Synthesis, Chiroptical Properties, and Configurational Assignment of Fulleroproline Derivatives and Peptides

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Abstract: 1,3-Dipolar cycloaddition of azomethine ylides to C_{60} leads to fulleroproline derivatives, in which a proline ring is fused on a 6,6-ring junction of the fullerene spheroid. This unnatural amino acid can be manipulated under standard coupling conditions to afford fulleroproline-containing peptides. All optically active fulleroproline derivatives and peptides display a characteristic maximum at 428 nm in CD spectra, which is diagnostic for the assignment of the absolute configuration of the C^{α} atom of the proline ring. Calculation of the CD spectra confirm the configurational assignment.

Introduction

In rapid succession, the discovery,[1] isolation,[2] characterization,[3] and study of physical and chemical properties of fullerene C_{60} have attracted the attention of an increasing number of scientists.[4] Postulated to be an unreactive species, C_{60} has instead been found to react with a wide range of nucleophiles, electrophiles, and free radicals.[5] The functionalization of C_{60} can produce stable derivatives, which, while retaining the main electronic features of C_{60}, may possess different properties, such as solubility in polar solvents.[6] Systematic investigations on the organic derivatization have resulted in the opening of new horizons in fullerene research, mainly concerned with practical applications of this new class of compounds, whose potential is to a large extent still unexplored. In this connection, one of the most exciting results is related to the biological activity exhibited by some C_{60} derivatives. In a fundamental contribution, Friedman *et al.*, on the basis of computational graphics simulation, suggested that the C_{60} sphere (about 10 Å in diameter) would perfectly fit the hydrophobic cleft that characterizes the active site of HIV-1 protease.[7] The possibility that fullerene derivatives might actually act as inhibitors of this important enzyme was verified by Sijbesma *et al.* on a methanofullerene compound containing a hydrophilic tail to ensure solubility for *in vitro* tests.[8] Other interesting reports on the preparation of molecules with potential biological applications and on the biological activity of fullerenes and fullerene derivatives have appeared in the literature,[9] but undoubtedly, the field will soon provide new exciting contributions.

In a research program aimed at the systematic investigation of the potential applications of fullerene derivatives, we have devised a general method for the functionalization of C_{60}, based on 1,3-dipolar cycloaddition of azomethine ylides.[10] This reaction leads to a class of stable and characterizable fullerene

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derivatives, commonly termed fulleropyrrolidines. Given the large number of methods used to generate the reactive azomethine ylides,[11] the ready availability of the starting materials, and the easy functionalization of the reaction products, a wide range of fulleropyrrolidines have already become available.[12]

In this paper we present a full account[13] on the preparation, characterization, resolution, and chiroptical properties of derivatives and peptides of 3,4-fulleroproline[14] (hereafter termed Fpr, using the three-letter code for amino acids), a new α-aminoacid (probably the largest unnatural α-amino acid[15]) in which the 3,4-bond of the proline ring is fused on a 6,6-ring junction of C_{60}. We also show that Fpr derivatives and peptides exhibit a particularly intense CD maximum at 428 nm, which we consider diagnostic for the determination of the absolute configuration of the C^{α} atom of the proline ring.

The combination of the fullerene C_{60} and the α-amino acid proline is expected to produce new and interesting structures. In fact, besides possessing the main fullerene properties, Fpr is strictly related to Pro, which is a structurally important amino acid, characterized by restricted conformational freedom. When a Pro residue is incorporated into a peptide chain, it produces an ensemble of conformers, due to the *cis*−*trans* isomerism about the Xxx−Pro tertiary amide bond. The relevant role played by the *cis*−*trans* isomerism in Pro-containing peptides and its effect on the slow step of protein folding and on the catalytic activity of peptidyl-prolyl-*cis*−*trans* isomerases (*e.g.*, cyclophilin, the target of the immunosuppressive drug cyclosporin A) are well established. Segments of homo-oligo $(Pro)_n$ are frequently exploited as semirigid molecular spacers. Pro is an effective α-helix and *β*-sheet structure breaker, but it is a strong *β*-turn inducer. An Xxx−Pro bond represents the primary cleavage site of the enzyme HIV-1 protease, and a Pro residue structurally characterizes the consensus triplet of the left-handed helical component of the collagen triple helix.[16]

Results and Discussion

Two straightforward approaches to Fpr give excellent results: the thermal ring-opening of 2-ester-substituted aziridines and the "tautomerization route" of α-amino esters to azomethine ylides.[11] However, given the chiral nature of α-amino acids and peptides, a practical entry to enantiomerically pure Fpr is of fundamental importance in the design of Fpr-containing peptides. In the present work, three different ways to chiral Fpr derivatives and peptides have been pursued: (1) chromatographic separation of racemic mixtures via enantioselective HPLC, (2) chromatographic separation of diastereomers on silica columns, and (3) use of a suitable chiral substrate to induce asymmetry in the cycloaddition step.

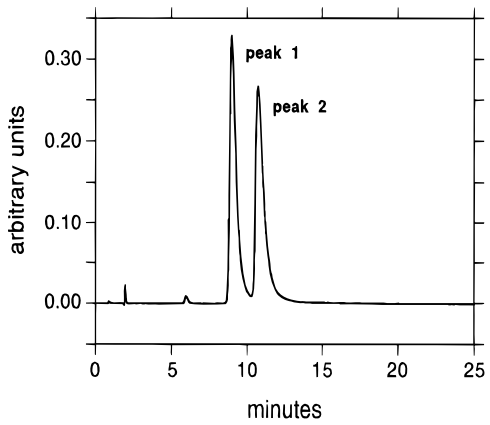
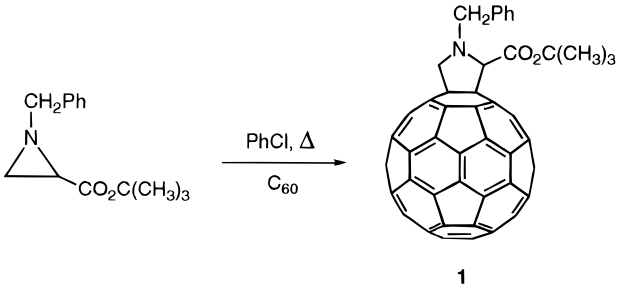


Figure 1. HPLC trace for the enantiomeric resolution of fulleroproline **1**.

Scheme 1



1

The synthesis of *N*-benzyl-3,4-fulleroproline methyl ester was described.[10] Under the same conditions, *N*-benzyl-3,4-fulleroproline *tert*-butyl ester (**1**) can be isolated in 36% yield after chromatography (72% based on C_{60} recovery) from *N*-benzyl-2-(*tert*-butoxycarbonyl)aziridine and C_{60} (Scheme 1).[17]

Fpr derivative **1** possesses no symmetry plane, so that 60 different signals are expected in the ^{13}C NMR spectrum for the fullerene moiety: 58 in the aromatic region and two in the aliphatic region. This is in fact the case when considering few incidental superpositions in the overcrowded aromatic region. In ^{1}H NMR the two methylene protons in the pyrrolidine ring are found at 4.92 and at 4.25 ppm as doublets (J = 9.2 Hz), whereas the methine proton appears as a singlet at 4.90 ppm. The UV−vis spectrum, typical of all fulleropyrrolidines,[10] shows that Fpr derivative **1** basically retains the electronic properties of C_{60}.[18]

Compound **1** was subjected to enantioselective HPLC, using a Whelk O-1[19] column and toluene/*n*-hexane as eluant. A typical chromatogram is shown in Figure 1.

The two well-resolved peaks were separated on an analytical column (batches of about 1 mg), affording enantiopure Fpr

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(14) The correct name for compound **1**, as reported by *Chemical Abstracts* is 2′H-[5,6]fullereno-C_{60}-I_h-[1,9-*c*]pyrrole-2′-carboxylic acid, 1′,5′-dihydro-1′-(phenylmethyl)-, 1,1-dimethylethyl ester. A short name for this derivative might be either fulleroproline or prolinofullerene. We have preferred the former, which is closer to the chemical *Chemical Abstracts* name.

(15) Holmes, A. B.; Stephenson, G. R. *Chem. Ind.* **1994**, 303.

(16) For a recent review article on Pro-dependent structural and biological properties of peptides and proteins, see: Yaron, A.; Naider, F. *Crit. Rev. Biochem. Mol. Biol.* **1993**, *28*, 31−81.

(17) In all of the additions to C_{60} reported in the present work, primary attention has been paid to the isolation of monoaddition products. We have always observed the formation of diadducts, which, in a few cases, were purified by chromatography and characterized by mass spectrometry. However, products of more than one addition to C_{60} are usually composed of complex mixtures of several isomers (Hirsch, A.; Lamparth, I.; Karfunkel, H. R. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 437−438). The isolation of these isomers as single compounds is far beyond the scope of this work. Accordingly, the additions have been carried out in such a way as to achieve the best combined yields of monoaddition product and recovered C_{60}.

