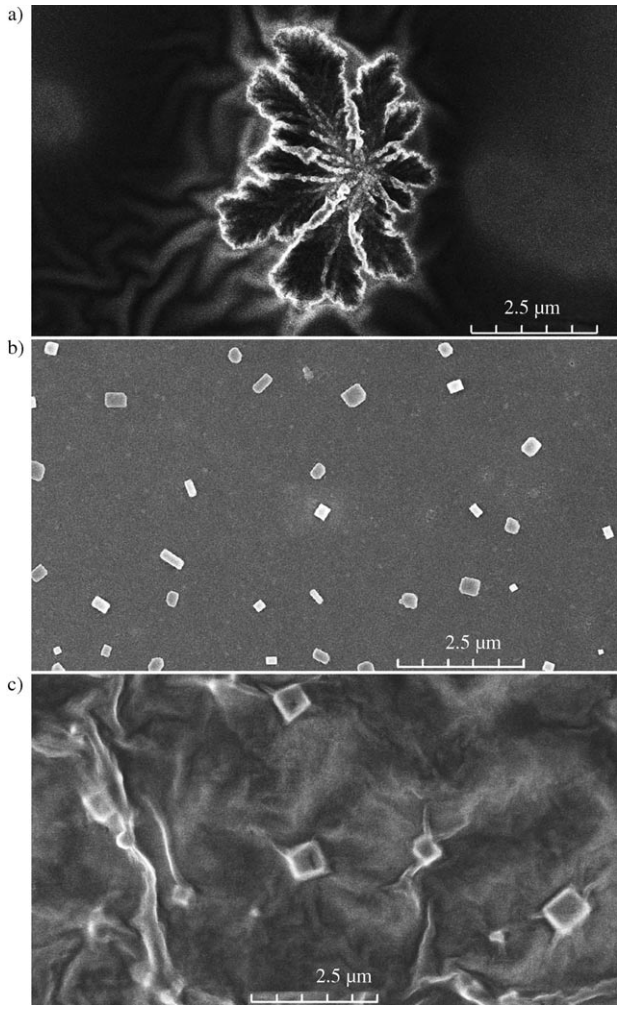


Figure 6. SEM images for a) pure functionalized peptide **3**, b) pure unfunctionalized peptide **1**, and c) unfunctionalized peptide **1** blended with 90 wt% of PVA.

bonding in complexing cyclic peptides with PVA. On the basis of the whole characterization results, the self-assembly behaviors revealed in Figures 4 and 5 is peculiar to the peptide **3**/polymer complex and should be mediated by the presence of PVA hydrogen bonded to the cyclic peptide.

The SEM results revealed that long-grain ricelike aggregates were obtained after drying a very dilute solution of the complex (0.1–0.5 mg mL^{-1}). The small amount of material and the small size of the aggregates made any molecular-level structural characterizations (by using X-ray diffraction, for example) difficult. At this time, we do not know how the peptide molecules and PVA chains are exactly organized in the aggregate. However, even without direct experimental evidence, the anisotropic form and the high aspect ratio of the long-grain ricelike aggregates of **3**/PVA suggests the organization of some kind of tubular nanostructures resulting from the stacking of cyclic peptides. This is qualitatively supported by the prominent amide I and amide II bands retained for the complexes (Figure 1). Considering the fact that, under the same conditions, the pure cyclic peptide

cannot lead to the formation of the anisotropic aggregates, the polymer must play an active role in mediating the self-assembly of the peptide. In a dilute aqueous solution, PVA backbones hydrogen bonded to peptide molecules may serve as a bridge to bring them into proximity for stacking, while preventing them from crystallizing.

Conclusion

The complexation of cyclic peptides with polymers through noncovalent interactions such as hydrogen bonding is a useful approach for using polymers to mediate the self-assembly and organization of cyclic peptides. The present study with carboxylic acid derivatized cyclic peptide **3** has revealed that with PVA as the complexing polymer, the cyclopeptide develops extensive, but nonspecific, hydrogen-bonding interactions with the polymer. A consequence of this is that even a small amount of polymer can significantly improve the mechanical strength of the peptide. In dilute solutions of such a peptide–polymer complex, the hydrogen bonding of the peptide to the polymer may allow the stacking of peptide molecules, driven by their intercycle hydrogen bonding, to occur. This self-assembly process gives rise to high aspect ratio anisotropic aggregates in solution, which can further assemble into long filaments on the substrate surface.

