Molecular Recognition by a Silica-Bound Fullerene Derivative

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Received February 17, 1997[⊗]

Abstract: Thermal ring opening of *N*-[3-(triethoxysilyl)propyl]-2-carbomethoxyaziridine in the presence of C_{60} produces a fulleropyrrolidine derivative which is then attached covalently to HPLC silica gel. The new chromatographic material is used to investigate binding affinities of potential hosts for the immobilized C_{60}. Exceptionally high size selectivities have been obtained for cyclic oligomeric compounds like calixarenes and cyclodextrins in organic and water-rich media, respectively. A number of rationally designed, helical-shaped peptides bind selectively to the grafted fullerene. The most tightly bound peptide carries two ferrocene moieties at the periphery of a hydrophobic binding cavity complementary in size to C_{60}.

Introduction

The chromatographic separation of complex fullerene mixtures, obtained by resistive heating of graphite, has required an increasing use of appropriate stationary phases. Size exclusion chromatography, or suitably modified silica columns, have been successfully employed. Accordingly, silica has been modified by introducing substituted arenes, phthalocyanins, or tetraphenylporphyrins.[1] The basic idea is that the aromatic portion of the stationary phase should efficiently interact with the fullerene cage, giving rise to supramolecular complexes whose relative stability, which determines the extent of the separations, is highly dependent on the electronic properties of the aromatic rings and on their spatial arrangement.

The opposite approach, namely, the use of fullerenes in the modification of HPLC chromatographic supports, has not received comparable attention. As a matter of fact, grafting a fullerene, or any suitable derivative, to a solid matrix not only would greatly facilitate the evaluation of relative affinities of potential hosts for the immobilized fullerene from the analysis of retention data but would also enable the use of solvents, in which fullerenes are not soluble, in the study of host−guest chemistry of fullerenes.

Since chromatography promptly responds to even subtle variations in the structure, shape, size, and dynamics of the analytes, C_{60}-based HPLC materials have practical implications in both separation science and supramolecular chemistry. Preliminary results in this direction have been recently reported.[2] Chlorosilane-functionalized C_{60} was used in the modification of silica for the preparation of an HPLC microcolumn, which was shown to separate a mixture of C_{60} and C_{70} and also a mixture of calixarenes.[2a,b] (3-Aminopropyl)silyl-bonded silica was employed to prepare a C_{60}-based stationary phase, which was utilized in the HPLC separation of haloaromatics.[2c]

In addition, recent reports on C_{60} derivatives as potential or effective antiviral agents and enzyme inhibitors suggest that C_{60}-containing chromatographic phases, tailored for biopolymers, may be useful as biological probes as well as in developing therapeutical agents.[3]

In this paper we report the preparation, characterization, and chromatographic applications of a new HPLC stationary phase, characterized by the presence of a fullerene derivative covalently linked to silica microparticles.

Results and Discussion

The potential utility of surface-linked fullerene materials[4] has recently resulted in the development of synthetic methodologies to covalently incorporate C_{60} into inorganic or organic matrices.[2,5] This has been achieved either by chemical modification of a matrix with a reagent able to add to an appropriate fullerene in solution or by preliminary introduction of a reactive function onto the C_{60} spheroid, followed by a bond-forming step between the modified fullerene and the solid support. We have adopted the second strategy employing a preformed, well-characterized fullerene-containing trialkoxysilane which smoothly reacts with silica microparticles, leading to a chemically homogeneous

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[⊗] Abstract published in *Advance ACS Abstracts,* July 1, 1997.

