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Diastereoselective Synthesis of C_{60}/Steroid Conjugates

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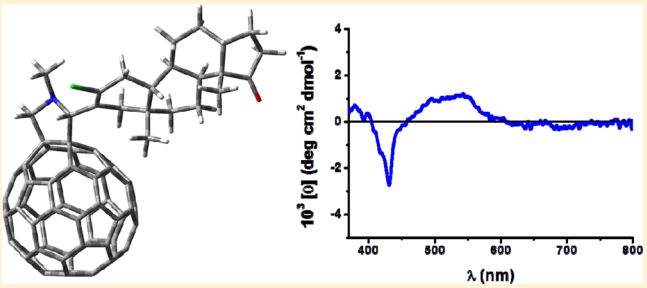
Supporting Information

ABSTRACT: The design and synthesis of fullerene−steroid hybrids by using Prato's protocol has afforded new fullerene derivatives endowed with epiandrosterone, an important naturally occurring steroid hormone. Since the formation of the pyrrolidine ring resulting from the 1,3-dipolar cyloaddition reaction takes place with generation of a new stereogenic center on the C2 of the five-membered ring, the reaction proceeds with formation of a diastereomeric mixture [compounds **6** and **7** in 70:30 ratio, **8** and **9** in 26:74 ratio (HPLC)] in which the formation of the major diasteroisomers **6** and **9** is consistent with an electrophilic attack of [60]fullerene on the *Re* face of the azomethine ylide directed by the steroidic unit. The chiroptical properties of these conjugates reveal typical Cotton effects in CD spectra that have been used to assign the absolute configuration of the new fulleropyrrolidines. The electrochemical study of the new compounds reveals the presence of four quasi-reversible reduction waves which are cathodically shifted in comparison with the parent C_{60}, thus ascertaining the proposed structures.



INTRODUCTION

Although a large number of reactions with C_{60} have been reported so far,[1] stereoselective additions have comparatively been less studied. The addition of azomethine ylides to C_{60} is one of the most powerful and versatile methods for derivatizing fullerenes.[2] In this regard, condensation of readily available starting materials such as α-amino acids and aldehydes gives rise to reactive 1,3-dipoles which efficiently add to C_{60} leading to a wide variety of functionalized fulleropyrrolidines.

Recently, we have carried out a straightforward procedure catalyzed by silver or copper acetate to efficiently obtain pyrrolidino[60]fullerenes with stereochemical control by enantioselective cycloaddition of *N*-metalated azomethine ylides to the C_{60} molecule.[3] This methodology was later extended to higher fullerenes, developing the first stereoselective cycloaddition of *N*-metalated azomethine ylides to C_{70}, thus achieving a selective control (site-, regio-, diastereo-, enantiocontrol) in the functionalization of higher fullerenes.[4] Furthermore, the stereoselective synthesis of 1,3-dipolar cycloadditions has also been extended to endohedral fullerenes, namely to a La@C_{72}($C_6H_3Cl_2$) derivative.[5] More recently, we have reported the stereodivergent syntheses of *cis*/*trans*-pyrrolidino[3,4:1,2]fullerenes and *endo*/*exo*-pyrrolidines with high enantioselectivity levels.[6]

The formation of fulleropyrrolidines by 1,3-dipolar cycloaddition reaction occurs through a planar intermediate azomethine ylides. Therefore, an additional chiral center on the α-amino acid or in the moiety containing the aldehyde group is thus required in order to achieve stereoselection. Thus, Prato et al. had previously applied this reaction to the preparation of chiral fulleroprolines, the absolute configurations of which were determined on the basis of experimental and calculated circular dichroism (CD) spectra.[7] The diastereoselective synthesis of fulleropyrrolidines endowed with enantiomerically pure functionalized cyclobutanes has also been previously reported.[8]

Recently, the synthesis of diastereoisomerically pure fulleropyrrolidines as chiral platforms for the design of optically active liquid crystals,[9] and the diasteroselective preparation of fulleroprolines by lithium salt-assisted cycloaddition of azomethine ylides have been reported.[10]

On the other hand, it is well-known that the main drawback for the potential use of C_{60} in biomedical applications stems from its lack of solubility in water and very poor solubility in most of the typical organic solvents.[11] The covalent linkage of

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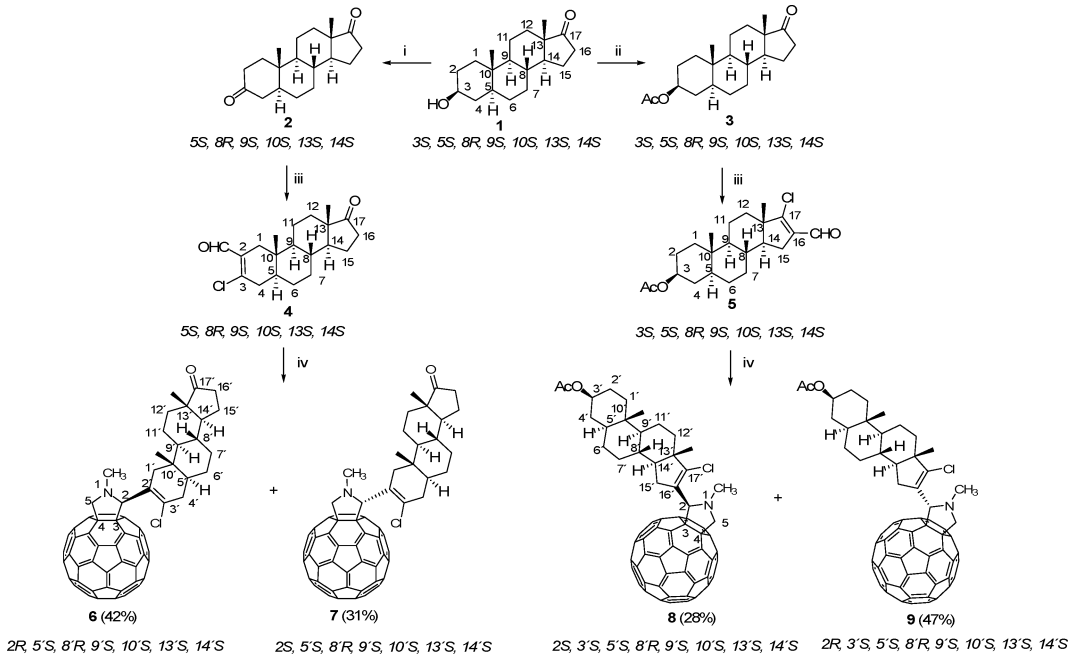
Scheme 1. Synthesis of Steroids−[60]Fullerenes 6−9[a]



[a]Key: (i) acetone, Jones' reagent, rt; (ii) acetic anhydride, pyridine, rt; (iii) $POCl_3$, DMF, reflux; (iv) C_{60}, *N*-methylglycine, toluene, reflux.