Experimental Section

Boc-Ser-OMe (5): A solution of K_2CO_3 (27.0 g, 195 mmol) in H_2O (100 mL) was added to a suspension of **4** (10.02 g, 95.4 mmol) in dioxane (100 mL). Boc_2O (22.9 g, 105 mmol) was added over a period of 10 min. The resulting mixture was stirred for 16 h at RT. The solvent was removed under reduced pressure and H_2O (150 mL) was poured onto the residue. Citric acid was added until pH 4 was reached. The solution was extracted with EtOAc (5×100 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The resulting yellow oil was dissolved into DMF (150 mL) containing K_2CO_3 (14.5 g, 105 mmol). MeI (27.0 g, 191 mmol) was then slowly added at 0°C. The mixture was allowed to warm to RT; it was stirred for 16 h then poured in H_2O (200 mL) and extracted with EtOAc (4×250 mL). The organic extracts were collected and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residual oil was purified by flash chromatography on silica gel eluting with hex/EtOAc (3:7 to 6:4) to yield **5** as a yellow oil (20.9 g, 100%). R_f=0.3 (1:1 hex/EtOAc); $[\alpha]_D^{20}$=−45.7 (c=1.13, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.42 (br, 1H), 4.39 (br, 1H), 3.95 (dd, 1H, *A*BX, J_{AB}=11.0 Hz, J_{AX}=4.0 Hz), 3.92 (dd, 1H, A*B*X, J_{AB}=11.0 Hz, J_{BX}=3.5 Hz), 3.79 (s, 3H), 1.45 ppm (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=171.4, 155.7, 80.2, 63.2, 55.7, 52.5, 28.2 ppm; IR (NaCl): $\tilde{\nu}$=3399, 2977, 1744, 1697, 1164, 1513 cm^{-1}; MS: m/z (%): 189 (10) $[M\mathrm{H}-\mathrm{OCH_3}]^+$, 160 (35) $[M-\mathrm{CO_2CH_3}]^+$, 133 (100); HRMS: m/z calcd for $C_8H_{15}O_4N$: 189.1001 $[M\mathrm{H}-\mathrm{OCH_3}]^+$; found: 189.1004 $[M\mathrm{H}-\mathrm{OCH_3}]^+$.

Oxazolidine 6: DMP (20.25 g, 194.5 mmol) and PTSA (254 mg, 1.3 mmol) were added to a solution of **5** (20.9 g, 95.3 mmol) in Bz (150 mL). The mixture was heated at reflux for 30 min and MeOH was distilled off at the same time. The final mixture was poured into Et_2O (100 mL) then washed with a saturated aqueous solution of $NaHCO_3$ (3×75 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The oily residue was purified by flash chromatography on silica gel eluting with hex/EtOAc (30:70 to 35:65) to give **6** as a yellow oil (20.6 g, 83%). R_f=0.45 (3:7 hex/EtOAc); $[\alpha]_D^{20}$=−53.2 (c=1.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ=4.49 (dd, 0.5H, AB*X*, J_{AX}=7.0 Hz, J_{BX}=3.0 Hz), 4.37 (dd, 0.5H, AB*X*, J_{AX}=7.0 Hz, J_{BX}=3.0 Hz), 4.2–4.0 (m, 2H), 3.75 (s, 3H), 1.7–1.4 ppm (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=171.7, 151.1, 95.0, 80.3, 66.2, 59.2, 52.2, 28.3, 25.9, 25.1, 24.9, 24.3 ppm; IR (NaCl): $\tilde{\nu}$=2975, 1748, 1699, 1211, 1179 cm^{-1}; MS: m/z (%): 244 (20) $[M-\mathrm{CH_3}]^+$, 144 (100); HRMS: m/z calcd for $C_{11}H_{18}O_5N$: 244.1185 $[M-\mathrm{CH_3}]^+$; found: 244.1189 $[M-\mathrm{CH_3}]^+$.

