A Platinum Open Access Journal for Organic Chemistry

Review

Free to Authors and Readers | DOAJ Seal | *Arkivoc* **2022**, part i, 140-164

Steroid-fullerene hybrids: a review

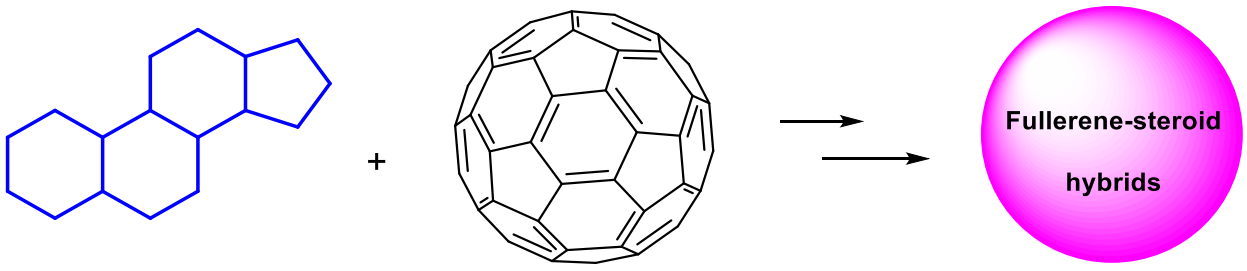
Malika Ibrahim-Ouali,*[a] and Frédéric Dumur[b]

[a]*Aix Marseille Univ, CNRS, Centrale Marseille, iSm2, F-13397 Marseille, France*
[b]*Aix Marseille Univ, CNRS, ICR, UMR 7273, F-13397 Marseille, France*
Email: malika.ibrahim@univ-amu.fr

Received 01-30-2022 **Accepted** 03-17-2022 **Published on line** 04-20-2022

Abstract

Among carbon-based materials, fullerenes have emerged as very fascinating nanocarbons on account of the interesting properties and applications of some of their derivatives in medicine or materials chemistry. The low solubility of C_{60}, the most famous family member, has limited its use in medicinal chemistry but its covalent functionalization with different moieties such as porphyrins or other bioactive molecules changes its physicochemical properties, such as its solubility and biocompatibility. New hybrid compounds, bearing fullerene and steroid units in the same molecule can be regarded as promising functional chimeras with encouraging biomedical applications and have become a topic of considerable interest in the last few years. Recent developments in the syntheses of steroid-fullerene hybrids are described herein.



Keywords: steroid; C_{60}-fullerene; hybrid molecules; linkage.

Table of Contents

1. Introduction

Steroids constitute an extensive and important class of biologically active polycyclic compounds that are widely used for therapeutic purposes.[1-3] Even after decades of research, the total synthesis of steroid nuclei by improved strategies continues to receive considerable attention. Numerous methods[4,5] have been exploited for the total synthesis of steroids which are widely distributed in nature, and which possess practical medical importance. Research into steroid total synthesis continues to this day.

The C_{60} fullerene is the most common fullerene. Since its discovery, it is at the origin of many studies in various fields.[6] Indeed, the family of fullerenes and especially its main representative, C_{60}, has exceptional physicochemical properties at the origin of their interest. These compounds have unique optical,[7-9] electrophysical,[10-12] mechanical,[13-15] tribological,[16-18] sorption[19-22] and biological[23-25] properties. New dyes and catalysts[26-30] have even been discovered among functionalized fullerenes. The use of functionalized fullerenes in the field of medical chemistry, in particular for the treatment of significant diseases, seems interesting because of their wide range of biological activity.

In recent years, hybrid systems have grown in importance due to their many applications in medicine and drug development. Hybrid systems are formed from different molecular entities, natural or not, to obtain functional molecules in which the characteristics of various moieties are amplified or modulated or lead to completely new properties. These hybrids can be made from carefully selected components, either through direct covalent bonds or through the integration of key functional characteristics.

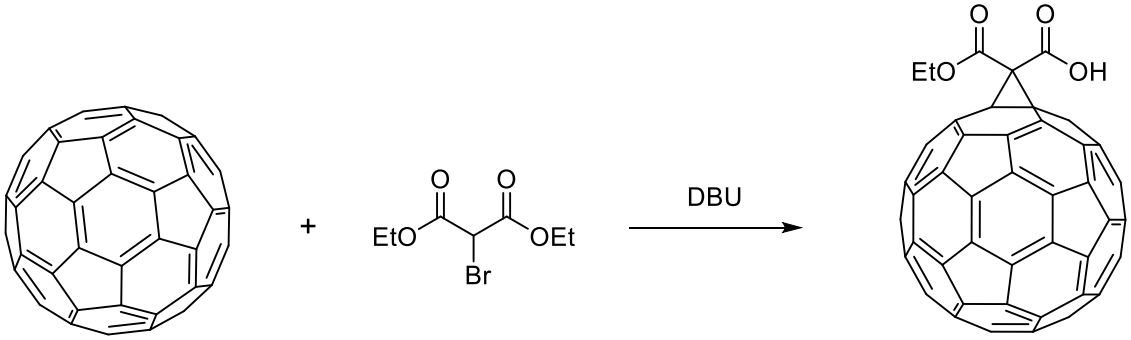
These promising molecules make it possible to generate new molecular entities by intelligently combining two or more different classes of compounds of synthetic or natural origin. The interest is to be able to mix the structural characteristics of two or more biologically active molecules in the same molecule or their covalent coupling can improve the characteristics of the individual components or reveal new properties. In all cases, this approach is interesting because it offers a multitude of possibilities for generating a wide range of molecules for applications in biology, medicine and in the field of materials.

This article provides an overview of the various syntheses of steroid-fullerene hybrids along with interesting biological activities. To the best of our knowledge and much to our surprise, there are no reports on this subject.

2. Synthesis of Steroid-Fullerene Hybrids

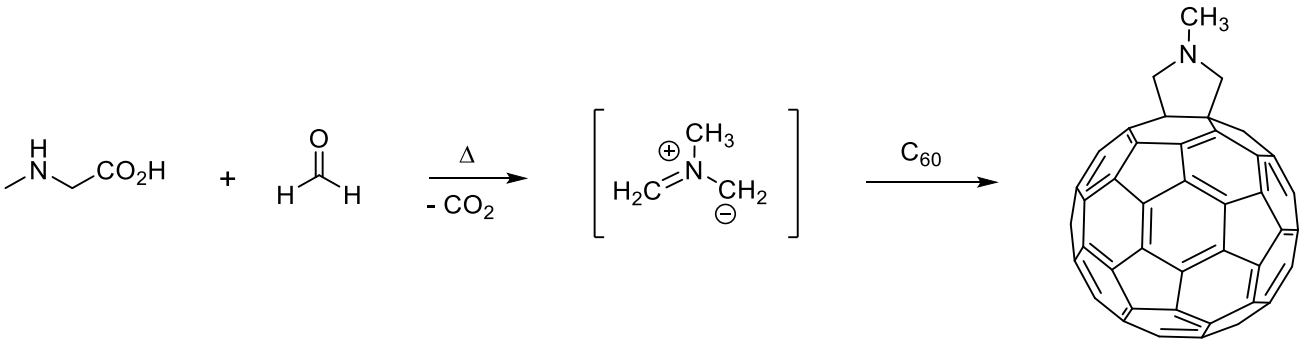
One of the main drawbacks to the potential use of C_{60} in medical applications is its lack of solubility in water and its very poor solubility in most conventional organic solvents.[31-32] Covalent binding of [60]fullerene to suitably functionalized molecules is an effective approach to overcome this problem of solubility. Thus, steroids which are bioactive molecules have been covalently connected to C_{60} fullerene by various authors.

Two main reactions were used to synthesize these systems: nucleophilic cyclopropanation using the Bingel protocol and thermal addition of steroidal compounds. The Bingel reaction is a fullerene cyclopropanation reaction to a methanofullerene, first discovered by C. Bingel[33] with the bromo derivative of diethyl malonate in the presence of a base such as sodium hydride or DBU (Scheme 1).



Scheme 1. The Bingel reaction.

In 1999, Schuster and coworkers[34] reported the synthesis of steroid-fullerene hybrids using the Prato reaction.[35] The Prato reaction is a particular example of the well-known 1,3-dipolar cycloaddition of azomethine ylides to olefins. The amino acid *N*-methylglycine reacts with paraformaldehyde when heated at reflux in toluene to provide an ylide which reacts with a double bond in a 6,6-ring position of the fullerene *via* a 1,3-dipolar cycloaddition to yield a pyrrolidinofullerene (Scheme 2).



Scheme 2. The Prato reaction.

Here, the Prato reaction[35] was used to synthesize the steroidal fulleropyrrolidine precursors **1**, **2**, and **3** (Figure 1).

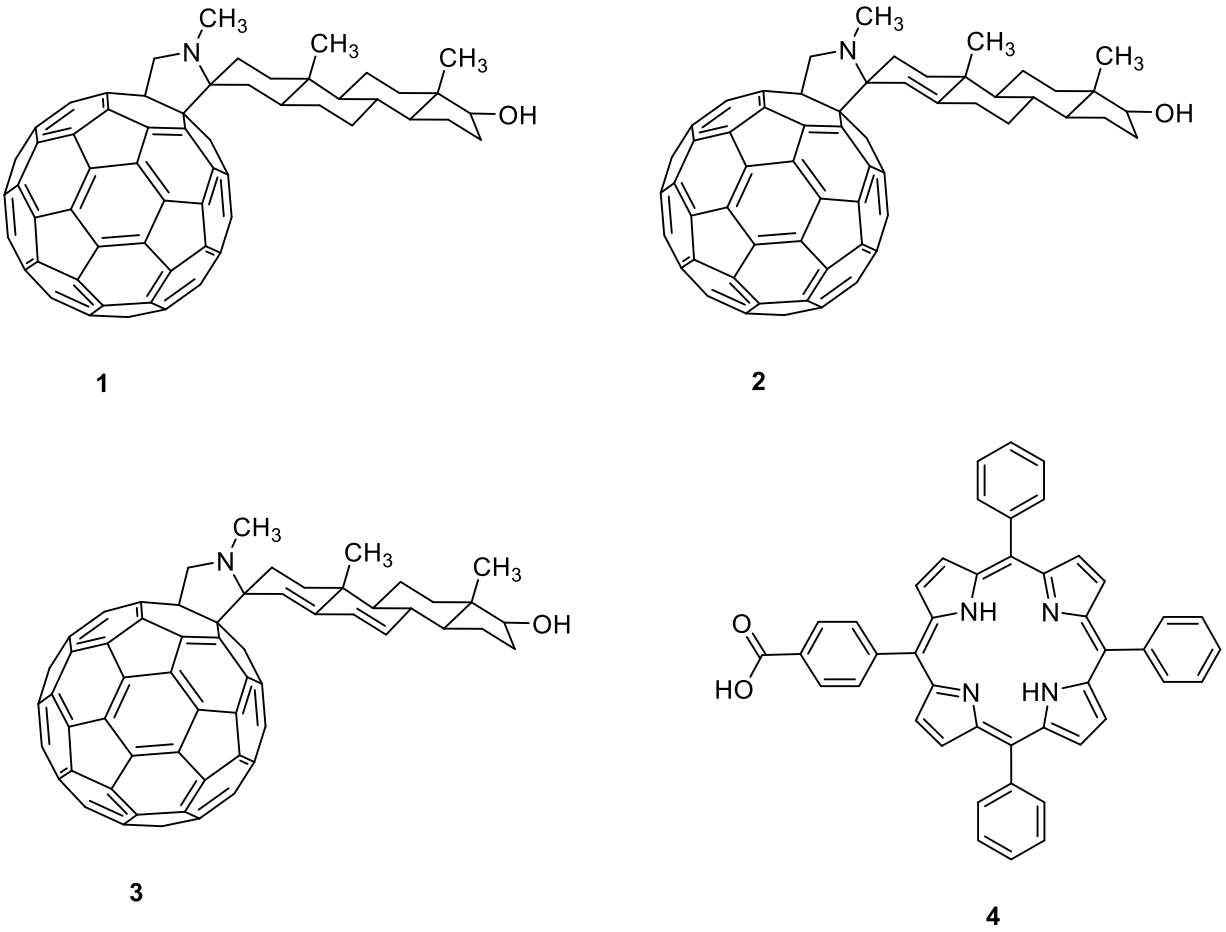


Figure 1. Synthesis of steroidal fulleropyrrolidine precursors **1**, **2**, and **3** and the tetraphenylporphyrin carboxylic acid **4**.

Union of these precursors with tetraphenylporphyrin carboxylic acid **4** was realized through a standard EDCI coupling to furnish the fullerene-steroid hybrids **5**-**7** (Figure 2).

