

SCIENTIFIC REPORT

Dendrimer-carbon nanostructure conjugates as drug delivery support

Phase 1 - period december 2013– december 2013

Phase III. Synthesis and physico-chemical characterization of dendrimer-carbon nanostructure-dendrimer complexes

At this stage was continued the investigation obtained in order to characterize their structures, so for the fullerene amino acid derivatives were used photoelectron emission methods (XPS), in order to confirm the reaction product. Based on obtained results a conversion rate of 74.5% for acyclic and 4.56% for cyclic structure. The total yield of functionalization reaction of fullerene alanine is 79.06%. The report for linked alanine / C60 (molar) is 0.89.

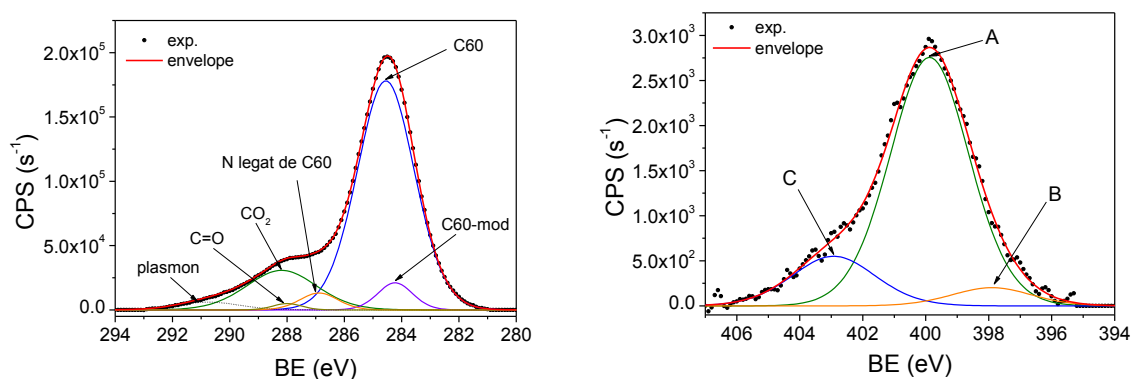
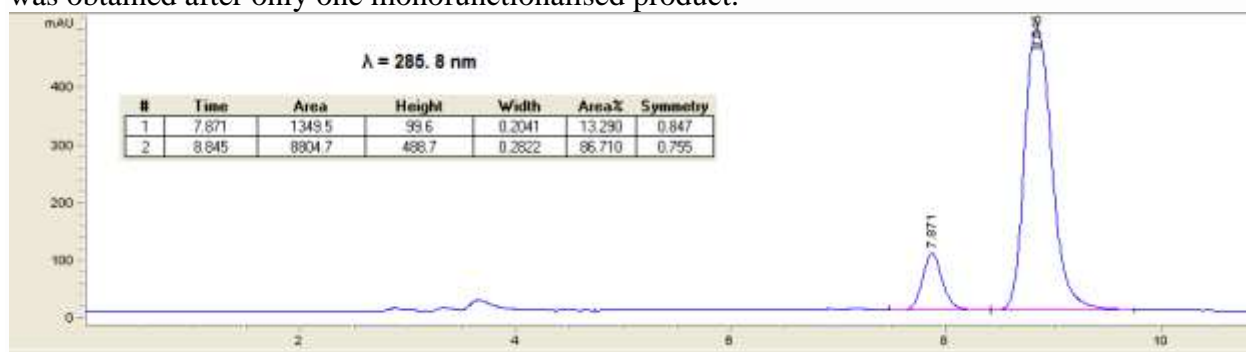