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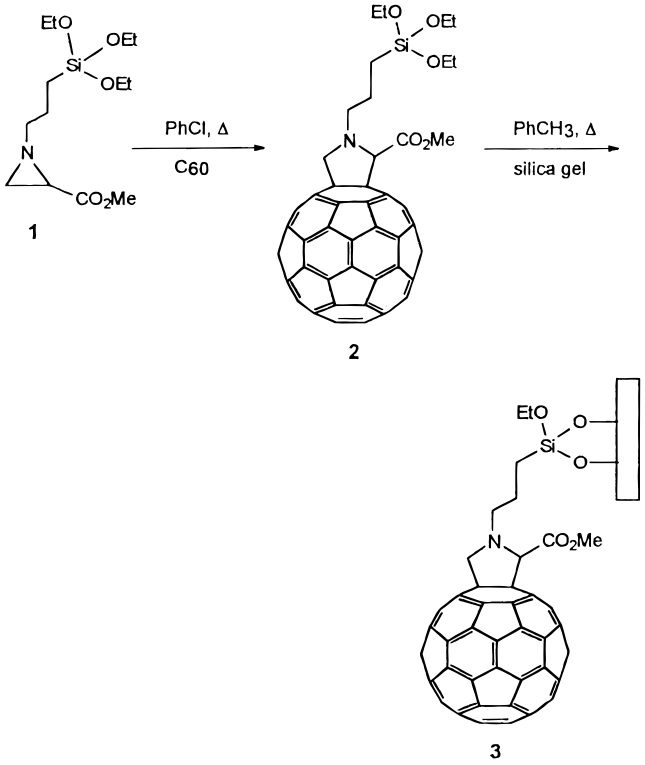
S0002-7863(97)00502-7 CCC: $14.00

Scheme 1



material with unequivocal structure (*vide infra*) that retains the inherent high chromatographic efficiency of the starting silica and presents only minimal modifications of the fullerene structure.

A versatile synthesis of fullerene derivatives relies on the 1,3-dipolar cycloaddition of azomethine ylides to C_{60}, a reaction that, under controlled conditions, affords fulleropyrrolidines as single and stable products of monoaddition across a 6,6 junction of C_{60}.[6] One way of generating azomethine ylides is the thermal ring opening of aziridines. This approach was chosen in the present work due to the easy scaling up of the reaction. Aziridine **1**, prepared from (3-aminopropyl)triethoxysilane and methyl 2,3-dibromopropionate (from methyl acrylate), was heated in chlorobenzene in the presence of C_{60}. The reaction gave rise to fulleropyrrolidine **2** in 36% isolated yield (71% based on C_{60} conversion), Scheme 1.

Compound **2** was fully characterized by IR, UV−vis, MALDI-MS, and ^{1}H and ^{13}C NMR techniques and its purity checked by HPLC and elemental analysis. In the proton spectrum, the pyrrolidine ring is readily recognized by the characteristic AB quartet of the methylene protons (4.1 and 5.0 ppm, 9 Hz) and by the singlet at 5.2 ppm for the methine proton. Due to the C_1 symmetry of **2**, all the carbon atoms of the fullerene spheroid are different. In the carbon spectrum, 39 (37 sp^2 and 2 sp^3) out of the possible 60 signals were detected due to some overlapping in the overcrowded aromatic region.

Reaction of derivative **2** with HPLC spherical silica particles in refluxing toluene afforded fulleropyrrolidine-functionalized silica **3** (Scheme 1). Elemental analysis of the vacuum-dried, dark brown silica **3** indicated a loading of 0.07 mmol (72 mg) of fulleropyrrolidine per gram of silica (based on 5.86% C) corresponding to a surface coverage of 0.4 μmol m^{-2}; FT-IR analysis of **3** showed distinctive bands at 2990−2940 cm^{-1} (C−H), 1738 cm^{-1} (CO_2Me), 1428 and 526 cm^{-1} (C_{60}), matching the corresponding absorptions of fulleropyrrolidine **2**. Thermal gravimetric analysis of **3** showed two initial weight losses (ca. 1.5% and 2.2%) in the 50−200 and 200−450 °C temperature ranges, the first due to removal of physically sorbed water and the second related to dehydroxylation of residual silanols and removal of unreacted Si-ethoxy groups; further heating to 950 °C resulted in a ca. 9.5% loss corresponding to degradation of the fulleropyrrolidine moiety, in fair agreement with elemental analysis.

Modified silica **3** was packed into a 250 × 1.8 mm stainless-steel column using standard procedures (see the Experimental Section). Preliminary chromatographic runs revealed some unique properties of **3** in that it allows efficient separations (*N/m* in the range (4−5) × 10^4) of simple aromatic solutes in both organic and water-rich media. With aqueous eluents, retention increases with the water content and solute hydrophobicity; i.e., the fulleropyrrolidine phase has a retention mode resembling that of typical reversed phase packings. However, different selectivities are expected toward more complex solutes capable of establishing multipoint contacts with the curved fulleroid surface of **3**.

