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Peptide Dendrimers Based on Polyproline Helices

Laia Crespo,[§] Glòria Sanclimens,[§] Beatriz Montaner,[‡] Ricardo Pérez-Tomás,[‡] Miriam Royo,*[,§] Miquel Pons,*[,§] Fernando Albericio,*[,§] and Ernest Giralt*[,§]

Contribution from the Departament de Química Orgànica, Universitat de Barcelona, Martí i Franquès 1, 08028-Barcelona, Spain, and Departament de Biologia Cel·lular i Anatomia Patològica, Universitat de Barcelona, Feixa Llarga s/n, 08907-L'Hospitalet de Llobregat, Spain

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Abstract: We present a new family of peptide dendrimers based on polyproline helices and *cis*-4-amino-L-proline as a branching unit. Dendrimers were synthesized by a convergent solid-phase peptide synthesis approach. The conformational transition between polyproline type I helix and polyproline type II helix was observed by circular dichroism in branched polyproline building blocks with more than 14 proline residues and in the resulting dendrimers. Both linear and dendritic polyprolines were found to be actively internalized by rat kidney cells. Preliminary results show that the antibiotic ciprofloxacin form complexes with branched polyproline chains in 99.5% propanol.

Introduction

During the past few years, dendrimers have emerged as a new class of macromolecules with interesting structural properties[1−11] and promising technological applications.[12−16] High generation dendrimers contain cavities that may host other molecules, while the properties of the dendrimer are mostly determined by its surface groups. As a consequence, biocompatible dendrimers are obvious candidates for drug delivery applications. Peptide-based dendrimers have been previously described,[17−26] and the pioneering work of the group of J. Tam[27−29] has demonstrated the application of lysine dendrimers as immunogens.

Proline is singular among the 20 genetically coded amino acids in that it contains both a cyclic backbone as well as a secondary, as opposed to primary, α-amino group. These structural features impart unique stereochemical properties to proline. Polyproline oligomers exist in two distinct conformations. In organic solvents, they adopt a conformation known as polyproline I (PPI), a right-handed helix in which all peptide bonds are cis-oriented ($\omega = 0°$).[30] In aqueous solvents, they adopt the conformation known as polyproline II (PPII), a left-handed helix in which all peptide bonds are trans-oriented ($\omega = 180°$).[31] The transition from PPI to PPII implies a considerable increase in the long dimension of the helix that changes from 1.9 to 3.1 Å per residue.

In the present article, we describe the solid-phase synthesis of dendrimers based on polyproline chains. Our final goal is to explore the use of the conformational plasticity of polyproline chains to modulate the dendrimer properties by changing the length of the branches. In this way, drugs could be trapped in organic solvents, where polyproline chains adopt a PPI conformation, and released under physiological conditions in which polyproline spacers form more extended PPII helices.

* To whom correspondence should be addressed. E-mail: egiralt@qo.ub.es.
[§] Departament de Química Orgànica, Universitat de Barcelona.
[‡] Departament de Biologia Cel·lular i Anatomia Patològica, Universitat de Barcelona.

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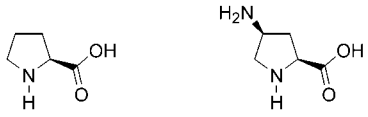
Scheme 1. Structures of L-Proline (Pro) and *cis*-4-Amino-L-proline (Amp)



L-proline (Pro) *cis*-4-amino-L-proline (Amp)

Most recent efforts to modulate the properties of dendrimers have focused primarily on the modification of the dendrimer surface.[32−35] Hence, our approach is novel in that the plasticity of the dendrimer is encoded within its polyproline branches, whereby a simple change of solvent environment is sufficient to induce conformational change.

The biocompatibility of peptide structures and the possibility of cellular internalization[36] of polyproline structures are additional potential benefits of this approach.

As a branching unit, we decided to use *cis*-4-amino-L-proline (Amp), a building block derived from the natural compound *trans*-4-hydroxy-L-proline[37,38] (Scheme 1). Amp is expected to cause minimal distortion to polyproline conformations because of its close structural relation to L-proline. In addition, Amp is commercially available in a suitable protected form.

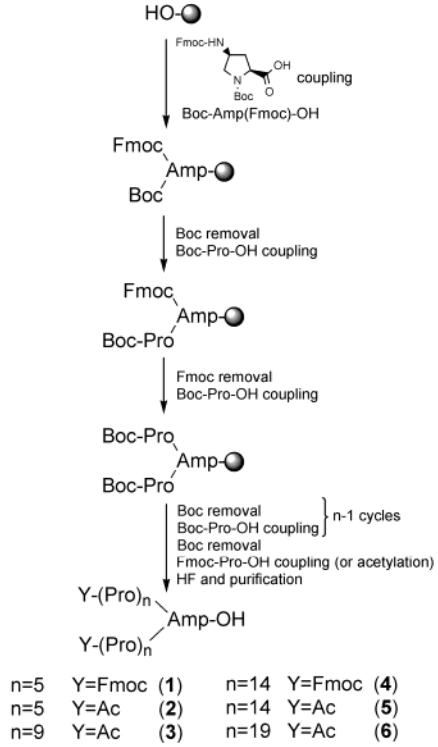
We present here the synthesis of polyproline-based dendrimers using a synthetic strategy totally based on solid-phase methods. Circular dichroism (CD) studies have been used to show that branched polyproline chains and the resulting dendrimers can undergo a conformational transition from PPII to PPI. We have also demonstrated that a polyproline dendrimer is actively internalized by rat kidney cells, and we report preliminary results on the formation of a complex between a polyproline dendrimer and the antibiotic ciprofloxacin in 99.5% propanol.

Results and Discussion

Synthesis and Purification of Building Blocks. Synthesis of branched polyproline peptides is not at all straightforward. After a careful study of several different approaches,[39] we have chosen the optimized synthetic route shown in Scheme 2.