(18) Wudl was the first to show that most derivatives retain the electronic and electrochemical properties of the original fullerene: Wudl, F. *Acc. Chem. Res.* **1992**, *25*, 157−161.

(19) Pirkle, W. H.; Welch, C. J.; Lamm, B. *J. Org. Chem.* **1992**, *57*, 3854−3860.

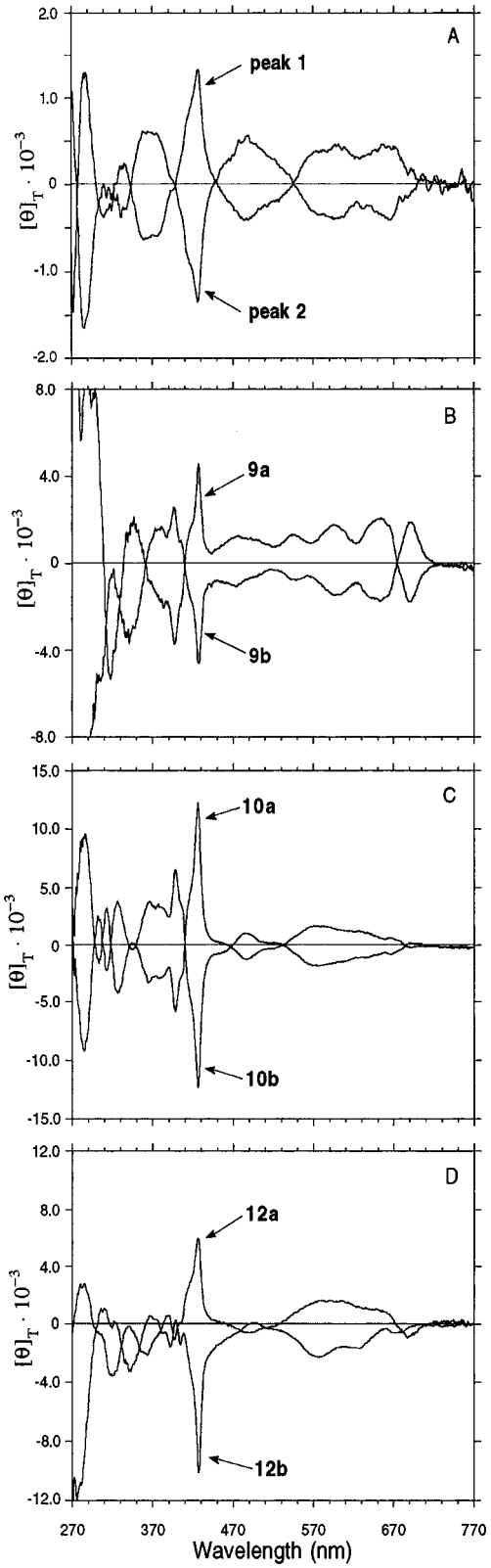
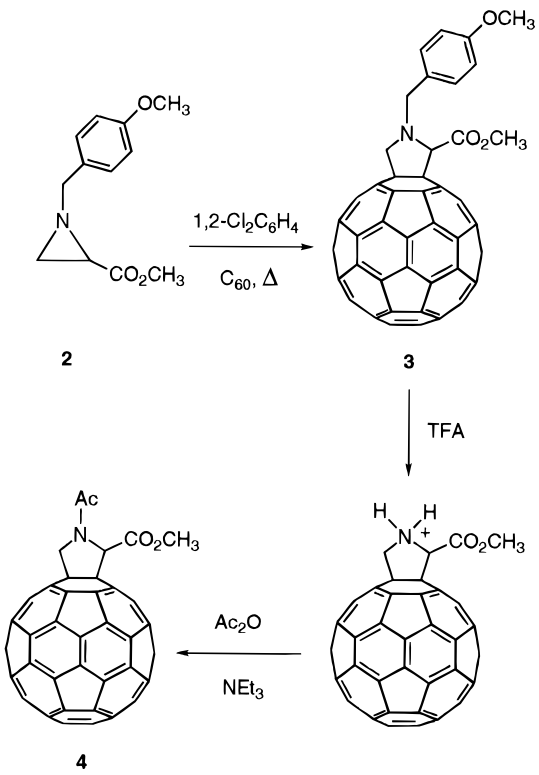


Figure 2. CD spectra for derivatives **1** (A) (4.0×10^{-4} M), **9** (B) (5.0×10^{-4} M), **10** (C) (4.5×10^{-4} M), and **12** (D) (4.0×10^{-4} M) in $CHCl_3$.

derivatives. The two enantiomers exhibit interesting CD spectra (Figure 2A). Optically active bands extend throughout the entire wavelength region from 270 to 700 nm, with a particularly intense maximum at 428 nm. The latter band is most probably due to the same electronic transition responsible for the band typically observed around 428 nm in the UV−vis spectra of monofunctionalized C_{60} derivatives.[9a,20] The high ellipticity of several bands in the low-energy region of the CD spectra reflects the consequence of the dissymmetric perturbation induced by the chiral Pro ring on the extended π-system of the modified fullerene. It is interesting to note that two different chiral systems can give rise to an optically active band in the CD spectra of fullerenes and fullerene derivatives. One case is that reported in the present paper, where the ellipticity is caused by a chirally functionalized fullerene. The other case, previously reported by Hawkins *et al.*,[21] is that where much higher ellipticities are due to chiral fullerene chromophores.[22]

Scheme 2



Whereas the *tert*-butyl ester group in **1** could be easily hydrolyzed by acidic treatment, any attempt to remove the *N*-benzyl group proved unsuccessful, due to the known reactivity of the C_{60} moiety under catalytic hydrogenation conditions.[6a,23]

As N-substituted aziridines can be relatively easily prepared, better N-protecting groups can be envisaged for the synthesis of the free amino acid. As a matter of fact, we have already obtained evidence that the 4-methoxybenzyl group, removable under acidic conditions, serves the purpose very well. The N-protected fulleroproline derivative **3**, prepared from the corresponding substituted aziridine **2**, was treated for 20 h in trifluoroacetic acid at 65 °C (Scheme 2). The resulting deprotected amino group was directly transformed into its *N*-acetyl derivative **4** and characterized.

However, while potentially useful for future work, the aziridine approach proved inadequate to achieve selective

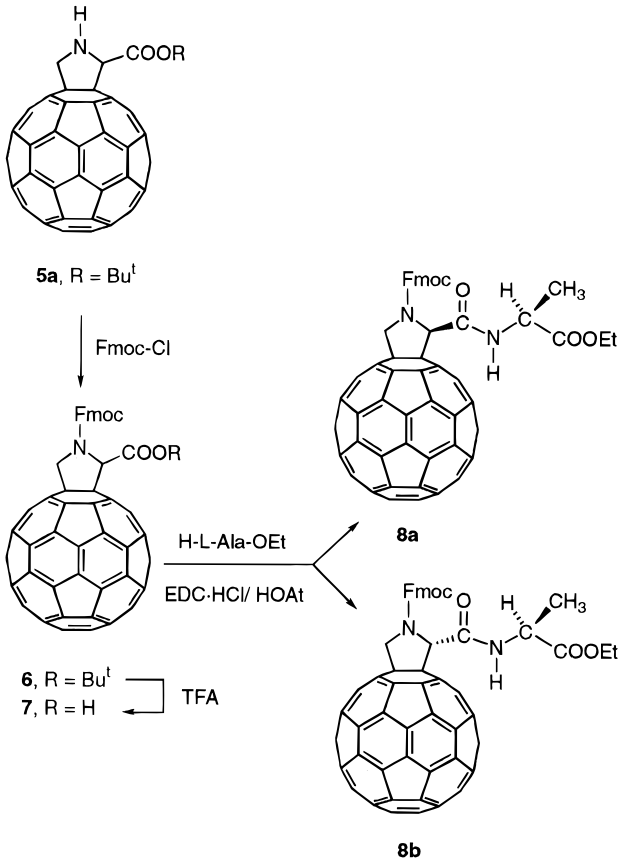
(20) The observation of a sharp UV−vis absorption and a small CD maximum at 431-435 nm "characteristic and even diagnostic of dihydrofullerenes" was reported: Wilson, S. R.; Wu, Y.; Kaprinidis, N. A.; Schuster, D. I.; Welch, C. J. *J. Org. Chem.* **1993**, *58*, 6548−6549.

(21) (a) Hawkins, J. M.; Meyer, A.; Nambu, M. *J. Am. Chem. Soc.* **1993**, *115*, 9844−9845. (b) Hawkins, J. M.; Meyer, A. *Science* **1993**, *260*, 1918−1920. (c) Hawkins, J. M.; Nambu, M.; Meyer, A. *J. Am. Chem. Soc.* **1994**, *116*, 7642−7645.

(22) For a comparison of the two effects, see: Herrmann, A.; Rüttimann, M.; Thilgen, C.; Diederich, F. *Helv. Chim. Acta* **1995**, *78*, 1673−1704.