Alcohol 7: A solution of **6** (18.12 g, 69.9 mmol) in Et_2O (100 mL) was slowly added to a suspension of $LiAlH_4$ (2.65 g, 69.9 mmol) in Et_2O (100 mL). The mixture was heated under reflux for 2 h, then EtOH (20 mL) and a saturated aqueous solution of NH_4Cl (70 mL) were added. The resulting solution was filtered over Celite and solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel eluting with hex/EtOAc (95:5 to 50:50) to give **7** as a colorless oil (11.5 g, 71%). R_f=0.2 (7:3 hex/EtOAc); $[\alpha]_D^{20}$=−23.8 (c=1.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ=4.15–3.95 (m, 2H), 3.8–3.55 (m, 3H), 1.65–1.45 ppm (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=153.9, 94.0, 81.0, 65.2, 64.7, 59.4, 28.3, 27.1, 24.5, 23.0 ppm; IR (NaCl): $\tilde{\nu}$=3438, 2979, 1698, 1678, 1392, 1366 cm^{-1}; MS: m/z (%): 216 (50) $[M-\mathrm{CH_3}]^+$, 200 (40) $[M-\mathrm{OCH_3}]^+$, 100 (100); HRMS: m/z calcd for $C_{10}H_{18}O_4N$: 216.1236 $[M-\mathrm{CH_3}]^+$; found: 216.1240 $[M-\mathrm{CH_3}]^+$.

Aldehyde 8: Acid **7** (600 mg, 2.79 mmol) was dissolved in CH_2Cl_2 (20 mL) and cooled with an ice bath. Then the other reagents were successively added in the following order: 1-hydroxybenzotriazole (HOBt; 452 mg, 3.35 mmol), 4-dimethylaminopyridine (DMAP; 851 mg, 6.97 mmol), *n*BuSH (313 μL, 2.93 mmol), and *N*′-(3-dimethylaminopropyl-*N*-ethylcarbodiimide (EDCI; 639 mg, 3.35 mmol). The flask was sealed with a rubber septum and purged with N_2. The resulting mixture was stirred until a clear homogenous solution was obtained and immediately placed in the freezer at −17°C for 18 h without stirring. Some starting material **7** was still present, and more *n*BuSH (60 μL, 0.56 mmol) and EDCI (106 mg, 0.56 mmol) were then quickly added to the reaction mixture placed in an ice bath. The flask was sealed and purged with nitrogen and set at −17°C for another 18 h, after which time the reaction was complete. The reaction mixture was poured directly from the freezer into a saturated aqueous solution of NH_4Cl (30 mL). The CH_2Cl_2 layer was isolated and the remaining aqueous solution was extracted with CH_2Cl_2 (3×20 mL). The combined organics layers were dried on Na_2SO_4, filtrated, and concentrated. The crude residue was purified by flash chromatography on silica gel eluting with Et_2O/hex (7:3) to yield **8** as a colorless oil (688 mg, 86%). R_f=0.80 (7:3 Et_2O/hex); ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.75–5.5 (m, 2H), 4.64 (br, 1H), 3.72 (br, 2H), 3.24 (d, 2H, 5.5 Hz), 2.84 (t, 2H, 7.5 Hz), 1.52 (m, 2H), 1.42 (s, 9H), 1.38 (m, 2H), 0.89 ppm (t, 3H, 7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ=197.5, 155.7, 131.9, 123.4, 79.4, 47.0, 42.1, 31.5, 28.7, 28.3, 21.9, 13.5 ppm; IR (NaCl): $\tilde{\nu}$=3355, 2965, 2930, 1695, 1515, 1250, 1170, 970 cm^{-1}; MS: m/z: 214 $[M-\mathrm{CH_3}]^+$; HRMS: m/z calcd for $C_{10}H_{16}O_4N$: 214.0902 $[M-\mathrm{CH_3}]^+$; found: 214.0909 $[M-\mathrm{C_4H_9O}]^+$.

Diazophosphonate 10: A solution of NaN_3 (936 mg, 14.4 mmol) in H_2O (4 mL) was quickly added to a suspension of *p*-toluenesulfonyl chloride (PTSCl) (2.3 g, 12 mmol) in isopropanol (7 mL). The reaction mixture was stirred for 1 h at RT, H_2O (75 mL) was added and the mixture stirred for another 1 h, then extracted with CH_2Cl_2 (4×25 mL). The combined organic extract was dried ($MgSO_4$) and solvent was evaporated under reduced pressure to yield $TosN_3$ as a colorless oil (2.36 g, 100%). ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.84 (d, 2H, J=8.5 Hz), 7.42 (d, 2H, J=8.5 Hz), 2.48 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=146.2, 135.5, 130.2, 127.5, 21.7 ppm; IR (NaCl): $\tilde{\nu}$=3060, 2930, 2128, 1595, 1371, 1168 cm^{-1}; MS: m/z (%): 197 (10) $[M]^+$, 155 (97), 91 (100); HRMS: m/z calcd for $C_7H_7O_2N_3S$: 197.0259 $[M]^+$; found: 197.0256 $[M]^+$.