The complete synthesis was carried out using solid-phase peptide synthesis methodology.[40] Starting from a hydroxymethyl resin (Merrifield resin), we incorporated the orthogonally protected Boc-L-Amp(Fmoc)-OH using *N*,*N*′-diisopropylcarbodiimide (DIPCDI) and catalytic amounts of 4-dimethylaminopiridine (DMAP) in dichloromethane (DCM). After removal of the *tert*-butoxycarbonyl (Boc) group, Boc-Pro-OH was coupled. The 9-fluorenylmethoxycarbonyl (Fmoc) group was then removed, and a second Boc-Pro-OH was coupled. The two

Scheme 2. Building Block Solid-Phase Synthesis



n=5	Y=Fmoc	(**1**)	n=14	Y=Fmoc	(**4**)
n=5	Y=Ac	(**2**)	n=14	Y=Ac	(**5**)
n=9	Y=Ac	(**3**)	n=19	Y=Ac	(**6**)

branches were elongated simultaneously by successive additions of two Boc-Pro-OH units at each step. Incorporation of Boc-Pro-OH to the Pro-Amp(Pro) resin-bound fragment was carried out by an in situ neutralization approach with *N*,*N*-diisopropylethylamine (DIEA), using benzotriazole-1-yl-oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) or benzotriazole-1-yl-oxytris(pyrrolidine)phosphonium hexafluorophosphate (PyBOP) as a coupling reagent and 1-hydroxybenzotriazole (HOBt) as an additive in *N*,*N*-dimethylformamide (DMF)[41,42] to avoid intramolecular cyclization to form a diketopiperazine (DKP). The remaining couplings were carried out using standard solid-phase peptide methods.[43] Using this procedure, we have been able to synthesize branched peptides of different lengths (n = 5, 9, 14, 19), acetylated or Fmoc-protected, with good yields. Chloranil[44] and ninhydrin tests[45] were used to ensure quantitative couplings. Cleavages of the Fmoc-protected or acetylated peptides from the resin were carried out by acidolysis with anhydrous HF with 10% anisole providing crude peptides 70−85% pure. Peptides were purified by semipreparative or preparative reversed-phase high performance liquid chromatography (RPHPLC), and purities of 96−98% were confirmed by RPHPLC and MALDI-TOF.

Synthesis and Purification of Dendrimers. Dendrimers were prepared on solid phase by successive addition of branched polyproline building blocks starting from one of the two different

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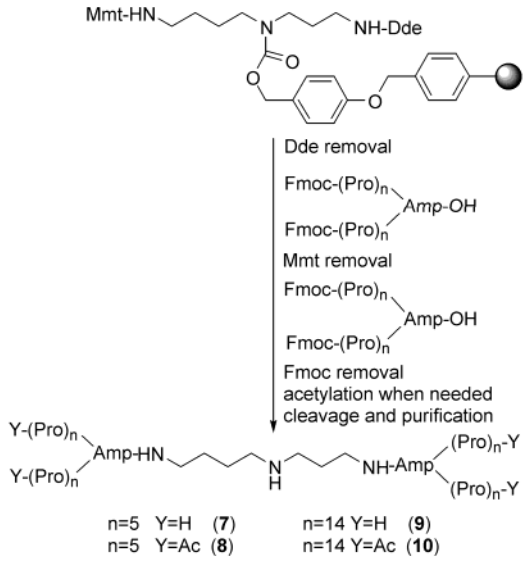
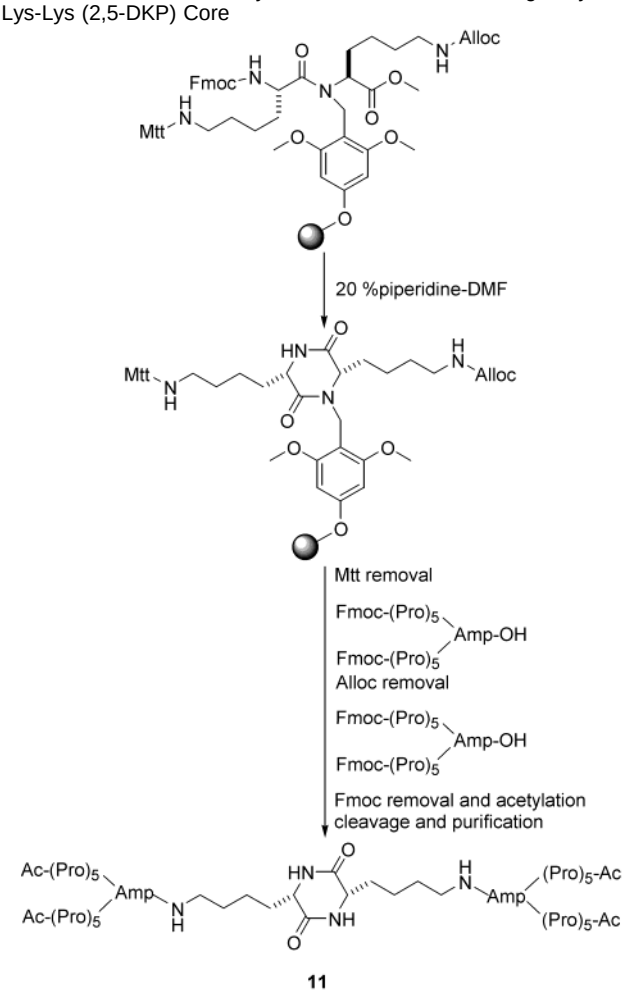
Scheme 3. Solid-Phase Synthesis of Dendrimers Using a Spermidine Core



Scheme 4. Solid-Phase Synthesis of Dendrimers Using a Cyclic Lys-Lys (2,5-DKP) Core



cores which are anchored to a solid support: spermidine and *c*[Lys(Alloc)-Lys(Mtt)]. The general strategy for both synthetic approaches is shown in Schemes 3 and 4.

The commercially available spermidine core (Scheme 3), orthogonally protected with 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde) and 4-methoxytrityl (Mmt) groups, is anchored by its central amino group to a Wang carbonate resin.