(23) Avent, A. G.; Darwish, A. D.; Heimbach, D. K.; Kroto, H. W.; Meidine, M. F.; Parsons, J. P.; Remars, C.; Roers, R.; Ohashi, O.; Taylor, R.; Walton, D. R. M. *J. Chem. Soc., Perkin Trans. 2* **1994**, 15−22.

Scheme 3

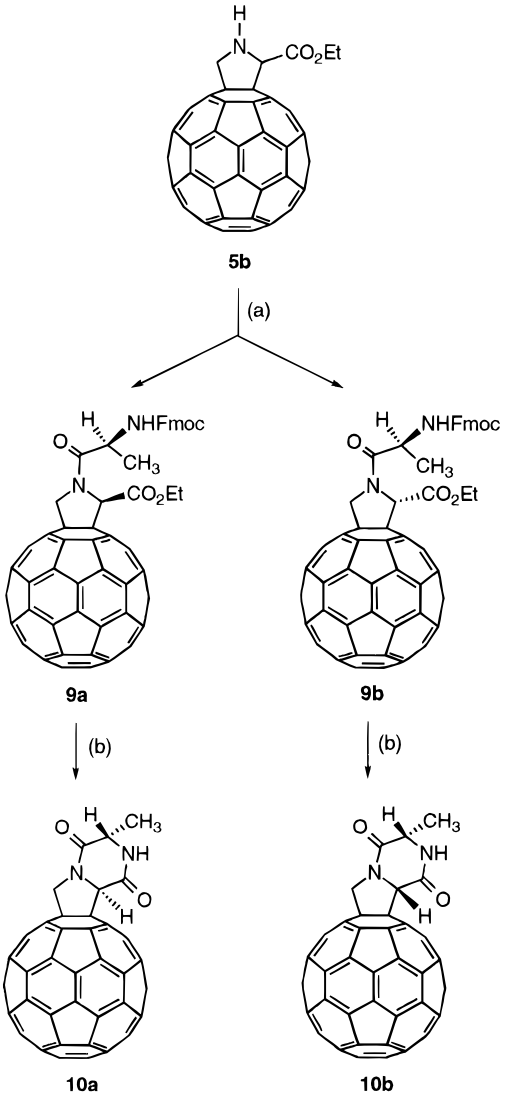


protection of both amino and carboxylic groups, due to the similar instability to acidic conditions of the *N*-(4-methoxybenzyl) and *tert*-butyl ester groups. To obtain a stable and characterizable Fpr derivative useful for peptide synthesis, the approach was then modified in the following way. A mixture containing glycine *tert*-butyl ester, paraformaldehyde, and C_{60} was heated to reflux in toluene for 1.5 h.[13] The N-deprotected Fpr ester **5a** (Scheme 3), which forms in the reaction, suffers the presence of the free amino group[24] and cannot be isolated as a pure solid. However, it can be purified from unreacted C_{60} by chromatography and can be kept in dilute solutions in the dark. For these reasons, **5a** was used without characterization. Functionalization at nitrogen can be achieved easily via standard acylation procedures with acid anhydrides or acid chlorides.

Reaction of **5a** with 9-fluorenylmethyl chloroformate (Fmoc-Cl) in toluene gave the Fmoc-Fpr-OBu^t (**6**) (Scheme 3) in 74% yield (based on recovered C_{60}). This compound is ideal for peptide synthesis, as the Fpr residue is protected with two groups removable under completely different conditions. The two enantiomers, separated by HPLC as in the case of **1**, gave CD spectra with strong positive and negative maxima at 428 nm. Exposure of **6** (racemic mixture) to trifluoroacetic acid allowed the quantitative hydrolysis of the *tert*-butyl ester, leaving unmodified the Fmoc group and the fullerene spheroid. The N-protected free acid **7** was subjected to peptide coupling conditions (EDC·Cl, HOAt, H-L-Ala-OEt), giving a mixture of the two diastereomeric dipeptides **8a** and **8b**, separable by semipreparative HPLC, using a reverse-phase (C_{18}) column and acetonitrile/chloroform as eluant. CD spectra of the two isomers show, again, a strong Cotton effect at 428 nm.

(24) Hirsch, A.; Li, Q.; Wudl, F. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1309−1310.

Scheme 4[a]



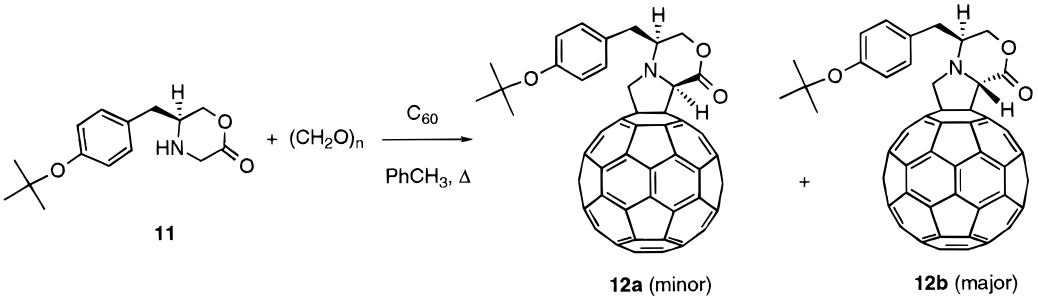
[a] (a) (Fmoc-L-Ala)$_2$O; (b) piperidine.

Reaction of **5b** (obtained from glycine ethyl ester similarly to **5a**[13]) with (Fmoc-L-Ala)$_2$O in toluene gave a mixture of the two diastereomeric dipeptides **9a** and **9b** (Scheme 4).

The two isomers formed at different rates, but the reaction was allowed to go to completion in order to obtain optimal yields for both compounds. Diastereomeric Fmoc-L-Ala-DL-Fpr-OEt **9a** and **9b** were separated on a silica column using mixtures of toluene and ethyl acetate as eluant. CD spectra of the two isomers show an almost mirror image behavior and the expected strong maximum at 428 nm (Figure 2B). In the ^{1}H NMR spectra, a *cis*−*trans* equilibrium was unraveled for both compounds, due to restricted rotation about the tertiary amide bond, each compound giving rise to a pair of rotamers detectable at room temperature. However, any attempt to establish the absolute configuration of the C$^\alpha$ atom of Fpr by NOE correlation to the known *S* configuration of the Ala moiety was unsuccessful. Transformation of **9a** and **9b** into the corresponding 2,5-dioxopiperazines gave a handle for the solution of the stereochemical problem. The Ala amino group was deprotected by addition of excess piperidine (10% in methylene chloride). This procedure did not interfere with the known reactivity of C_{60} with secondary amines,[25] as no trace of piperidine addition to any double bond of C_{60} was detected. The two open-chain

(25) Hirsch, A. *Synthesis* **1995**, 895−913.

Scheme 5



H-L-Ala-D-Fpr-OEt and H-L-Ala-L-Fpr-OEt dipeptides were not isolated, as cyclization occurred *in situ*, giving rise to diastereomeric 2,5-dioxopiperazines **10a** and **10b** in 43% and 35% yields (from **9a** and **9b**, respectively). The relatively poor solubility of these compounds in the most common solvents raised some experimental problems during the spectroscopic characterization. However, in this case, the cyclic dipeptide backbone is conformationally restricted enough to allow a clear detection of the NOE enhancement derived from spatial proximity of the C^{α} proton of D-Fpr and the L-Ala β-CH_3 group in **10a**. Conversely, an NOE between the C^{α} protons of L-Ala and L-Fpr was observed in **10b**. Configurations *R* and *S* were thus firmly assigned to the C^{α} atom of Fpr in **10a** and **10b**, respectively (and, consequently, in **9a** and **9b**). The CD spectra of diastereomers **10a** and **10b** (Figure 2C) are in good approximation mirror images of each other and exhibit an extraordinary increase in the intensity of the 428 nm maximum. This Cotton effect has been seen in all Fpr derivatives and peptides and appears to be diagnostic for the assignment of the absolute configuration of the C^{α} atom of Fpr. *A negative Cotton effect at 428 nm corresponds to the S configuration, whereas a positive Cotton effect is associated with the R configuration.*

To confirm this hypothesis, we have synthesized enantiopure Fpr derivatives with definable stereochemistry via chiral azomethine ylide cycloaddition.[26] Morpholin-2-one **11** (Scheme 5), synthesized in 10 steps from L-tyrosine (see supporting information), was condensed with paraformaldehyde in toluene at reflux in the presence of C_{60}. The reaction proceeded smoothly, affording a mixture of two products, **12a** and **12b**, in 44% overall yield and in a 6:94 ratio (diastereomeric excess 88%, HPLC), along with unreacted C_{60} (36%).