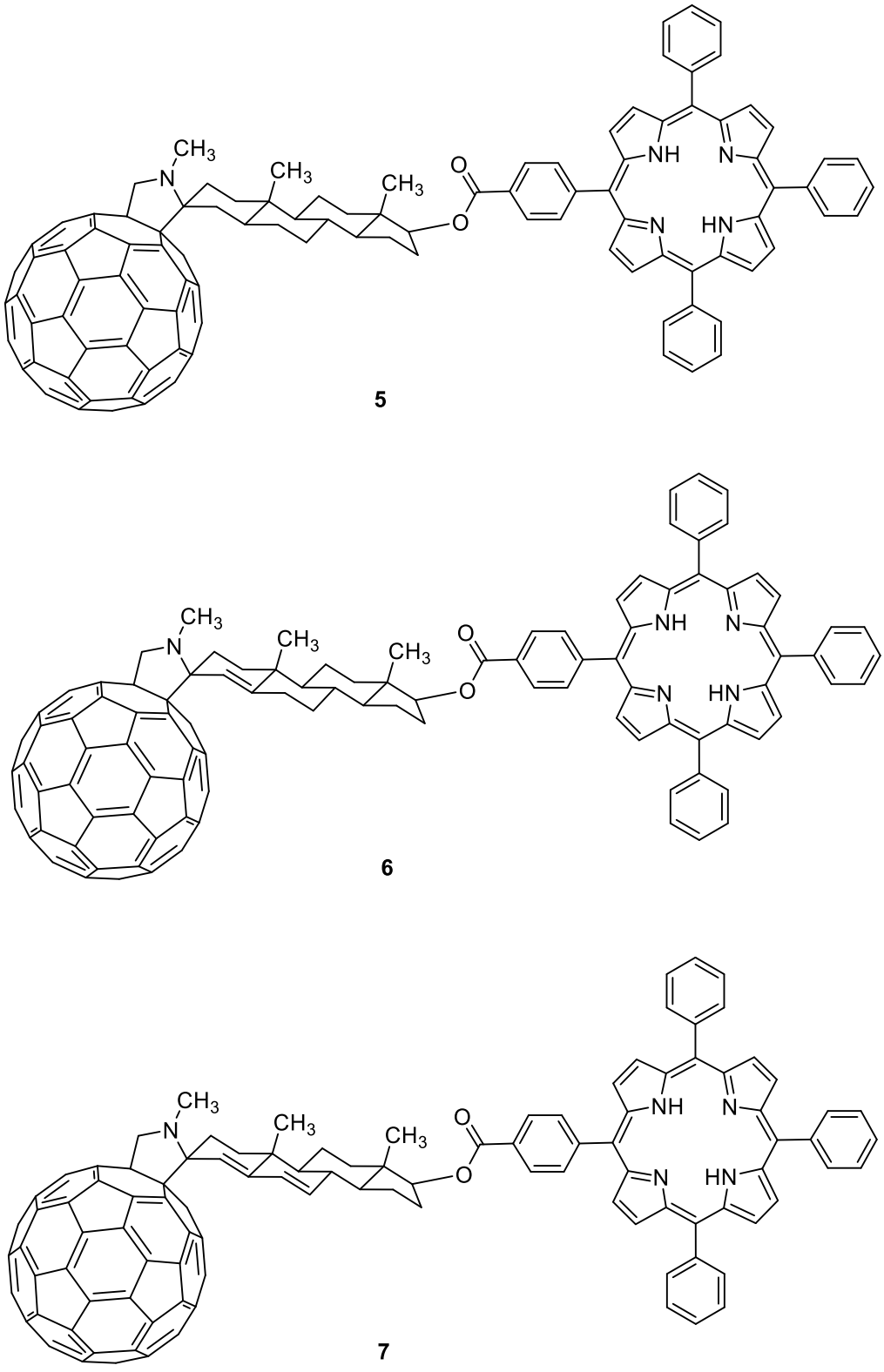
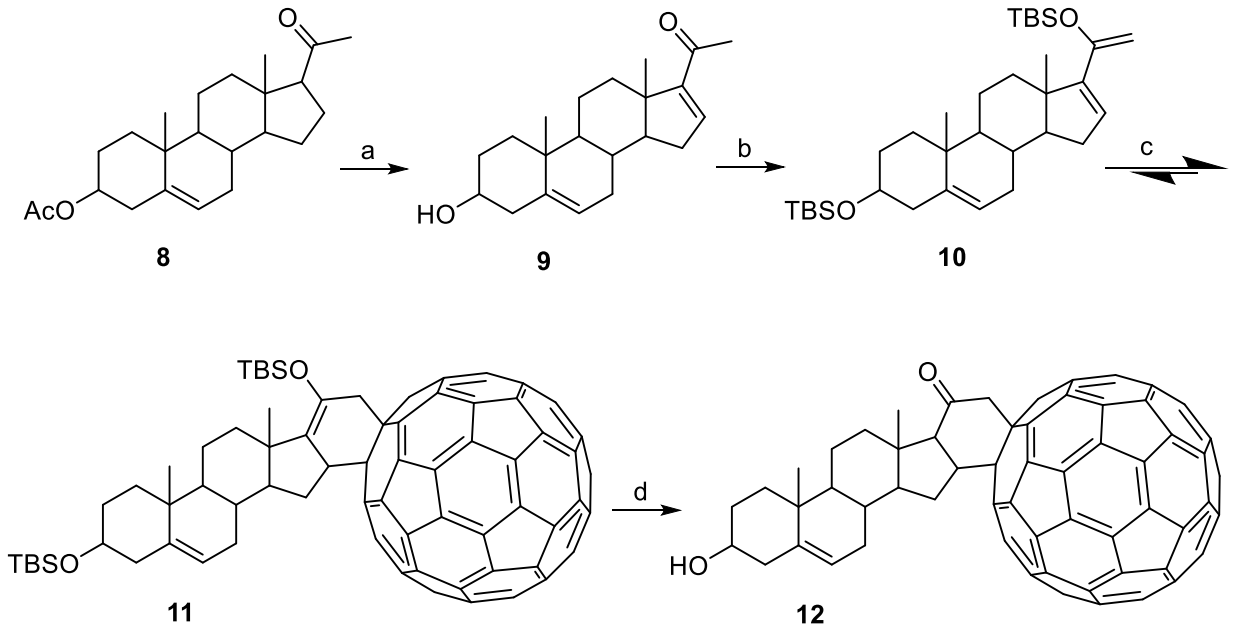


Figure 2. Synthesis of steroid-fullerene hybrids **5**, **6**, and **7**.

In 2001, Yang et al.[36] described the synthesis of two new steroid-fullerene hybrids by a Diels–Alder reaction as the key step.

The synthetic pathway is depicted in Scheme 3. Treatment of the available steroid 3'-acetoxypregna-5,16-dien-20-one **8** with LiOH led to **9** in 90% yield. This latter was easily transformed by reaction with *tert*-butyldimethylsilyl triflate into the desired silyloxydiene **10** in an isolated yield of 85%. Reaction of C_{60} with **10** in toluene at 90 °C for 2 h led to the desired molecule **12** in a yield of 30% (in two steps, 40% yield based on C_{60} consumed) after hydrolysis of the enol silyl ether and deprotection of the hydroxy group at C-3 using classic acid conditions.

A biological study on the cytotoxic effects of hybrid **12** has been carried out at the cellular level. The authors observed its effect on the transmembrane membrane of the reconstituted sarcoplasmic reticulum Ca^{2+}-ATPase (SR Ca^{2+}-ATPase) in soybean phospholipid liposomes. Thus, they were able to conclude that compound **12** can inhibit the enzyme. In addition, they also observed its effect on the survival of human lung adenocarcinoma A549 cells. The authors find that the survival of A549 cells after treatment with the steroid-C_{60} **12** is reduced. Eukaryotic cells are rich in membrane structures. Steroid-fullerene hybrids could be the cause and lead to abnormal cell functions and therefore decrease the survival of A549 cells.



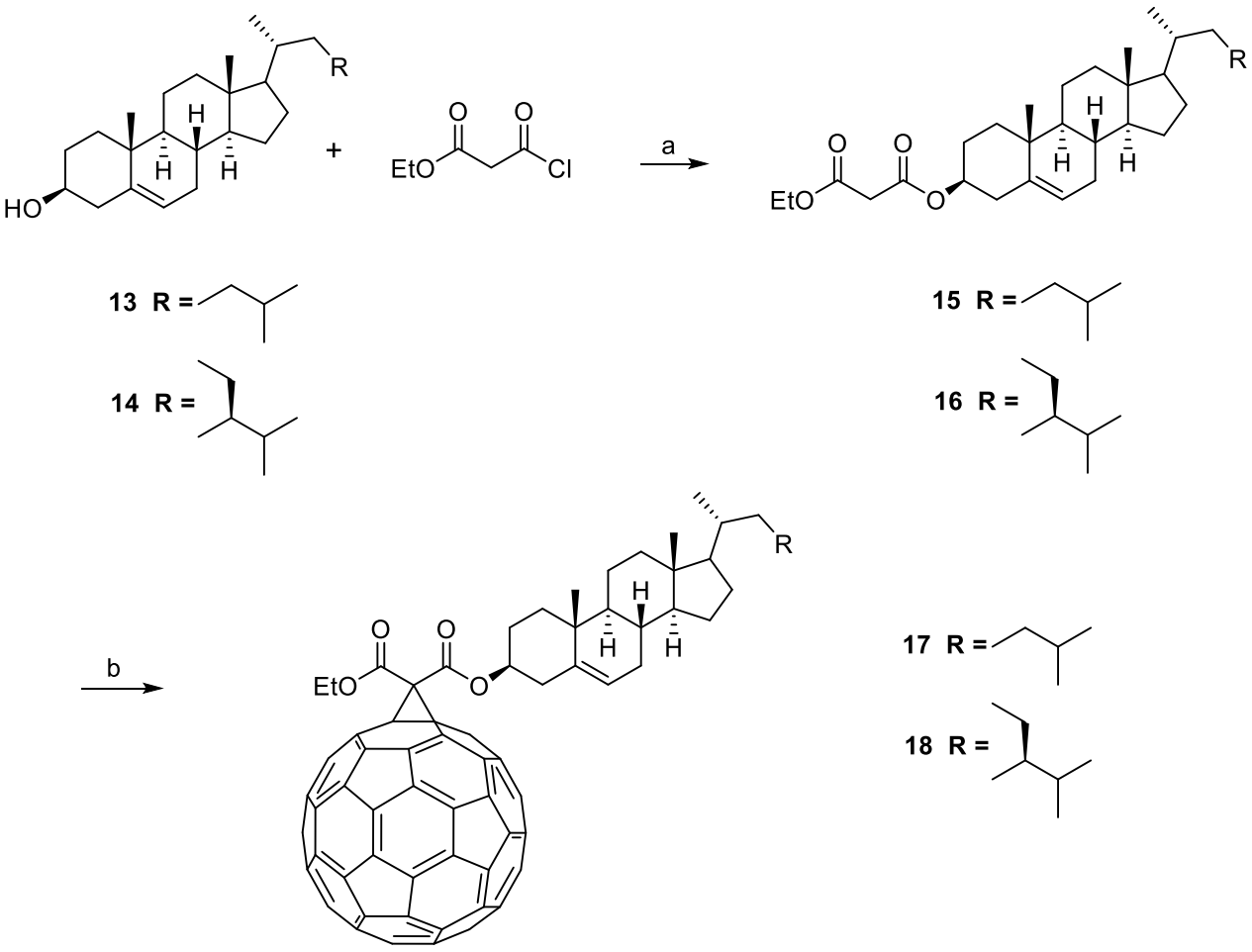
Reagents : a-THF/H_2O, LiOH, 90%; b- TBSOTf, NEt_3, 85%;
c- C_{60}, toluene, Δ; d- *p*TsOH, Δ, 40% (two steps).

Scheme 3. Synthesis of steroid-fullerene hybrid **12**.

In 2009, Martin et al.[37] reported the synthesis of a new series of hybrid functionalized chimeras **17**-**18** (Scheme 4) and **21** (Scheme 5) by cyclopropanations between C_{60} and readily available malonates bearing different steroid moieties **15**-**16**, and **20** using the Bingel–Hirsch protocol.

The synthesis of these hybrid molecules is shown in Schemes 4 and 5. The malonates **15**, **16** and **20** necessaries for the synthesis of these hybrid systems were prepared by treating three different sterols - cholesterol **13**, β-sitosterol **14**, and ergosterol **19** - with acetyl chloride (ethoxycarbonyl) commercially available. The reactions, carried out in anhydrous dichloromethane and pyridine at 0 °C, gave the desired malonates **15**,

16 (Scheme 4), and **20** (Scheme 5) with respective yields of 85%, 74% and 70%. Cyclopropanation under Bingel-Hirsch conditions, by treatment of **15**, **16**, or **20** with C_{60} in the presence of CBr_4 and DBU allowed the covalent binding of malonates **15**-**16**, and **20** previously synthesized with C_{60} fullerene. The expected hybrid systems **17**, **18**, and **21** were obtained very quickly (2 h) with moderate yields (50 to 58%) as stable brown solids.