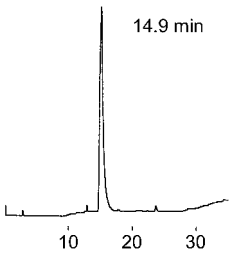


Figure 1. Analytical RPHPLC on a C_4 column of pure dendrimer **9**. Elution conditions: A, 0.045% TFA in H_2O; B, 0.036% TFA in CH_3CN; linear gradient from 0 to 100% in 30 min; flow rate 1 mL/min, $\lambda = 220$ nm.

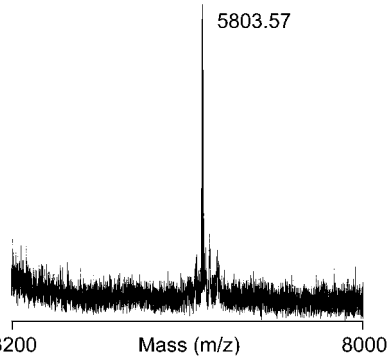


Figure 2. MALDI-TOF mass spectrum of pure dendrimer **9** using linear mode and sinapinic acid as matrix.

The first step of this synthesis involved Dde removal with a solution of N_2H_4-DMF (2:98) and coupling of the first building block ($n = 5$, 14) using 7-azabenzotriazol-1-yl-oxytris(pyrrolidino)phosphonium hexafluorophosphate (PyAOP)/DIEA.[46] After Mmt removal with a solution of trifluoroacetic acid (TFA)-triisopropylsilane-DCM (1:5:94), the second building block ($n = 5$, 14) was introduced using the same conditions. The ninhydrin test[45] was used to monitor the progress of the couplings. Fmoc groups were removed, and the N-terminus was acetylated or left in its free amino form. Dendrimers were released from the resin by acidolysis with TFA-water (95:5) providing crude dendrimers that were 65−75% pure and were purified by semipreparative RPHPLC leading to dendrimers 96−98% pure. The products were homogeneous by analytical RPHPLC (Figure 1) and showed correct MALDI-TOF spectra (Figure 2).

The cyclic Lys-Lys (2,5-DKP) core (Scheme 4), with ϵ-amino groups protected by allyloxycarbonyl (Alloc) and 4-methyltrityl (Mtt) groups and attached to a solid support through a backbone amide, was prepared using a synthesis previously developed in our laboratory.[47]

Starting with backbone amide linker (BAL)-4-methylbenzhydrylamine (MBHA)-polyethylene glycol (PEG)-resin, we carried out a reductive amination using HCl·H_2N-Lys(Alloc)-OMe with $NaBH_3CN$ in DMF. The secondary α-amino group attached to the BAL resin was acylated with Fmoc-L-Lys(Mtt)-OH using PyAOP as a coupling reagent and DIEA in DMF. Next, treatment of the Fmoc-dipeptidyl-BAL-resin with piperidine-DMF led to quantitative formation of Lys-Lys 2,5-DKP

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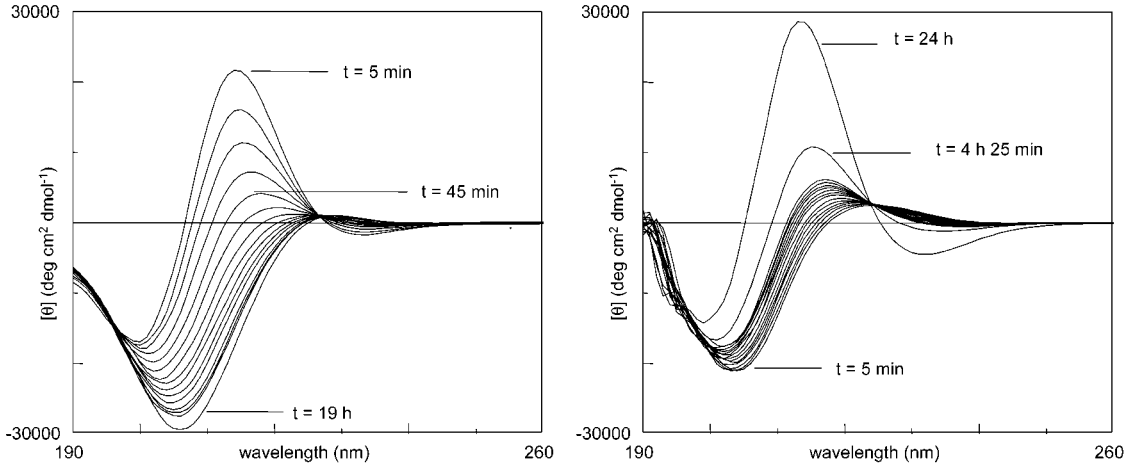

Figure 3. Representative CD spectra of dendrimer **9** recorded during left, PPI → PPII isomerization in water at 25 °C; right, PPII → PPI isomerization in *n*-propanol/water (99.5:0.5, v/v) at 25 °C.

orthogonally protected with Alloc and Mtt. The first step of the dendrimer synthesis involved Mtt removal with a solution of TFA-H_2O-DCM (2:1:97) and coupling of the first building block ($n = 5$) with PyAOP/DIEA. After Alloc removal with catalytic amounts of tetrakis (triphenylphosphine) palladium(0) in the presence of triphenylsilane as allyl scavenger in DCM, the second building block ($n = 5$) was introduced using the same conditions. The ninhydrin test[45] was used to monitor the progress of the couplings. After Fmoc removal, acetylation, and TFA-water (95:5) acidolytic cleavage, the final compound was 40% pure. Purification was carried out by semipreparative RPHPLC, and purity of 95% was confirmed by analytical RPHPLC and MALDI-TOF.

Taking into account the number of synthetic steps required for the preparation of polyproline dendrimers with the cyclic Lys-Lys (2,5-DKP) core and the low yields obtained, we carried out the structural studies with the spermidine core polyproline dendrimers.