The ^{1}H NMR spectrum of the major isomer **12b** shows a singlet at 5.59 ppm for the Fpr C^{α} proton and a multiplet at 4.91 ppm for the tyrosinol α proton. On the other hand, in **12a** the same protons resonate at 4.78 and 3.61 ppm, respectively. The lower field chemical shift of these protons in **12b** with respect to those of **12a** may be due to a different distance from the fullerene spheroid.[27]

Both compounds were subjected to NOE analysis. In **12a** a strong NOE enhancement between Fpr and tyrosinol α protons (15%), absent in **12b**, is highly diagnostic of a *cis* relationship, allowing a firm determination of the absolute configuration of the C^{α} atom in the Fpr residues of **12a** and **12b**, consistent with the depicted structures. These results are in agreement with the expected attack of the fullerene from the less sterically hindered side of the intermediate 1,3-dipole during cycloaddition.

The CD spectra of **12a** and **12b**, reported in Figure 2D, fully confirm the usefulness of the sign of the 428 nm maximum for the assignment of the configuration of the Fpr C^{α} atom. Again, L-Fpr (**12b**) gives a negative Cotton effect, whereas D-Fpr (**12a**) shows a positive CD band at 428 nm.

In order to get more information on the electronic transitions involved and theoretical support to the proposed configurational assignments, a calculation of the CD spectra of **1** and **10** was carried out. Both simulations give similar results, so that the following discussion will focus only on 2,5-dioxopiperazines **10**.

Calculation of the CD Spectra

The general expression for the rotational strength of a transition $0 \rightarrow a$ is written as:

$$R_{0a} = \mathrm{Im}(\langle\psi_0|\boldsymbol{\mu}|\psi_a\rangle\cdot\langle\psi_a|\mathbf{m}|\psi_0\rangle) \qquad (1)$$

where $\boldsymbol{\mu}$ is the electric dipole operator and $\mathbf{m} = (e/2mc)(\mathbf{r} \times \mathbf{p})$ is the magnetic dipole operator, proportional to the vector product of the position operator **r** and momentum operator **p**. The rotational strength of a transition is in turn related to the molar ellipticity $[\theta]$ through the expression:

$$R_{0a} = (3hc/8\pi^3\mathbf{N}_0)\int[\theta(\lambda)]/\lambda\ \mathrm{d}\lambda \qquad (2)$$

The molecular constants appearing in eq 1 were evaluated using the CNDO/S Hamiltonian, with the Coulomb integrals computed in the Mataga−Nishimoto approximation. The electric dipole integrals were calculated within the dipole velocity operator method, whereas for the magnetic integrals the complete angular momentum operator was used.[28] Taking into account previous work on semiempirical calculations of the permitted[29] and vibronic[30] magnetic circular dichroism (MCD) spectrum of C_{60}, the configuration interaction (CI) was fixed to all the singly excited promotions in the space of 18 × 19 molecular orbitals. The results, graphically displayed in Figure 3 for **10a** and **10b**, were obtained through simulation of simulating the spectral bands by means of Gaussians centered on the calculated wavelength according to the following expressions,[31] where *a* is a constant and Θ_i is the half-width of the band with maximum at λ_i.

$$[\theta_i] = [\theta_i^{\,0}]\ \exp[-(\lambda_i-\lambda)^2/\Theta_i^{\,2}]$$

$$[\theta_i^{\,0}] = a(\lambda_i/\Theta_i)\mathrm{R}_{0i} \qquad (3)$$

The experimental CD spectra of diastereomers **10a** and **10b**, reported in Figure 2C, show a range of mirror image signals in

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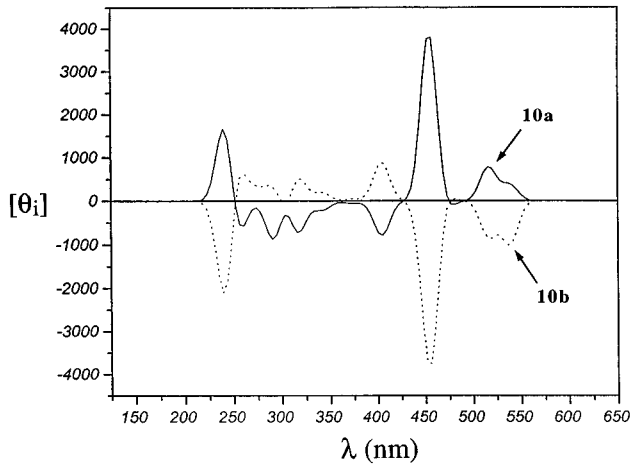

Figure 3. Calculated CD spectra for 2,5-dioxopiperazines **10a** (solid line) and **10b** (dotted line).

the 750−270 nm interval, with a distinctive maximum at 428 nm. The calculated spectra reproduce in a satisfactory way the sequence and the signs of the main bands of the two diastereomers, although the calculated energies appear overestimated, especially for the lower energy part of the spectrum (700−500 nm). This fact is due to the limitations of the semiempirical method used, which, for the parent C_{60}, calculates the energy of the lowest excited state at 2.30 eV,[32] whereas the highest frequency band of the fluorescence spectrum is detected at 1.87 eV.[33] The sign of the diagnostic band at 455 nm is calculated positive for **10a** and negative for **10b**, and the whole CD spectrum results in a sequence of quasispecular bands: *the theoretical method recognizes the absolute configuration of this class of compounds in full agreement with the experiments.*

As for the electronic structure of the Fpr peptides examined, inspection of the molecular orbitals involved in the low-lying excited states indicates that these can be described as fullerene states, slightly perturbed by the Pro group. In fact, the calculated electron densities are limited to a few percent on the nitrogen of the Pro ring and its substituents. Breaking of the high symmetry of C_{60} (I_h), appears to be instrumental in lending allowed character to states otherwise electrically and magnetically forbidden. In parent C_{60}, the lowest excited states deriving from the promotion HOMO (h_u) → LUMO (t_{2u}) transform as the T_{1g}, T_{2g}, and G_g irreducible representations in the I_h point symmetry. Among these three states, which are calculated almost degenerate,[32] only G_g and T_{1g} can bear on intrinsic magnetic moment. The latter, which has been indicated as responsible for the fluorescence and a remarkable *A* term in the MCD spectrum in the region of ∼600 nm, is calculated with a large magnetic dipole moment.[30] Therefore, we interpret the low-energy part of the spectrum, characterized by a strong positive and a weak negative band for **10a** (and *vice versa* for **10b**), as due to the energy splitting of two perturbed C_{60} states, T_{1g} and G_g.

The most distinctive part of the spectrum, however, is given by the sharp maximum at 428 nm, which can be taken as diagnostic for this class of compounds. The theory is in fairly good agreement with the experiments, giving the state with the largest rotational strength at 455 nm (21 965 cm^{-1}) for compounds **10**. Since no other states with T_{1g} symmetry are calculated below 310 nm for C_{60},[32] it turns out that the band at about 430 nm must result from a strong perturbation of a fullerene state in this region, brought about by the presence of the chiral Pro moiety. Indeed, inspection of molecular orbitals shows that the transition calculated at 455 nm corresponds to a state with relatively larger participation of the proline ring, with subsequent borrowing of electric moment (the dipole strength is 8×10^{-2} D^2, much larger than that of the lower lying states and consistent with the appearance of a small but sharp band in the absorption spectrum). The high-energy part of the CD spectrum shows a complicated pattern of bands, reflecting the overcrowded density of states of these large molecules at those energies.

Conclusions

The synthesis of derivatives of the unnatural amino acid Fpr can be achieved by means of 1,3-dipolar cycloadditions of azomethine ylides to C_{60}. Among others, a fully protected Fpr is easily prepared and considered an ideal starting material for peptide synthesis. In fact, under standard conditions of peptide synthesis, dipeptides that include Fpr have been prepared. The Fpr derivatives and peptides examined in this work have been separated into optically pure enantiomers via enantioselective HPLC or diastereomers via preparative reverse-phase HPLC or silica column separation. The CD spectrum of each compound shows a wide range of optically active bands due to the large window of absorptions of the fullerene moiety coupled with the dissymmetric perturbation induced by the chiral Pro ring. The most distinctive feature in the CD spectra of these compounds is a sharp maximum at about 428 nm. The sign of this Cotton effect offers a strong clue to the determination of the absolute configuration of the newly-generated Pro chiral C^α atom: a positive maximum is associated with the *R* configuration, whereas the *S* isomer gives a negative maximum. The configurational assignment has been confirmed by quantum chemical computations, and the nature of the electronic transitions has been determined.

More complex compounds with Fpr incorporated into biologically active peptides are currently being synthesized in our laboratories.