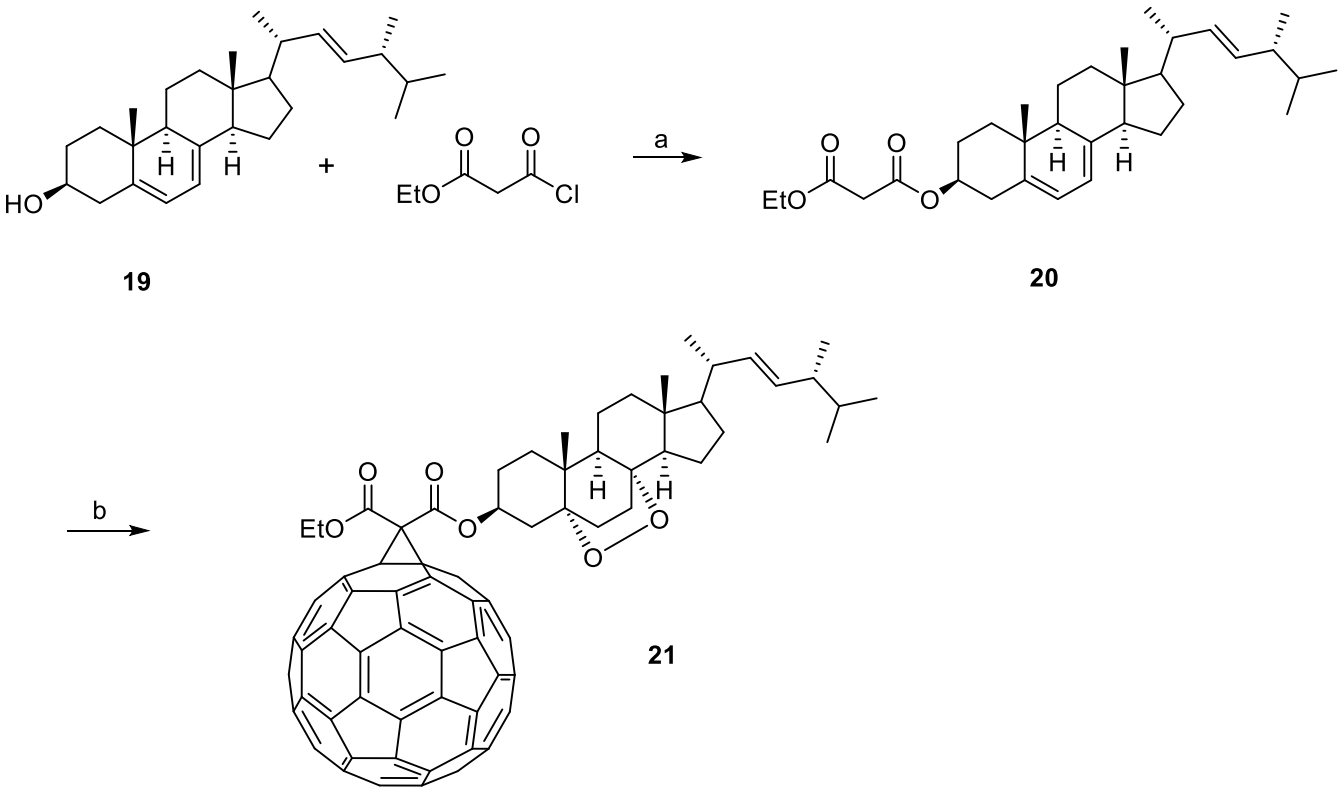
Reagents : a- CH_2Cl_2, pyridine, 0°C, 24 h; b- C_{60}, DBU/CBr_4, toluene, r.t.

Scheme 4. Synthesis of steroid-fullerene hybrids **17** and **18**.

The authors were able to observe the formation of product **21** containing two additional oxygen atoms resulting from the Bingel-Hirsch reaction between compound **20** and C_{60} (see scheme 5). Another Diels-Alder cycloaddition reaction with $^1O_2$, like that observed for ergosterol itself,[38] takes place at the 5,6-diene moiety of ring B. It is interesting to note that this cycloaddition is observed only when the diene system is in an environment containing oxygen and an effective triplet sensitizer, such as C_{60}. Thus, molecular oxygen, generally in a triplet ground state, is excited in a singlet state and reacts as a dienophile with the diene unit of the steroid leading to an endoperoxide following a Diels-Alder cycloaddition reaction.

The proposed structures are confirmed by their electronic spectra as well as by cyclic voltammetry, which allows concluding to the formation only of mono adducts, without observation of any formation of bis-cycloadducts which would come from the intramolecular Diels-Alder cycloaddition of the steroid diene part of ergosterol at C-7 to the fullerene double bond.

These new hybrid derivatives **17**, **18**, and **21**, consisting of both fullerene and steroid units, can be considered as promising functional systems. The potential biomedical applications of these hybrid molecules have not been reported to date.



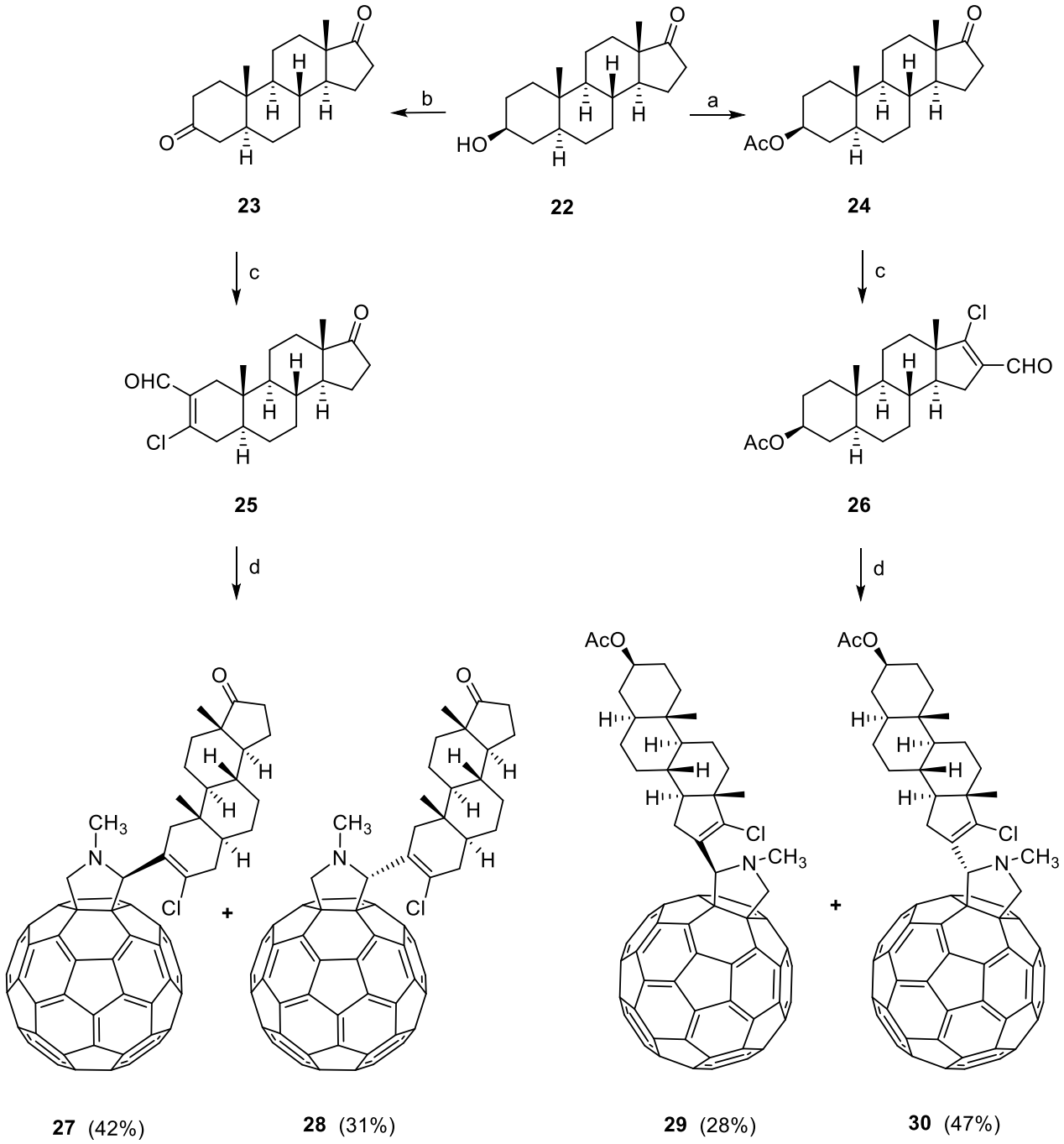
Reagents : a- CH_2Cl_2, pyridine, 0°C, 24 h; b- C_{60} / O_2, DBU/CBr_4, toluene, r.t.

Scheme 5. Synthesis of steroid-fullerene hybrid **21**.

In 2013, the same authors[39] used epiandrosterone which is an important steroid to synthesize steroid-fullerene hybrids. Indeed, this hormone is the natural metabolite of dehydroepiandrosterone *via* the enzyme 5α-reductase and is known for its low androgenic activity. In addition, it is present in most mammals, including pigs.[40]

Prato's method was here used to synthesize potentially biologically active fullerene-steroid hybrids from formyl-substituted steroid derivatives. The fullerene-steroid conjugates **27**-**30** 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 **25** and **26** as depicted in Scheme 6. The convenient transformation of the hydroxyl group at C-3 of the epiandrosterone **22** by oxidation gave the corresponding 5α-androstan-3,17-dione **23**, whereas acetylation of **22** afforded 3β-acetoxy-5α-androstan-17-one **24**, both in yields like those previously reported. The corresponding formyl derivatives, 3-chloro-2-formyl-17-oxo-5α-androstan-2-ene **25** and 3β-acetoxy-17-chloro-16 -formyl-5'-androstan-16-ene **26**, were obtained respectively by a Vilsmeier-Haack reaction of **23** and **24** with phosphorus oxychloride and dimethylformamide in dichloromethane. The yields obtained are good (78% and 50%, respectively) and the products crystallize after basic hydrolysis with aqueous sodium acetate.

Finally, 1,3-dipolar cycloaddition reactions of azomethine ylides generated *in situ* with C_{60} according to the Prato protocol leads to the production of *N*-methyl-2-substituted pyrrolidino [3,4: 1,2] [60] fullerenes. Thus, the reaction is carried out by mixing the chloroformyl derivative (**25** or **26**), C_{60} and sarcosine (*N*-methylglycine), the whole brought to reflux in toluene under an argon atmosphere for 6 h (see Scheme 6). The formation of the products is confirmed by the color change of the solution from purple to brown.



Scheme 6. Synthesis of steroid-fullerene hybrids **27**-**30**.

Flash chromatography makes it possible to easily separate the diastereoisomers and to obtain compounds **27**, **28**, **29** and **30**, in the form of stable brown solids, with respective yields of 42%, 31%, 28% and 47%. The stereoselectivity, which provides diastereomers **27** and **30** as main products, is relatively modest. This result agrees with the fact that the electrophile, the C_{60} attacks preferentially on the *Re* face of the 1,3-dipole.

The absolute configuration of fulleropyrrolidines was easily determined using chiroptic properties with Cotton-typical effects in CD spectra of chiral adducts. Thus, the absolute C2 configuration for compounds **27**-**30** was determined to be: (*2R*) for **27**, (*2S*) for **28**, (*2S*) for **29** and (*2R*) for **30**.

Finally, the authors observed that the conjugation of epiandrosterone derivatives with C_{60} increases the solubility of these new hybrid systems (**27**-**30**) in organic solvents such as chloroform, dichloromethane and dimethylformamide, among others. It is therefore undeniable that the presence of the steroid unit in the new hybrid compounds (**27**, **28**, **29** and **30**) improves their solubility. This result allowed the authors to consider other investigations on these structures, especially biological ones.

In 2014, the same authors[41] described the multistep synthesis of a fullerene hybrid dumbbell having two fullerene units connected through an epiandrosterone molecule.