[60]fullerene to suitably funtionalized molecules is an efficient approach to generate functional entities in which each moiety modulates the properties and increases the solubility of the final compounds. Thus, [60]fullerene has been covalently connected to different bioactive entities such as aminoacids and peptides,[12] oligonucleoides,[13] steroids,[14] and imidazonium salts,[15] among others.

In a previous work, we have reported the design of hybrid fullerene−steroid derivatives by using the Bingel−Hirsch protocol. Interestingly, treatment of [60]fullerene with malonates bearing cholesterol, β-sitosterol, and ergosterol moieties afforded a variety of methanofullerene conjugates exhibiting solubility in different organic solvents.[16]

Continuing our research on the synthesis of new functionalized steroid−fullerene hybrids, in this paper we have chosen an important steroid to be used as steroid moiety, namely epiandrosterone. This steroid hormone has a weak androgenic activity, is the natural metabolite of dehydroepiandrosterone via the 5-α reductase enzyme, and has been shown to naturally occur in most mammals, including pigs.[17]

Furthermore, recent studies have shown that epiandrosterone inhibits the pentose phosphate pathway (PPP) and dilates isolated blood vessels precontracted by partial depolarization. These results suggest that it may act as an antagonist of L-type Ca^{2+} channel with properties similar to those of 1,4-dihydropyridine (DHP) Ca^{2+} channel blockers.[18] Also, this steroid can be used as a synthetic intermediate for obtaining a wide range of compounds with biological activity, such as aminosteroids, which find application as antiarhythmic, hypotensive, anti-inflammatory, and fungicidal activity.[19] Another example is the development of steroid-heterocyclic hybrid systems (pyridine, thiophene, thiazole, and pyrazole) which have potential antiepileptic, antiprotozoal, antitumor, and hypoglycemic bactericidal activities.[20]

Remarkably, there are very few examples of steroid−fullerene hybrids reported so far. One of the first reports being the synthesis of fullerene bisadducts endowed with a steroid molecule.[21] Other hybrids have also been prepared by Diels−Alder reaction from an steroidal diene and C_{60}[22] and by 1,3-dipolar cycloaddition to give fulleropyrrolidine derivatives.[14,23−25] The present investigation is directed to the search for functional hybrid molecules in which the characteristics of fullerenes may be changed, improving their solubility and biocompatibility, thus enabling further biological investigations.

Here we report on the design of a diasteroselective synthesis of new molecular hybrids through 1,3-dipolar cycloaddition between azomethine ylides (generated in situ) and C_{60} by treatment of the corresponding formyl-substituted steroid with sarcosine and [60]fullerene using Prato's protocol.

The aim is the preparation of new biological active fullerene−steroid hybrids which is an ongoing theme of research in our group.

RESULTS AND DISCUSSION

The fullerene steroids conjugates (**6**−**9**) were prepared in a multistep synthetic procedure in which the C_{60} unit has been connected to the steroid unit by Prato reaction from pristine [60]fullerene and the respective formyl-containing steroids. Thus, the first step required the preparation of formyl derivatives **4** and **5** as depicted in Scheme 1.

The convenient transformation of the hydroxyl group on C3 of the espiandrosterone (**1**) by oxidation gave the corresponding 5α-androstan-3,17-dione (**2**), whereas acetylation of **1** afforded 3β-acetoxy-5α-androstan-17-one (**3**), both in yields similar to those previously reported.[26,27] The Vilsmeier−Haack reaction of **2** and **3** with phosphorus oxychloride and dimethylformamide in dichloromethane led to the corresponding 3-chloro-2-formyl-17-oxo-5α-androstan-2-ene (**4**) and 3β-acetoxy-17-chloro-16-formyl-5α-androstan-16-ene (**5**), respectively. The reactions were monitored by TLC, and the final compounds were obtained as white solids in good and moderate yields (78% and 50%, respectively) after basic

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hydrolysis with aqueous sodium acetate and purification by flash chromatography with a cyclohexane−ethyl acetate (4:1) mixture as the eluent. The new compounds were fully characterized by analytical and spectroscopic techniques (see the Experimental Section and Supporting Information).

The ^{1}H NMR spectra of compounds **4** and **5** show the signals corresponding to the proton of the formyl group at ∼10 ppm. The principal differences in the spectrum of **4** in comparison with the unmodified steroid **2** resides in the disappearance of the signals corresponding to the methylene protons in C2 of the A ring, while the protons on C1 appear as two doublets at 1.75 and 2.56 ppm (J = 17 Hz), which are deshielded in comparison with the same protons in compound **2**. The rest of the signals are quite similar in position and multiplicity. The ^{13}C NMR spectrum of **4** showed the carbon of the formyl group at 191.35 ppm and the two new Csp^2 on ring A at 133.00 (C2) and 149.57 (C3) ppm.

NMR spectra of compound **5** showed the chemical transformation occurred in ring D. Signals corresponding to the protons attached to C16 in the ^{1}H NMR spectrum and the signal of the C═O group on C17 in the ^{13}C NMR spectrum disappear. Two new signals assignable to C16 and C17 at 136.42 and 162.60 ppm, respectively, are observed in the ^{13}C NMR spectrum, as well as the signal corresponding to the CHO group which appears at 188.15 ppm.

The chemical structures of the new compounds were ascertained by mass spectrometry. Under ESI conditions, compounds **4** and **5** show sodium adduct peaks at m/z = 357.2 $[M + Na]^+$ and 401.2 $[M + Na]^+$, respectively.

The synthesis of *N*-methyl-2-substituted pyrrolidino[3,4:1,2][60]fullerenes was carried out by 1,3-dipolar cycloaddition of the in situ generated azomethine ylides to C_{60} following Prato's procedure. Thus, a mixture of the corresponding chloroformyl steroid (**4** or **5**), C_{60}, and sarcosine (*N*-methylglycine) was heated at reflux in toluene under argon atmosphere for 6 h (see Scheme 1). The color of the solution changed from purple to brown, which confirms the formation of the products.

The HPLC chromatogram of the reaction mixture (toluene/acetonitrile 9:1; 1 mL/min) when using starting enantiopure **4** shows peaks at 8.71 and 9.33 min. As the cyclization to afford the pyrrolidine ring takes place with generation of a new stereogenic center on the C2 of the five-membered ring, and the configuration of the steroid is fixed, the reaction gives rise to a diasteromeric mixture of compounds **6** and **7** in 70:30 ratio (HPLC).

Under the same reaction conditions, **5** afforded the mixture of the diastereomers **8** and **9** with retention times under the same HPLC conditions of 5.29 and 7.03 min in 26:74 ratio (HPLC). In each of these chromatograms an additional peak, assigned to the C_{60} starting material, appears at 9.84 min, together with other minor peaks attributed to bis-adducts.

The mixture of diasteromers was easily separated by flash chromatography, initially with carbon disulfide, to elute unreacted C_{60}, followed by dichloromethane or toluene. Compounds **6**, **7**, **8**, and **9** were obtained in 42%, 31%, 28%, and 47% yields, respectively, as stable brown solids.