Circular Dichroism Measurements. The conformational properties of polyproline oligomers are well known.[1,2,48] They can adopt two helical conformations, PPI and PPII, with very different physicochemical and spectroscopic properties. PPI helices are more compact than PPII helices[49] and are favored in *n*-propanol and other aliphatic alcohols. Water, acetic acid, and other acidic solvents favor the formation of PPII helix.

The presence of PPI and PPII forms and their interconversion in solution can be analyzed by CD spectroscopy.[50−52] The CD spectrum of poly-L-proline type I helix is characterized by a medium intensity negative band at 199 nm, a strong positive band at 215 nm, and a weak negative band at 232 nm. The CD spectrum of poly-L-proline type II helix exhibits a strong negative band at 206 nm and a weak positive band at 226 nm.

We first studied the conformation of branched polyproline building blocks of different lengths in water and 99.5% propanol.

CD spectra of acetylated branched peptides **2**, **3**, **5**, and **6** (see Supporting Information) in water were characteristic of PPII helix exhibiting a strong negative band at 206 nm and a weak positive band at 228 nm. CD spectra of **2** and **3** ($n = 5, 9$) in PPI promoting conditions, 99.5% propanol, did not show the characteristic PPI CD signature. In contrast, in the same experimental conditions, the CD spectrum of **5** ($n = 14$) showed a large degree of PPII to PPI conversion. Finally, the CD spectrum of **6** ($n = 19$) in 99.5% propanol is characteristic of 100% PPI helix with a medium intensity band at 199 nm, a strong positive band at 214 nm, and a weak negative band at 232 nm. Thus, while all branched polyproline peptides can adopt a PPII conformation in water, only the longer peptides ($n = 14$ and $n = 19$) form PPI helices in 99.5% propanol. The threshold for adoption of PPI conformation by linear proline oligomers is between 3 and 5 residues,[50−51] depending on the nature of the N- and C-terminal groups.

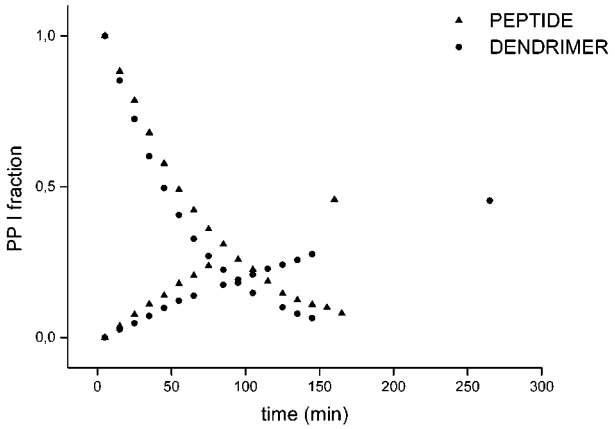

Figure 4. PPI fractional population for dendrimer **9** and for a linear reference H-Pro_{14}-OH during PPI → PPII and PPII → PPI isomerizations. The PPI → PPII isomerization was completed at 1125 min for dendrimer **9** and at 1120 min for H-Pro_{14}-OH. The PPII → PPI isomerization was completed at 1455 min for dendrimer **9** and at 1610 min for H-Pro_{14}-OH.

CD spectra of dendrimers **7**−**10** (see Supporting Information) in water were characteristic of PPII helix, with a strong negative band at 205 nm and a weak positive band at 229 nm. In contrast to branched peptide **5** ($n = 14$), no PPI conformation is detectable for acetylated dendrimer **10** ($n = 14$) in 99.5% propanol. Thus, increasing ramification hinders the adoption of PPI conformation, although this can be partially offset by increasing the length of the proline spacer.

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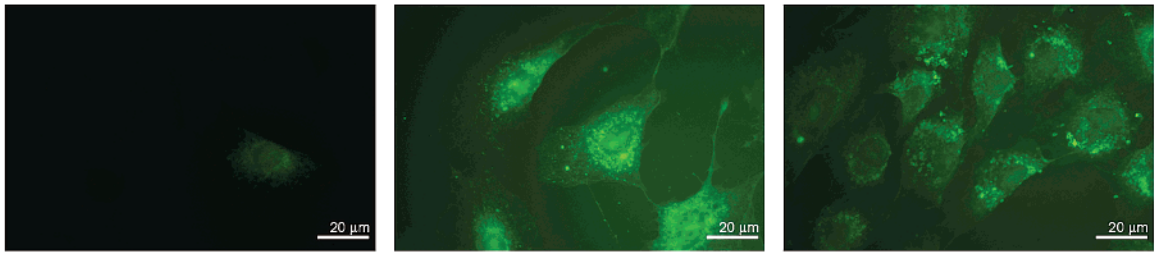

Figure 5. Fluorescence microscopy. NRK cells were (left) untreated at 37 °C, (center) treated with 60 μM fluoresceine-labeled linear peptide **12** at 37 °C for 1 h, and (right) treated with 60 μM fluoresceine-labeled dendrimer **9** at 37 °C for 1 h.

The N-terminal group also has an influence in the ability to adopt a PPI conformation. Thus, the nonacetylated dendrimer with $n = 14$ (**9**) shows a typical PPI CD spectrum in 99.5% propanol. Although all dendrimers show spectra typical of PPII in water, the intensity of the characteristic 205 nm band is higher in the acetylated dendrimers (e.g., **10** > **9**, **8** > **7**), and this is also the case when acetylated and nonacetylated linear proline oligomers are compared (e.g., Ac-Pro$_{14}$-OH > H-Pro$_{14}$-OH). Thus, acetylation increases PPII conformation in water and decreases the stability of PPI in propanol.

The kinetics of isomerization of the two PP helical forms can easily be followed by CD.[53−56] Figure 3 displays representative CD spectra for PPI → PPII and PPII → PPI isomerizations of dendrimer **9** in water and 99.5% propanol, respectively, at 25 °C.

Figure 4 shows the changes in the fractional population of PPI extracted from CD data during the PPI → PPII transition in water and during the PPII → PPI interconversion in 99.5% propanol. The corresponding data for a linear reference H-Pro$_{14}$-OH are included as a reference.