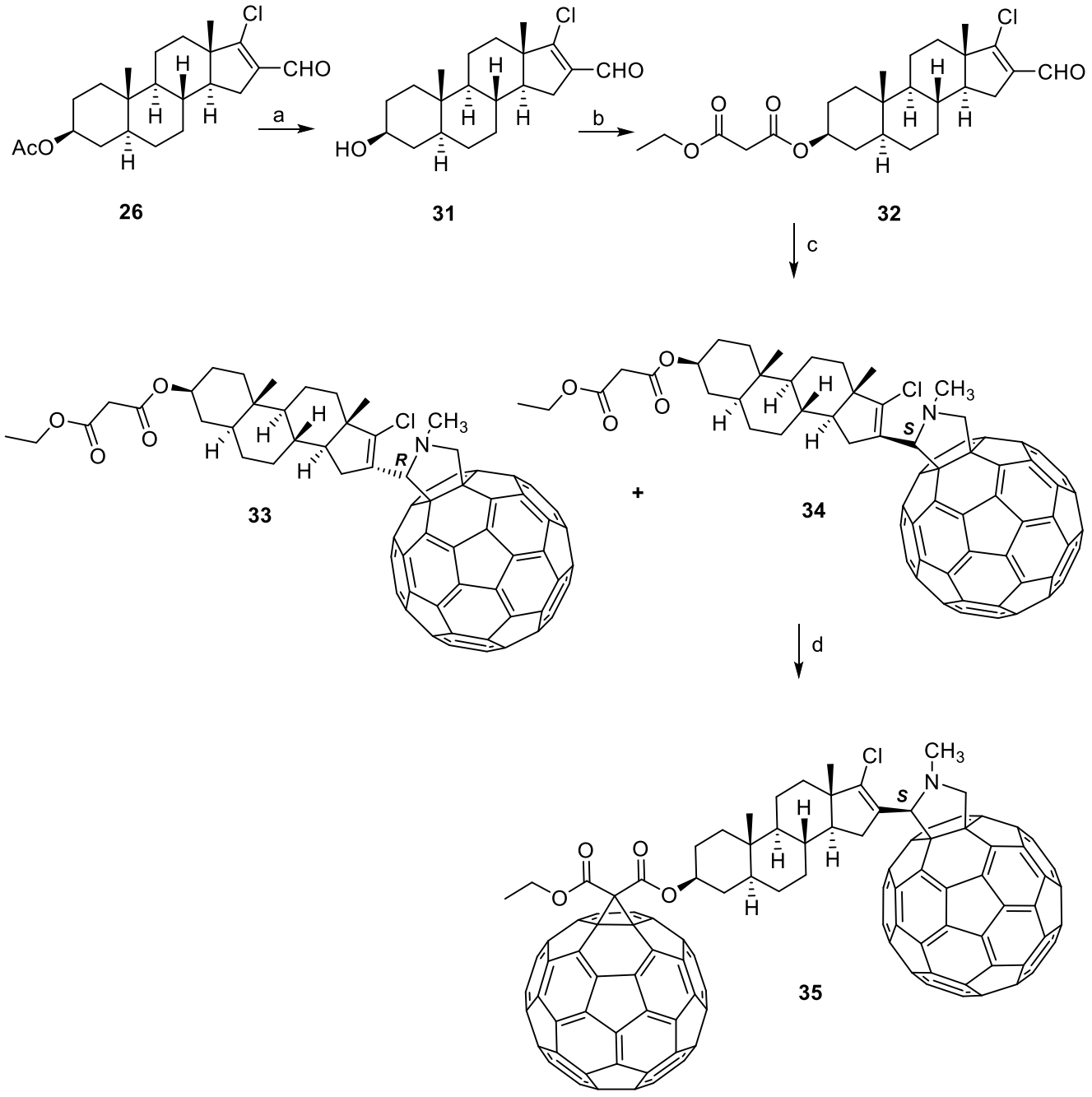
Thanks to the presence of a formyl group at the C-16 position of ring D of the starting product **26**, the first C_{60} unit was introduced by a 1,3-dipolar cycloaddition reaction. And after the transformation of the acetate group attached to position C-3 in ring A into the corresponding malonate, a cyclopropanation reaction made it possible to introduce the second unit of C_{60} (see Scheme 7).

After hydrolysis with potassium carbonate in methanol of the acetate group in C-3 position of the 3β-acetoxy-17-chloro-16-formyl-5α-androstan-16-ene **26**, the 17-chloro-16-formyl-3β-hydroxy-5α-androstan-16-ene **31** was isolated in good yield (92%) and allowed the incorporation of the malonate moiety by reaction of **31** with (ethoxycarbonyl)acetyl chloride in anhydrous dichloromethane and pyridine at 0 °C. This latter bearing a formyl group was obtained as a pale-yellow solid in 82% yield. The 1,3-dipolar cycloaddition of **32** was done by reaction with [60]fullerene and *N*-methylglycine in refluxing toluene leading to *N*-methyl-2-(3'β-ethylmalonate-17'-chloro-5'α-androstan-16'-ene)pyrrolidine-[3,4:1,2][60]fullerenes **33** and **34**. These molecules present a new stereocenter at C-2 in the pyrrolidine ring, and therefore, two diastereomers are formed. These stereoisomers **33** and **34** were easily separated by flash chromatography leading to stable amorphous brown solids **33** and **34** with moderate yields (**34**, 34%; **35**, 27%).

The fullerene dumbbell derivative **35** was obtained by reaction of diastereomer **34** bearing a malonate moiety to [60]fullerene under Bingel− Hirsch conditions. Treatment of **34** with C_{60} in the presence of CBr_4 and DBU led after 4 h, to the formation of the dumbbell derivative **35**. The pure fullerene dimer **35** was isolated after purification by flash chromatography as a stable brown solid in 55% yield (see Scheme 7).

The new compounds **33**-**35** have been characterized by spectroscopy and their redox potentials, determined by cyclic voltammetry, reveal three reversible reduction waves for hybrids **33** and **34**, whereas these signals are split in dumbbell **35**. The most stable conformations for the hybrid compounds (**33**−**35**) were determined by theoretical calculations at semiempirical (AM1) and single point B3LYP/6-31G(d) levels, and show the importance of the chlorine atom on the D ring of the steroid. Indeed, it seems to be clear that the existence of a hydrogen bond C−H···Cl contributes to the stabilization of the hybrid systems.

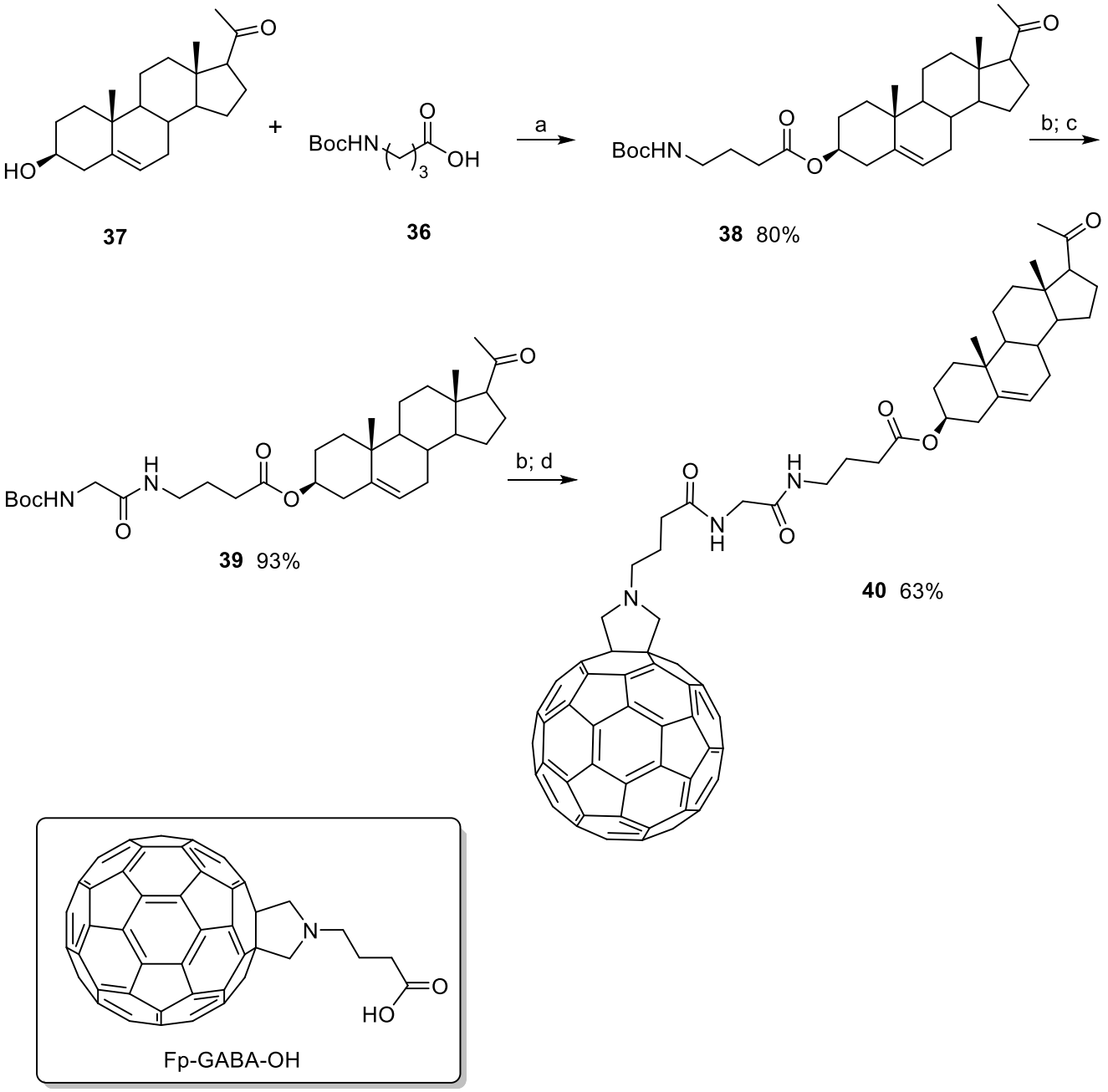
The same year, Bjelakovic et al.[42] reported the synthesis, morphological study, and preliminary *in vitro* antioxidant capacity of new fullerene-steroid hybrids.



Reagents : a- K_2CO_3 (5%), MeOH, r.t.; b- (ethoxycarbonyl)acetyl chloride, DCM, pyridine,0 °C;
c- C_{60}, *N*-methylglycine, toluene, reflux; d- C_{60}, CBr_4, DBU, toluene, r.t.

Scheme 7. Synthesis of steroid-fullerene hybrids **33**-**35**.

The synthetic strategy to obtain the fullerene-peptide-steroid hybrids **40** and **42** are depicted in Schemes 8 and 9 and show firstly the synthesis of a steroid peptide dyad and then its conjugation with the fullerene subunit based on esterification and amidation reactions with protection-deprotection steps required. Two different activating systems, DCC/DMAP and EDC/HOBt in dichloromethane were used to optimize these reactions. The DCC/DMAP-assisted esterification of *N*-protected GABA **36** with sterol **37** led smoothly to the ester **38** in very good yield (80%), while in the presence of EDC/HOBt no product was isolated.



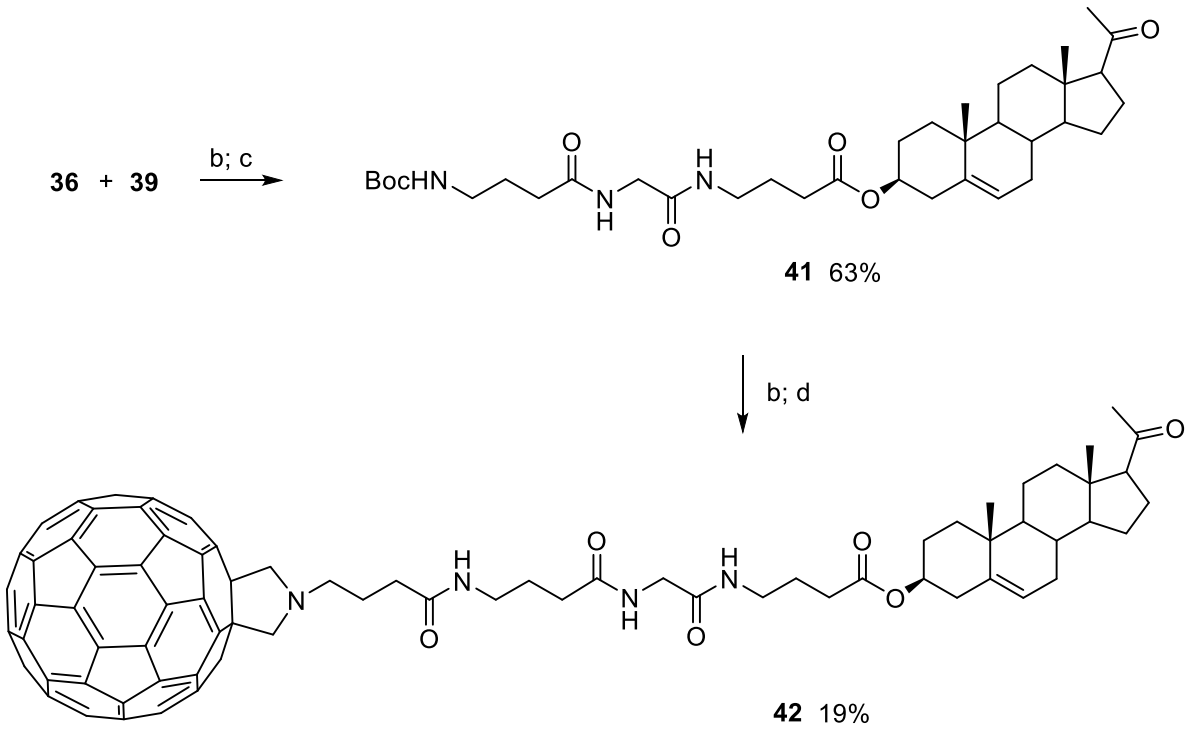
Reagents : a- DCC/DMAP, CH_2Cl_2, 0°C to r.t., 24 h; b- TFA, CH_2Cl_2, r.t., 2 h;
c- Boc-Gly-OH, TEA, DCC/DMAP, CH_2Cl_2, 0°C to r.t., 24 h;
d- Fp-GABA-OH, TEA, DCC/DMAP, CH_2Cl_2, 0°C to r.t., 24 h.