The relatively modest stereoselectivity (de = 42 and 47%), which provides diasteroisomers **6** and **9** as main products, is consistent with preferred electrophilic attack of [60]fullerene onto the *Re* face of the 1,3-dipole (Figure 1). This is probably due to a more stable and planar arrangement of the π conjugated system, dipole and steroid double bond, in a W shape with respect to the S conformation. In this W

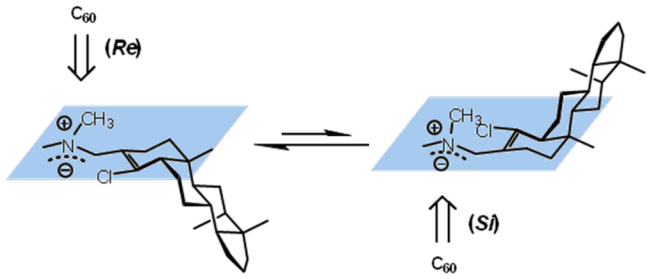

Figure 1. Plausible attack of C_{60} onto the *Re* less hindered face of the azomethine−steroid ylide.

conformation, the presence of the steroid group hampers the addition from the *Si* face and favors the attack of C_{60} onto the *Re* face giving rise to the corresponding two diastereoisomers in a 6:4 ratio.

^{1}H NMR spectroscopy revealed the formation of C_{60}−steroid hybrids. Besides the disappearance of the formyl proton (signal at δ ∼10 ppm) in the fulleropyrrolidines **6**, **7**, **8**, and **9**, new signals corresponding to the protons of the pyrrolidine ring appear at δ 4.16 and 4.90 (**6**), δ 4.18 and 4.94 (**7**), δ 4.65 and 4.90 (**8**), and δ 4.08 and 4.18 (**9**) as doublets ($J \approx 9$ Hz; geminal protons). The proton attached to C2 of the pyrrolidine ring resonated at δ 5.30 for **6** and **7** and ∼5.05 ppm for **8** and **9**. The *N*-methyl protons appear at 2.75 (**6**), 2.84 (**7**), 2.83 (**8**), and 2.80 (**9**) ppm. The other signals are in agreement with the data reported for steroids **4** and **5** (see the Experimental Section).

The number of signals observed in the ^{13}C NMR spectra reveal the lack of symmetry in these compounds. The ^{13}C NMR spectra of **6**−**9** show the presence of the signals of the 6,6-ring junction of the C_{60} framework at ∼69 ppm and ∼75 ppm. For compounds **6** and **7**, the signals of the carbons of the fulleropyrrolidine ring appear at ∼79 ppm (C2) and ∼70 ppm (C5), while these signals for compounds **8** and **9** appear at ∼76 and ∼69 ppm, respectively. The positions of the remaining steroid carbon atoms are relatively insensitive to the presence of the C_{60} cage, and there are no significant variations in the chemical shifts.

The structure of all compounds reported in this paper was determined unequivocally by combined NMR spectroscopic data from ^{1}H, ^{13}C, COSY, DEPT, HMQC, and HMBC experiments. Because we had achieved unambiguous assignments for the ^{1}H NMR resonances, the ^{13}C NMR resonances were assigned in a straightforward manner by analysis of the HMQC spectra for the protonated carbon atoms on the basis of chemical shift theory, substituent effects, and DEPT data. Quaternary carbon atoms were assigned by analysis of the HMBC spectra. It is interesting to note that the compounds showed similar trends in the chemical shifts of the common moiety of the molecular backbone, thus confirming their chemical structures (see the Experimental Section and Supporting Information).

MS allowed the proposed structures to be verified. The ESI spectrum for compound **6** shows a peak at m/z = 1082.2 which corresponds to protonated molecule $[M + H]^+$. The isolation and subsequent fragmentation of this ion under CDI conditions reveals that the retro-Prato reaction cannot take place due to the protonation of the nitrogen atom of the pyrrolidine ring, similarly to other results recently reported by us.[28] However, in the negative mode of detection, the corresponding odd-electron molecular ion $M^{\bullet-}$ at m/z = 1081.2 undergoes two different

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fragmentations. The first one corresponds to a loss of HCl forming a fragment at m/z 1045.2. The second fragmentation takes place via a retrocycloaddition process to form the parent fullerene ion, $C_{60}^{\bullet -}$, at m/z 719.9.

In the ESI positive mode of ionization, compound **7** showed a peak at 1082.2 corresponding to $[M + H]^+$. The retrocycloaddition reaction is not observed due to the protonation of the pyrrolidine nitrogen atom. The MALDI-TOF and ESI mass spectra for compounds **8** and **9** showed peaks at m/z = 1126.2, both corresponding to the $[M + H]^+$. The MS^2 spectrum of the both protonated molecule ions revealed a total inhibition of the retrocycloaddition reaction.[28] (For accurate mass values, see the Experimental Section and Supporting Information).

We have also investigated the possible existence, for each diasteroisomer, of atropoisomers as a result of a restricted rotation around the pyrrolidine−steroid bond. To this aim, we performed computational studies and variable-temperature NMR experiments on compound **6**. The ^1H NMR spectra recorded in CD_2Cl_2 over a range of temperatures (from −60 to 25 °C) showed only slight changes in the chemical shift of some signals, and no dynamic process could be appreciated. Even at −60 °C, the dynamic exchange between the two potential atropoisomers is fast enough on the NMR time scale so that the resulting spectrum represents the time average of both rotamers.

We have calculated by means of accurate quantum chemical methods (density functional theory) the torsion energy profiles resulting from single bond rotation around the pyrrolidine-steroid bond (fully relaxed and with a tight grid), which clearly allows distinguishing the existence of a pair of energy minima, **A** and **B**, at approximately 42 and 190° (see Figure 2). Note that intramolecular (noncovalent) dispersion interactions,[29,30] which are expected to highly influence the mutual orientation between the fullerene and steroid moieties, are explicitly considered in these calculations and that the cost-effective SVP basis set was considered here although the conclusions are not expected to vary upon further basis set extensions.

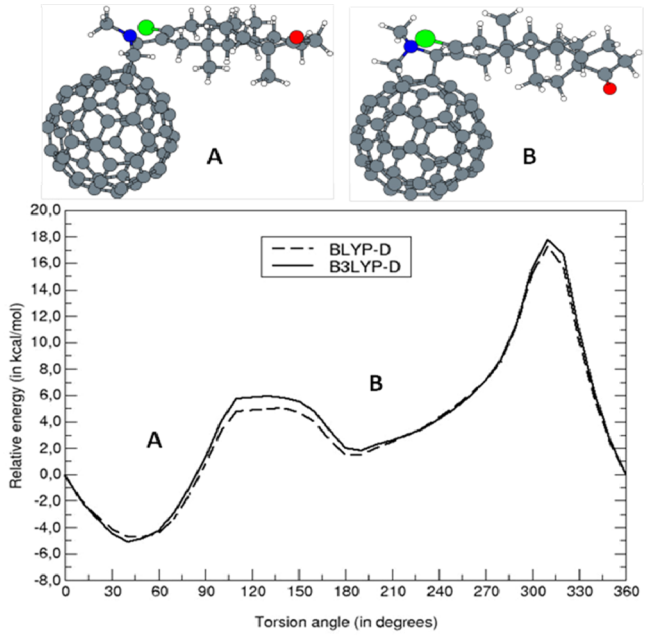

Figure 2. Torsion energy profiles of compound **6** calculated with various DFT methods.