A solution of phosphonate **9** (3.3 g, 20 mmol) in Bz (15 mL) was slowly added to suspension of NaH (839 mg, 21 mmol) in an anhydrous mixture of Bz (75 mL) and THF (12 mL). The reaction was stirred at 0°C for 1 h before addition of freshly prepared $TosN_3$ (4.13 g, 21 mmol) in Bz (15 mL). The resulting solution was stirred at RT for 2 h, filtered over

Celite and solvent was removed under reduced pressure. The oily residue was purified by flash chromatography on silica gel eluting with hex/EtOAc (5:5 to 3:7) to give **10** as a yellow oil (3.78 g, 98%). R_f=0.2 (1:1hex/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.85 (d, 6H, J=12.0 Hz), 2.26 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=189.7, 129.4, 126.2, 53.5, 27.0 ppm; IR (NaCl): $\tilde{\nu}$=2960, 2127, 1660 cm^{-1}; MS: m/z (%): 192 (80) $[M]^+$, 109 (100); HRMS: m/z calcd for $C_5H_9O_4N_2P$: 192.0300 $[M]^+$, found: 192.0304 $[M]^+$.

Alkyne 11: Trichloroisocyanuric acid (1.02 g, 4.4 mmol) was added to a solution of **7** (1.0 g, 4.3 mmol) in anhydrous CH_2Cl_2 (7 mL). A solution of TEMPO (6 mg, 0.04 mmol) in CH_2Cl_2 (1 mL) was then added to the resulting mixture at 0°C. The reaction mixture was stirred for 30 min at 0°C, filtered over silica eluting with hex/EtOAc (7:3), and the solvent was removed under reduced pressure. EtOAc (15 mL) was added and the organic layer was washed with a saturated aqueous solution of $NaHCO_3$ (3×5 mL); it was dried ($MgSO_4$) and removal of solvent under reduced pressure gave **8** (991 mg, 100%) as a yellow oil.

K_2CO_3 (1.19 g, 8.6 mmol) was added to an ice-cooled solution of **8** (991 mg, 4.3 mmol) and **10** (1.24 g, 6.5 mmol) in MeOH (15 mL). The resulting solution was stirred for 1 h at 0°C then for 16 h at RT. The mixture was treated with a saturated aqueous solution of NH_4Cl (4 mL). MeOH was removed under reduced pressure and H_2O (5 mL) was added prior to extraction with EtOAc (3×10 mL). The combined organic extract was dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel eluting with hex/EtOAc (95:5) to give **11** as a colorless oil (652 mg, 67%). R_f=0.3 (95:5 hex/EtOAc); $[\alpha]_D^{20}=-1$ (c=1.25, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ =4.61 (br, 0.33H, ABX), 4.50 (br, 0.66H, ABX), 4.1–4.0 (m, 2H), 2.27 (br, 1H), 1.7–1.45 ppm (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=151.3, 94.4, 82.7, 80.3, 70.1, 68.7, 48.8, 28.4, 26.8, 25.8, 25.1, 24.3 ppm; IR (NaCl): $\tilde{\nu}$=3260, 2980, 2360, 1703, 1378, 1263, 1097 cm^{-1}; MS: m/z (%): 210 (45) $[M-CH_3]^+$, 110 (100); HRMS: m/z calcd for $C_{11}H_{16}O_3N$: 210.1130 $[M-CH_3]^+$; found: 210.1133 $[M-CH_3]^+$.