Experimental Section

Instrumentation. ^1H and ^{13}C NMR spectra were recorded on Bruker AC 200, AC 250, and AM 400 spectrometers. Chemical shifts are given in parts per million (δ) relative to tetramethylsilane. Nuclear Overhauser effect determination (^1H NMR NOE): observed nucleus H_a:{saturated nucleus H_b}, percent enhancement. UV−vis absorption spectra were taken on a Perkin-Elmer Lambda 5 spectrophotometer. FT-IR spectra were recorded on a Perkin-Elmer 1720 X spectrophotometer. MALDI (matrix-assisted laser desorption ionization) mass spectra were obtained in positive linear mode at 15 kV acceleration voltage on a Reflex time of flight mass spectrometer (Bruker), using 2,5-dihydroxybenzoic acid as matrix. Circular dichroism measurements were performed on a Jasco J-500 spectropolarimeter, using quartz Hellma cells of 1, 2, and 10 mm path length. The values are given as $[\Theta]_T$ (total molar ellipticity, degree × cm^2 × $dmol^{-1}$). A 250 × 4.6 mm (*S*,*S*) Whelk O-1[19] column (Regis Chemical Co., Morton Grove, IL) was used in the enantiomeric separation of fulleroprolines **1** and **6**. Isocratic elution was performed on a Waters HPLC unit at a flow rate of 1.5 mL/min with HPLC-grade *n*-hexane and toluene as the mobile phase. **1**: 75:25 ($K'_1 = 3.77$, $\alpha = 1.25$). **6**: 50:50 ($K'_1 = 6.52$, $\alpha = 1.26$). The elution was monitored on a Waters Model M490 spectrophotometric detector at 320 nm. HPLC separation of diastereoisomeric fulleroprolines **8a** and **8b** was accomplished on a Pharmacia LKB-LCC 2252 unit employing a 250 × 10 mm Vydac reverse-phase column (C_{18}, Model 218TP510). Gradient elution was performed with HPLC-grade $CH_3CN/CHCl_3$ mixtures as the mobile phase (from 75:25 to 65:35 in 40 min at a flow rate of 2 mL/min). After filtration of

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the mixture with a Teflon 0.2 μm membrane filter, 0.2 mL portions of the brown solution were injected. The elution was monitored on a Pharmacia UV Uvicord SD spectrophotometric detector at 280 nm. Determination of the diastereoisomeric excess for cyclic fulleroproline derivatives **12a** and **12b** was accomplished on the previously described Pharmacia HPLC unit, detector, and Vydac column using $CH_3CN/CHCl_3$ mixtures as the mobile phase (from 80:20 to 40:60 in 20 min at a flow rate of 2 mL/min). Reactions were monitored by thin-layer chromatography using Merck precoated silica gel 60-F_{254} (0.25 mm thickness) plates. Flash column chromatography was performed employing 230−400 mesh silica gel (ICN Biomedicals). Reaction yields were not optimized and refer to pure, isolated products. All calculations were performed on a Dec Alpha AXP 3000/500 station.

Symbols and Abbreviations. All optically active amino acids employed have L-chirality. Symbols and abbreviations used for amino acids and peptides follow the recommendations of the IUPAC−IUB Joint Commission on Biochemical Nomenclature (JCBN); see, for example, *Eur. J. Biochem.* **1984**, *138*, 5−37. Fpr: fulleroproline, Ac: acetyl, Fmoc: fluorenyl-9-methyloxycarbonyl, Z: benzyloxycarbonyl, TEA: triethylamine, NMM: *N*-methylmorpholine, TFA: trifluoroacetic acid, EDC·HCl: *N*-ethyl-*N*′-((3-dimethylamino)propyl)carbodiimide hydrochloride, HOAt: 1-hydroxy-7-azabenzotriazole, Ac_2O: acetic anhydride, TBAF: tetrabutylammonium fluoride.

Materials. C_{60} was purchased from Hoechst A.G. (gold grade). All other reagents were used as purchased from Fluka and Aldrich. *N*-benzyl-2-(*tert*-butoxycarbonyl)aziridine was prepared as described in the literature.[34] All solvents were distilled prior to use. Chloroform and cyclohexane, employed for UV−vis and CD measurements, were commercial spectrophotometric grade solvents.

$PhCH_2$-Fpr-OBu^t (1). A solution of 100 mg (0.14 mmol) of C_{60} and 130 mg (0.56 mmol) of N-benzyl-2-carbo-*(tert*-butoxycarbonyl)-aziridine in 100 mL of chlorobenzene was stirred at reflux temperature for 15 h, and then the solvent was removed *in vacuo*. The residue was purified by flash chromatography (eluant: toluene/petroleum ether), affording 24 mg (36%) of **1** along with 18 mg (36%) of unreacted C_{60}: IR (KBr) (cm^{-1}) 1743, 1725, 1453, 1367, 1249, 1144, 575, 528; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.70−7.08 (m, 5H), 4.92 (d, J = 9.2 Hz, 1H), 4.90 (s, 1H), 4.62 (d, J = 13.8 Hz, 1H), 4.25 (d, J = 9.2 Hz, 1H), 4.10 (d, J = 13.8 Hz, 1H), 1.51 (s, 9H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 168.46, 154.68, 154.55, 153.78, 151,15, 147.22, 147.11, 146.35, 146.28, 146.24, 146.13, 146.08, 145.91, 145.65, 145.58, 145.47, 145.38, 145.28, 145.15, 145.10, 144.51, 144.38, 144.30, 142.96, 142.92, 142.53, 142.50, 142.48, 142.12, 142.09, 142.03, 141.99, 141.95, 141.87, 141.84, 141.66, 140.16, 140.11, 139.65, 139.16, 137.56, 137.11, 136.37, 135.89, 135.42, 128.85, 128.57, 128.06, 127.60, 125.15, 82.44, 76.27, 72.59, 69.21, 64.64, 55.86, 28.15; MALDI-MS $C_{74}H_{19}NO_2$ (MW = 953), *m/z* 953 (M^+); UV−vis ($CHCl_3$) λ_{max} (ϵ) 701 (363), 429 (1950), 308 (20 700); CD ($CHCl_3$) λ_{max} ($\theta_T \times 10^{-3}$) 665 (−0.41, 0.47), 598 (−0.40, 0.46), 489 (0.56, −0.41), 428 (−1.32, 1.33), 360 (0.61, −0.63), 286 (−1.65, 1.30). Anal. Calcd for $C_{74}H_{19}NO_2$: C, 93.17; H, 2.01; N, 1.47. Found: C, 92.15; H, 1.94; N, 1.41.

***N*-(4-Methoxybenzyl)-2-carbomethoxyaziridine (2).** To a cooled solution (ice bath) of 1.9 g (7.70 mmol) of methyl 2,3-dibromopropionate in 6 mL of dry benzene was added a solution of 1 mL (7.70 mmol) of (4-methoxybenzyl)amine and 2.15 mL (15.4 mmol) of TEA in 9 mL of dry benzene dropwise over a period of 30 min. When the addition was complete, the suspension was heated to reflux for 3 h, then brought to room temperature, and filtered with suction over a pad of Celite. The filtrate was diluted with toluene (50 mL) and washed with brine. The organic phase, dried over Na_2SO_4, was evaporated under reduced pressure, and aziridine **2** was purified by flash chromatography (eluant: toluene/methanol, 9:1), affording 1.6 g (93%) of a pale yellow oil: IR (neat) (cm^{-1}) 1744, 1611, 1512, 1461, 1439, 1247; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.77 (d, J = 8.3 Hz, 2H), 7.17 (d, J = 8.3 Hz, 2H), 3.68 (s, 3H), 3.60 (s, 3H), 3.37 (m, 2H), 2.13−2.10 (m, 2H), 1.66 (d, J = 7.3 Hz, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 170.93, 158.68, 129.51, 129.13, 113.54, 62.98, 54.91, 51.88, 36.87, 34.13; EI-MS (MW = 221), *m/z* 221 (5), 206 (13), 162 (6), 135 (30), 121 (100). Anal. Calcd for $C_{12}H_{15}NO_3$: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.05; H, 6.84; N, 6.22.

4-$MeOC_6H_4CH_2$-Fpr-OMe (3). A solution of 100 mg (0.14 mmol) of C_{60} and 112 mg (0.54 mmol) of *N*-(4-methoxybenzyl)-2-carbomethoxyaziridine (**2**) in 70 mL of 1,2-dichlorobenzene was stirred at reflux temperature for 2.5 h, and then the solvent was removed *in vacuo*. The residue was purified by flash chromatography (eluant: toluene/petroleum ether, then toluene), affording 39 mg (30%) of **1** along with 42 mg (42%) of unreacted C_{60}: IR (KBr) (cm^{-1}) 1753, 1736, 1611, 1511, 1463, 1431, 1247, 576, 528; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.58 (d, J = 8.4 Hz, 2H), 7.01 (d, J = 8.4 Hz, 2H), 5.05 (s, 1H), 4.90 (d, J = 9.5 Hz, 1H), 4.56 (d, J = 13.5 Hz, 1H), 4.28 (d, J = 9.5 Hz, 1H), 4.14 (d, J = 13.5 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 170.33, 159.25, 154.71, 154.43, 153.32, 151.10, 147.38, 147.28, 146.35, 146.30, 146.25, 146.21, 146.05, 145.78, 145.73, 145.67, 145.62, 145.56, 145.46, 145.39, 145.34, 145.29, 145.28, 145.24, 144.65, 144.50, 144.46, 144.39, 143.08, 143.03, 142.65, 142.62, 142.25, 142.18, 142.11, 142.08, 141.98, 141.95, 141.91, 141.79, 141.76, 140.24, 140.19, 139.80, 139.67, 130.50, 128.40, 114.09, 76.22, 72.72, 69.20, 64.70, 55.39, 55.32, 52.36; MALDI-MS $C_{72}H_{15}NO_3$ (MW = 941), *m/z* 942 ($M + H^+$); UV−vis ($CHCl_3$) λ_{max} (ϵ) 698 (507), 429 (3500), 309 (32 300), 254 (111 400); Anal. Calcd for $C_{72}H_{15}NO_3$: C, 91.81; H, 1.61; N, 1.49. Found: C, 91.49; H, 1.64; N, 1.38.