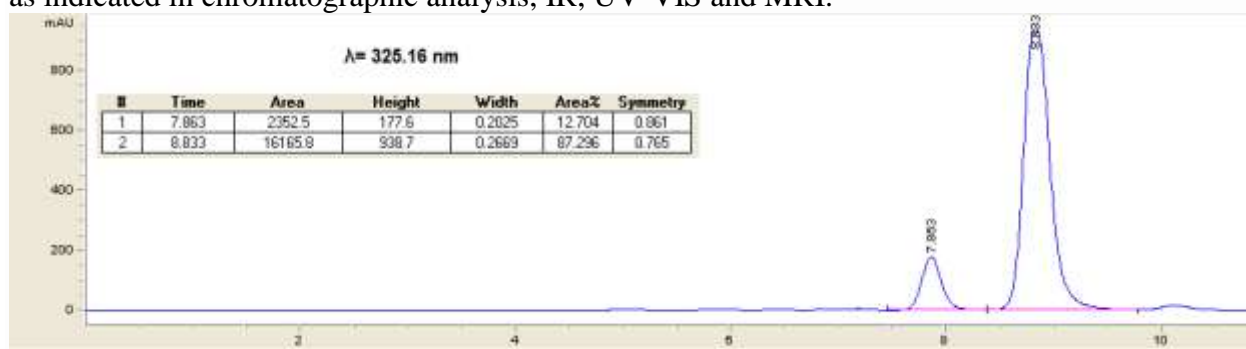
Figure 1. C1s XPS and N1s spectra for C₆₀-alanine (A)- N type NH₂; (B)- N bonded on C₆₀; (C)- N alanine NH₃;

In parallel to vary the types of nanostructures on the surface functions were carried out various functionalization reactions as follows:

1) functionalization of C₆₀ fullerene with phenol in the presence of a catalyst yields structures with functionalized phenoxide groups. Chromatographic analysis of a sample concentration of 1 mg / ml using a column Cosmosil thermostat to 25 ° C, eluent toluene (100%), pressure column 60 bars at 3 different wavelengths: 285.8, 312.16 respectively 325.16 nm indicates that reaction was obtained after only one monofunctionalised product.



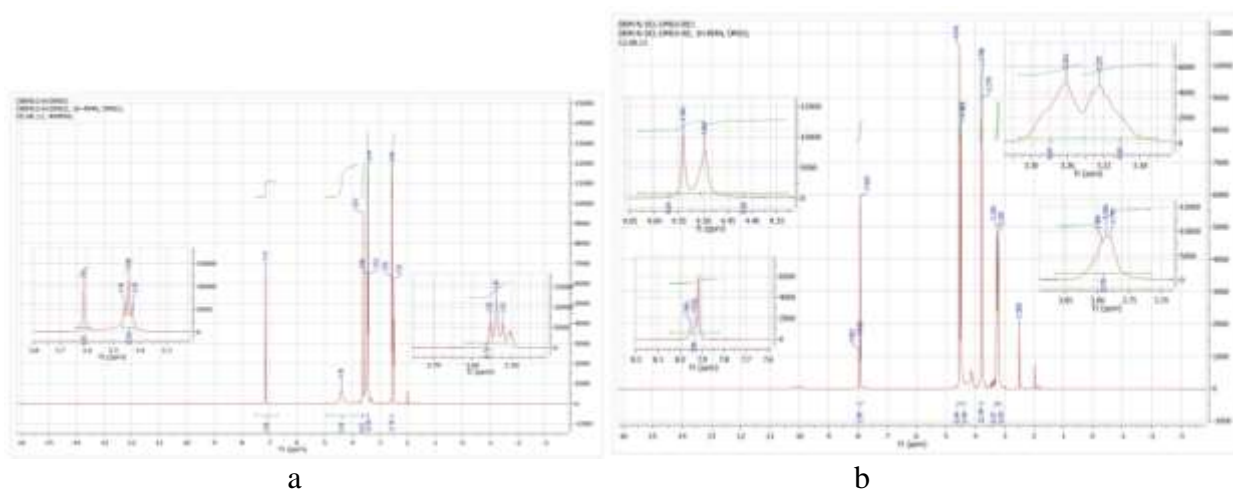
2) Functionalization of C60 fullerene with 4-methyl aminopyridine in the presence of a catalyst, resulting in binding the reactant on the fullerene surface by amine group having a single product, as indicated in chromatographic analysis, IR, UV-VIS and MRI.