The transition from PPI → PPII in water is much faster than the opposite interconversion in 99.5% propanol. This is also true for the reference peptide. However, while in water the PPI → PPII interconversion of dendrimer **9** is around 20% faster than that in the linear reference peptide, the opposite is true for the PPII → PPI conversion, which is ca. twice as fast for H-Pro$_{14}$-OH than for dendrimer **9**. Thus, dendrimer peptides not only have lower tendencies to adopt PPI conformation than the linear peptides, but the rate of formation, from PPII, is also slower than for the reference linear peptides.

Cell Uptake and Localization. Dendrimer **9** and a linear polyproline peptide ($n = 14$), **12**, were labeled with fluoresceine. Intracellular fluorescence was examined by confocal fluorescence microscopy after incubation of normal rat kidney cells (NRK-49F) with the fluoresceine-labeled peptides.

As seen in Figure 5, untreated cells showed only a light autofluorescence (control). However, exposure to labeled linear peptide **12** or dendrimer **9** at 37 °C resulted in extensive uptake of the fluorescent label, with many cells showing strong cytoplasm staining with a vesicular distribution. Control experiments, with fluoresceine at 37 °C, showed no accumulation. By contrast, cells incubated at 4 °C with the same molecules showed very low levels of cellular staining (see Supporting Information). This behavior suggests that the cellular uptake takes place via an endocytosis mechanism. No cell morphology modification was observed in either treated or control cells.

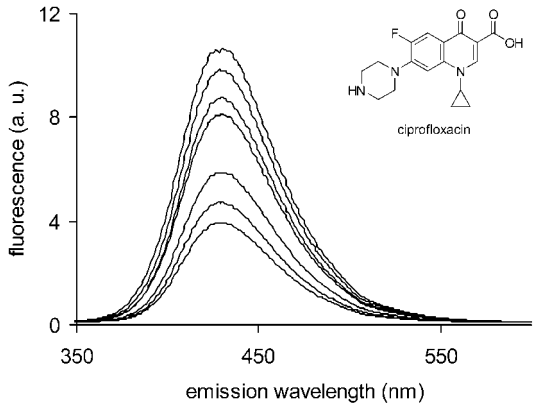

Figure 6. Fluorescence binding titration of dendrimer **9** with ciprofloxacin (20 μM, *n*-propanol/water (99.5:0.5, v/v), 25 °C, 330 nm).

In the experiments above, both dendrimer **9** and peptide **12** were in PPII conformation. The cellular uptake of both compounds in PPI conformation could not be studied because of the long incubation times required.

Fluorescence Binding Studies (Assay). Ciprofloxacin is a synthetic 6-fluoroquinolone antibiotic currently in clinical use for the treatment of infections by Gram-positive and Gram-negative bacteria.

Binding of ciprofloxacin to dendrimer **9** was studied by fluorescence.[57] Titration of a solution of ciprofloxacin in 99.5% propanol with dendrimer **9** caused an increase in the fluorescence quantum yield of the antibiotic (Figure 6). In contrast, fluorescence enhancement was not observed when both molecules were dissolved in water. However, the slow interconversion kinetics between PPII and PPI allowed the identification of complexes between ciprofloxacin and both PPI and PPII conformations in 99.5% propanol.

Analogous experiments using H-Pro$_{14}$-OH (**12**) showed no changes in the fluorescence of ciprofloxacin, indicating that a polyproline motif is not the only requirement for ciprofloxacin binding, but that branching is essential.

The graphical method of continuous variation (Job plot) revealed a 1:2 stoichiometry (see Supporting Information). This is equivalent to binding one molecule of ciprofloxacin in each building block. Binding data were fit to a bimolecular model[58] for dendrimer−ciprofloxacin binding as described in Materials and Techniques, where each dendrimer molecule was assumed to have two identical and noninteracting binding sites for

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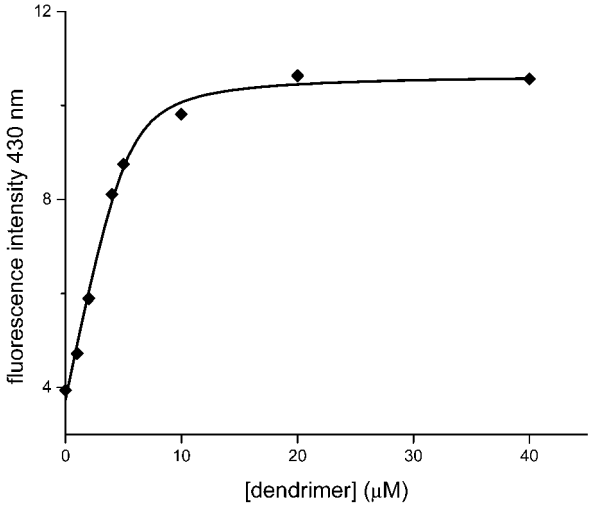

Figure 7. Plot of ciprofloxacin fluorescence against total dendrimer concentration. The line through the experimental points is the best fit obtained according to the equation described in the Experimental Section.

ciprofloxacin. The association constant to each binding site is $2.0 \times 10^6 \pm 0.46 \times 10^6$ M^{-1} (Figure 7).

Conclusions

We have established an efficient synthetic protocol for the preparation of polyproline dendrimers on the basis of a convergent solid-phase peptide synthesis strategy. Pure Fmoc-Pro$_n$-L-Amp(Fmoc-Pro$_n$)-OH ($n = 5$, 14) building blocks were assembled on two different orthogonally protected cores: spermidine and cyclic Lys-Lys (2,5-DKP) using the highly efficient coupling agent PyAOP. This approach provides a new method for the synthesis of other peptide dendrimers and demonstrates the suitability of convergent solid-phase synthesis for the preparation of these compounds.

CD analysis shows that branched polyproline peptides and dendrimers with $n = 14$ can adopt both PPII and PPI conformations and suggests that acetyl groups at the periphery of dendrimers make PPI formation difficult.