The energy separation between these two rotamers converges to a value of 7 kcal/mol, independent of the method chosen, including highly sophisticated DFT-based extentions.[31,32] This is well below of the value reported for related fullerene derivatives which displayed the presence of atropoisomers. Indeed, Nierengarten et al. reported a slow dynamic exchange, observable with NMR, between two atropoisomers only at −40 °C for an energy barrier of 15 kcal/mol.[33]

The solution electrochemistry of the fullerene−steroid hybrids **6**−**9** was investigated by cyclic voltammetry (CV) and Osteryoung square wave voltammetry (OSWV). The redox potentials are summarized in Table 1.

Table 1. Redox Potentials of fullerene derivatives 6−9 vs Ferrocene in THF (V)[a]

compd	$E^1_{1/2,red}$	$E^2_{1/2,red}$	$E^3_{1/2,red}$	$E^4_{1/2,red}$
6	−1.02	−1.60	−2.25	−2.78[b]
7	−0.98	−1.55	−2.16	−2.65[b]
8	−0.98	−1.54	−2.17	−2.64[b]
9	−0.94	−1.51	−2.16[b]	−2.67[b]
C_{60}[c]	−0.90	1.49	−2.06	−2.56

[a]Working electrode: glassy carbon electrode; counter electrode: Pt; reference electrode: $Ag/AgNO_3$. Supporting electrolyte: $TBAPF_6$. Scan rate: 0.1 V s^{-1}. $E^{1/2} = (E^{pa} + E^{pc})/2$, where E^{pc} and E^{pa} = cathodic and anodic peak potentials, respectively. [b]From OSWV. [c]From ref 34, using $TBAClO_4$ as supporting electrolyte.

All fulleropyrrolidine derivatives exhibit four quasi-reversible reduction waves in the investigated solvent window (Figure 3).

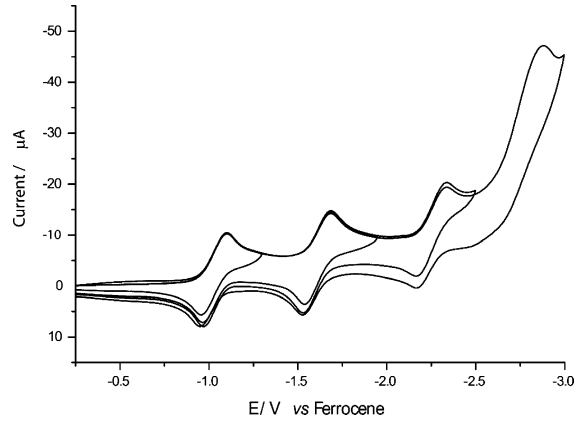

Figure 3. Cyclic voltammograms of **6** in THF, 0.1 M $TBAPF_6$, with a scan rate of 0.1 V/s.

As expected, these reduction values are cathodically shifted (ΔE = 0.12−0.04 V) in comparison with the parent C_{60},[34] a consequence of saturating a double bond on the C_{60} core, which raises the LUMO energy. Thus, the electrochemical studies further demonstrated the formation of fulleropyrrolidine monoadducts.

Furthermore, the absence of significant potential shifts with respect to other fulleropyrrolidine monoadducts indicate that there is essentially no effect of the steroidic unit on the electronic properties of the C_{60} core.[35]

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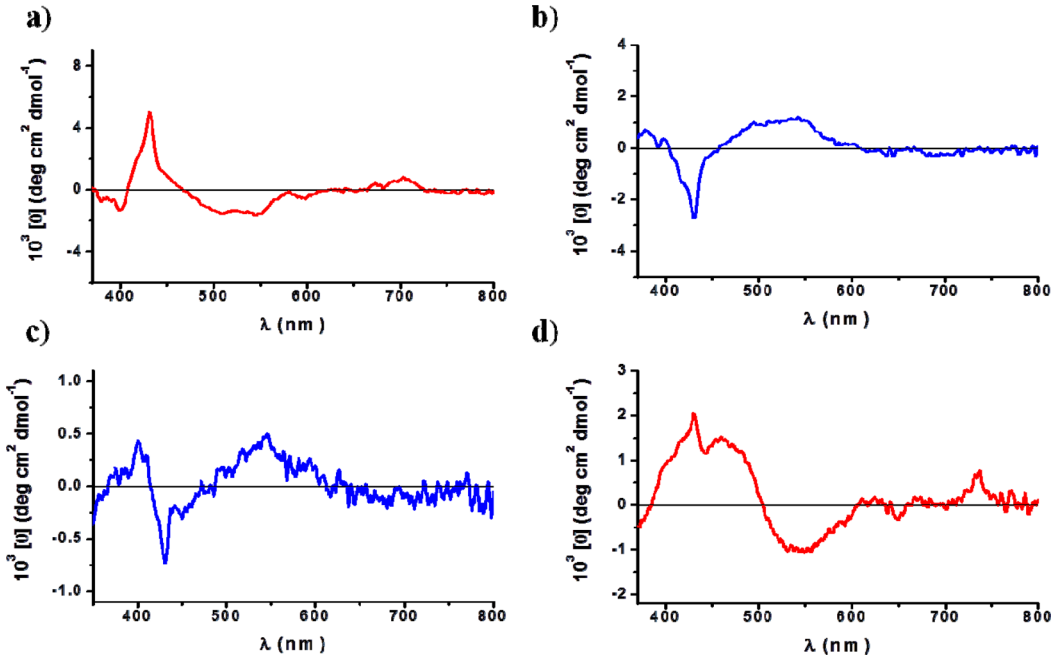

Figure 4. CD spectra of fulleroyrrolidines **6**–**9** (a–d) in CH_2Cl_2 (conc, 4×10^{-4} M).

Inspection of the CD spectra shows that diastereoisomeric pairs **6**, **7** and **8**, **9** give nicely opposite signed curves in the 430 nm region, thus confirming opposite configurations at the new stereogenic center (see Figure 4). This UV–vis band is considered to be the fingerprint for all fullerene monoadducts at 6,6 junctions (between two fused hexagons) regardless of the nature of the organic addend saturating the double bond.

In order to assign the absolute configuration at the new stereogenic carbon formed on C2 of the pyrrolidine ring, we used the sector rule,[36] proposed for fullerene derivatives,[37,38] which links the Cotton effect (CE) associated with this UV–vis band and the stereochemical environment around the 6,6 junction. This rule consists in a plane tangent to the C_{60} sphere at the attacked 6–6 single bond. This plane is in turn divided in four sectors by two other planes: one that goes through the 6–6 bond and the second one which bisects the 6–6 single bond (see Figure S45 in the Supporting Information). Because of the spheric symmetry of fullerenes, the "sector rule" can be easily used on mono functionalized fullerenes.