3) Functionalization of C60 fullerene with N- (3 nitro benzyl) methylamine and ethylamine in the presence of a catalyst that has been achieved both classic and microwave field, resulting in a microwave field method to obtain larger quantities of final product.

4) Functionalization of carbon nanotubes with enzymes for use in the transport of enzymes. Single-wall nanotubes were suspended in phosphate buffer and sonicated 10 minutes. To this mixture *Candida Antarctica* Lipase B was added under continuous stirring 3h at 1500rpm and 200C. The precipitate was washed and filtered and determined protein content (approx. 1%). Enzyme was tested in capacity preservation of the synthesis of optically pure enantiomers, resulting in maintaining the enzymatic capacity over 10 cycles of use.

5) Obtaining 1 generation dendrimer. I generation dendrimers have been made towards increasing the number of nanostructures that can bind to the surface of the dendrimer. Thus it was created and investigated the conformational properties using various different techniques for NMR in deuterated solvents different generation dendrimers I and derivative hydrochloride and trifluoroacetate.



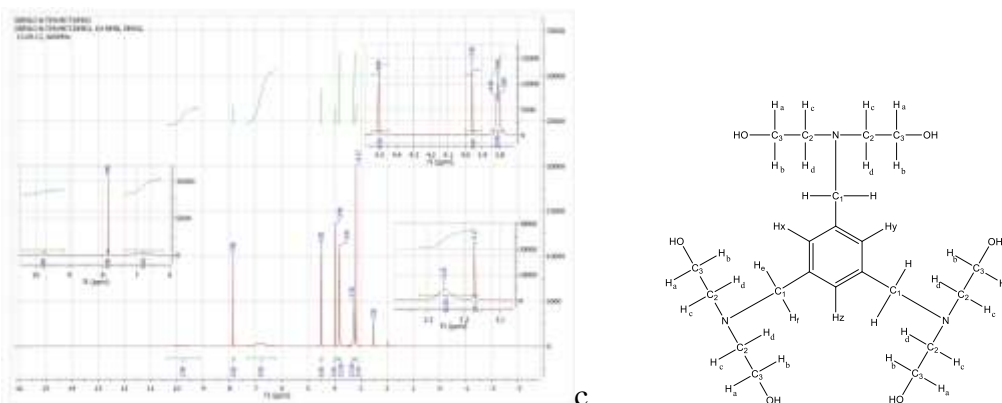
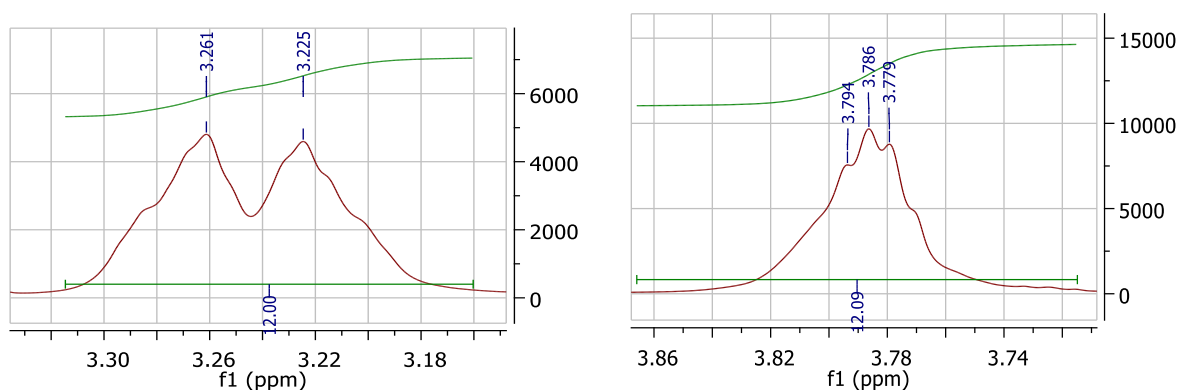
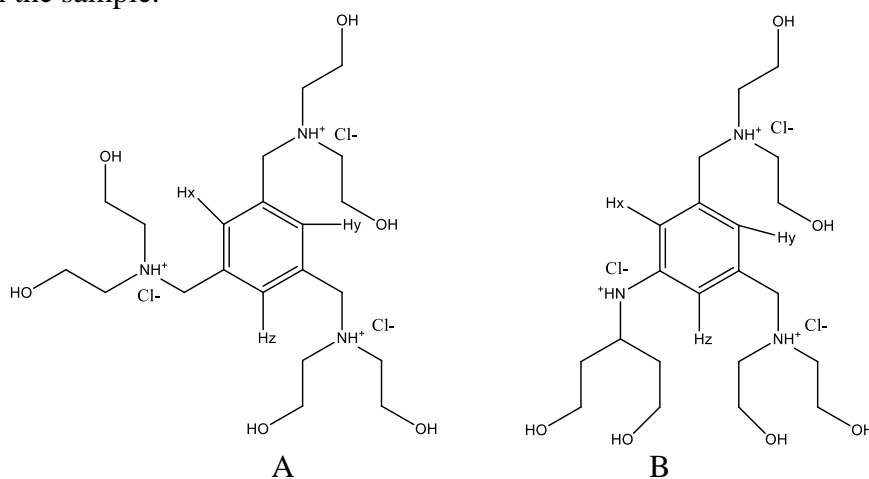