Scheme 8. Synthesis of steroid-fullerene hybrid **40**.

The steroid peptide dyad **39** was coupled with Boc-Gly-OH[43] after a quantitative Boc-deprotection with TFA. This latter was isolated with an excellent yield (93%) and used for two different syntheses of steroidal hybrids. The reaction of **40** with Fp-GABA-OH led to fullerene-peptide-steroid hybrid **40** in a 63% yield and reaction with GABA afforded a steroid dipeptide dyad **41** presenting an elongation of the peptide chain (Scheme 9). Finally, triad **42** was isolated in a poor yield of 19% after Boc-removal and a subsequent coupling with a fullerene unit. The yield is lower than this obtained using a shorter peptide. The DCC/DMAP activating system seems to be

somewhat more efficient for amidations including both Boc-Gly-OH and Fp-GABA-OH (reactions 3/4 and 4/5, respectively) than for esterification of Boc-GABA **36**.

This synthetic approach can be used to generate different steroid-fullerene derivatives with potential biological activity. High antioxidant activity of these new hybrids, twice and 13-fold better than fullerene C_{60} and standard antioxidant agent vitamin C, respectively, was observed after an *in vitro* study using the Ferrous ion Oxidation-Xylenol orange (FOX) method.

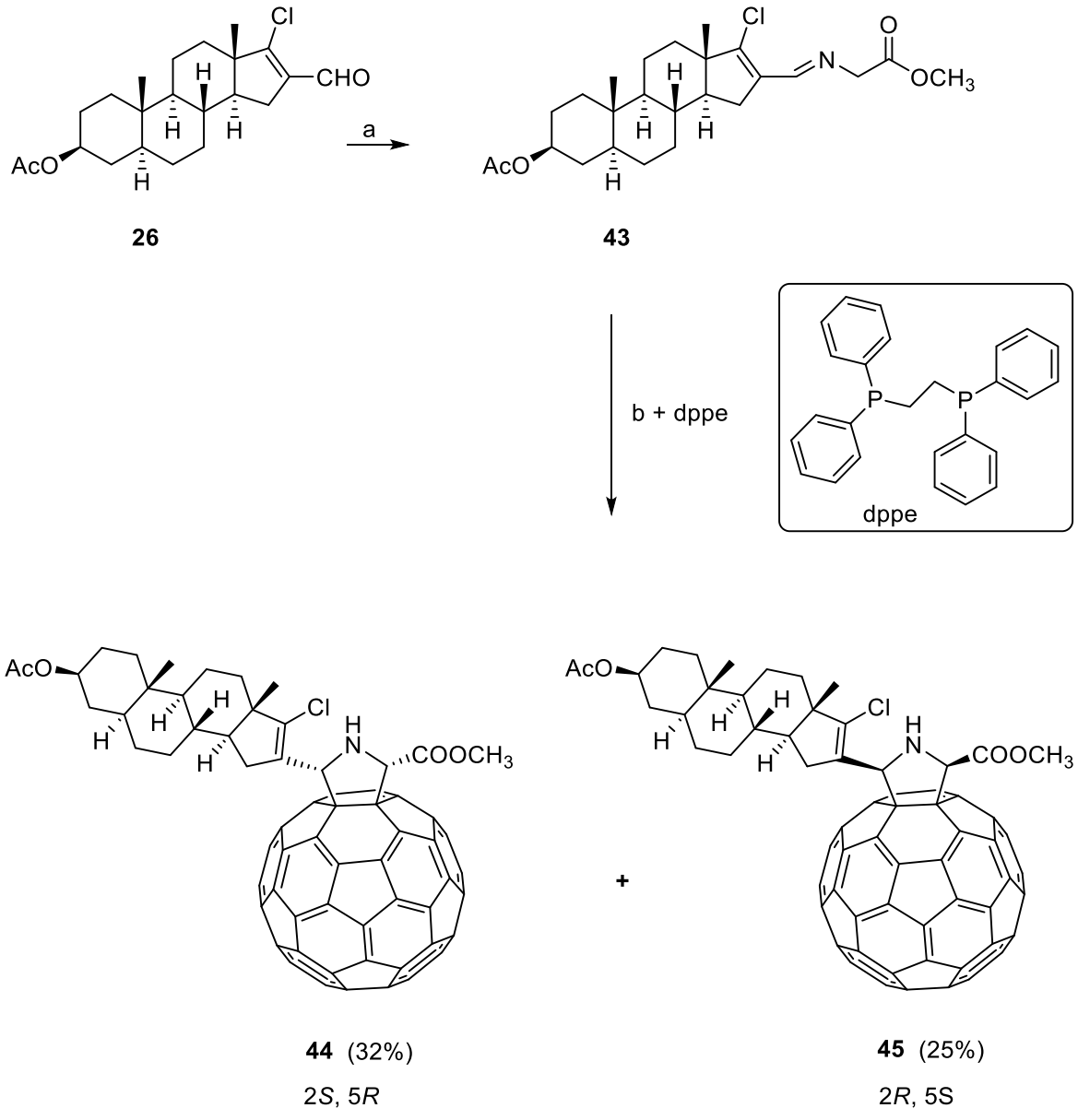


Scheme 9. Synthesis of steroid-fullerene hybrid **42**.

In 2017, Martin and coworkers[44] reported for the first time a controlled diastereoselective synthesis of *cis* and *trans* steroid−fulleropyrrolidines hybrids by reaction of *N*-metallated azomethine ylides [Cu(II) or Ag(I)] with the appropriate chiral ligand and C_{60}.

To obtain the target steroid-fullerene hybrids, the authors first synthesized the imine **43** by reaction of the previously reported[39] 3-chloro-2-formyl-17-oxo-5α-androstan-2-ene **26** and the glycine methyl ester hydrochloride using triethylamine as a base and dichloromethane as solvent at room temperature (see Scheme 10). This imine was isolated in a good yield (77%) as a yellow oil. The coupling of this latter with C_{60} using anhydrous silver acetate along with the achiral ligand [dppe, 1,2-bis(diphenylphosphino)ethane] in toluene at room temperature led to a mixture of the two *cis*-diastereomers **44** and **45**. It is worth pointing out that the stabilization of the *N*-metallated azomethine ylide is provided by the allylic moiety of the steroid unit. The two products were separated by flash chromatography to give **44** in a yield of 32% and **45** in a yield of 25% (see

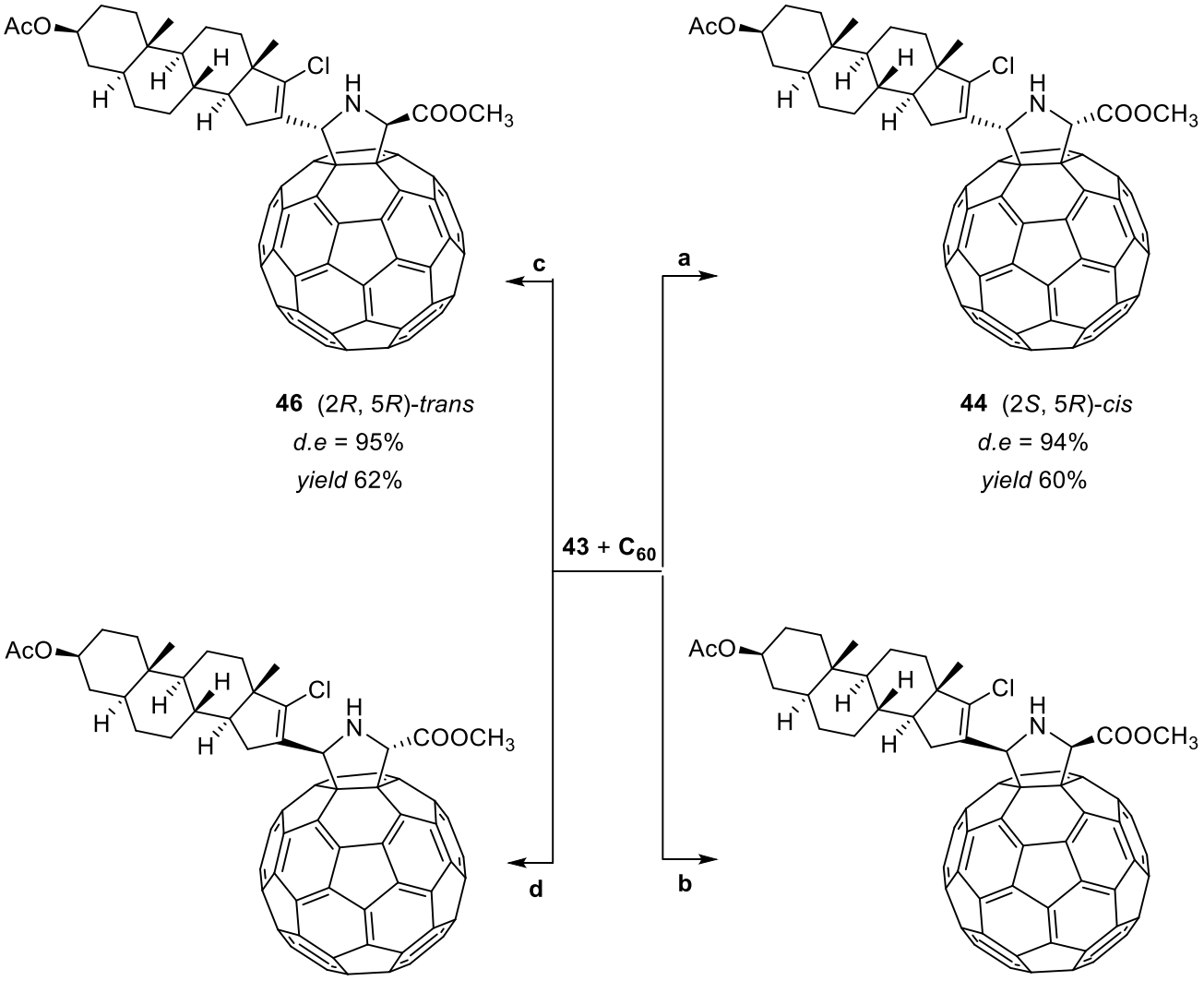
Scheme 10). We can see that the stereoselectivity is modest due to poor chiral induction of the steroid moiety on the attack to one of the faces of the dipole in the fullerene derivative.



Reagents : a- Cl^-,^+H_3N-CH_2COOCH_3, NEt_3, DCM,r.t.; b- C_{60}, AgOAc, dppe, toluene, r.t.

Scheme 10. Synthesis of steroid-fullerene hybrids **44** and **45**.

The same reaction was done with other catalytic systems to ascertain their stereocontrol in the 1,3-dipolar cycloaddition of azomethine ylides onto C_{60} even in the presence of a chiral moiety, such as the imine **43**.



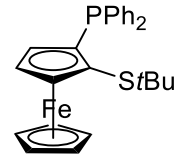
46 (2*R*, 5*R*)-*trans*
d.e = 95%
yield 62%

44 (2*S*, 5*R*)-*cis*
d.e = 94%
yield 60%

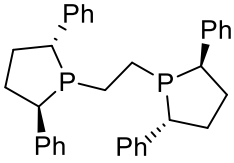
47 (2*S*, 5*S*)-*trans*
d.e = 93%
yield 63%

45 (2*R*, 5*S*)-*cis*
d.e = 93%
yield 57%

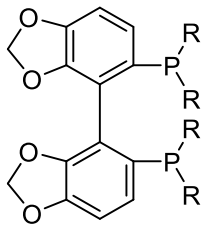
Reagents : a-$Cu(AcO)_2$, FeSulphos, 0 °C, 2 h; b- AgOAc,BPE,0 °C, 2 h; c- $Cu(OTf)_2$,
R-DTBM-Segphos, NEt_3, 2 h, r.t.; d- $Cu(OTf)_2$, *S*-DTBM-Segphos, NEt_3, 2 h, r.t.



FeSulPhos



BPE



R = 2,3-*t*Bu-3-OMe-Ph
DTBM-Segphos

Scheme 11. Synthesis of steroid-fullerene hybrids **44**-**47**.

Thus, the reaction of steroid iminoester **43** onto C_{60} with the catalytic complex Cu(II)−Fesulphos at 0 °C led to cis-(*2S*,*5R*)-2-methoxycarbonyl-5-(3'β-acetoxy-17'-chloro-5'α-16'-androstene) pyrrolidino [3,4:1,2] [60]fullerene **44** with an excellent diastereomeric induction (see Scheme 11). Using the catalytic system AgOAc/(*R*,*R*)-BPE at the same temperature, the *cis* diastereoisomer (*2R,5S*) (*cis*-2-methoxycarbonyl-5-(3'β-acetoxy-17'-chloro-5'α-16'-androstene)pyrrolidino[3,4:1,2]-[60]-fullerene **45** showing an opposite configuration in the pyrrolidine ring is obtained, also with excellent diasteroselectivity. For both catalysts, the counterion of the metal salt which is acetate reacts like a base and is probably located in the empty metal coordination sphere, which is the origin of this good stereodifferentiation.[45] This diastereodivergent study was completed using bulky chiral ligand DTBM-Segphos[46,47] to obtain the *trans*-diastereomers. Thus, the use of the Cu(II)-(*R*)-DTBM-Segphos complex in the presence of triethylamine as the base at room temperature leads to the *trans*-diastereoisomer **46** with (*2R*, *5R*) configuration. And, the same reaction carried out with the Cu(II)-(*S*)-DTBM-Segphos complex under the same conditions leads to the *trans*-diastereomer **47** with a (*2S, 5S*) configuration.