Labeled forms of a linear oligoproline peptide ($n = 14$) and polyproline dendrimer **9** are actively internalized by rat kidney cells.

Fluorescence spectroscopy experiments proved that there is an interaction of polyproline dendrimer **9** and ciprofloxacin in 99.5% propanol with a 1:2 stoichiometry and an association constant of 2.0×10^6 M^{-1}. The complex is not observed in water.

These results show the feasibility of using polyproline dendrimers as new drug delivery systems.

Experimental Section

Materials and Techniques. Protected amino acids were from Calbiochem-NovaBiochem AG (Läufelfingen, Switzerland) and Neosystem (Strasbourg, France). DIPCDI, DMAP, and HOBt were from Fluka Chemika (Buchs, Switzerland); BOP, PyBOP, and solid supports for peptide synthesis were supplied from Calbiochem-NovaBiochem AG. These reagents were used without further purification. Solvents for peptide synthesis and RPHPLC were from Scharlau (Barcelona, Spain). Trifluoroacetic acid was from KaliChemie (Bad Wimpfen, FRG). Other chemicals from Aldrich (Milwaukee, WI) were used and of the highest purity commercially available. Peptide syntheses were performed manually in a polypropylene syringe fitted with a polyethylene disk. HF was from Air Products and Chemicals, Inc. (Allentown, Canada), and the equipment was from Peptide Institute Inc., Minoh, Osaka, Japan. Analytical RPHPLC was performed in Waters (Milford, MA) or Shimadzu (Kyoto, Japan) chromatography systems using a reversed-phase Nucleosil C_4 (0.4 × 25 cm, 10 μm) column. Semipreparative RPHPLC was performed in a Waters (Milford, MA) chromatography system using a Vydac C_8 (1 × 25 cm, 10 μm) column. Compounds were detected by UV absorption at 220 nm. Preparative RPHPLC was performed in a Waters (Milford, MA) chromatography system using a Vydac C_8 (5 × 25 cm, 15 μm) column. Compounds were detected by UV absorption at 220 nm. Mass spectra were recorded on a MALDI Voyager DE RP time-of-flight (TOF) (Applied Biosystems, Framingham). α-Cyano-4-hydroxycinnamic acid and sinapinic acid were used as matrixes and were purchased from Aldrich.

General Procedures. Coupling of the First Amino Acid in a Hydroxymethyl Resin. Amino acid (3 equiv), DIPCDI (3 equiv), and DMAP (0.3 equiv) in DMF. After double coupling of 1 h each, the resin was washed with DMF (4 × 1 min) and DCM (4 × 1 min). The possible remaining hydroxyl groups on the resin were blocked by acetylation with HOAc (5 equiv), DIPCDI (5 equiv), and DMAP (0.5 equiv) in DCM (1 × 20 min).

Boc Group Removal (except for the Second Proline Residue). (1) DCM (4 × 1 min); (2) TFA-DCM (4:6) (1 × 1 min + 2 × 15 min); (3) DCM (4 × 1 min); (4) DIEA-DCM (5:95) (4 × 3 min); (5) DCM (4 × 1 min).

Fmoc Group Removal. (1) DMF (4 × 1 min); (2) piperidine-DMF (2:8) (1 × 1 min + 2 × 10 min); (3) DMF (4 × 1 min).

Incorporation of Boc-Pro-OH (except for the Third Proline Residue). (1) Boc-Pro-OH (5 equiv) and *N*,*N*′-dicyclohexylcarbodiimide (DCC) (5 equiv) in DCM (1 × 2 h); (2) DCM (4 × 1 min); (3) DMF (4 × 1 min).

In Situ Neutralization Coupling of Boc-Pro-OH (Third Proline). (1) Boc-Pro-OH (5 equiv), DIEA (10 equiv), BOP (5 equiv), or PyBOP (5 equiv) and HOBt (5 equiv) in DMF (1 × 2 h); (2) DMF (4 × 1 min); (3) DCM (4 × 1 min).

Incorporation of Fmoc-Pro-OH. (1) Fmoc-Pro-OH (5 equiv), DIPCDI (5 equiv), and HOBt (5 equiv) in DMF (1 × 2 h); (2) DMF (4 × 1 min); (3) DCM (4 × 1 min).

Coupling Efficiencies. These were monitored using the chloranil test for secondary amines and the ninhydrin test for primary amines.

Capping with Acetic Anhydride. Ac_2O (240 μL; 5 mmol) and DIEA (425 μL; 5 mmol) in DCM (1 × 15 min).

Acidolytic Cleavage with HF. The peptide resin was washed with MeOH (3 × 1 min + 1 × 15 min), dried, and treated with HF in the presence of 10% anisole for 1 h at 0 °C. The crude peptide was precipitated with anhydrous diethyl ether, dissolved in HOAc, and lyophilized.

Acidolytic Cleavage with TFA. The peptide resin was washed with DCM (3 × 1 min + 1 × 15 min), dried, and treated with TFA-H_2O (95:5) for 3 h at 25 °C. The crude peptide was precipitated with anhydrous diethyl ether, dissolved in HOAc, and lyophilized.