Figure 2. $^1\text{H-NMR-DMSO-}d_6\text{-400MHz, 298K}$ spectrum for 1 generation dendrimers (a), hydrochlorurated derivatives (b) and trifluoroacetat derivatives (c).

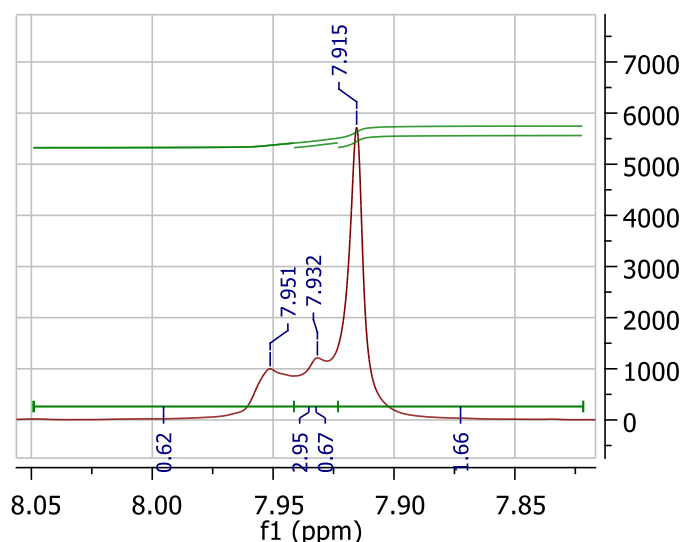
From RMN spectra analyses results existence of several diastereotopic protons (Ha, Hb si Hc, Hd).



Analyzing the signal in the field of aromatic region observed three peaks, which confirms the existence of two isomeric structure type propeller. Based on integrals areas can deduct their concentration in the sample.