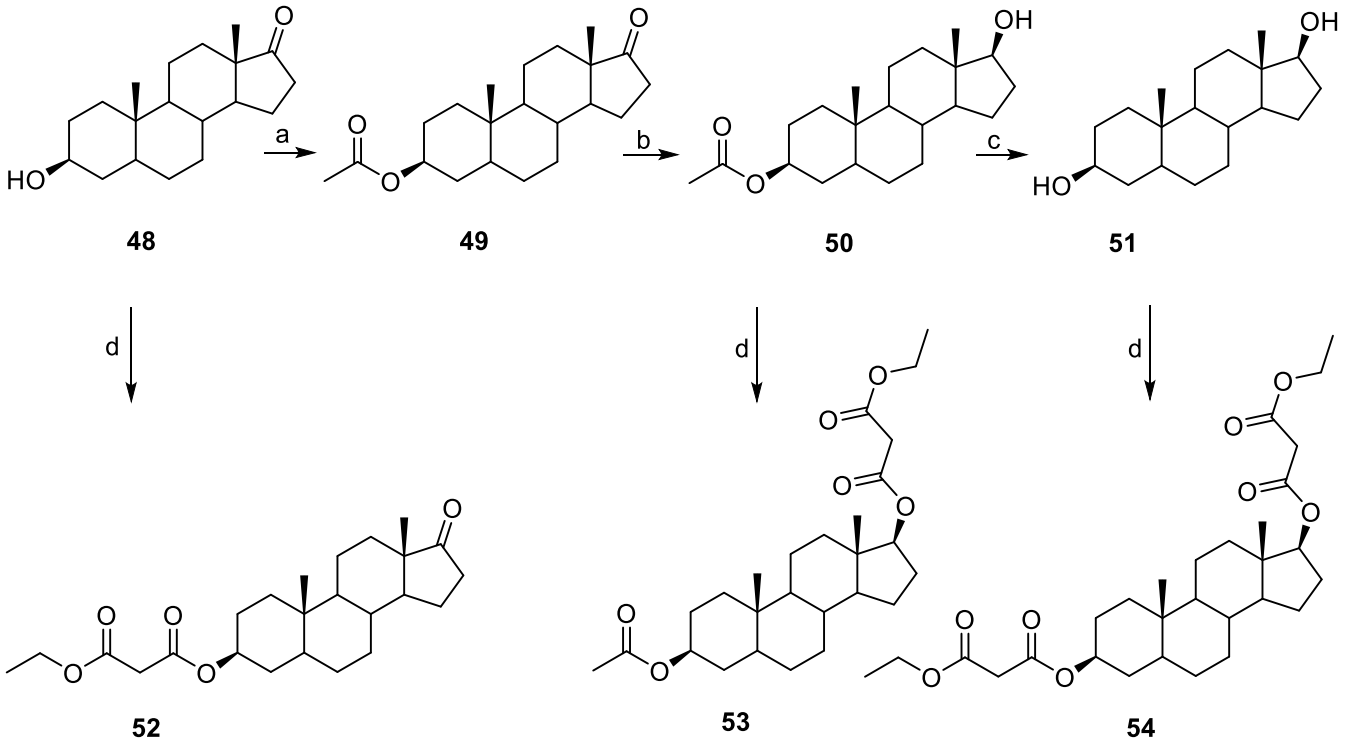
This is an efficient stereodivergent protocol to synthesize hybrid systems in a diastereoselective manner allowing access to a variety of optically active fullerene hybrids.

In 2018, the same authors[48] described the synthesis of new [60]fullerene-steroid hybrids by Bingel–Hirsch protocol of the corresponding steroid containing malonates with C_{60}.

The synthesis started with the preparation of compounds **52**, **53**, and **54** from commercially available epiandrosterone **48**, in which functional groups in C3 and C17 positions were transformed. Thus, the steroid derivative **48** was treated with acetic anhydride in pyridine and led to the corresponding 3β-acetoxy-5α-androstan-17-one **49** in a good yield. As expected, the reduction of compound **49** with sodium borohydride in methanol afforded with stereoselective control the 3β-acetoxy-5α-androstan-17β-ol **50**. In the following step, 5α-androstan-3β,17β-diol **51** was isolated after basic hydrolysis of the previous compound in a similar yield as this reported.[49,50]

The sterol derivatives **48**, **50**, and **51** were transformed into the corresponding malonates **52**-**54** by reaction with the commercially available (ethoxycarbonyl)acetyl chloride (Scheme 12). The reaction was carried out in anhydrous dichloromethane and pyridine at 0°C and led to the derivatives **52**, **53**, and **54** in 80, 72, and 75% yields, respectively.

The steroid-fullerene hybrids **52**, **53**, or **54** were synthesized using the Bingel–Hirsch conditions by reaction of C_{60} and the malonyl steroids **52**, **53**, or **54**, with CBr_4 and DBU at room temperature (see Scheme 13). The cyclopropanation process was confirmed by the change of the color of the solution (purple to brown) leading to the [6,6] mono-adduct. In the case of compounds **55** and **56**, the reaction was finished in 2 h and furnished after purification by flash chromatography, compounds **55** and **56** in 68 and 58% yields, respectively, as stable brown solids. While, the reaction between C_{60} and dimalonate **54** led to three hybrid systems **57**, **58**, and **59** in 42%, 12%, and 11% yields, respectively.

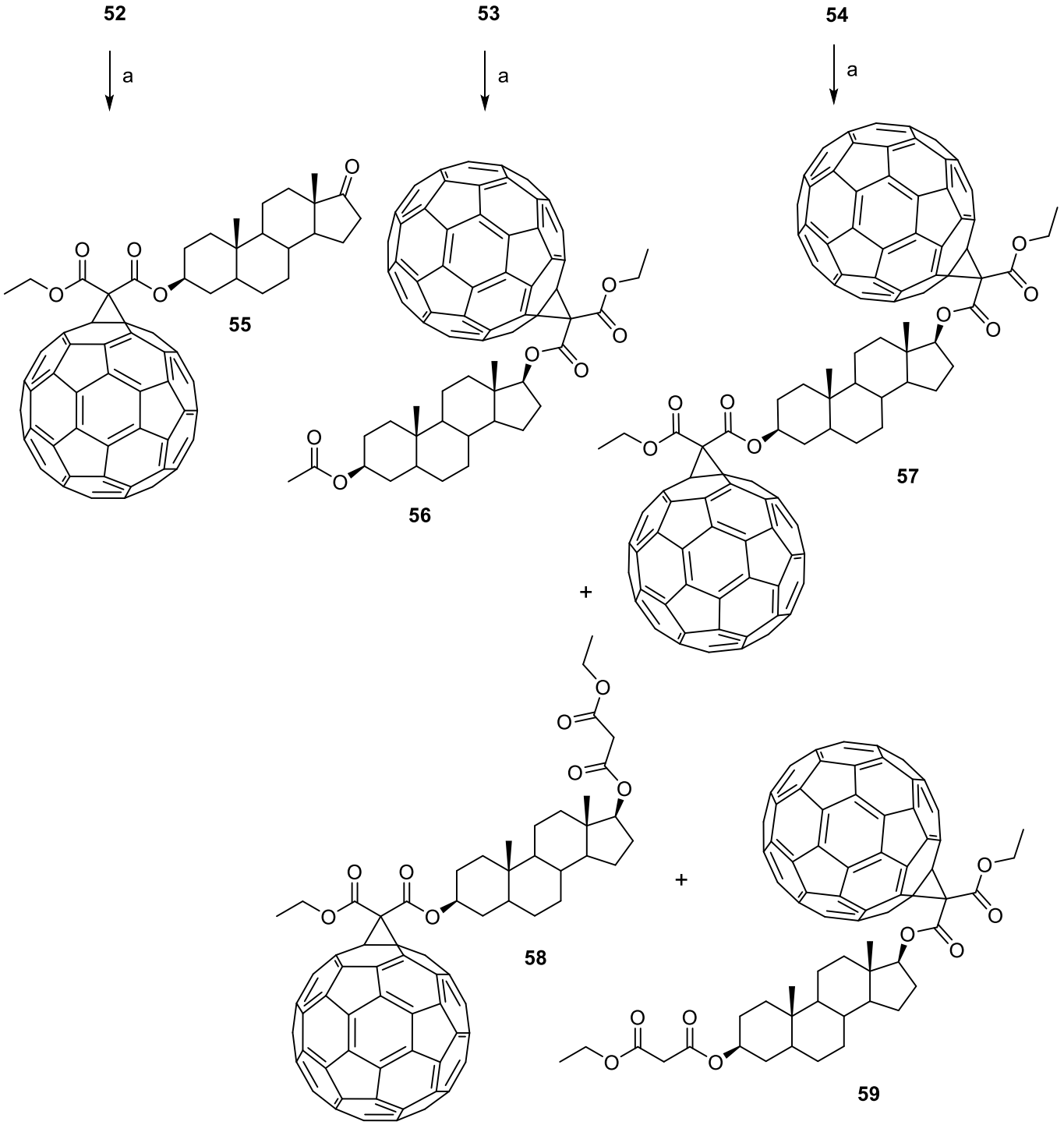


Reagents : a- acetic anhydride, pyridine, 5 h, r.t.; b- $NaBH_4$, MeOH, r.t., 25 min;
c- K_2CO_3, 5%, MeOH, r.t., 24 h; d- $CH_3CH_2OCOCH_2COCl$, pyridine, DCM,0 °C.

Scheme 12. Synthesis of steroid derivatives **52**-**54**.

In 2020, Martin and coworkers[51] developed a synthetic strategy based on a multistep approach to synthesize novel fullerene-steroid hybrids. The synthesis required the obtention of malonyl derivatives **61** and **62** to fix the [60]fullerene cage by a Bingel–Hirsch reaction (Scheme 14). Thus, dehydroepiandrosterone **60** (DHEA) was transformed into malonates **61** and **62** by reaction with commercially available (ethoxycarbonyl)acetyl chloride and malonyl chloride, respectively. These reactions were carried out in anhydrous dichloromethane (DCM) and pyridine at 0 °C. The purified compounds **61** and **62** were obtained with 82% and 78% yields, respectively. The Bingel–Hirsch protocol was used to synthesize the novel methano[60]fullerenes **63** and **64**, by reaction of either **61** or **62** with [60]fullerene in the presence of CBr_4 and DBU at room temperature. After 2 h and purification by flash chromatography, compounds **63** and **64** were isolated as stable brown solids in 74 and 51 % yields, respectively.

It is interesting to note that the DHEA moiety in the [60]fullerene systems **63**, and **64** increases the solubility in organic solvents, such as chloroform, dichloromethane, dimethylformamide, and dimethylsulfoxide. This fact might be useful since biological studies could be realized in some of these solvents. Indeed, in general, *in vitro* tests with the HIV-1 protease are performed in DMSO[52] due to the poor solubility of fullerene derivatives in water.