The pyrrolidinofullerene **6** endowed with and steroid moiety on C2 displays a positive Cotton effect at 430 nm. That means that the steroid moiety is located on the upper right or down left sector. The absolute configuration of the sterogenic carbon corresponds to *R* (Figure 5a and Figure S46, Supporting Information). On the other hand, a negative Cotton effect for **7** is consistent with the configuration having the biggest substituent on the upper left or down right sector. In this case, the configuration of the stereogenic carbon C2 can be designed by *S* (Figure 5b and Figure S47, Supporting Information). In this particular case, the steroid on the C-2 atom of the pyrrolidine ring allows the unambiguous application of this empirical rule, since this moiety has the greatest number of atoms.

A similar analysis was carried out with the steroid–fullerenes **8** (Figure S48, Supporting Information) and **9** (Figure S49, Supporting Information). Using this rule, we have determined the absolute configuration at C2 for compounds **6** (2*R*), **7** (2*S*), **8** (2*S*), and **9** (2*R*) (see Scheme 1 and the Supporting Information).

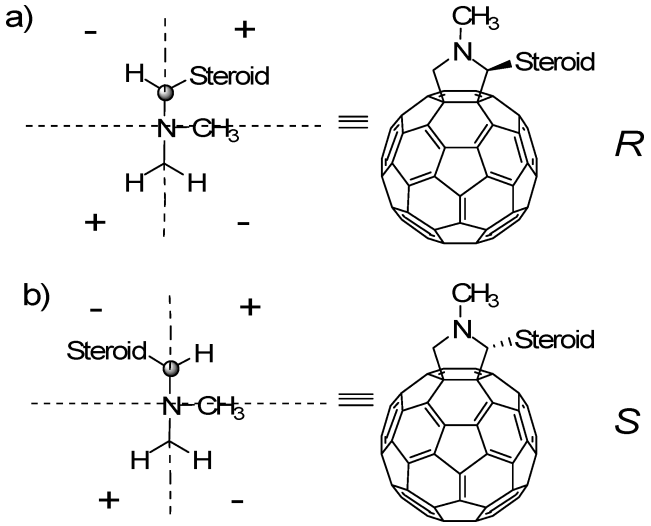

Figure 5. Assigned absolute configuration at C2 in compounds **6** (a) and **7** (b) using the sector rule.

Finally, we found that the conjugation of epiandrosterone derivatives to C_{60} improves the solubility to these new hybrids compounds (**6**–**9**) in organic solvents such as chloroform, dichloromethane, and dimethylformamide, among others. This fact will enable us to carry out further biological investigations.

CONCLUSIONS

We have carried out the synthesis of new [60]fullerene–steroid conjugates as functional hybrids (**6**, **7**, **8**, and **9**) by using a Prato reaction of the formyl group on steroids **4** and **5**. The reactions are sensitive to steric factors and C_{60} reacts with moderate diastereoselectivity with bulky chiral reagents. A thorough spectroscopic and analytical study has allowed the chemical structures of the new fulleropyrrolidines to be

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unambiguously determined. The resulting chiral adducts exhibit chiroptical properties, with typical Cotton effects in CD spectra that can be used to assign the absolute configuration of the fulleropyrrolidines. The presence of the steroid moiety in the new hybrid compounds (**6**, **7**, **8**, and **9**), improving their solubility, allows further biological investigations on these structures.

EXPERIMENTAL SECTION

General Methods. All reactions were performed using an atmosphere of argon and oven-dried glassware. Solvents were dried by standard procedures. All reagents were of commercial quality and were used as supplied unless otherwise specified. Reactions were monitored by thin-layer chromatography carried out on 0.25 mm silica gel plates (230−400 mesh). Flash column chromatography was performed using silica gel (60 Å, 32−63 μm). FTIR spectra were recorded in $CHCl_3$. ^{1}H NMR spectra were recorded at 700 MHz and ^{13}C NMR at 175 MHz; the one-bond heteronuclear correlation (HMQC) and the long-range ^{1}H−^{13}C correlation (HMBC) spectra were obtained by use of the inv4gs and the inv4gslplrnd programs. All HRMS-ESI and HRMS-MALDI (dithranol as matrix) experiments were carried out in negative and positive modes of detection. UV/vis spectra were recorded in $CHCl_3$. A high-performance liquid chromatography (HPLC) system (column dimensions, 4.6 × 250 mm; flow rate 1.0 mL min^{-1}, injection volume 15 μL, eluent toluene:acetonitrile 9:1) was used to determine the purity of the compounds synthesized. The retention times (t_R) reported were determined at a wavelength of 320 nm. Electrochemical measurements were performed with a three-electrode configuration system. The measurements were carried out with THF solutions [0.1 M in tetrabutylammonium hexafluorophosphate ($TBAPF_6$)]. A glassy carbon electrode (3 mm diameter) was used as the working electrode, and a platinum wire and an $Ag/AgNO_3$ electrode were employed as the counter and the reference electrode, respectively. Ferrocene (Fc) was added as an internal reference, and all of the potentials were determined relative to the Fc/Fc^+ couple. Both the counter and the reference electrodes were directly immersed in the electrolyte solution. The surface of the working electrode was polished with commercial alumina prior to use. Solutions were stirred and deaerated by bubbling argon for a few minutes prior to each measurements. Unless otherwise specified, the scan rate was 100 mV/s.

Theoretical Results. All calculations shown here were performed with the ORCA 2.8.0 package[39] and employ larger-than-default numerical thresholds. Furthermore, the RI- and COSX-based techniques were also used to alleviate the computational cost when needed. The BLYP, B3LYP, and B2-PLYP exchange-correlation functional, which allows to explore the dependence of the results upon sophistication of the underlying expressions, were always used with the corresponding dispersion correction (-D) for weakly overlapping molecular fragments.

Synthesis of Compounds. *5α-Androstane-3,17-dione (**2**).* This compound was prepared by following the method previously reported in the literature in 85% yield (1.7 g, 5.9 mmol), mp 130−132 °C (lit.[14] mp 129−131 °C).

*3β-Acetoxy-5α-androstan-17-one (**3**).* This compound was prepared by following the method previously reported in the literature in 80% yield (1.8 g, 5.4 mmol), mp 103−104 °C (lit.[26] mp 106−108 °C).