It is known that calix[8]arenes form insoluble complexes with C_{60} from toluene, benzene or CS_2 solutions.[7] This prompted us to investigate the interactions between *tert*-butylcalix[*n*]arenes (hereafter calix[n]arenes) and **3** in organic solvents. When a mixture of calix[*n*]arenes (n = 4, 6, 8) was chromatographed on **3** using 100% toluene as eluent the six- and eight-membered calixarenes were almost coeluted (retention times 7.3 and 7.9 min, respectively) and well separated from calix[4]arene (retention time 2.0 min). By contrast, with a CH_2Cl_2/*i*-PrOH (99.5/0.5) mixture as eluent, **3** shows extraordinary size-selectivity in the form of a marked preference for calix[8]arene over the four and six-membered oligomers (Figure 1). It is interesting to note that, under these conditions, the underlying silica does not contribute to the observed selectivity, as all the solutes are front-eluted when chromatographed on a bare silica column.[8] In a study of the influence of eluent composition on retention, we noted that incremental additions of alcoholic modifiers (2-propanol or methanol) to the eluent cause the retention of calix-

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(8) Attemped resolutions of the three calixarenes on unmodified Hypersil silica were unsuccessful. However, we found that on LiChrosorb SiO_2 Si 100 (hexane/CH_2Cl_2 (50/50) as eluent) the capacity factors for the calix-[8]-, -[6]-, and -[4]arenes are 0.07, 0.27, and 0.47, respectively; i.e., the elution order is inverted compared to **3** for calix[4]- and calix[8]arenes.

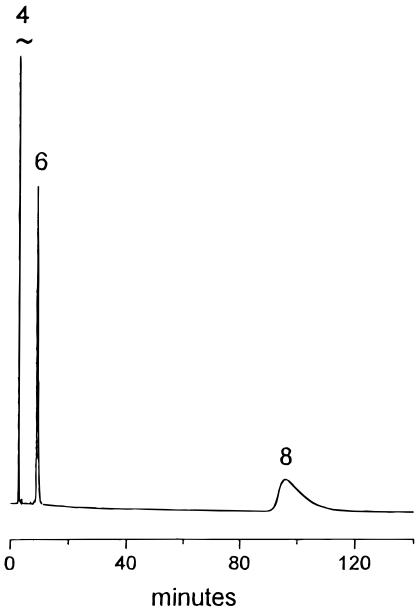

Figure 1. HPLC trace for the separation of *tert*-butylcalix[*n*]arenes on **3**. Eluent CH_2Cl_2/*i*-PrOH (99.5/0.5), flow rate 0.3 mL/min; $T = 25$ °C; UV detection at 280 nm. Numbers on top of the peaks denote the solute's ring size.

[8]arene to steadily *increase*, while retention of the other calixarenes was almost unaffected by the amount of alcohol in the 0−10% range in toluene or CH_2Cl_2 (Figure 2). These results can be best interpreted considering the structures of calix[8]-arene and of its complex with C_{60}: in the solid state, and presumably in solution as well, calix[8]arene adopts a nearly planar, "pleated-loop" conformation with the phenolic OH engaged in a circular array of H-bonds.[9] In the solid state complex between C_{60} and calix[8]arene,[7] the latter adopts an alternate-cone conformation with the original H-bond network partly disrupted. Assuming similar conformations in the solid state and in solution, the chromatographic behavior of calix[8]-arene on **3**, at variable alcohol concentrations, may be due to a favorable effect of the polar modifier on the conformational changes[10] and reorganization of the intramolecular H-bond system required for complexation with the grafted C_{60}.