Alkene 12: A stream of Ar was passed though a solution of alkyne **11** (497 mg, 2.2 mmol) and quinoline (64 mg, 0.5 mmol) in EtOH for 5 min. A suspension of Lindlar catalyst (5%, 110 mg, 0.05 mmol) in EtOH (2 mL) was added. The mixture was purged with H_2 (3×) and stirred for 16 h at RT. The precipitate was filtered off over Celite and the solvent was removed under reduced pressure. The oily residue was purified by flash chromatography on silica gel eluting with hex/EtOAc (100:0 to 93:7) to afford **12** as a colorless oil (433 mg, 86%). R_f=0.3 (85:15 hex/EtOAc); $[\alpha]_D^{20}=+11.9$ (c=1.04, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.80 (br m, 1H), 5.3–5.05 (m, 2H), 4.39 (br, 0.33H, ABX), 4.26 (br, 0.66H, ABX), 4.04 (dd, 1H, ABX, J_{AB}=9.0 Hz, J_{AX}=6.0 Hz), 3.74 (dd, 1H, ABX, J_{AB}=9.0 Hz, J_{BX}=2.0 Hz), 1.7–1.35 ppm (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=159.1, 137.3, 115.7, 93.8, 80.1, 68.0, 59.6, 28.3, 26.4 ppm; IR (NaCl): $\tilde{\nu}$=2980, 1699, 1383, 1175 cm^{-1}; MS: m/z (%): 212 (45) $[M-CH_3]^+$, 156 (100), 57 (100); HRMS: m/z calcd for $C_{11}H_{18}O_3N$: 212.1287 $[M-CH_3]^+$, found: 212.1290 $[M-CH_3]^+$.

Ester 14: A solution of benzyl chloroformate (7.02 g, 41.1 mmol) in anhydrous CH_2Cl_2 (30 mL) was added dropwise to a solution of **13** (3.22 g, 37.4 mmol) and pyridine (7.16 g, 90.5 mmol) in anhydrous CH_2Cl_2 (20 mL). The resulting mixture was stirred for 16 h at RT. The white precipitate was filtered off on Celite and the solution was washed with a saturated aqueous $CuSO_4$ (3×30 mL), dried ($MgSO_4$), and the solvent was removed under reduced pressure. The crude oil was purified by flash chromatography on silica gel eluting with hex/Et_2O (100:0 to 95:5) to afford **14** as a yellowish oil (4.77 mg, 72%). R_f=0.3 (9:1 hex/Et_2O); IR (NaCl): $\tilde{\nu}$=3065, 3039, 2956, 1737, 1164 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.45–7.3 (m, 5H), 5.95 (m, 1H), 5.25–5.15 (m, 4H), 3.15 ppm (d, 2H, J=7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ=171.3, 135.9, 130.2, 128.6, 128.3, 118.7, 66.4, 39.1 ppm; MS: m/z (%): 176 (10) $[M]^+$, 91 (100); HRMS: m/z calcd for $C_{11}H_{12}O_2$: 176.0837 $[M]^+$; found: 176.0831 $[M]^+$.

Alkene 15: The 2nd generation Grubbs catalyst (199 mg, 0.03 mmol) was added to anhydrous CH_2Cl_2 (0.8 mL) under an Ar atmosphere. The suspension was purged with Ar for 2 min. Solutions of **12** (1.78 g, 7.8 mmol) in anhydrous CH_2Cl_2 (1.5 mL) and **14** (1.65 g, 9.4 mmol) in anhydrous CH_2Cl_2 (1.5 mL) were concomitantly added to the Grubbs catalyst. The reaction mixture was purged with Ar for 5 min and heated at reflux for 12 h. The solvent was removed under reduced pressure and the resulting oil was purified by flash chromatography on silica gel eluting with hex/EtOAc (100:0 to 95:5) to afford **15** as a yellowish oil (1.68 g, 58%). R_f=0.1 (9:1 hex/EtOAc); $[\alpha]_D^{20}=-6.9$ (c=1.15, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.4–7.3 (m, 5H), 5.85–5.65 (m, 1H), 5.65–5.5 (m, 1H), 5.12 (s, 2H), 4.40 (br, 0.33H, ABX), 4.26 (br, 0.66H, ABX), 4.01 (dd, 1H, ABX, J_{AB}=9.0 Hz, J_{AX}=6.0 Hz), 3.73 (dd, 1H, ABX, J_{AB}=9.0 Hz, J_{BX}=2.0 Hz), 3.13 (d, 2H, J=7.0 Hz), 1.65–1.35 ppm (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=171.2, 151.9, 138.2, 128.6, 128.2, 123.7, 68.1, 66.5, 58.8, 37.6, 28.8 ppm; IR (NaCl): $\tilde{\nu}$=3015, 2978, 1738, 1694, 1384, 1159 cm^{-1}; MS: m/z (%): 360 (5) $[M-CH_3]^+$, 260 (100); HRMS: m/z calcd for $C_{20}H_{26}O_5N$: 360.1811 $[M-CH_3]^+$; found: 360.1815 $[M-CH_3]^+$.