Ac-Fpr-OMe (4). A solution of 13 mg (0.0138 mmol) of **3** in 2 mL of TFA was heated at 65 °C for 20 h. The solvent was removed under reduced pressure, and the residue, suspended in 4 mL of CH_2Cl_2, was treated with 200 μL of TEA followed by excess Ac_2O. The mixture was stirred at room temperature for 10 h, and then the solvent was removed *in vacuo*. The solid residue was purified by flash column chromatography (eluant: toluene, then toluene/ethyl acetate, 9:1), affording 9 mg (76%) of **4**: IR (KBr) (cm^{-1}) 1745, 1672, 1430, 1402, 575, 527; ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.63 and 6.25 (two s, 1H, *cis*/*trans*), 6.05 and 5.81 (two d, J = 11.6 Hz, 1H, *cis*/*trans*), 6.52 and 5.47 (two d, J = 11.6 Hz, 1H, *cis*/*trans*), 3.98 and 3.90 (two s, 3H, *cis*/*trans*), 2.58 and 2.53 (two s, 3H, *cis*/*trans*); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 170.67, 170.26, 154.05, 153.85, 152.21, 147.54, 147.47, 146.48, 146.43, 146.36, 146.24, 146.20, 146.15, 145.72, 145.69, 145.65, 145.61, 145.44, 145.39, 145.37, 144.74, 144.64, 144.49, 144.46, 143.19, 143.08, 142.79, 142.75, 142.71, 142.23, 142.14, 142.09, 142.03, 141.88, 141.86, 141.79, 140.32, 139.79, 129.02, 128.21, 125.28, 71.01, 69.77, 68.85, 59.94, 52.74, 22.75; MALDI-MS $C_{66}H_9NO_3$ (MW = 863), *m/z* 864 [$(M + H)^+$]; UV−vis ($CHCl_3$) λ_{max} (ϵ) 693 (215), 428 (2620), 311 (29 600). Anal. Calcd for $C_{66}H_9NO_3$: C, 91.78; H, 1.05; N, 1.62. Found: C, 90.38; H, 1.03; N, 1.59.

Fmoc-Fpr-OBu^t (6). A solution of 72 mg (0.10 mmol) of C_{60}, 15.7 mg (0.52 mmol) of paraformaldehyde, and 26.3 mg (0.20 mmol) of glycine *tert*-butyl ester, prepared by catalytic hydrogenation of the corresponding Z derivative, in 100 mL of toluene was stirred at reflux temperature for 1.5 h. The solution was then poured on top of a SiO_2 column and eluted with toluene and toluene/ethyl acetate, 95:5. The fractions containing unreacted C_{60} were concentrated, affording 45 mg (66%) of pure material. The fractions containing amine **5a** were concentrated to ca. 20 mL and added to a solution of 27.5 mg (0.11 mmol) of Fmoc-Cl and 12 μL (0.11 mmol) of NMM in 1 mL of CH_2Cl_2. The mixture was stirred at room temperature for 12 h, and then the solvents were removed *in vacuo*. The solid residue was purified by flash chromatography (eluant:toluene), affording 9 mg (8%) of **6**: IR (KBr) (cm^{-1}) 1738, 1716, 1450, 1421, 1368, 1332, 756, 576, 528; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.81−7.75 (m, 4H), 7.44−7.35 (m, 4H), 6.27 and 6.04 (two s, 1H, *cis*/*trans*), 5.76−5.54 (m, 2H), 4.79−4.72 (m, 2H), 4.50−4.44 (m, 1H), 1.52 (s, 9H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 169.01, 168.96, 167.73, 167.44, 154.74, 154.55, 154.48, 152.76, 147.53, 147.39, 146.47, 146.36, 146.31, 146.19, 146.18, 146.11, 145.93, 145.76, 145.65, 145.53, 145.48, 145.43, 145.33, 145.31, 144.97, 144.60, 144.53, 144.49, 144.28, 143.74, 143.56, 143.19, 143.19, 143.08, 142.75, 142.70, 142.33, 142.22, 142.18, 142.13, 142.09, 142.06, 141.94, 141.77, 141.66, 141.40, 140.29, 140.25, 139.58, 137.05, 136.97, 135.86, 135.31, 135.14, 127.89, 127.21, 125.06, 120.14, 120.12, 84.03, 69.83, 69.61, 68.67, 68.32, 68.18, 59.01, 58.55, 47.26, 28.20; MALDI-MS $C_{82}H_{23}NO_4$ (MW = 1085), *m/z* 1086 [$(M + H)^+$]; UV−vis ($CHCl_3$) λ_{max} (ϵ) 694 (273), 429 (3130), 310 (35 800), 301 (38 200), 257 (123 600); CD ($CHCl_3$) λ_{max} ($\theta_T \times 10^{-3}$) 690 (2.27, −1.68), 656 (−2.11,

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1.97), 601 (−1.45, 1.31), 549 (−0.79, 0.66), 428 (−3.87, 3.32), 396 (2.88, −2.62), 343 (−5.38, 3.65), 319 (2.82, −2.96); Anal. Calcd for $C_{82}H_{23}NO_4$: C, 90.68; H, 2.13; N, 1.29. Found: C, 89.51; H, 2.02; N, 1.12.

Fmoc-Fpr-L-Ala-OEt (8a and 8b). To a solution of 27.8 mg (0.0256 mmol) of **6** in 2 mL of CH_2Cl_2 was added 2 mL of TFA, and the mixture was stirred at ambient temperature for 4 h. The solvent was removed *in vacuo*, and the residue was washed several times with methanol in a centrifuge tube and finally dried under reduced pressure. Carboxylic acid **7** was obtained in nearly quantitative yield: IR (KBr) (cm^{-1}) 3435, 1718, 1638, 1450, 1424, 1222, 1151, 757, 576, 528; ^{1}H NMR (250 MHz, DMSO/$CDCl_3$, 5:2) δ 7.86−7.80 (m, 4H), 7.42−7.36 (m, 4H), 6.19 (s, 1H), 5.75−5.42 (m, 2H), 4.86−4.40 (m, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 170.99, 154.57, 154.43, 150.72, 146.99, 146.89, 146.21, 145.94, 145.88, 145.84, 145.67, 145.60, 145.26, 145.12, 145.10, 145.03, 145.00, 144.90, 144.83, 144.79, 144.42, 144.14, 144.01, 143.87, 143.51, 143.42, 142.68, 142.55, 142.24, 142.19, 141.93, 141.78, 141.66, 141.59, 141.48, 141.25, 140.84, 139.73, 139.64, 139.09, 136.31, 135.70, 134.72, 127.69, 127.08, 125.14, 120.03, 67.65, 58.51, 57.90, 46.75; MALDI-MS $C_{78}H_{15}NO_4$ (MW = 1029), *m/z* 1075 [$(M + 2Na)^+$].

To a suspension of 26.4 mg (0.0256 mmol) of **7** in 5 mL of CH_2Cl_2 were added 5.2 mg (0.0384 mmol) of HOAt and 7.4 mg (0.0384 mmol) of EDC·HCl, and the mixture was stirred at room temperature for 15 min. A solution of 4.3 mg (0.0282 mmol) of HCl·H-L-Ala-OEt and 3 μL (0.0282 mmol) of NMM in 0.5 mL of CH_2Cl_2 was added and the solution stirred for 30 min. The solvent was removed under reduced pressure and the mixture of dipeptides **8a** and **8b** purified by flash column chromatography (eluant: toluene/ethyl acetate, 98:2). The fractions containing the dipeptide were concentrated, and the two diastereoisomers were separated by HPLC (C_{18}, see Instrumentation).