*3-Chloro-2-formyl-17-oxo-5α-androstan-2-ene (**4**).* To a stirred cold (0−5 °C) mixture of phosphorus oxychloride (1.6 mL) and dimethylformamide (1.3 mL) was added dropwise a solution of 5α-androstan-3,17-dione (**2**) (1.25 g, 4.3 mmol) in dichloromethane (20 mL). The reaction mixture was allowed to attain room temperature under argon for 18 h. The mixture was added to a solution of sodium acetate (40 g in 100 mL of water) and stirred at room temperature for 1 h. The mixture was extracted with dichloromethane, and the organic phase was washed twice with water and subsequently dried with anhydrous sodium sulfate. The organic extract was concentrated, and the solid obtained was purified by column chromatography using a mobile phase, cyclohexane−-ethyl acetate (4:1). The product was isolated as a white solid: yield 1.1 g, (3.3 mmol, 75%); mp 140−142 °C; IR ($CHCl_3$) ν 2923, 1738, 1676, 1625, 1448, 1149 cm^{-1}; ^{1}H NMR (700 MHz, $CDCl_3$) δ 0.76 (s, 3H, CH_3−C10), 0.83 (m, 1H, H9), 0.88 (s, 3H, CH_3−C13), 1.27 (m, 1H, H12), 1.27 (m, 1H, H14), 1.27 (m, 1H, H6), 1.42 (m, 1H, H11), 1.51 (m, 1H, H15), 1.57 (m, 1H, H5), 1.58 (m, 1H, H6), 1.62 (m, 1H, H8), 1.75 (d, J = 17.0 Hz,1H, H1), 1.75 (m, 1H, H11), 1.80 (m, 1H, H7), 1.84 (m, 1H, H12), 1.84 (m, 1H, H7), 1.90 (m, 1H, H15), 2.07 (m, 1H, H16), 2.35 (m, 1H, H4), 2.45 (m, 1H, H4), 2.44 (m, 1H, H16), 2.56 (d, J = 17.0 Hz,1H, H1), 10.18 (s, 1H, CHO); ^{13}C NMR (175 MHz, $CDCl_3$) δ 11.66 (CH_3-C10), 13.73 (CH_3-C13), 20.43 (C11), 21.73 (C15), 27.35 (C6), 30.18 (C7), 31.37 (C12), 34.88 (C8), 34.30 (C10), 35.76 (C16), 37.78 (C1), 40.24 (C4), 42.42 (C5), 46.63 (C13), 51.19 (C14), 56.43 (C9), 133.00 (C2), 149.57 (C3), 191.35 (CHO), 220.89 (C17); MS (ESI) 357.2 [M + Na]$^+$. Anal. Calcd for $C_{20}H_{27}ClO_2$: C, 71.73; H, 8.13. Found: C, 71.67; H, 8.20.

*3β-Acetoxy-17-chloro-16-formyl-5α-androstan-16-ene (**5**).* To a stirred cold (0−5 °C) mixture of phosphorus oxychloride (10 mL) and dimethylformamide (10 mL) was added dropwise a solution of 3β-acetoxy-5α-androstan-17-one (2 g, 6 mmol) in chloroform (40 mL). The reaction mixture was allowed to attain room temperature and then heated at reflux under argon for 5 h. The mixture was added to a solution of sodium acetate (40g in 100 mL of water) and stirred at room temperature for 1 h. The mixture was extracted with dichloromethane, and the organic phase was washed twice with water and subsequently dried with anhydrous sodium sulfate. The organic extract was concentrated, and the solid obtained was purified by column chromatography using as mobile phase cyclohexane−ethyl acetate (4:1). The product was isolated as a white solid: yield 1.5 g (3.9 mmol, 65%); mp 130−132 °C; IR ($CHCl_3$) ν 2939, 1732, 1673, 1587, 1243, 1025 cm^{-1}; ^{1}H NMR (700 MHz), $CDCl_3$) δ 0.78 (m, 1H, H9), 0.87 (s, 3H, CH_3-C10), 0.97 (s, 3H, CH_3−C13), 0.98 (m, 1H, H7), 1.05 (m, 1H, H1), 1.20 (m, 1H, H5), 1.30 (m, 1H, H6), 1.33 (m, 1H, H6), 1.37 (m, 1H, H4), 1.41 (m, 2H, H11, H12), 1.50 (m, 1H, H14), 1.51 (m, 1H, H2), 1.60 (m, 1H, H8),1.61 (m, 1H, H4), 1.72 (m, 1H, H1), 1.73 (m, 1H, H7), 1.83 (m, 1H, H2), 2.03 (s, 3H, CH_3-CO), 1.84 (m, 1H, H12), 2.04 (m, 1H, H15), 2.53 (m, 1H, H15), 4.70 (m, 1H, H3), 10.02 (s, 1H, CHO); ^{13}C NMR (175 MHz, $CDCl_3$) δ 12.30 (CH_3-C10), 15.20 (CH_3-C13), 20.60 (C11), 21.46 (CH_3-CO), 27.37 (C4), 27.37 (C2), 28.20 (C6), 28.30 (C15), 31.00 (C7), 32.90 (C12), 33.38 (C8), 35.80 (C10), 36.46 (C1), 44.76 (C5), 50.90 (C13), 53.61 (C14), 54.42 (C9), 73.43 (C3), 136.42 (C16), 162.60 (C17), 170.68 (COO), 188.15 (CHO); MS (ESI): 401.2 [M + Na]$^+$. Anal. Calcd for $C_{22}H_{31}ClO_3$: C, 69.73; H, 8.25. Found: C, 69.66; H, 8.18.

Synthesis of *N*-Methyl-2-substituted-pyrrolidino[3,4:1,2]-[60]fullerenes 6−9. A mixture of C_{60} (0.15 mmol), *N*-methylglycine (0.76 mmol), and the corresponding chloroformyl steroid **4** or **5** (0.17 mmol) in toluene (250 mL) under argon atmosphere was heated at reflux for 6 h. The color of the solution changed from purple to brown. The solvent was removed under reduced pressure, and the solid residue thus obtained was purified by column chromatography on silica gel, using CS_2 to elute unreacted C_{60} and dichloromethane to elute the corresponding pyrrolidino[3,4:1,2][60]fullerene. Additional purification of these compounds was carried out by repetitive precipitation and centrifugation using hexane, methanol, and diethyl ether as solvents.

*N-Methyl-2(R)-(3′-chloro-17′-oxo-(5α-antrostan-2′-en-2-yl)-pyrrolidino[3,4:1,2][60]fullerene (**6**).* HPLC: toluene/acetonitrile (9:1), flow rate 1 mL/min, t_R = 8.71 min. The purification of **6** was performed by column chromatography on silica gel with CS_2 and dichloromethane as the eluents: yield 63 mg (0.06 mmol, 42%); $[\alpha]^{20}_D$ = +185 (c 2 × 10^{-4} CH_2Cl_2); brown solid; UV/vis λ_{max} (log ε) = 425 (3.70), 325 (4.31), 258 (4.52) nm; IR ($CHCl_3$) ν 2961, 2919, 2850, 1737, 1447, 1261, 1098, 802, 753, 665 cm^{-1}; ^{1}H NMR (700 MHz, $CDCl_3$) δ 0.74 (m, 1H, H9′), 0.93 (s, 3H, CH_3-C13′), 0.96 (m, 1H, H7′), 1.06 (s, 3H, CH_3-C10′), 1.29 (m, 1H, H6′), 1.30 (m, 1H, H14′), 1.32 (m, 1H, H12′), 1.44 (m, 1H, H5′), 1.53 (m, 1H, H15′), 1.54 (m, 1H, H6′), 1.55 (m, 1H, H11′), 1.58 (m, 1H, H8′), 1.82 (m, 1H, H7′), 1.90 (m, 1H, H12′), 1.91 (m, 1H, H11′), 1.96 (m, 1H, H15′), 2.03 (d,