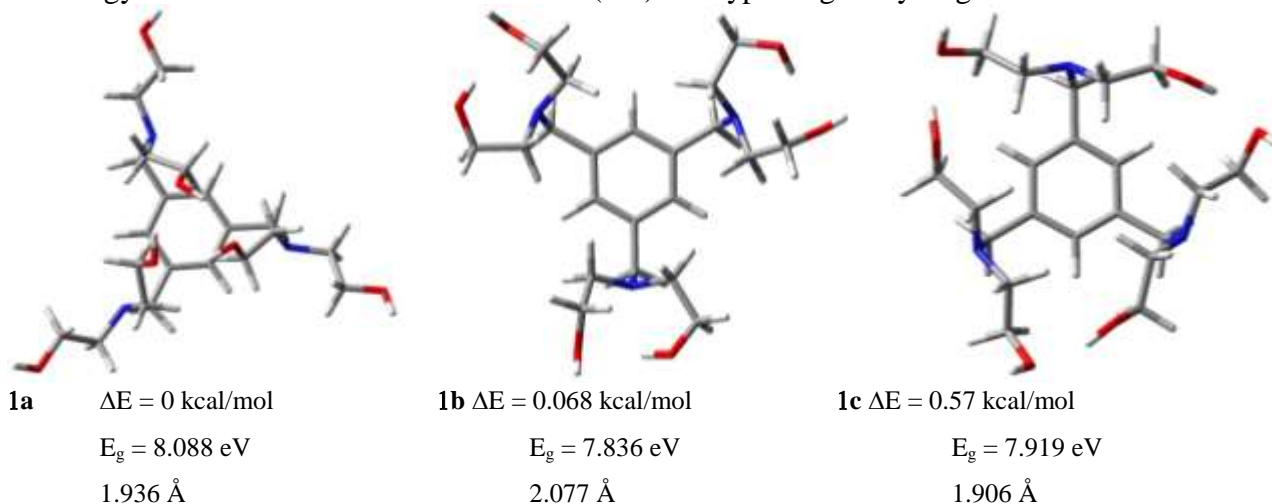


Semnal (ppm)	Aria	Atomi de H
7.951	0.62	y
7.932	0.67	y
7.915	1.66	x+y
Total	2.95	

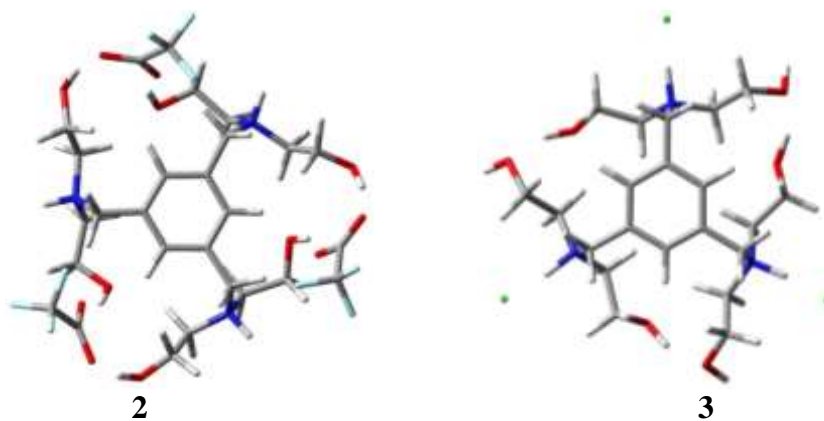


From calculation results that the A structure is in proportion of 33.8% and structure B in proportion of 66.2%.

To confirm the structures obtained and a theoretical study was done in parallel. Structures 1 to 3 was geometrically optimized with DFT method using density functional M06-2X, which is a hybrid exchange functional correlation (54% instead HF) correction dispersant basic set 6-31G (d, p). Functional M06-2X was used successfully in the study of systems where there are links from hydrogen, Thus is suitable for analysis of interactions in 1-3 structures. 1-3 Since there is the possibility of a large number of conformers initially were considered geometries corresponding topological symmetry (C3v). But this configuration is not a local minimum because the vibration spectrum appear more frequently negative, resulting in a distortion in geometry with C3 symmetry. Thus they were considered more C3 symmetry geometries with different kind of possible interactions of hydrogen. That all it represents a local minimum, and the difference in energy is very small. It can be seen in the table below between 6 OH groups as there are multiple ways to stabilize the molecule through the formation of intramolecular H-type connections. It shows the optimized geometry of the most stable conformers with the energy difference total to the compound most stable, the energy difference between orbitals border (EG) and type lengths hydrogen interactions.