Fmoc-D-Fpr-L-Ala-OEt (8a): 7 mg (24%); IR (KBr) (cm^{-1}) 3405, 1718, 1688, 1509, 1221, 1151, 757, 576, 528; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.78−7.69 (m, 4H), 7.43−7.29 (m, 4H), 6.20 (broad s, 1H), 5.72 (d, J = 11.33 Hz, 1H), 5.59 (d, J = 11.33 Hz, 1H), 4.80−4.67 (m, 3H), 4.44 (t, J = 6.21 Hz, 1H), 4.28−4.20 (q, J = 7.31 Hz, 2H), 1.32 (d, J = 6.94 Hz, 3H), 1.30 (t, J = 7.31 Hz, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 172.36, 168.78, 154.59, 154.17, 152.63, 150.34, 147.54, 147.40, 146.42, 146.38, 146.34, 146.16, 146.12, 146.09, 145.83, 145.63, 145.60, 145.57, 145.52, 145.40, 145.37, 145.33, 144.98, 144.65, 144.58, 144.55, 144.39, 144.33, 143.70, 143.64, 143.14, 142.73, 142.65, 142.33, 142.28, 142.21, 142.19, 142.10, 142.04, 142.01, 141.95, 141.83, 141.73, 141.47, 141.40, 140.35, 140.23, 140.12, 139.66, 137.91, 136.74, 135.38, 134.99, 127.93, 127.89, 127.28, 127.24, 125.04, 120.18, 72.44, 70.22, 69.67, 68.17, 61.88, 58.89, 48.56, 47.30, 18.12, 14.17; MALDI-MS $C_{83}H_{24}N_2O_5$ (MW = 1128), *m/z* 1151 [$(M + Na)^+$]; UV−vis ($CHCl_3$) λ_{max} (ϵ) 696 (319), 429 (3070), 312 (34 300), 301 (36 800), 257 (115 300); CD ($CHCl_3$) λ_{max} ($\theta_T \times 10^{-3}$) 695 (−0.96), 658 (3.12), 605 (2.38), 515 (−0.86), 428 (3.78), 396 (−3.12), 384 (−2.36), 343 (6.80), 319 (−5.84).

Fmoc-L-Fpr-L-Ala-OEt (8b): 6 mg (21%); IR (KBr) (cm^{-1}) 3423, 1718, 1689, 1635, 1510, 1222, 1151, 757, 576, 528; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.77−7.69 (m, 4H), 7.43−7.27 (m, 4H), 6.05 (broad s, 1H), 5.73 (d, J = 11.70 Hz, 1H), 5.57 (d, J = 11.70 Hz, 1H), 4.90−4.66 (m, 3H), 4.41 (t, J = 6.21 Hz, 1H), 4.15−4.07 (q, J = 6.94 Hz, 2H), 1.50 (d, J = 7.31 Hz, 3H), 1.21 (t, J = 6.94 Hz, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 172.09, 168.96, 154.99, 154.05, 152.53, 150.28, 147.50, 147.40, 146.35, 146.33, 146.16, 146.09, 145.58, 145.56, 145.52, 145.37, 145.27, 144.90, 144.63, 144.54, 144.51, 144.40, 143.74, 143.60, 143.19, 143.07, 142.70, 142.67, 142.57, 142.29, 142.21, 142.10, 142.04, 141.98, 141.91, 141.80, 141.48, 141.38, 140.31, 140.17, 140.04, 139.76, 138.00, 136.88, 135.36, 134.88, 127.99, 127.94, 127.32, 127.27, 125.03, 120.14, 72.36, 70.50, 69.65, 68.16, 61.70, 58.81, 48.30, 47.30, 18.57, 14.16; MALDI-MS $C_{83}H_{24}N_2O_5$ (MW = 1128), *m/z* 1151 [$(M + Na)^+$]; UV−vis ($CHCl_3$) λ_{max} (ϵ) 696 (391), 429 (3620), 308 (38 000), 300 (41 000), 257 (127 900); CD ($CHCl_3$) λ_{max} ($\theta_T \times 10^{-3}$) 695 (0.85), 658 (−2.14), 603 (−2.00), 515 (0.58), 428 (−4.71), 394 (0.98), 383 (1.26), 343 (−7.12), 317 (3.79). Anal. Calcd for $C_{83}H_{24}N_2O_5$: C, 88.29; H, 2.14; N, 2.48. Found: C, 86.32; H, 1.96; N, 2.24.

Fmoc-L-Ala-Fpr-OEt (9a and 9b). A solution of H-Gly-OEt, prepared by treating 120 mg (0.86 mmol) of HCl·H-Gly-OEt with 95 μl (0.86 mmol) of NMM in 5 mL of CH_2Cl_2 at room temperature, was added to a solution of 300 mg (0.416 mmol) of C_{60} and 50 mg (1.67 mmol) of paraformaldehyde. The mixture was heated to reflux for 1 h, then brought to ambient temperature, poured on top of a chromatographic column, and eluted with toluene and then toluene/ethyl acetate, 95:5. The fractions containing unreacted C_{60} were concentrated, affording 120 mg (40%) of pure material. The fractions containing **5b** were concentrated to ca. 50 mL and added to a solution of 700 mg (1.16 mmol) of (Fmoc-L-Ala)$_2$O and 127 μL (1.16 mmol) of NMM in 40 mL of CH_2Cl_2. The mixture was stirred at room temperature for 3 days, and then the solvent was removed *in vacuo*. Diastereomeric dipeptides **9a** and **9b** were separated by flash column chromatography (eluant: toluene/ethyl acetate, 98:2).

Fmoc-L-Ala-D-Fpr-OEt (9a): 57 mg (12%); IR (KBr) (cm^{-1}) 3420, 1742, 1724, 1667, 1503, 1449, 1426, 1191, 758, 528; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80−7.30 (m, 8H), 6.62 (s, 1H, *cis*); 6.54 (s, 1H, *trans*), 6.15 (d, J = 11.7 Hz, 1H, *cis*), 5.90 (d, J = 8.0 Hz, 1H), 5.83 (d, J = 11.0 Hz, 1H, *trans*), 5.60 (d, J = 11.0 Hz, 1H, *trans*), 5.52 (d, J = 11.7 Hz, 1H, *cis*), 5.10−5.00 (m, 1H), 4.53−4.25 (m, 5H), 1.74 (d, J = 7.3 Hz, 3H), 1.27 (t, J = 6.6 Hz, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 172.26, 169.51, 155.61, 153.93, 153.52, 151.95, 151.89, 149.60, 147.56, 147.45, 147.06, 146.50, 146.40, 146.22, 146.15, 145.76, 145.62, 145.53, 145.44, 145.37, 144.70, 144.60, 144.54, 144.47, 144.37, 144.33, 143.87, 143.82, 143.64, 143.10, 142.76, 142.72, 142.23, 142.18, 142.09, 141.82, 141.32, 140.41, 140.34, 139.70, 136.07, 127.76, 127.11, 125.17, 120.02, 70.65, 69.56, 68.97, 67.20, 62.30, 58.89, 48.77, 47.17, 19.99, 14.20; MALDI-MS $C_{83}H_{24}N_2O_5$ (MW = 1128), *m/z* 1151 [$(M + Na)^+$], 1129 [$(M + H)^+$]; UV−vis ($CHCl_3$) λ_{max} (ϵ) 428 (3080), 312 (37 600), 301 (39 600), 256 (128 800); CD ($CHCl_3$) λ_{max} (θ_T x 10^{-3}) 690 (−1.71), 654 (2.07), 595 (1.77), 544 (1.34), 477 (1.28), 428 (4.55), 397 (−3.72), 347 (2.13).

Fmoc-L-Ala-L-Fpr-OEt (9b): 57 mg (12%); IR (KBr) (cm^{-1}) 3417, 1742, 1728, 1670, 1501, 1449, 1424, 1194, 758, 528; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82−7.30 (m, 8H), 6.66 (s, 1H, *trans*), 6.28 (s, 1H, *cis*), 6.02 (d, J = 11.7 Hz, 1H, *cis*), 5.94 (d, J = 8.0 Hz, 1H, *trans*), 5.77 (d, 1H, *cis*), 5.76 (s, 2H, *trans*), 5.56 (d, J = 11.7 Hz, 1H, *cis*), 5.15−5.10 (m, 1H, *trans*), 4.98−4.90 (m, 1H, *cis*), 4.53−4.25 (m, 5H), 1.72 (d, J = 7.3 Hz, 3H), 1.27 (t, J = 6.6 Hz, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 171.90, 169.54, 155.64, 153.79, 153.31, 152.00, 149.74, 147.56, 147.44, 146.48, 146.38, 146.35, 146.21, 146.14, 145.75, 145.59, 145.55, 145.45, 145.37, 145.32, 144.69, 144.58, 144.51, 144.45, 144.35, 144.29, 143.88, 143.70, 143.08, 142.78, 142.73, 142.69, 142.22, 142.17, 142.10, 142.07, 142.03, 141.83, 141.79, 141.31, 140.29, 139.68, 136.63, 135.94, 135.57, 135.26, 127.77, 127.11, 125.17, 120.03, 70.53, 69.87, 68.76, 67.27, 62.30, 58.83, 48.59, 47.13, 18.47, 14.18; MALDI-MS $C_{83}H_{24}N_2O_5$ (MW = 1128), *m/z* 1166 [$(M + K)^+$], 1151 [$(M + Na)^+$], 1129 [$(M + H)^+$]; UV−vis ($CHCl_3$) λ_{max} (ϵ) 428 (3380), 311 (34 800), 301 (36 800), 256 (118 200); CD ($CHCl_3$) λ_{max} ($\theta_T \times 10^{-3}$) 690 (1.88), 653 (−1.70), 596 (−1.42), 548 (−0.74), 474 (−1.03), 428 (−4.63), 396 (2.58), 341 (−3.71). Anal. Calcd for $C_{83}H_{24}N_2O_5$: C, 88.29; H, 2.14; N, 2.48. Found: C, 85.65; H, 1.83; N, 2.40.