Acid 16: A 0.5 M aqueous solution of NaOH (4.8 mL) was added to a solution of **15** (596 mg, 1.6 mmol) in MeOH (8 mL). The reaction mixture was stirred at RT for 16 h. MeOH was removed under reduced pressure, H_2O (10 mL) was added, and the mixture was extracted with CH_2Cl_2 (3×10 mL). A 1 M aqueous solution of HCl was added to the aqueous phase until pH 4 was reached and it was extracted with EtOAc (4×15 mL). The combined EtOAc extract was dried ($MgSO_4$) and solvent was removed under reduced pressure to afford **16** as a dark yellow solid (435 mg, 96%).

Pentafluorophenyl ester 17: A solution of DCC (267 mg, 1.3 mmol) in EtOAc (2 mL) was added dropwise to a solution of acid **16** (351 mg, 1.2 mmol) and PfpOH (238 mg, 1.3 mmol) in EtOAc (6 mL). The resulting mixture was stirred at RT for 3 h. The white urea precipitate was filtered off and the solvent was removed under reduced pressure. The oily residue was purified was by flash chromatography on silica gel eluting with hex/EtOAc (100:0 to 93:7) to yield **17** as a colorless oil (525 mg, 95%). R_f=0.2 (93:7 hex/EtOAc); $[\alpha]_D^{20}=-17$ (c=0.43, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.85–5.65 (br, 2H), 4.45 (br, 0.33H, ABX), 4.32 (br, 0.66H, ABX), 4.05 (dd, 1H, ABX, J_{AB}=9.0 Hz, J_{AX}=6.0 Hz), 3.76 (d, 1H, ABX, J_{AB}=9.0 Hz), 3.44 (d, 2H, J=5.0 Hz), 1.65–1.4 ppm (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=167.3, 150.7, 142.8, 139.3, 134.9, 121.6, 120.1, 93.7, 79.8, 68.0, 58.6, 36.2, 28.3 ppm; IR (NaCl): $\tilde{\nu}$=2981, 1686, 1521, 1385, 1366 cm^{-1}; MS: m/z (%): 452 (10) $[M-CH_3]^+$, 413 (100); HRMS: m/z calcd for $C_{20}H_{23}F_5O_5N$: 452.1496 $[M-CH_3]^+$; found: 452.1486.

Tripeptide 20: TFA (5 mL) was added to a suspension of **18** (817 mg, 2.6 mmol) in CH_2Cl_2 (50 mL). The reaction mixture was stirred for 1 h at RT; it was then azeotroped with toluene (3×50 mL) to yield the TFA salt **19** as an orange oil. This oil and **17** (1.16 g, 2.6 mmol) were dissolved in MeAc (30 mL). A solution of K_2CO_3 (889 mg, 6.4 mmol) in H_2O (7 mL) was added and the reaction mixture was stirred at RT for 16 h. The orange solution was concentrated under reduced pressure, poured into H_2O, and extracted with CH_2Cl_2 (3×15 mL). The aqueous phase was acidified with a 1 M aqueous solution of HCl until pH 4 was reached, then it was extracted with EtOAc (4×15 mL). The combined EtOAc extract was dried ($MgSO_4$) and solvent was removed under reduced pressure. The resulting oil was purified was by flash chromatography on silica gel eluting with CH_2Cl_2/MeOH (100:0 to 90:10) to give **20** as a viscous oil (690 mg, 56%). R_f 0.15 (95:5 CH_2Cl_2/MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ=8.15–7.9 (br, 2H), 5.8–5.55 (m, 6H), 4.30 (br, 1H), 4.05 (dd, 1H, J=9.0 Hz, J=6.0 Hz), 3.8–3.65 (m, 5H), 3.04 (d, J=7.0 Hz, 2H), 3.0–2.9 (m, 2H), 1.6–1.4 ppm (m, 15H); ^{13}C NMR (75 MHz, CD_3OD): δ=174.0, 172.1, 132.4, 129.6, 129.1, 125.5, 125.2, 124.5, 93.6, 67.8, 59.0, 40.7, 40.6, 40.0, 36.9, 27.3 ppm; IR (NaCl): $\tilde{\nu}$=3307, 2980, 2934, 2472, 1676, 1639, 1394 cm^{-1}; MS: m/z (%): 479 (%) $[M]^+$, 267 (100); HRMS: m/z calcd for $C_{24}H_{37}O_7N_3$: 479.2631 $[M]^+$, found: 479.2622 $[M]^+$.