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J = 17.7 Hz,1H, H1′), 2.08 (m, 1H, H16′), 2.30 (m, 1H, H4′), 2.39 (m, 1H, H4′), 2.46 (m, 1H, H16′), 2.75 (s, 3H, CH_3-N), 2.95 (d, J = 17.7 Hz,1H, H1′), 4.18 (d, J = 9.4 Hz, H5 pyrrolidine ring), 4.90 (d, J = 9.4 Hz, H5 pyrrolidine ring), 5.30 (s, 1H, H2-pyrrolidine ring); ^{13}C NMR (175 MHz, $CDCl_3$) δ 11.98 (CH_3-C10′), 13.78 (CH_3-C13′), 20.74 (C11′), 21.80 (C15′), 27.54 (C6′), 30.12 (C7′), 31.44 (C12′), 35.36 (C10′), 35.80 (C8′), 35.85 (C16′), 39.33 (C4′), 40.39 (CH_3-N), 41.11 (C1′), 43.08 (C5′), 47.67 (C13′), 51.16 (C14′), 53.75 (C9′), 69.74 (Csp^3 C_{60}), 69.68 (C5 pyrrolidine ring), 75.45 (Csp^3 C_{60}), 79.67 (C2 pyrrolidine ring), 128.32 (C2′), 133.43 (C3′), 135.65, 134.92, 136.77, 139.72, 139.98, 140.05, 140.27, 141.74, 141.83, 141.93, 142.08, 142.15, 142.17, 142.19, 142.23, 142.25, 142.60, 142.65, 144.37, 142.69, 143.17, 143.06, 144.43, 144.53, 144.68, 145.16, 145.26, 145.31, 145.39, 145.48, 145.52, 145.65, 145.80, 146.01, 146.03, 146.06, 146.08, 146.19, 146.22, 146.39, 146.35, 146.64, 147.30, 153.75, 154.51, 154.54, 156.15, 221.14 (C17′); HRMS (ESI) $[M + H]^+$ = 1082.22424, calcd for $C_{82}H_{33}ClNO$ 1082.22451.

*N-Methyl-2(S)-(3′-chloro-17′-oxo-(5α-antrostan-2′-en-2-yl)-pyrrolidino[3,4:1,2][60]fullerene (**7**).* HPLC: toluene/acetonitrile (9:1), flow rate 1 mL/min, t_R = 9.33 min. The purification of **7** was performed by column chromatography on silica gel with CS_2 and dichloromethane as the eluents: yield 47 mg (0.04 mmol, 31%); $[\alpha]^{20}_D$ = +75 (c 2 × 10^{-4} CH_2Cl_2); brown solid; UV/vis λ_{max} (log ε) = 425 (3.70), 325 (4.31), 258 (4.52) nm; IR ($CHCl_3$) ν 2925, 2850, 1734, 1544, 1460, 1373, 1081, 1022, 868, 801, 667 cm^{-1}; ^{1}H NMR (700 MHz, $CDCl_3$) δ 0.56 (s, 3H, CH_3-C10′), 0.87 (s, 3H, CH_3-C13′), 0.90 (m, 1H, H9′), 1.04 (m, 1H, H7′), 1.25 (m, 1H, H6′), 1.31 (m, 1H, H12′), 1.32 (m, 1H, H11′), 1.32 (m, 1H, H14′), 1.51 (m, 1H, H8′), 1.52 (m, 1H, H15′), 1.57 (m, 1H, H6′), 1.72 (m, 1H, H5′), 1.84 (m, 1H, H7′), 1.85 (m, 1H, H11′), 1.87 (m, 1H, H12′), 1.97 (m, 1H, H15′), 2.10 (m, 1H, H16′), 2.23 (m, 1H, H1′), 2.29 (m, 1H, H4′), 2.41 (m, 1H, H4′), 2.48 (m, 1H, H16′), 2.84 (s, 3H, CH_3-N), 3.12 (d, J = 16.5 Hz,1H, H1′), 4.18 (d, J = 9.3 Hz, H5 pyrrolidine ring), 4.94 (d, J = 9.3 Hz, H5 pyrrolidine ring), 5.30 (s, 1H, H2 pyrrolidine ring); ^{13}C NMR (175 MHz, $CDCl_3$) δ 11.68 (CH_3-C10′), 13.86 (CH_3-C13′), 20.46 (C11′), 21.73 (C15′), 27.26 (C6′), 30.29 (C7′), 31.49 (C12′), 34.94 (C10′), 35.10 (C8′), 35.80 (C16′), 39.33 (C4′), 41.37 (CH_3-N), 41.41 (C1′), 43.10 (C5′), 47.70 (C13′), 51.70 (C14′), 54.30 (C9′), 69.89 (Csp^3 C_{60}), 70.02 (C5 pyrrolidine ring), 75.59 (Csp^3 C_{60}), 79.26 (C2 pyrrolidine ring), 128.50 (C2′), 133.40 (C3′), 135.41, 134.93, 136.34, 137.01, 139.87, 139.35, 140.11, 140.33, 141.52, 141.81, 141.88, 141.90, 142.06, 142.10, 142.13, 142.17, 142.19, 142.22, 142.26, 142.28, 142.61, 142.66, 142.70, 143.06, 143.11, 144.37, 144.44, 144.59, 145.17, 145.23, 145.32, 145.38, 145.42, 145.44, 145.65, 145.67, 145.88, 146.03, 146.06, 146.12,146.19, 146.23, 146.29, 146.39, 146.48, 146.95, 147.28, 147.33, 152.92, 154.69, 154.80, 156.02, 221.17 (C17′); HRMS (ESI) $[M + H]^+$ = 1082.22414; calcd for $C_{82}H_{33}ClNO$ 1082.22451.

