Steroids conjugated to carbon nanoforms as potential viral proteases inhibitors, Synthesis, DFT calculations, and Molecular Docking

Reinier Lemos,[a] Kamil Makowski,[b] Margarita Suárez,[a]
[a]Laboratorio de Síntesis Orgánica, Facultad de Química, Universidad de la Habana, 10400 La Habana (Cuba)
[b]Departamento de Surfactantes y Nanobiotecnología, IQAC-CSIC, Madrid (España)
reylemos81@gmail.com

Introduction

The synthesis of hybrid molecules where [60]fullerene is covalently linked to biologically active compounds allows to increase their biocompatibility and bioavailability. In addition, the efficient antiviral capacity of some of these derivatives has been recognized, being used as inhibitors of the protease of HIV-1, of the Ebola virus, and recently their potential use against SarsCov-2 has begun to be evaluated.[1] Steroids constitute a large and important class of biologically active polycyclic compounds and are widely used for medical purposes. Hybridization of steroids with other molecules could allow the preparation of compounds with completely new properties and functions. Hybrids of fullerenes with sugars or steroids have previously shown interesting biological activities.[2] Therefore, hybrid systems combining C_{60} with steroids and carbohydrates could have potential antiviral applications.

Results

The methodology used to obtain the hybrids basically consisted of three steps. At first, the transformation of the starting steroids to generate functionalized malonates followed by their coupling to previously protected monosaccharides. C_{60} was finally covalently linked to the monosaccharide-steroid conjugate by cycloaddition using the Bingel-Hirsch methodology.

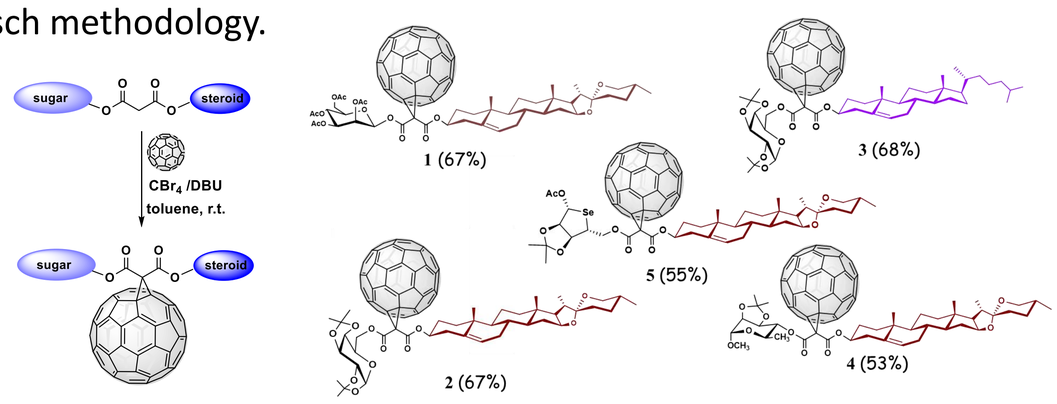

Figure 1. Synthesis of sugar-steroid methano[60]fullerene.

All hybrid molecules were recovered as stable solids in good yield. The structural elucidation of the purified methane[60]fullerenes was carried out by combining different spectroscopic techniques. Although all derivatives are novel, the first selenium-sugar fused to a steroid system and C_{60} was generated.

The thermal stability of the new hybrids was evaluated by thermogravimetric analysis. Figure 4 shows the representative thermograms of **3** and **4**. It was appreciated that the hybrids exhibit a slight weight loss between 100 and 150 °C. Compounds **3** and **4** show an abrupt loss of mass associated with the breakdown of the steroid fraction where **4** loses 22% of its total mass at 500 °C. Considering the temperature of the consecutive mass losses, it can be concluded that the nature of the non-steroidal substituents of the hybrids have a marked influence on their thermal behavior for future applications.

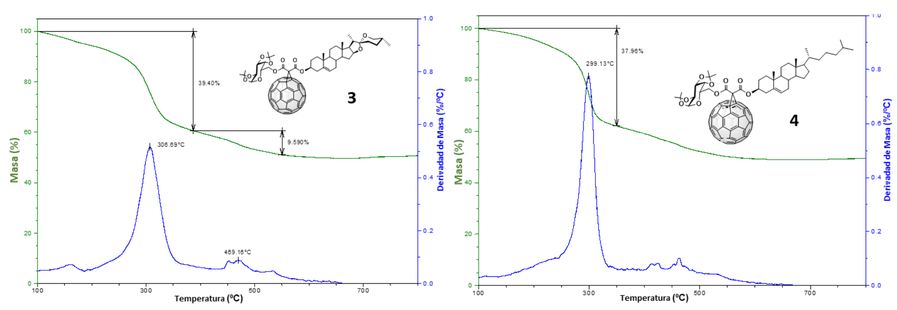

Figure 4. Thermograms of methanefullerenes **3** and **4**.