The fulleropyrrolidine-bonded phase displays large size selectivities also in aqueous media as demonstrated by the separation of cyclodextrins shown in Figure 3. Several groups[11] have proved that γ-cyclodextrin is able to form a water soluble complex with C_{60}, while the smaller α- and β-cyclodextrins are not. Spectroscopic data are in favor of a 2:1 complex in which C_{60} is sandwiched between two γ-cyclodextrin molecules. The steric bulk of the substituted pyrrolidine ring in **3** and low concentration of cyclodextrin in the mobile phase make this kind of complex unlikely to form in our chromatographic system, where presumably complexation takes place with a 1:1 stoichiometry; despite this, **3** maintains a strong preference for the γ-cyclodextrin over the α- and β-cyclodextrins (Figure 3).

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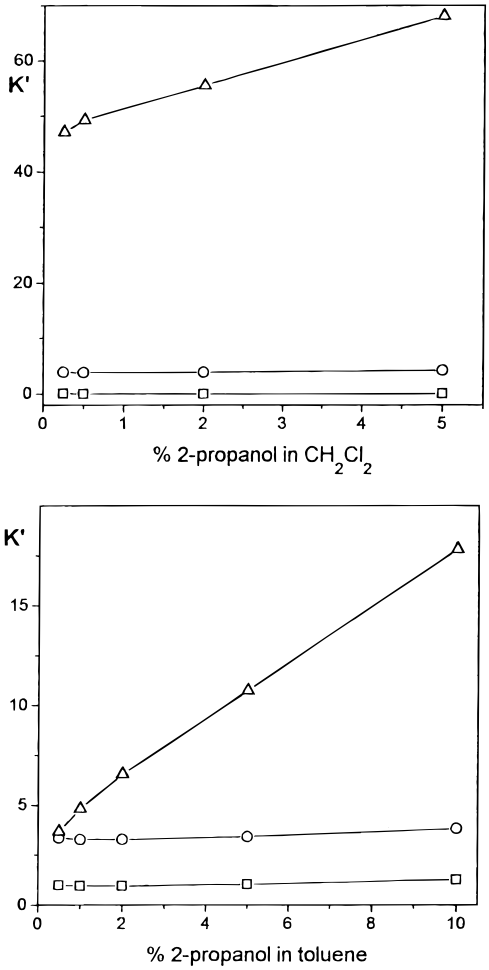

Figure 2. Retention as a function of the amount of 2-propanol in the eluent for calix[*n*]arenes on **3**. *K′* (capacity factor) defined as $(t - t_0)/t_0$ where t and t_0 are the retention time of the analyte and the void time, respectively. △, calix[8]arene; ○, calix[6]arene; □, calix[4]arene.

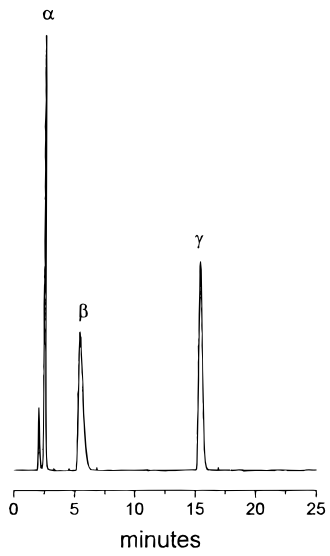

Figure 3. HPLC trace for the separation of α-, β-, and γ-cyclodextrins on **3**. Eluent 3 min isocratic H_2O/MeOH/THF (80/10/10) and then linear gradient to 40/30/30 in 25 min, flow rate 0.25 mL/min; $T = 25$ °C; evaporative light scattering detector, $P_{(air)} = 2$ atm, $T = 60$ °C.

As expected, none of the three cyclodextrins were retained on the octadecyl-bonded phase (ODS Hypersil) under the same conditions.

Since the fulleropyrrolidine-modified stationary phase size selectively recognizes tight-fitting partners in both organic and

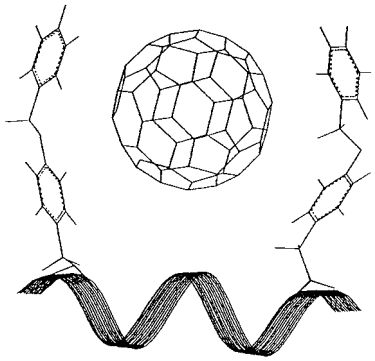

Figure 4. Schematic representation of the **P2**−C_{60} complex (see the text) obtained with the help of the Insight II program running on a Silicon Graphics workstation. The helical stripe is an exact geometrical reproduction of the 3_{10}-helical peptide backbone.