*N-Methyl-2(R)-(3′β-acetoxy-17′-chloro-5α-androstan-16-en-2-yl)pyrrolidino[3,4:1,2][60]fullerene (**8**).* HPLC: toluene/acetonitrile (9:1), flow rate 1 mL/min−1, t_R = 5.29 min. The purification of **8** was performed by column chromatography on silica gel with CS_2 and toluene as the eluents: yield 44 mg (0.04 mmol, 28%); $[\alpha]^{20}_D$ = +90 (c 2 × 10^{-4} CH_2Cl_2); brown solid; UV/vis λ_{max} (log ε) = 425 (3.70), 325 (4.31), 258 (4.52) nm; IR ($CHCl_3$) ν 2923, 2852, 2786, 1730, 1628, 1245, 1028, 1245, 1026, 757, 660 cm^{-1}; ^{1}H NMR (700 MHz, $CDCl_3$) δ 0.67 (m, 1H, H9′), 0.76 (m, 1H, H7′), 0.87 (s, 3H, CH_3−C10′), 1.01 (td, J = 14.3 Hz, J = 3.7 Hz, 1H, H1′), 1.08 (s, 3H, CH_3−C13′), 1.16 (m, 1H, H5′), 1.20 (m, 1H, H12′), 1.30 (m, 2H, H6′), 1.30 (m, 1H, H14′), 1.35 (m, 1H, H4′), 1.37 (m, 1H, H11′), 1.48 (m, 1H, H2′), 1.59 (m, 1H, H4′), 1.60 (m, 1H, H8′), 1.61 (m, 1H, H11′), 1.68 (dt, J = 13.3 Hz, J = 3.3 Hz, 1H, H1′), 1.73 (m, 1H, H7′), 1.77 (m, 1H, H12′), 1.80 (m, 1H, H2′), 2.02 (s, 3H, CH_3-CO), 2.42 (m, 1H, H15′), 2.80 (m, 1H, H15′), 2.83 (s, 3H, CH_3−N), 4.18 (d, J = 9.7 Hz, 1H, H5 pyrrolidine ring), 4.65 (m, 1H, H3), 4.90 (d, J = 9.7 Hz, 1H, H5 pyrrolidine ring), 5.05 (s, 1H, H2 pyrrolidine ring); ^{13}C NMR (175 MHz, $CDCl_3$) δ 12.17 (CH_3-C10′), 15.54 (CH_3-C13′), 20.69 (C11′), 21.46 (CH_3-CO), 27.38 (C2′), 28.30 (C6′), 31.16 (C7′), 32.07 (C15′), 33.90 (C4′), 33.80 (C8′), 34.23 (C12′), 35.70 (C10′), 36.30 (C1′), 44.62 (C5′), 48.60 (CH_3-N), 48.90 (C13′), 54.45 (C9′), 54.96 (C14′), 69.76 (Csp^3 C_{60}), 69.99 (C5, pyrrolidine ring), 73.42 (C3), 75.95 (Csp^3 C_{60}), 76.16 (C2 pyrrolidine ring), 133.60 (C16′), 135.31, 135.75, 136.37, 136.70, 139.42, 140.07, 140.23, 141.59, 141.79, 141.86, 141.99, 142.05, 142.08, 142.14, 142.15, 142.19, 142.21, 142.24, 142.60, 142.66, 142.71, 143.05, 143.19, 144.39, 144.45, 144.65, 144.71, 145.19, 145.28, 145.35, 145.38, 145.39, 145.42, 145.45, 145.46, 145.51, 145.61, 145.80, 146.00 (C17′), 146.05, 146.08, 146.13, 146.21, 146.22, 146.26, 146.34, 146.38, 146.45, 146.60, 146.67, 147.31, 147.36, 152.89, 153.96, 154.14, 155.96, 170.64 (COO); HRMS-MALDI-TOF $[M + H]^+$ = 1126.245; HRMS-ESI $[M + H]^+$ = 1126.25446, calcd for $C_{84}H_{37}ClNO_2$ 1126.25128.

*N-Methyl-2(S)-(3′β-acetoxy-17′-chloro-5α-androstan-16-en-2-yl)pyrrolidino[3,4:1,2][60]fullerene (**9**).* HPLC: toluene/acetonitrile (9:1), flow rate 1 mL/min, t_R = 7.03 min. The purification of **7** was performed by column chromatography on silica gel with CS_2 and toluene as the eluents: yield 73 mg (0.06 mmol, 47%); $[\alpha]^{20}_D$ = +65 (c 2 × 10^{-4} CH_2Cl_2); brown solid; UV/vis λ_{max} (log ε) = 425 (3.70), 325 (4.31), 258 (4.52) nm; IR ($CHCl_3$) ν 2923, 2853, 2789, 1734, 1628, 1258, 1092, 1024, 801, 706 cm^{-1}; ^{1}H NMR (700 MHz, $CDCl_3$) δ 0.78 (s, 3H, CH_3-C13′), 0.86 (s, 3H, CH_3−C10′), 0.88 (m, 1H, H9′), 1.10 (m, 1H, H1′), 1.26 (m, 1H, H5′), 1.29 (m, 2H, H6′), 1.32 (m, 1H, H7′), 1.38 (m, 1H, H11′), 1.39 (m, 1H, H4′), 1.45 (m, 1H, H12′), 1.53 (m, 1H, H2′), 1.58 (m, 1H, H8′), 1.64 (m, 1H, H4′), 1.67 (m, 1H, H12′), 1.68 (m, 1H, H14′), 1.71 (m, 1H, H11′), 1.77 (m, 1H, H1′), 1.84 (m, 1H, H7′), 1.85 (m, 1H, H2′), 2.05 (s, 3H, CH_3-CO), 2.15 (m, 1H, H15′), 2.80 (m, 1H, H15′), 2.80 (s, 3H, CH_3-N), 4.72 (m, 1H, H3′), 4.08 (d, J = 9.0 Hz, 1H, H5-pyrrolidine ring), 4.18 (d, J = 9.0 Hz, 1H, H5-pyrrolidine ring), 5.02 (s, 1H, H2 pyrrolidine ring); ^{13}C NMR (175 MHz, $CDCl_3$) δ 12.29 (CH_3-C10′), 16.18 (CH_3-C13′), 20.94 (C11′), 21.43 (CH_3-CO), 27.54 (C2′), 29.94 (C6′), 32.17 (C15′), 31.54 (C7′), 33.77 (C12′), 34.07 (C4′), 34.16 (C8′), 35.85 (C10′), 36.70 (C1′), 40.38 (CH_3-N), 45.06 (C5′), 49.19 (C13′), 55.68 (C14′), 54.80 (C9′), 69.84 (Csp^3 C_{60}), 69.67 (C5 pyrrolidine ring), 71.51 (C3′), 75.54 (Csp^3 C_{60}), 76.49 (C2 pyrrolidine ring), 133.36 (C16′), 135.47, 135.80, 136.38, 136.88, 139.50, 140.11, 140.14, 140.32, 141.85, 141.90, 141,95, 142.05, 142.14, 142.20, 142.23, 142.30, 142.64, 142.68, 142.71, 142.73, 143.08, 144.39, 144.47, 144.61, 144.72, 145.19, 145.25, 145.35, 145.41, 145.48, 145.53, 145.64, 145.78, 146.04, 146.06, 146.09, 146.12, 146.23, 146.31, 146.35, 146.37, 146.54, 147.31 (C17′), 147.49, 153.36, 154.07, 154.31, 155.09, 170.45 (COO); HRMS-ESI $[M + H]^+$ = 1126.25072, calcd for $C_{84}H_{37}ClNO_2$ 1126.25128.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Spectral data for all compounds, HPLC chromatograms of the reactions mixture and pure products, absolute configuration assignement, and *xyz* coordinates (B3LYP-D3/SVP) of **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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