In addition, to predict the intramolecular and intermolecular electrostatic interactions in the hybrids, the molecular electrostatic potential map and selected descriptors and properties were calculated.

Table 1. Electronic properties determined using molecular descriptors.

Property	1	2	3	4	5
SASA (Å²)	1305,37	1232,75	1113,65	1260,53	**1270,74**
logP	13,65	17,35	22,43	21,93	**18,75**
Hy	-6,026	-6,156	-6,244	-6,165	**-6,134**
TPSA (Å²)	185,49	117,21	98,75	107,98	**107,98**
Dipole moment	3,01732	5,03789	7,05372	5,86538	**5,86538**

Considering these results, it was expected that these hybrid molecules could be able to penetrate through lipid membranes and the blood-brain barrier, which would be of paramount importance for prospective biological applications. These findings are in good agreement with the results reported for similar compounds.[2]

The interaction of the fullerene derivatives against the HIV-1 protease (PR) and the main protease of Sars-Cov-2 (Mpro) was studied from Molecular Docking. Negative binding energies were obtained and hydrogen bonds were established between the residues of the active site and the oxygen atoms of the ligands. The complexes of hybrid **5** are representatively shown in Figure 5 and the interactions with proteases residues in Table 2.

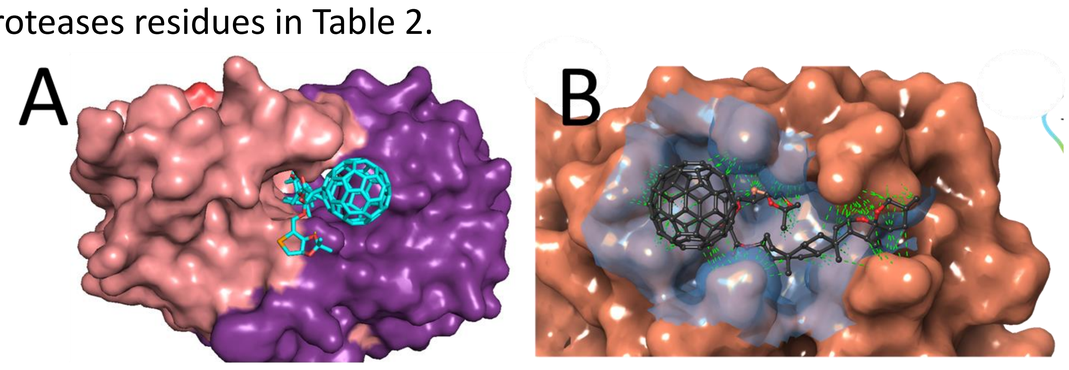

Figure 5. Representative binding modes of **5** with PR (A) and Mpro (B).

Table 2. Protease residues involved in the interaction with **5** and crystallization ligand.

Ligand	PR	Mpro
5	ARG8, ASP25, GLY27, ALA28, GLY48, GLY49, ILE50, LEU23', ASP25', GLY27', ALA28', ASP29', ASP30', ILE47', GLY48', GLY49', ILE50', PHE53', PRO81', VAL82', ILE84'	THR24, THR25, HIS41, THR45, SER46, GLU47, MET49, ASN142, MET165, GLU166, LEU167, PRO168, ASP187, ARG188, GLN189'
Cryst. ligand	LEU23, ASP25, GLY27, ALA28, ASP29, ASP30, VAL32, ILE47, GLY48,GLY49, ILE50, VAL82, ILE84, LEU23', ASP25', GLY27', ALA28', ASP29', ASP30', VAL32', ILE47', GLY48', GLY49', ILE50', LEU76', PRO81', VAL82', ILE84'	THR24, THR25, HIS41, CYS44, THR45, SER46, MET49, TYR54, ASN142, CYS145, HIS164, MET165, GLU166, VAL186, ASP187, ARG188, GLN189, GLN192

Conclusions

The synthesis of a new type of C_{60} derivatives was carried out using the Bingel-Hirsch methodology. Experimental and theoretical studies of [60]fullerene derivatives led to a better understanding of their properties. All theoretical physicochemical parameters allow us to infer the behavior of the new hybrid in the environment of biological fluids and its potential to cross biological membranes. The interaction of the hybrids with the residues of the viral proteases studied suggest that they could be used as potential inhibitors to initiate viral inhibition studies.

References

1- T.D. Marforio, E. Mattioli, F. Zerbetto, M. Calvaresi, *Molecules,* **2022**, 27(6), 1916-1927.
2-a) L. Almagro, R. Lemos, K. Makowski, H. Rodríguez, O. Ortiz, W. Cáceres, M.Á. Herranz, D. Molero, R. Martínez-Álvarez, M. Suárez, N. Martín, *Eur. J. Org. Chem*. **2020** (2020), 5926–5937. b) G. Guerrero-Luna, M. G. Hernández-Linares, S. Bernès, A. Carrasco, D. Montalvo-Guerrero, M. A. Fernández-Herrera, J. Sandoval-Ramírez, *Molecules* **2020**, *25*, 1213.