aqueous eluents, we were ready to explore the recognition ability of **3** toward small peptides containing hydrophobic cavities. These compounds, in fact, could be considered suitable models for the interactions between **3** and more complex biological systems possessing hydrophobic clefts.[3a] The peptides investigated were the side-chain-modified tyrosine nonapeptides **P1**-**P4**:

P1: Bz-Aib-L-Tyr(Me)-Aib_2-Gly-Aib_2-L-Tyr(Me)-Aib-OMe

P2: *p*BrBz-Aib-L-Tyr(Bzl)-Aib_2-Gly-Aib_2-L-Tyr(Bzl)-Aib-OMe

P3: *p*BrBz-Aib-L-Tyr(Me)-Aib_2-Gly-Aib_2-L-Tyr(Bzl)-Aib-OMe

P4: Bz-Aib-L-Tyr(ferrocenoyl)-Aib_2-Gly-Aib_2-L-Tyr(ferrocenoyl)-Aib-OMe

where Bz is benzoyl, *p*BrBz *p*-bromobenzoyl, Me methyl, Bzl benzyl, OMe methoxy, and Aib α-aminoisobutyric acid.

These terminally-blocked peptides are all characterized by a high content (66%) of the C^{α}-tetrasubstituted Aib residue which is known to significantly stabilize 3_{10}- and α-helical structures.[12] The 3_{10}-helix is preferred over the α-helix when the Aib content exceeds 50%.[12a] This expectation was fully confirmed by the X-ray diffraction analysis of **P1**.[13] The two Tyr residues, separated by five intervening amino acids, are located on the same side of the ternary, right-handed helix after two complete turns. This scaffold generates a cavity characterized by a distance between the two Tyr C^{β} atoms of 11.5 Å, where a C_{60} molecule may be accommodated (Figure 4). To avoid unfavorable steric interactions, we have incorporated a Gly residue, the least sterically demanding α-amino acid, on the same face of the helix between the two Tyr residues. Using this bistyrosyl, 3_{10}-helical peptide template, for the construction of C_{60} receptors, we also decided to exploit the known affinity of C_{60} for the (benzyloxy)benzyl[14] and ferrocenyl[15] moieties. Therefore, we have prepared the [Tyr(Bzl)^{2,8}]- and the [Tyr(ferrocenoyl)^{2,8}]-nonapeptides **P2** and **P4**, respectively (see the Supporting Information). For comparison, the related **P1** and **P3** nonapeptides were also synthesized.

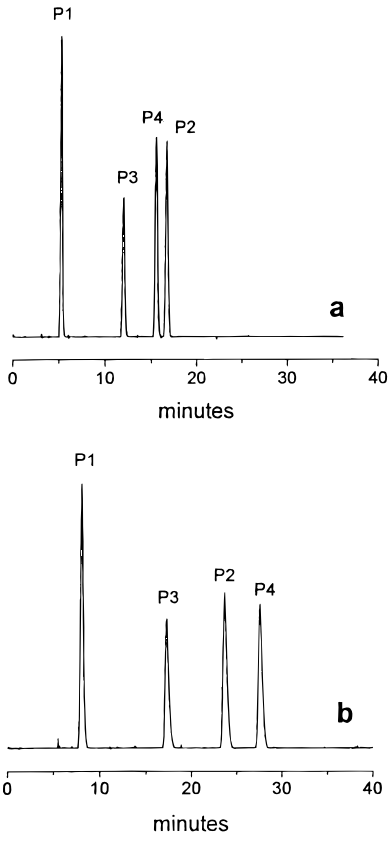

Figure 5. HPLC traces for the separation of peptides **P1**−**P4** on Hypersil ODS (a) and **3** (b). Eluent linear gradient from 50/25/25 to 10/45/45 $H_2O/CH_3CN/THF$ in 45 min, flow rate 0.25 mL/min; $T = 25$ °C; evaporative light scattering detector, $P_{(air)} = 2$ atm, $T = 60$ °C.