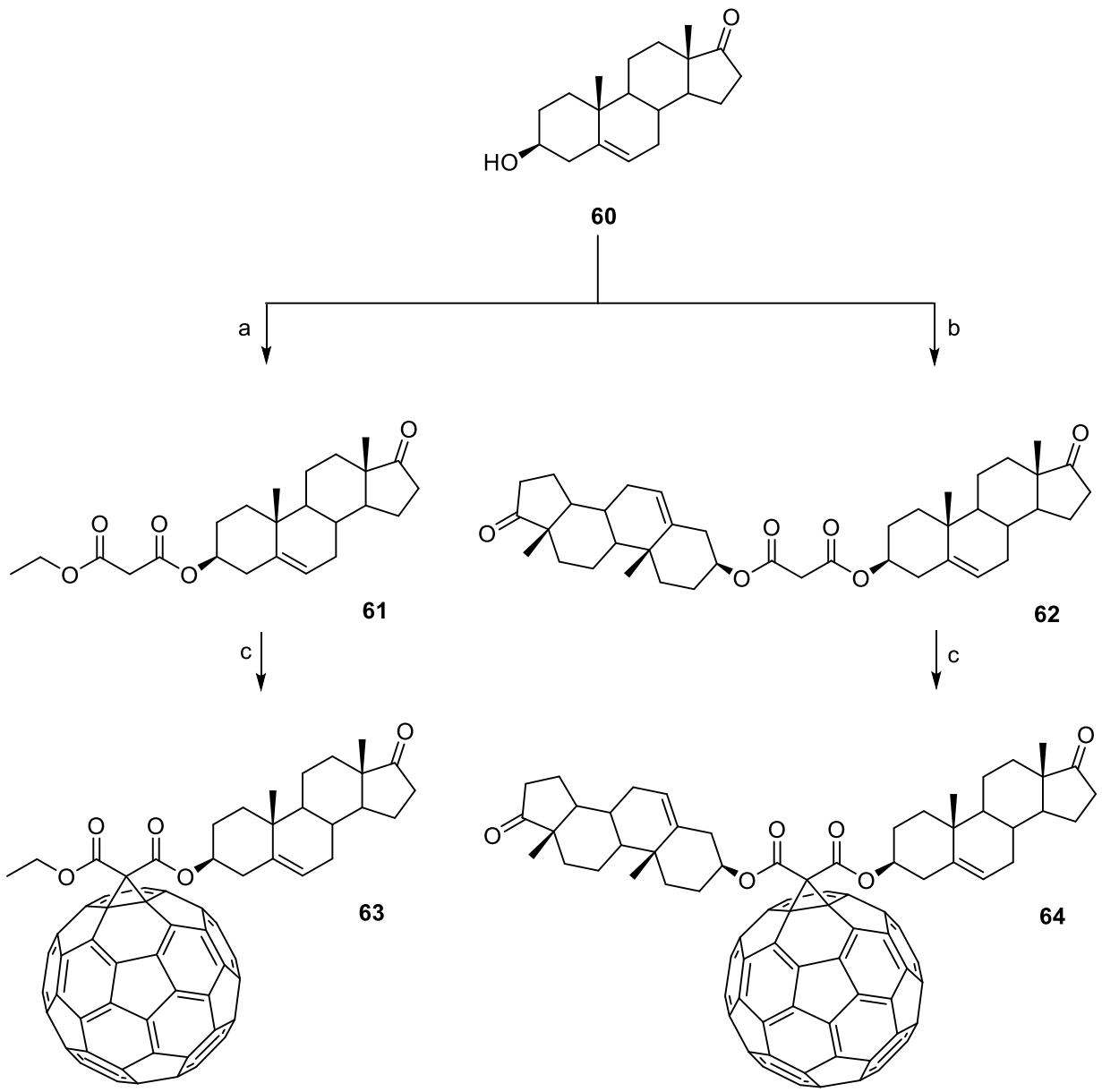


Reagents : a- C_{60}, DBU/CBr_4, toluene, r.t.

Scheme 13. Synthesis of steroid-fullerene hybrids **55**-**59**.

The TEM analysis showed that steroid-fullerene hybrids in water give nano-sized spherical vesicles with highly heterogeneous size populations. Moreover, DFT theoretical calculations revealed that hydrogen bonds play a major role in the geometry of these hybrid systems. And finally, these derivatives could have an application as HIV-1 protease inhibitors as suggested by molecular docking studies. All the characteristics of C_{60}-

steroid derivatives (including polarity, lipophilicity) constitute promising results to further explore the biomedical aspects of these molecular chimeras.



Reagents : a- $CH_3CH_2OCOCH_2COCl$, pyridine, DCM,0 °C; b- $ClOCOCH_2COCl$, pyridine, DCM,0 °C; b- C_{60}, DBU, CBr_4, toluene, r.t.

Scheme 14. Synthesis of steroid-fullerene hybrids **63** and **64**.

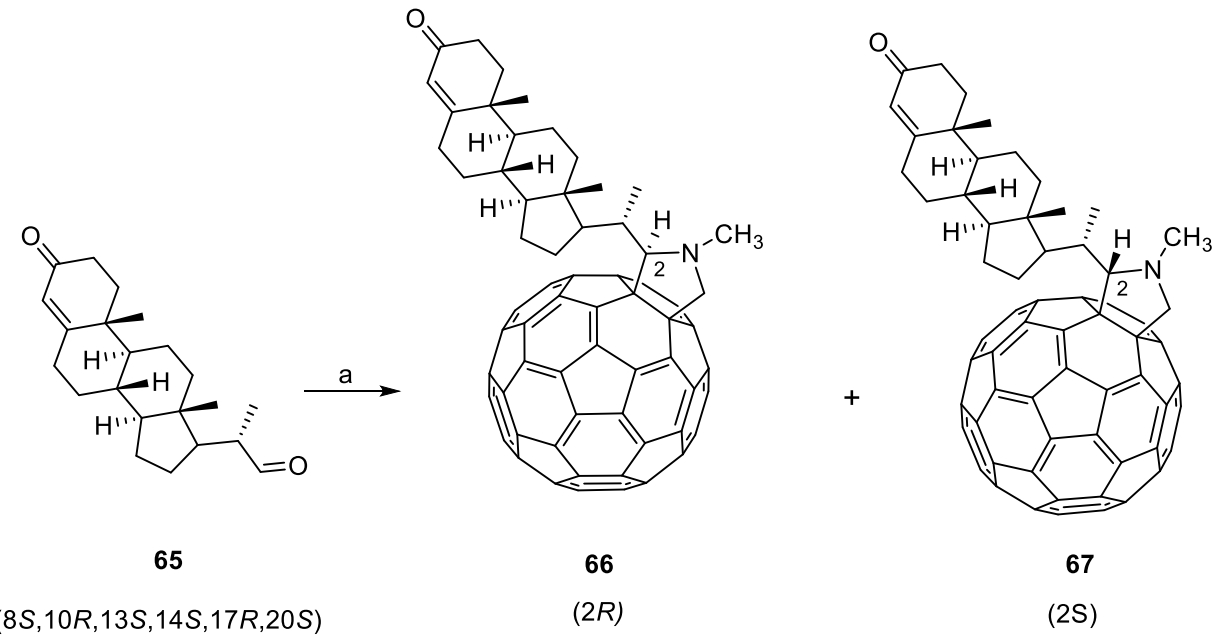
The same year, Martin and coworkers[53] also reported the synthesis of two new diastereomeric steroid [60]fullerene hybrids in a single 1,3-dipolar cycloaddition reaction.

The synthetic pathway is depicted in Scheme 15. A 1,3-dipolar cycloaddition reaction of steroidal derivatives with C_{60} *via* azomethine ylides generated *in situ* led to *N*-methyl-2-substituted pyrrolidino-[3,4:1,2][60]fullerenes possessing steroidal moieties. Thus, the reaction of the enantiopure formyl steroidal

derivative (*8S,9S,10R,13S,14S,17R,20S*)-3-oxopregn-4-ene-20-carboxaldehyde, **65**, C_{60}, and sarcosine in toluene under argon atmosphere led to the corresponding fullerene cycloadducts **66** and **67** after 5 h of reflux (Scheme 15).

The formation of the five-membered ring occurred with the formation of a new stereogenic center on the C-2 of the pyrrolidine ring by a stereospecific *syn* process. Since the configuration of the stereogenic centers of the steroid always remains the same, the reaction led to a mixture of diastereoisomers of derivatives **66** and **67**, which were separated by flash chromatography. Thus, products **66** and **67** were isolated as stable brown solids with yields of 68% and 13%, respectively. The diastereoselectivity of the reaction could be explained by a preferential electrophilic attack of C_{60} on the *Re* face of the 1,3-dipole. As a result, the steric interactions due to the presence of the methyl group at C-20 are at the origin of the attack on the *Si* face. Thus, the 5:1 ratio (*Re:Si*) observed is explained by an attack on the [60]fullerene on the *Re* face of the 1,3-dipole due to lower steric hindrance.

Here too, it is worth noting the increase in the solubility of derivatives **66** and **67** in organic solvents. This fact is very interesting to consider possible biological studies on materials. Additionally, these hybrids can potentially be used as HIV-1 protease inhibitors. These are very encouraging results that could be the source of many applications (biology, materials, etc.).



65

(8*S*,10*R*,13*S*,14*S*,17*R*,20*S*)

66

(2*R*)

67

(2S)

Reagents : a- C_{60}, *N*-methylglycine, toluene,reflux, under argon.

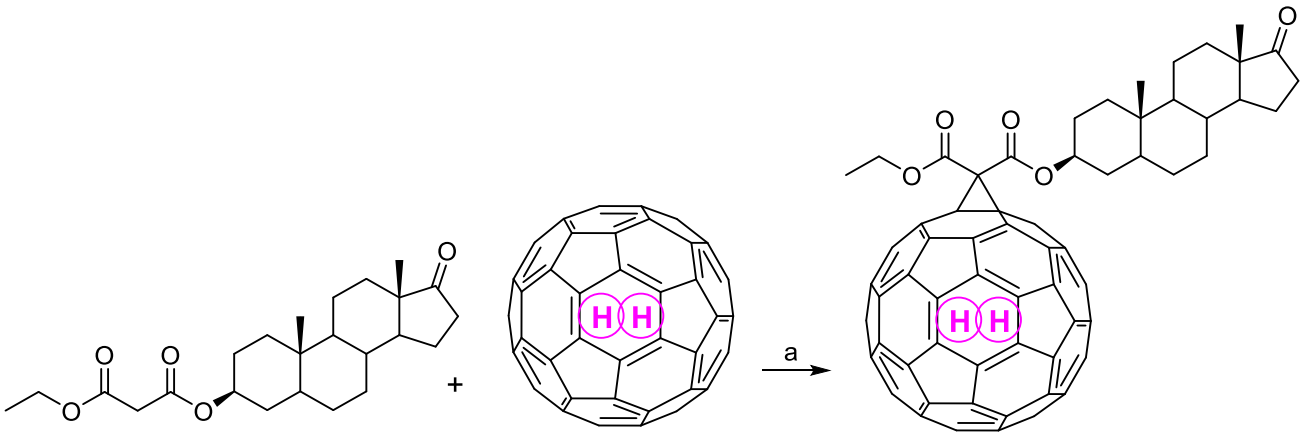
Scheme 15. Synthesis of steroid-fullerene hybrids **66** and **67**.

Recently, the same authors[54] reported the synthesis and characterization of a fullerene-steroid hybrid that contains $H_2@C_{60}$ and a dehydroepiandrosterone moiety synthesized by a cyclopropanation reaction.

The authors observed that the encapsulation of a hydrogen molecule does not in any way modify the reactivity or the stereoselectivity of the C_{60} hollow fullerene.[24] The synthesis is depicted in Scheme 16. The new endohedral steroid-fullerene hybrid **69** was prepared by applying the Bingel-Hirsch protocol between 3β-ethyl

malonate-5-androsten-17-one **52**[51] and H_2@C_{60}. The H_2@[60]fullerene, compound **52**, CBr_4, and DBU mixture produced at room temperature very quickly leads to a change in the color of the solution from purple to brown, identical to that observed when the reaction is carried out using unmodified C_{60}[51]and indicative of the success of the reaction. Thus, the [6,6]-closed mono-adduct **69** was isolated in a good yield (76%) as a stable brown solid. The endohedral hybrid is obtained with yields and reaction times similar to those obtained during hollow 60]fullerene reactions with steroids.[51]

It is therefore interesting to note that the presence of the H_2 molecule inside the cavity of the fullerene does not affect the reactivity of the hollow fullerene. Furthermore, the authors have shown that in the hybrid system **69**, the hydrogen molecule does not present a clear perpendicular disposition to one of the hexagonal rings of the fullerene cage.



52 **68** **69**

Scheme 16. Synthesis of steroid-fullerene hybrid **69**.

Theoretical studies were considered on the new endohedral steroid-fullerene hybrid **69** to evaluate its biological potential. It was thus observed that it would have higher antiviral properties, in particular against Covid-19, than its hollow counterparts. Of course, these preliminary and encouraging results will have to be verified by future *in vitro* and *in vivo* tests.

3. Conclusions

The present review offers up-to-date literature on the syntheses of steroid-fullerene hybrids reported during the last years. The coupling of two or more natural products to make hybrids makes it possible to obtain new types of compounds with various structures, at the origin of improved properties compared to the starting molecules or at the origin of new properties. The biological aspect and all the properties of these molecules are still not well-known. But it is expected that the synthesis of fullerene derivatives will continue in the years to come as well as the still current interest in the chemistry of steroids and their derivatives given the applications (biological or in the chemistry of materials) inherent to this family of compounds.

4. Acknowledgements

This work has been financially supported by the CNRS and the Ministère de l'Enseignement Supérieur et de la Recherche.