Cyclopeptide 21: A solution of DIC (132 mg, 1.0 mmol) in EtOAc (5 mL) was added to a solution of acid **20** (477 mg, 0.99 mmol) and PfpOH (192 mg, 1.0 mmol) in EtOAc (30 mL). The resulting mixture was stirred at RT for 12 h. The white urea precipitate was filtered off and the solvent was removed under reduced pressure. The residue was dissolved in AcOH (5 mL). PTSA (1.8 mg, 0.01 mmol), Bz (5 mL), and a few drops of H_2O were added. The mixture was stirred at RT for 12 h. The solution was concentrated under reduced pressure to give an oil that was dissolved in CH_2Cl_2 (5 mL) and TFA (5 mL). The mixture was stirred for

40 min at RT; it was then azeotroped with toluene (3×10 mL). The residue was dissolved in dioxane (200 mL), NMM (201 μL, 2.0 mmol) was added, and the resulting mixture was stirred for 48 h at 80 °C. The solvent was removed under reduced pressure and the resulting oil was purified by flash chromatography on silica gel eluting with CH_2Cl_2/MeOH (100:0 to 92:8) to afford **21** as a dark yellow oil (141 mg, 44 %). R_f 0.15 (92:8 CH_2Cl_2/MeOH); $[\alpha]_D^{20}=+3.7$ ($c=1.78$, MeOH); ^{1}H NMR (300 MHz, CD_3OD): $\delta=7.96$ (br t, 1H), 7.88 (br t, 1H), 7.83 (br d, $J=8.0$ Hz, 1H), 5.7–5.55 (m, 6H), 4.39 (br, 1H), 3.9–3.8 (*A*BX, 2H), 3.75–3.65 (A*B*X, 2H), 3.6–3.55 (m, 2H), 3.1–2.85 ppm (m, 6H); ^{13}C NMR (75 MHz, CD_3OD): $\delta=172.6$, 131.5, 130.4, 130.0, 124.8, 124.3, 124.2, 63.2, 53.1, 53.0, 40.1, 39.4, 39.0 ppm; IR (NaCl): $\tilde{\nu}=3292$, 2926, 1629, 1458 cm^{-1}; MS: *m*/*z* (%): 322 (30) $[M-H]^+$, 321 (35) $[M]^+$, 98 (100); HRMS: *m*/*z* calcd for $C_{16}H_{23}O_4N_3$: 321.1688 $[M]^+$, found: 321.1681.

Succinate 3: DMAP resin (6 mg, 0.02 mmol; 3 mmol of DMAP/g of resin) and succinic anhydride (36 mg, 0.36 mmol) were added to a solution of **21** (58 mg, 0.18 mmol) in MeCN (2 mL) and pyridine (2 mL). The mixture was stirred at RT for 16 h, the resin was filtered off and washed with pyridine. The filtrate was concentrated under reduced pressure and the residue was dissolved in MeCN (2 mL) and pyridine (2 mL). Benzyl alcohol resin (350 mg, 0.7 mmol; 2 mmol of BnOH/g resin) was added to the solution, which was then stirred for 16 h at RT. The resin was filtered off and washed with pyridine. The filtrate was concentrated under reduced pressure to yield **3** as a dark-yellow oil (69 mg, 91 %). $[\alpha]_D^{20}=+34$ ($c=1.31$, MeOH); ^{1}H NMR (300 MHz, CD_3OD): $\delta=5.8$–5.55 (m, 6H), 4.63 (br m, 1H), 4.19 (dd, 1H, *A*BX, J(A,B)=11.0, $J_{AX}=5.0$ Hz), 4.08 (dd, 1H, A*B*X, J(A,B)=11.0, J(B,X)=7.0 Hz), 3.9–3.8 (*A*BX, 2H), 3.75–3.65 (A*B*X, 2H), 3.1–2.85 (m, 2H), 2.6–2.55 ppm (m, 4H); ^{13}C NMR (75 MHz, CD_3OD): $\delta=174.5$, 172.5, 172.4, 130.4, 129.8, 125.8, 124.3, 124.4, 64.8, 49.9, 40.3, 39.9, 39.4, 39.3, 28.5, 28.3 ppm; IR (NaCl): $\tilde{\nu}=$ 3530, 3330, 2954, 1731, 1713, 1648, 1641, 1161 cm^{-1}; MS: *m*/*z* (%): 322 (20) $[M-COC_2H_4CO_2H]^+$, 290 (78), 96 (100); HRMS: *m*/*z* calcd for $C_{16}H_{23}O_4N_3$: 321.1688 $[M-COC_2H_4CO_2]^+$; found: 321.1684 $[M-COC_2H_4CO_2]^+$.