***c*-(L-Ala-D-Fpr) (10a).** A solution of 17 mg (0.015 mmol) of **9a** and 0.9 mL of piperidine in 6 mL of CH_2Cl_2 was stirred at room temperature for 30 min, and then the solvent was removed *in vacuo*. The residue was purified by flash chromatography (eluant: toluene/2-propanol, 95:5), affording 5.7 mg (43%) of **10a**: IR (KBr) cm^{-1} 3435, 3378, 3255, 1678, 1431, 575, 528; ^{1}H NMR (200 MHz, $CDCl_3$/DMSO, 5:1) δ 8.67 (s, 1H), 6.09 (d, J = 12.20 Hz, 1H), 5.85 (s, 1H), 4.76 (d, J = 12.20 Hz, 1H), 4.39 (m, 1H), 1.66 (d, J = 6.93 Hz, 3H); ^{1}H NMR NOE (200 MHz, $CDCl_3$/DMSO, 5:1) H($^{\alpha}$CH-Fpr):{H($^{\beta}CH_3$-Ala)}, 2.0; H($^{\beta}CH_3$-Ala):{H($^{\alpha}$CH-Fpr)}, absent; MALDI-MS $C_{66}H_{88}N_2O_2$ (MW: 860), *m/z* 861 [$(M + H)^+$]; UV−vis ($CHCl_3$) λ_{max} (ϵ) 688 (234), 428 (3260), 311 (31 000), 255 (89 500); CD ($CHCl_3$) λ_{max} ($\theta_T \times 10^{-3}$) 574 (1.71), 481 (−1.26), 428 (12.28), 398 (−5.79), 364 (−3.31), 326 (−4.22), 311 (3.30), 303 (−1.62), 287 (9.59); λ_{calcd} 538, 516, 455, 4.04, 341, 315, 275, 253, 242.

***c*-(L-Ala-L-Fpr) (10b).**The synthesis was carried out as described for compound **10a**, starting from 17 mg (0.015 mmol) of **9b**. Derivative **10b**: 4.5 mg (35%); IR (KBr) (cm^{-1}) 3435, 3386, 3259, 1686, 1439, 575, 528; ^{1}H NMR (200 MHz, $CDCl_3$/DMSO, 5:1) δ 8.73 (s, 1H), 6.15 (d, J = 12.10 Hz, 1H), 5.95 (s, 1H), 4.85 (d, J = 12.10 Hz, 1H), 4.54 (m, 1H), 1.75 (d, J = 6.89 Hz, 3H); ^{1}H NMR NOE (200 MHz, $CDCl_3$/DMSO, 5:1) H($^{\alpha}$CH-Fpr):{H($^{\alpha}$CH-Ala)}, 2.3; H($^{\alpha}$CH-Ala):{H-($^{\alpha}$CH-Fpr)}, absent; MALDI-MS $C_{66}H_8N_2O_2$ (MW: 860), *m/z* 861 [(M

+ H)$^+$]; UV−vis ($CHCl_3$) λ_{max} (ϵ) 693 (279), 428 (3360), 312 (31 800); CD ($CHCl_3$) λ_{max} ($\theta_T \times 10^{-3}$) 575 (−1.81), 483 (1.11), 428 (−12.33), 398 (6.56), 366 (3.89), 326 (3.90), 312 (−2.27), 302 (2.58), 285 (−9.17); λ_{calcd} 538, 516, 455, 4.04, 341, 315, 275, 253, 242. Anal. Calcd for $C_{66}H_{88}N_2O_2$: C, 92.09; H, 0.94; N, 3.25. Found: C, 89.05; H, 0.93; N, 3.27.

Fulleroproline Derivatives 12a and 12b. A solution of 33 mg (0.046 mmol) of C_{60}, 5.5 mg (0.183 mmol) of paraformaldehyde, and 24.3 mg (0.091 mmol) of compound **11** (see supporting information for synthetic details) in 35 mL of toluene was heated to reflux for 30 min. The solvent was removed *in vacuo*, and the crude was subjected to HPLC analysis (see Instrumentation) for the determination of the diastereomeric excess: 94:6 ratio (de 88%). Purification by flash column chromatography (eluant: toluene/ethyl acetate, 95:5) afforded 1 mg (2%) of **12a**, 19 mg (42%) of **12b**, and 12 mg (36%) of unreacted C_{60}.

Derivative 12a: ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.30 (d, J = 8.54 Hz, 2H), 7.06 (d, J = 8.54 Hz, 2H), 5.06 (d, J = 8.96 Hz, 1H), 4.78 (s, 1H), 4.59 (dd, J =10.67 and 10.24 Hz, 1H), 4.43 (dd, J = 10.67 and 3.84 Hz, 1H), 4.10 (d, J = 8.96 Hz, 1H), 3.61 (m, 1H), 3.56 (m, 1H), 3.05 (dd, J = 13.23 and 8.54 Hz, 1H), 1.39 (s, 9H); ^{1}H NMR NOE (200 MHz, $CDCl_3$): H($^\alpha$CH-Fpr):{H($^\alpha$CH-tyrosinol)}, 15.6; H($^\alpha$CH-tyrosinol):{H($^\alpha$CH-Fpr)}, 6.0; MALDI-MS $C_{76}H_{21}NO_3$ (MW = 995), *m/z* 995 (M^+); CD ($CHCl_3$) λ_{max} ($\theta_T \times 10^{-3}$) 688 (−0.90), 590 (1.61), 490 (−0.59), 428 (6.00), 397 (−1.10), 388 (0.57), 363 (−2.14), 340 (−0.18), 321 (−3.53), 285 (2.80).

Derivative 12b: IR (KBr) (cm^{-1}) 1749, 1608, 1506, 1462, 1364, 1236, 1161, 576, 528; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.23 (d, J = 8.32 Hz, 2H), 7.00 (d, J = 8.32 Hz, 2H), 5.59 (s, 1H), 5.38 (d, J = 12.68 Hz, 1H), 4.91 (m, 1H), 4.74 (d, J = 12.68 Hz, 1H), 4.56 (dd, J = 11.10 and 3.96 Hz, 1H), 4.48 (dd, J = 11.10 and 8.72 Hz, 1H), 3.55 (dd, J = 13.87 and 5.15 Hz, 1H), 2.94 (dd, J = 13.87 and 8.72 Hz, 1H), 1.35 (s, 9H); ^{1}H NMR NOE (200 MHz, $CDCl_3$) H($^\alpha$CH-Fpr):{H($^\beta$CH$_2$-tyrosinol)}, 3.3; H($^\beta$CH$_2$-tyrosinol):{H($^\alpha$CH-Fpr)}, 1.0; ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 166.68, 155.46, 154.63, 153.41, 153.36, 151.43, 147.11, 147.07, 146.70, 146.31, 146.23, 146.18, 146.15, 146.02, 145.97, 145.91, 145.64, 145.56, 145.48, 145.44, 145.39, 145.31, 145.14, 145.12, 145.05, 144.49, 144.44, 144.41, 144.26, 143.10, 143.04, 142.75, 142.73, 142.66, 142.64, 142.39, 142.27, 142.12, 142.09, 142.07, 141.95, 141.91, 141.71, 141.36, 140.57, 140.20, 140.01, 139.72, 136.42, 135.11, 135.08, 135.03, 130.83, 129.68, 124.67, 78.69, 73.79, 73.01, 72.86, 69.88, 64.46, 52.49, 35.01, 28.86; MALDI-MS $C_{76}H_{21}NO_3$ (MW = 995), *m/z* 995 (M^+); UV−vis ($CHCl_3$) λ_{max} (ϵ) 694 (468), 429 (3990), 313 (55 400), 255 (162 000); CD ($CHCl_3$) λ_{max} ($\theta_T \times 10^{-3}$) 670 (−0.61), 627 (−1.68), 577 (−2.29), 428 (−9.80), 404 (−1.40), 391 (−1.58), 367 (0.56), 343 (−3.27), 309 (1.10), 276 (−11.97). Anal. Calcd for $C_{76}H_{21}NO_3$: C, 91.65; H, 2.13; N, 1.41. Found: C, 91.36; H, 2.14; N, 1.69.

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Supporting Information Available: Synthetic details for the preparation of chiral morpholin-2-one **11** (5 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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