5. References

1. Zeelen, J. F. *Medicinal chemistry of steroids*; Elsevier: Amsterdam, Netherlands, 1990.
2. Trager, L. F. *Steroidhormone*; Springer: Berlin, 1977.
3. Biellmann, J. F. *Chem. Rev*. **2003**, *103*, 2019. http://doi.org/10.1021/cr020071b
4. Nising, C. F.; Bräse, S. *Angew. Chem. Int. Ed.* **2008**, *47*, 9389. https://doi.org/10.1002/anie.200803720
5. Hanson, J. R. *Nat. Prod. Reports* **2010**, *27*, 887. https://doi.org/10.1039/C001262A
6. Tzirakis, M. D.; Orfanopoulos, M. *Chem. Rev.* **2013**, *113*, 5262. https://doi.org/10.1021/ja808658b
7. Lu, X.; Feng, L.; Akasaka, T.; Nagase, S. *Chem. Soc. Rev.* **2012**, *41*, 7723. https://doi.org/10.1039/C2CS35214A
8. Das, S.; Presselt, M. *J. Mater. Chem. C* **2019**, *7*, 6194. https://doi.org/10.1039/C9TC00889F
9. Kang, S.; Zhang, J.; Sang, L.; Shrestha, L. K.; Zhang, Z.; Lu, P.; Li, F.; Li, M.; Ariga, K. *ACS Appl. Mater Interfaces* **2016**, *8*, 24295. https://doi.org/10.1021/acsami.6b06221
10. Badamshina, E.; Estrin, Y.; Gafurova, M. *J. Mater. Chem. A* **2013**, *1*, 6509. https://doi.org/10.1039/C3TA10204A
11. Stroyuk, O.; Raevskaya, A.; Gaponik, N. *Chem. Soc. Rev.* **2018**, *47*, 5354. https://doi.org/10.1039/C8CS00029H
12. Martynov, I. V.; Akkuratov, A. V.; Luchkin, S. Y.; Tsarev, S. A.; Babenko, S. D.; Petrov, V. G.; Stevenson, K. J.; Troshin, P. A. *ACS Appl. Mater. Interfaces* **2019**, *11*, 21741. https://doi.org/10.1021/acsami.9b01729
13. Zhu, S.-E.; Li, F.; Wang, G.-W. *Chem. Soc. Rev.* **2013**, *42*, 7535. https://doi.org/10.1039/C3CS35494F
14. Badamshina, E.; Gafurova, M. *J. Mater. Chem.* **2012**, *22*, 9427. https://doi.org/10.1039/C2JM15472B
15. Cao, Z.; Zhao, W.; Liu, Q.; Liang, A.; Zhang, J. *Adv. Mater. Interfaces* **2018**, *5*, 1701303. https://doi.org/10.1002/admi.201701303
16. Sun, J.; Du, S. *RSC Adv.* **2019**, *9*, 40642. https://doi.org/10.1039/C9RA05679C
17. Zhang, R.; Xiong, L.; Pu, J.; Lu, Z.; Zhang, G.; He, Z. *Adv. Mater. Interfaces* **2019**, *6*, 1901386. https://doi.org/10.1002/admi.201901386
18. Wu, K.; Qiang, L.; Gong, Z.; Zhao, G.; Gao, K.; Zhang, B.; Zhang, J. *Surf. Interface Anal.* **2015**, *47*, 903. https://doi.org/10.1002/sia.5793

19. Uchikawa, S.; Kawasaki, A.; Hoshino, N.; Takeda, T.; Noro, S.I.; Takahashi, K.; Nakamura, T.; Sato, N.; Kokubo, K.; Sakurai, H.; Akutagawa, T. *J. Phys. Chem. C* **2019**, *123*, 23545. https://doi.org/10.1021/acs.jpcc.9b06951
20. Velzeboer, I.; Kwadijk, C. J. A. F.; Koelmans, A. A. *Environ. Sci. Technol.* **2014**, *48*, 4869. https://doi.org/10.1021/es405721v
21. Avanasi, R.; Jackson, W. A.; Sherwin, B.; Mudge, J. F.; Anderson, T. A. *Environ. Sci. Technol.* **2014**, *48*, 2792. https://doi.org/10.1021/es405306w
22. Zhang, M.; Tao, S.; Wang, X. *Environ. Sci.: Nano* **2020**, *7*, 2486. https://doi.org/10.1039/D0EN00515K
23. Nierengarten, I.; Nierengarten, J.-F. *Chem. Asian J.* **2014**, *9*, 1436. https://doi.org/10.1002/asia.201400133
24. Hashimoto, A.; Takamura-Enya, T.; Oda, Y. *Photochem. Photobiol.* **2019**, *95*, 1403. https://doi.org/10.1111/php.13138
25. Bianco, A.; Da Ros, T. *Biological Applications of Fullerenes. Fullerenes: Principles and Applications*; The Royal Society of Chemistry: London, UK, 2011, Vol. 2, 507-545. https://doi.org/10.1039/9781849732956-00507
26. Plehn, T.; Megow, J.; May, V. *Phys. Chem. Chem. Phys.* **2014**, *16*, 12949. https://doi.org/10.1039/C4CP01081G
27. Chen, T.; Zou, H.; Wu, X.; Chen, Y.; Situ, B.; Zheng, L.; Yang, G. *ACS Biomater. Sci. Eng.* **2019**, *5*, 3079. https://doi.org/10.1021/acsbiomaterials.9b00372
28. Zhai, Y.; Zhu, Z.; Dong, S. *ChemCatChem* **2015**, *7*, 2806. https://doi.org/10.1002/cctc.201500323
29. Shah, S.; Tiwari, N.; Kumar, Y.; Jha, S. N.; Auroux, A.; Pandey, J. K.; Chowdhury, B. *ChemistrySelect* **2017**, *2*, 5640. https://doi.org/10.1002/slct.201700203
30. Gopiraman, M.; Saravanamoorthy, S.; Ullah, S.; Ilangovan, A.; Kim, I. S.; Chung, I. M. *RSC Adv.* **2020**, *10*, 2545. https://doi.org/10.1039/C9RA09244G
31. Beck, M. T.; Mandi, G.; Keki, S. *Proc. Electrochem. Soc.* **1995**, 95. https://doi.org/10.1080/1536383X.2010.481063
32. An, Y. Z.; Ellis, G. A.; Viado, A. L.; Rubin, Y. *J. Org. Chem.* **1995**, *60*, 6353. https://doi.org/10.1021/jo00125a022
33. Bingel, C. *Chem. Ber.* **1957**, *8*, 126. https://doi.org/10.1002/cber.19931260829
34. Fong, R.; Schuster, D. I.; Wilson, S. R. *Org. Lett.* **1999**, *1*, 729. https://doi.org/10.1021/ol990722z
35. Maggini, M.; Scorrano, G.; Prato, M. *J. Am. Chem. Soc.* **1993**, *115*, 9798. https://doi.org/10.1021/ja00074a056
36. Li, L. S.; Hu, Y. J.; Wu, Y.; Wu, Y. L.; Yang, F. *J. Chem. Soc., Perkin Trans. 1*, **2001**, 617. https://doi.org/10.1039/B007498P
37. Coro, J.; Rodríguez, H.; Rivera, D. G.; Suárez, M.; Molero, D.; Herranz, M. Á.; Martínez-Álvarez, R.; Filippone, S.; Martín, N. *Eur. J. Org. Chem.* **2009**, 4810. https://doi.org/10.1002/ejoc.200900583

38. Kim, D. S.; Baek, N.; Oh, S. R.; Jung, K. Y.; Lee, I. S.; Kim, J. H.; Lee, H. *Arch. Pharmacol Res.* **1997**, *20*, 201. https://0-doi-org.library.scad.edu/10.1007/BF02976145
39. Ruiz, A. ; Coro, J.; Almagro, L.; Ruiz, J. A. ; Molero, D.; Maroto, E. E.; Filippone, S.; Herranz, M. Á.; Martínez-Álvarez, R.; Sancho-García, J. C.; Di Meo, F.; Suárez, M.; Martín, N. *J. Org. Chem.* **2013**, *78*, 2819. https://doi.org /10.1021/jo302528t
40. Raeside, J. I.; Renaud, R. L.; Marshall, D. E. *J. Steroid Biochem. Mol. Biol.* **1992**, *42*, 113. https://doi.org /10.1016/0960-0760(92)90017-d
41. Ruiz, A. ; Coro, J.; Almagro, L.; Ruiz, J. A. ; Molero, D.; Maroto, E. E.; Filippone, S.; Herranz, M. Á.; Martínez-Álvarez, R.; Sancho-García, J. C.; Di Meo, F.; Suárez, M.; Martín, N. *J. Org. Chem.* **2014**, *79*, 3473. https://doi.org/10.1021/jo5005907
42. Bjelakovic, M. S.; Kop, T. J.; Vlajic, M.; ĐorCevic, J.; Milic, D. R. *Tetrahedron* **2014**, *70*, 8564. https://doi.org/10.1016/j.tet.2014.09.070
43. Schwarc, S.; Csuk, R. *Bioorg. Med. Chem.* **2010**, *18*, 7458. http://dx.doi.org/10.1016/j.bmc.2010.08.054
44. Suárez, M.; Ruiz, A.; Almagro, L.; Coro, J.; Maroto, E. E.; Filippone, S.; Molero, D.; Martínez-Álvarez, R.; Martín, N. J. Org. Chem. 2017, 82, 4654. http://dx.doi.org/10.1021/acs.joc.7b00286
45. Filippone, S.; Maroto, E. E.; Martín-Domenech, A.; Suárez, M.; Martín, N. *Nat. Chem.* **2009**, *1*, 578. http://dx.doi.org/10.1038/nchem.361
46. Maroto, E. E.; Filippone, S.; Martín-Domenech, A.; Suárez, M.; Martín, N. *J. Am. Chem. Soc.* **2012**, *134*, 12936. https://doi.org/10.1021/ja306105b
47. Maroto, E. E.; Filippone, S.; Suárez, M.; Martínez-Álvarez, R.; de Cózar, A.; Cossío, F. P.; Martín, N. *J. Am. Chem. Soc.* **2014**, *136*, 705. https://doi.org/10.1021/ja410408c
48. Ruiz, A. ; Coro, J.; Almagro, L.; Ruiz, J. A. ; Molero, D.; Maroto, E. E.; Filippone, S.; Herranz, M. Á.; Martínez-Álvarez, R.; Sancho-García, J. C.; Di Meo, F.; Suárez, M.; Martín, N. *Eur. J. Org. Chem.* **2018**, *33*, 4512. http://dx.doi.org/10.1002/ejoc.201800622
49. Reyes-Moreno, M.; Fuente-Hernández, A.; Ruiz-García, J. A.; Álvarez-Ginarte, Y. M.; Vélez-Castro, H.; Fernández-Villalobo, A.; Montiel-Smith, S.; Meza-Reyes, S.; Sandoval-Ramírez, J. *J. Mex. Chem. Soc.* **2007**, *51*, 232. https://doi.org/10.1021/ja074536x
50. Reyes, M.; Ruiz, J. A.; Ibarra, Y.; Fuente, A.; Vélez, H.; Hernández, I.; Martínez, I.; Rodeiro, I.; Sandoval, J.; Meza, S.; Montiel, S. *Eur. J. Med. Chem.* **2009**, *44*, 4567. https://doi.org/10.1016/j.ejmech.2008.03.003
51. Ruiz, A.; Lemos, R.; Makowski, K.; Rodríguez, H.; Ortiz, O.; Cáceres, W.; Ángeles Herranz, M.; Molero, D.; Martínez-Álvarez, R.; Suárez, M.; Martín, N. *Eur. J. Org. Chem.* **2020**, *37*, 5926. https://doi.org/10.1002/ejoc.202000989
52. Castro, E.; Martinez, Z. S.; Seong, C. S.; Cabrera-Espinoza, A.; Ruiz, M. A.; García, H.; Valdez, F.; Llano, M.; Echegoyen, L. *J. Med. Chem.* **2016**, *59*, 10963. https://doi.org/10.1021/acs.jmedchem.6b00994
53. Alonso, D.; Hernández-Castillo, D.; Almagro, L.; González-Alemán, R.; Molero, D.; Ángeles Herranz, M; Medina-Páez, E.; Coro, J.; Martínez-Álvarez, R.; Suárez, M.; Martín, N. *J. Org. Chem.* **2020**, *85*, 2426. https://dx.doi.org/10.1021/acs.joc.9b03121

54. Suárez, M.; Makowski, K.; Lemos, R.; Almagro, L.; Rodríguez, H.; Herranz, M. A.; Molero, D.; Ortiz, O.; Maroto, E.; Albericio, F.; Murata, Y.; Martín N. *ChemPlusChem* **2021**, *86*, 972. https://doi.org/10.1002/cplu.202000770

Authors' Biographies

Malika Ibrahim-Ouali carried out her Ph.D. under the supervision of Professor Gramain in Clermont-Ferrand (France) in 1996. The work was focused on the synthesis of alkaloids. She was a postdoctoral fellow with Prof. Knochel (Humboldt fellowship) in 1996 in Germany where she got her first training in organometallic chemistry. In 1997, she joined Professor H. P Husson's group at the ICSN (Paris) as a postdoctoral researcher. Since 1998, she is currently an Associate Professor at Aix-Marseille University, and her field of interest remains the total synthesis of natural compounds.

Frédéric Dumur received his Ph.D. in chemistry in 2002 from the University of Angers (France) under the supervision of Professor Pietrick Hudhomme. After Post-Doctoral studies at the University of Groningen (The Netherlands), Reims Champagne-Ardennes (France) and Versailles Saint-Quentin-en-Yvelines (France), he joined the Faculty of Sciences at Aix-Marseille University in 2008, where he is currently working as an Associate Professor. His research interests include the synthesis of phosphorescent dopants for OLEDs and photoinitiators of polymerization. He co-authored about 300 publications and 5 book chapters.