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[1] M. R. Ghadiri, J. R. Granja, R. A. Milligan, D. E. McRee, N. Khazanovich, *Nature* **1993**, *366*, 324.
[2] D. T. Bong, T. D. Clark, J. R. Granja, M. R. Ghadiri, *Angew. Chem.* **2001**, *113*, 1016; *Angew. Chem. Int. Ed.* **2001**, *40*, 988.
[3] D. Ranganathan, C. Lakshmi, I. L. Karle, *J. Am. Chem. Soc.* **1999**, *121*, 6103.
[4] D. Gauthier, P. Baillargeon, M. Drouin, Y. L. Dory, *Angew. Chem.* **2001**, *113*, 4771; *Angew. Chem. Int. Ed.* **2001**, *40*, 4635.
[5] S. Leclair, P. Baillargeon, R. Skouta, D. Gauthier, Y. Zhao, Y. L. Dory, *Angew. Chem.* **2004**, *116*, 353; *Angew. Chem. Int. Ed.* **2004**, *43*, 349.
[6] T. Kato, N. Mizoshita, K. Kishimoto, *Angew. Chem.* **2006**, *118*, 44; *Angew. Chem. Int. Ed.* **2006**, *45*, 38.
[7] J. Couet, M. Biesalski, *Macromolecules* **2006**, *39*, 7258.
[8] M. G. J. ten Cate, N. Severin, H. Börner, *Macromolecules* **2006**, *39*, 7831.
[9] L. Ayres, G. M. Grotenbreg, G. A. van der Marel, H. S. Overkleeft, M. Overhand, J. C. M. van Hest, *Macromol. Rapid Commun.* **2005**, *26*, 1336.
[10] L. Cui, Y. Zhao, *Chem. Mater.* **2004**, *16*, 2076.
[11] P. Garner, *Tetrahedron Lett.* **1984**, *25*, 5855.
[12] L. De Luca, Giacomelli, G. A. Porcheddu, *Org. Lett.* **2001**, *3*, 3041.
[13] P. L. Beaulieu, J. S. Duceppe, C. Johnson, *J. Org. Chem.* **1991**, *56*, 4196.
[14] T. Moriwake, S. Hamano, S. Saito, S. Torii, *Chem. Lett.* **1987**, 2085.
[15] P. Callant, L. D'Haenens, M. Vandewalle, *Synth. Commun.* **1984**, *14*, 155.
[16] P. Meffre, L. Gauzy, E. Branquet, P. Durand, F. Le Goffic, *Tetrahedron* **1996**, *52*, 11215.
[17] J. C. Gilbert, U. Weerasooriya, *J. Org. Chem.* **1982**, *47*, 1837.
[18] J.-F. Poisson, J. Normant, *J. Org. Chem.* **2000**, *65*, 6553.
[19] G. Sun, P. S. Savle, R. D. Gandour, N. Níca' Bhaírd, R. R. Ramsay, F. R. Froncsek, *J. Org. Chem.* **1995**, *60*, 6688.
[20] T. M. Trnka, R. H. Grubbs, *Acc. Chem. Res.* **2001**, *34*, 18.
[21] U. Schmidt, A. Liebernecht, H. Griesser, J. Talbiersky, *J. Org. Chem.* **1982**, *47*, 3261.
[22] P. Baillargeon, S. Bernard, D. Gauthier, R. Skouta, Y. L. Dory, *Chem. Eur. J.* **2007**, *13*, 9223.
[23] T. Kato, J. M. J. Frechet, P. G. Wilson, T. Saito, T. Uryu, A. Fujishima, C. Jin, F. Kaneuchi, *Chem. Mater.* **1993**, *5*, 1094.
[24] J. Bandekar, *Biochim. Biophys. Acta* **1992**, *123*, 1120.

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