Synthesis and Purification of Y-Pro$_n$-L-Amp(Y-Pro$_n$)-OH Y = Fmoc or Ac; $n = 5$, 9, 14, 19 (1, 2, 3, 4, 5, and 6). The first Pro residue was incorporated on a hydroxymethyl resin (2.0 g; 0.9 mmol/g) as Boc-L-Amp(Fmoc)-OH using the methodology described in the general procedure section. After the Boc removal, Boc-Pro-OH was coupled, then the Fmoc group was removed, and another Boc-Pro-OH was coupled. The desired branched peptide was obtained by simultaneous introduction of two Boc-Pro-OH units at every step following the program sequence described in the general procedure section. The last amino acid for each chain was introduced as an Fmoc-Pro-OH. When acetylated branched peptides were needed, acetylating reactions were carried out with Ac_2O. Peptides were cleaved from the resin with HF and lyophilized providing crude peptides 70−85% pure. Crude

peptides were purified by semipreparative or preparative RPHPLC using different linear gradients of acetonitrile (ACN) (containing 1% TFA) and H_2O (containing 1% TFA). Purity of the fractions was verified by analytical RPHPLC providing peptides 96−98% pure. For N-terminally Fmoc protected ($n = 5$) (**1**): MALDI calcd [M + H] 1546.5; found [M + Na] 1567.9. For N-terminally acetylated ($n = 5$) (**2**): MALDI calcd [M + H] 1186.1; found [M + Na] 1208.2. For N-terminally acetylated ($n = 9$) (**3**): MALDI calcd [M + H] 1962.9; found [M + Na] 1984.5. For N-terminally Fmoc protected ($n = 14$) (**4**): MALDI calcd [M + H] 3294.3; found [M + Na] 3317.3. For N-terminally acetylated ($n = 14$) (**5**): MALDI calcd [M + H] 2933.9; found [M + Na] 2954.9. For N-terminally acetylated ($n = 19$) (**6**): MALDI calcd [M + H] 3904.9; found [M + Na] 3927.9.

Synthesis and Purification of Dendrimers 7, 8, 9, 10, and Fluoresceine-Labeled Dendrimer 9. The first step of this synthesis was the removal of the Dde group with (1) DMF (4 × 1 min); (2) hydrazine-DMF (2:98) (3 × 4 min); (3) DMF (4 × 1 min) of the 1*N*-Dde, 8*N*-Mmt-spermidine-4-yl-carbonyl Wang resin (100 mg; 0.4 mmol/g). An Fmoc-Pro$_n$-L-Amp(Fmoc-Pro$_n$)-OH was then coupled (for $n = 5$, 125 mg; 1.5 equiv and for $n = 14$, 266 mg; 1.5 equiv) with PyAOP (33 mg; 1.5 equiv) (double addition) and DIEA (12 μL; 1.5 equiv) (double addition) in DMF (3 × 8 h) at 25 °C. The Mmt group was removed with (1) DCM (4 × 1 min); (2) TFA-triisopropylsilane-DCM (1:5:94) (4 × 2 min); (3) DCM (4 × 1 min); (4) DMF (4 × 1 min), and a second Fmoc-Pro$_n$-L-Amp(Fmoc-Pro$_n$)-OH was coupled using the same procedure. The dendrimer resin for $n = 5$ was divided into two aliquots. The first one was treated with piperidine-DMF (2:8). The second one was treated with piperidine-DMF to remove the Fmoc group and acetylated. The dendrimer resin for $n = 14$ was divided into three aliquots. The first one was treated with piperidine-DMF (2:8). The second one was treated with piperidine-DMF to remove the Fmoc group and acetylated. The third one was labeled, after previous removal of the Fmoc group, with fluoresceine (7 mg; 2 equiv), PyBOP (11 mg; 2 equiv), and DIEA (8 μL; 4 equiv) in DMF (1 × 2 h). Peptide resins were cleaved with TFA providing dendrimers 65−75% pure. Crude dendrimers were purified by semipreparative RPHPLC using different linear gradients of ACN (containing 1% TFA) and H_2O (containing 1% TFA). Purity of the fractions was verified by analytical RPHPLC providing dendrimers 96−98% pure. For N-terminally free ($n = 5$) (**7**): MALDI calcd [M + H] 2311.2; found [M + H] 2311.5. For N-terminally acetylated ($n = 5$) (**8**): MALDI calcd [M + H] 2479.2; found [M + H] 2479.6. For N-terminally free ($n = 14$) (**9**): MALDI calcd [M + H] 5807.8; found [M + H] 5803.6. For N-terminally acetylated ($n = 14$) (**10**): MALDI calcd [M + H] 5975.8; found [M + H] 5977.8.

Synthesis and Purification of Dendrimer 11. 5-(4-Formyl-3,5-dimethoxyphenoxy)valeric acid (136 mg; 5 equiv) and 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) (154 mg; 5 equiv) were dissolved in DMF, then DIEA (167 μL; 10 equiv) was added, and after 1 min of preactivation, this solution was added to amino-functionalized MBHA-PEG-resin (300 mg; 0.3 mmol/g) containing Ile as an internal reference amino acid. Coupling was allowed to proceed at 25 °C for 2 h. HCl·H_2N-Lys(Alloc)-OMe (270 mg; 10 equiv) was then coupled with $NaBH_3CN$ (60 mg; 10 equiv) in DMF (1 × 2 h), and after washing the resin with piperidine-DMF (2:8) (3 × 1 min), for neutralizing the hydrochloride, DMF (4 × 1 min) and DCM (4 × 1 min) Fmoc-Lys(Mtt)-OH (300 mg; 5 equiv) were coupled with PyAOP (250 mg; 5 equiv), DIEA (167 μL; 10 equiv) in DMF (2 × 2 h). Treatments of the Fmoc-dipeptidyl-BAL-resin with piperidine-DMF (2:8) (3 × 1 min, 3 × 5 min, 2 × 1 min) followed by washes with DMF (3 × 1 min) led to formation of cyclic Lys-Lys (2,5-DKP) orthogonally protected with Alloc and Mtt groups.