Binding affinities of **3** were screened against nonapeptides **P1**−**P4** in aqueous media using gradient elution. As shown in Figure 5b, the fulleropyrrolidine phase easily recognizes solute hydrophobicities and shows a marked preference for **P2** and **P4**, the peptides characterized by a hydrophobic cavity (see Figure 4). Of the latter two peptides, the ferrocene-containing **P4** is more retained, as expected on the basis of its stronger donor ability with respect to the benzyl group. Under identical conditions, an ODS phase revealed lower affinities for all the peptides and a preference for **P2** over **P4** (Figure 5a).

The above results indicate that the C_{60} spheroid of **3** interacts with (and discriminates between) the model peptide substrates more effectively than the ODS alkyl chains do. This effect can be expected considering the differences in the total area of hydrocarbons engaged in the binding processes. Moreover, the two phases show different selectivities for **P2** and **P4** which are distinguished, on the fulleropyrrolidine phase, by the π-bonding attitudes of the tyrosyl side-chain protecting groups. The novel shape and functional group selectivities of **3** underscored by these experiments originate from the ability of the curved surface of C_{60} to simultaneously establish $\pi-\pi$ and hydrophobic interactions.

Conclusions. We have described a new HPLC material incorporating a fullerene derivative that combines the unique size, shape, and function selectivity of C_{60} with the high efficiency and versatility of modern chromatographic media. On the new phase, solute dimensions dictate the retention behavior of macrocyclic compounds like calixarenes and

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cyclodextrins, while shape and functionality modulate the relative retentions of a series of protected peptides. Given the discrimination capabilities shown by **3**, we anticipate it will find additional applications in molecular recognition studies of C_{60} as well as in analytical issues requiring nonconventional selectivities.

As a final remark, the easy synthesis of optically pure fulleropyrrolidines[16] should allow the preparation of chiral fullerene-modified stationary phases potentially useful in enantioselective separations.

Experimental Section

Materials and Methods. Details regarding instrumentation used in this paper have been described elsewhere.[6] C_{60} was purchased from Bucky USA (99.5%). HPLC grade spherical silica (Hypersil, 5 μm particle diameter, 100 Å pore diameter, 170 $m^2\ g^{-1}$) was purchased from Shandon, U.K. All other reagents were used as purchased from Fluka and Aldrich. All solvents were distilled prior to use. Methyl 2,3-dibromopropionate was prepared by allowing methyl acrylate to react with bromine, according to published methodologies.[17] Tetrahydrofuran (THF), employed for UV−vis measurements, was distilled from $LiAlH_4$ prior to use.

***N*-[3-(Triethoxysilyl)propyl]-2-carbomethoxy aziridine (1).** A solution of 1.05 g (4.27 mmol) of methyl 2,3-dibromopropionate in 3.5 mL of dry benzene was cooled in an ice bath under nitrogen atmosphere. A solution of 1.0 mL (4.26 mmol) of (3-aminopropyl)-triethoxysilane and 1.2 mL (8.6 mmol) of triethylamine in 5 mL of dry benzene was added dropwise over a period of 20 min. The ice bath was removed and the mixture refluxed for 2 h, then filtered with suction over a pad of Celite, and washed with toluene. The filtrate was washed with water, and the organic layer, dried over Na_2SO_4, was concentrated under reduced pressure, affording 1.0 g (77%, purity >95% by GC−MS) of **1** as a clear oil that was used for the next reaction without further purification. ^{1}H NMR (200 MHz, C_6D_6): δ 0.76−0.82 (m, 2H), 1.06 (dd, 1H, J = 1.5 Hz, J = 6.2 Hz), 1.19 (t, 9H, J = 6.9 Hz), 1.78−1.88 (m, 2H), 1.94−2.16 (m, 1H), 2.08−2.10 (m, 2H), 3.35 (s, 3H), 3.81 (q, 6H, J = 6.9). ^{13}C NMR (62.5 MHz, C_6D_6): δ 8.48, 18.53, 23.48, 34.04, 37.37, 51.32, 58.44, 63.69, 170.96. GC−MS ($C_{13}H_{27}NO_5Si$ (MW = 305)): *m/z* 79 (76), 119 (64), 163 (86), 200 (46), 218 (100), 259 (26), 274 (8).

***N*-[3-(Triethoxysilyl)propyl]-2-carbomethoxy-3,4-fulleropyrrolidine (2).** A solution of 100 mg (0.14 mmol) of C_{60}, 167.6 mg (0.55 mmol) of *N*-[3-(triethoxysilyl)propyl]-2-carbomethoxyaziridine (**1**) in 100 mL of chlorobenzene was stirred at reflux temperature for 30 h, and then the solvent was removed in vacuo. The residue was purified by flash chromatography (eluant toluene, then toluene/ethyl acetate, (9/1), affording 51.5 mg (36%) of **2** along with 49.3 mg (49%) of unreacted C_{60}. FT-IR (KBr): 2972, 2924, 2886, 1755, 1736, 1462, 1431, 1387, 1167, 1103, 1087, 957, 575, 527 cm^{-1}. ^{1}H NMR (200 MHz, C_6D_6): δ 1.04−1.17 (m, 2H), 1.29 (t, 9H, J = 6.9 Hz), 2.20−2.27 (m, 2H), 2.91−2.95 (m, 1H), 3.33−3.40 (m, 1H), 3.47 (s, 3H), 3.95 (q, 6H, J = 6.9 Hz), 4.06, (d, 1H, J = 9.1 Hz), 5.01 (d, 1H, J = 9.1 Hz), 5.16 (s, 1H). ^{13}C NMR (62.5 MHz, C_6D_6): δ 8.65, 18.68, 22.61, 51.50, 55.04, 58.66, 65.0, 70.0, 73.20, 77.69, 126.97, 135.79, 136.50, 137.0, 138.23, 140.0, 140.19, 140.49, 140.56, 142.08, 142.11, 142.25, 142.36, 142.42, 142.59, 142.65, 142.93, 142.99, 143.39, 144.73, 144.78, 144.89, 144.94, 145.32, 145.58, 145.68, 145.75, 145.94, 146.16, 146.31, 146.39, 146.57, 147.55, 147.64, 151.86, 154.48, 155.17, 155.48, 170.34. UV−vis (THF): λ (ϵ) 254 (105 200), 308 (33 900), 429 (3300), 629 (230). MALDI MS ($C_{73}H_{27}NO_5Si$ (MW = 1025)) *m/z* 1025 [M^+]. Anal. Calcd for $C_{73}H_{27}NO_5Si$: C, 85.45; H, 2.65; N, 1.36. Found: C, 85.57; H, 2.51; N, 1.40.

Fulleropyrrolidine-Based Silica 3. A suspension of Hypersil, 5 μm (900 mg), in 20 mL of toluene was heated with stirring under a continuous stream of argon until the volume was reduced to ca. 15 mL. To this azeotropically dried mixture solid *N*-[3-(triethoxysilyl)-propyl]-2-carbomethoxy-3,4-fulleropyrrolidine (**2**) (185 mg, 0.18 mmol) was added, and the mixture was refluxed for 6 h. After being cooled to room temperature, the modified silica was collected by filtration and sequentially washed with 200 mL portions of toluene, chloroform, methanol, chloroform, and *n*-hexane and dried at 60 °C under reduced pressure. FT-IR (KBr): 2941, 2859, 1748, 1469, 527 cm^{-1}. Anal. Found: C, 5.86; H, 0.51; N, 0.10. Fulleropyrrolidine-based silica was packed in a 250 × 1.8 mm i.d. glass-lined, stainless steel column by the slurry procedure ($CHCl_3$, 600 atm); efficiency tests gave N/m > 47 000 for 1,3-dinitrobenzene eluting with hexane/$CHCl_3$ (90/10) and N/m > 40 000 for anthracene eluting with H_3O/CH_3CN (50/50) at a flow rate of 0.2 mL/min at 25 °C.

Acknowledgment. We thank Dr. Roberta Seraglia for MALDI-MS data and Mr. Stefano Zanellato for his help in working out detailed synthetic procedures. This work was in part developed within the *Progetto Strategico Materiali Innovativi* of the CNR.

Supporting Information Available: Characterization of compounds **P1**−**P4** (2 pages). See any current masthead page for ordering and Internet access instructions.

JA970502L

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