The Mtt group of the *c*[Lys(Alloc)-Lys(Mtt)]-BAL-MBHA-PEG resin (50 mg; 0.3 mmol/g) was removed with (1) DCM (4 × 1 min); (2) TFA-H_2O-DCM (2:1:97) (3 × 10 min); (3) DIEA-DCM (5:95) (4 × 3 min); (4) DCM (4 × 1 min). An Fmoc-Pro$_5$-L-Amp(Fmoc-Pro$_5$)-OH was then coupled (35 mg; 1.5 equiv) with PyAOP (13 mg; 1.5 equiv) (double addition), DIEA (4 μL; 1.5 equiv) (double addition) in DMF (2 × 8 h) at 25 °C. The Alloc group was removed with (1) DCM (4 × 1 min); (2) triphenylsilane (20 μL; 10 equiv), tetrakis (triphenylphosphine) palladium(0) (2 mg; 0.1 equiv) in anhydrous DCM (3 × 15 min) under argon atmosphere; (3) DCM (4 × 1 min) and a second Fmoc-Pro$_5$-L-Amp(Fmoc-Pro$_5$)-OH were coupled using the same procedure. The dendrimer resin was treated with piperidine-DMF (2:8) to remove the Fmoc groups and acetylated. The peptide resin was cleaved with TFA providing a crude dendrimer 40% pure. The crude peptide was purified by semipreparative RPHPLC using a linear gradient of ACN (containing 1% TFA) and H_2O (containing 1% TFA). Purity of the fractions was verified by analytical RPHPLC providing a dendrimer 95% pure. MALDI calcd [M + H] 2591.6; found [M + Na] 2614.7.

Synthesis and Purification of H-Pro$_{14}$-OH (12), Ac-Pro$_{14}$-OH (13), and Fluoresceine-Pro$_{14}$-OH (14). Starting with 100 mg of a hydroxymethyl resin (0.7 mmol/g), we synthesized a Boc-Pro$_{14}$-resin using the methodology described in the general procedure section. The peptide resin was divided into three aliquots. The first one was treated with TFA-DCM (4:6). The second one was treated with TFA-DCM (4:6) and acetylated. The third one was treated with TFA-DCM (4:6), and the peptide resin was labeled with fluoresceine (45 mg; 4 equiv), PyBOP (70 mg; 4 equiv), and DIEA (50 μL; 8 equiv) in DMF (1 × 2 h). Acidolytic cleavages with HF were carried out. Purities were verified by analytical RPHPLC providing peptides 97% pure. For H-Pro$_{14}$-OH (**12**): MALDI [M + H] calcd 1378.4; found 1378.0. For Ac-Pro$_{14}$-OH (**13**): MALDI [M + H] calcd 1420.4; found 1419.8. For fluoresceine-Pro$_{14}$-OH (**14**): MALDI [M + H] calcd 1692.7; found 1692.5.

CD Spectropolarimetry. CD spectra were recorded at 25 °C with a Jasco J700 spectropolarimeter at a spectral bandwidth of 2 nm, with a time constant of 4 s (scan speed 10 nm/min) and a step resolution of 1 nm. Concentrations were 95 μM for dendrimers **7** and **8**, 100 μM for building blocks of different length, and 60 μM for dendrimers **9** and **10**. The spectrum of the solvent was subtracted from the spectra of the samples.

PPII samples were dissolved in Milli-Q reagent grade water (conductivity 18.2 MΩ cm^{-1}). PPI samples were prepared by first dissolving them in water (0.5 part), then diluting them with spectral-grade *n*-propanol (99.5 parts), and allowing interconversion to form I to occur for at least 6 days before recording the CD spectrum. Cylindrical quartz cells with a path length of 1 mm were used. Data are presented in residue molar ellipticities (θ, deg cm^2 dmol^{-1}).

Fluorescence Microscopy. Normal rat kidney cells NRK-49F were obtained from the American Type Culture Collection (Rockville, MD). Cells were cultured in Dulbecco's modified eagle's medium (Biological Industries, Beit Haemek, Israel) supplemented with 10% fetal calf serum, 4 mM L-glutamine (Sigma), 100 U/mL penicillin G, 100 μg/mL streptomycin, and 1% nonessential amino acids all from Invitrogen (Barcelona, Spain) at 37 °C in 5% CO_2 atmosphere. Cells were cultured in a 24-well plate containing glass coverslips. After 24 h, the medium in each well was replaced by fresh medium, and cells were incubated in the absence (control cells) or in the presence of fluoresceine, fluoresceine-Pro$_{14}$-OH, and fluoresceine-labeled dendrimer **9**. After 1 h of incubation at 37 °C in 5% CO_2 atmosphere, or 1 h at 4 °C (previous preincubation for 30 min at 4 °C), cells were washed three times with phosphate buffer solution (PBS) and fixed by adding 0.5 mL of paraformaldehid-H_2O (1:99) for 15 min. Cells were washed three times with PBS, and then additionally washed with distilled water. Sections were examined with a Nikon Eclipse E800 microscope.

Fluorescence Measurements. Fluorescence data were collected with an Aminco Bowman Series2 fluorescence spectrophotometer with a scan resolution of 1 nm/s.

A set of 400 μL solutions of ciprofloxacin in titration solvent was prepared, and small aliquots of a concentrated dendrimer **9** solution or a concentrated peptide H-Pro$_{14}$-OH (**12**) solution were added. Solutions

were mixed well. The volume of the aliquots was small enough to ensure that dilution was less than the 5%. The ciprofloxacin contained in a 1 cm path length fluorescence cuvette was irradiated at 330 nm, and fluorescence emission was recorded from 345 to 600 nm.

Data at 430 nm were extracted and fit to a 1:1 binding curve by nonlinear least squares techniques to the following equation:

$$F = F_0 + (F_\infty - F_0) \times \frac{[C] + [D] + 1/K_a - \sqrt{([C] + [D] + 1/K_a)^2 - 4[C][D]}}{2[C]}$$

where F_0, F_∞, F, [C], [D], and K_a are the fluorescence with no added dendrimer, the fluorescence at saturation, and the fluorescence recorded during titration, the concentration of total ciprofloxacin, the concentration of total dendrimer, and the association constant, respectively.

A 1:2 binding stoichiometry was confirmed by a Job plot. A titration of continuous variation was conducted where the concentration of both ciprofloxacin and dendrimer **9** was varied between 0 and 20 μM such that the number of moles in solution stayed constant. A plot of the mole fraction of ciprofloxacin in the solution versus fluorescence at 430 nm gives a plot that indicates the stoichiometry of the complex.

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Supporting Information Available: CD spectra of building blocks and dendrimers, fluorescence Job Plot, and fluorescence microscopy photographs (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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