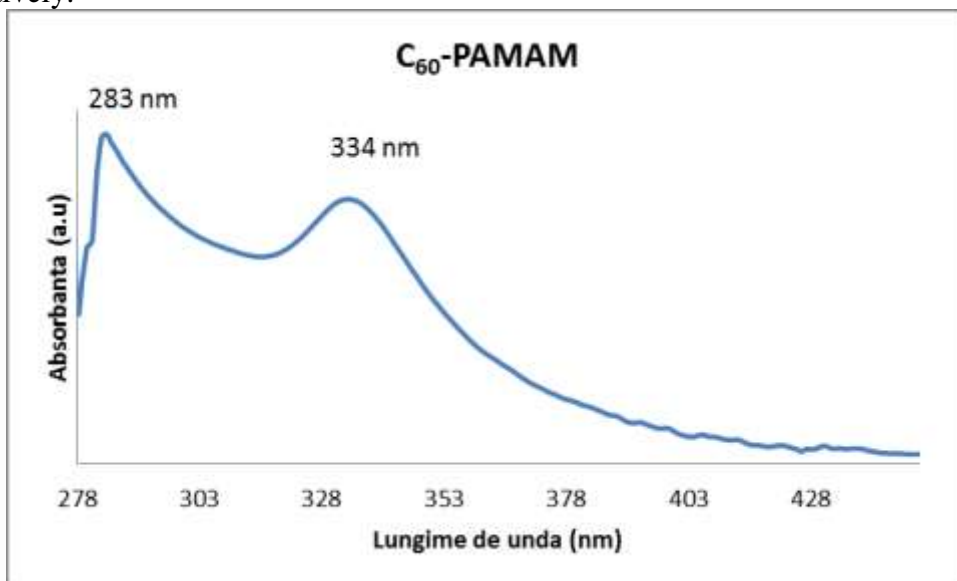
In the case of compound 2 there are two OH groups and interactions between atoms of the anion A CF_3COO^- . Cl atom (compound 3) preferred to form a bond with the proton linked to nitrogen and intermolecular hydrogen bonds type survived.

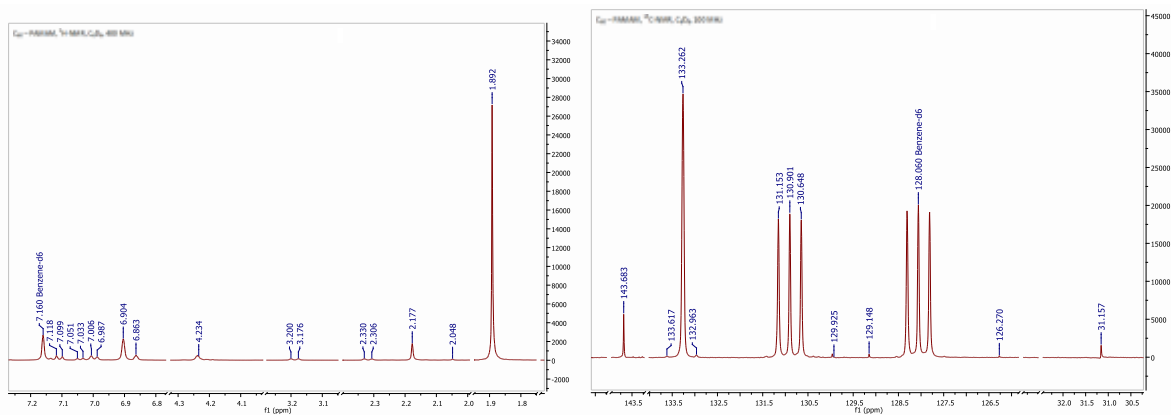


6) C₆₀ fullerene functionalization with PAMAM dendrimer in the presence of catalyst

Based on the results on coupling reactions using amino group in the presence of catalysts and microwaves field was achieved functionalization amine groups of the dendrimer PAMAM, resulting in a product dark. Procedure are: in a round bottom flask of 100 ml provided Refrigerated, C₆₀ fullerene (350 mg) was dissolved in toluene (C₇H₈, 60 ml). PAMAM dendrimer generation 0.0 (C₂₂H₄₈N₁₀O₄, 50 mg) with the ethylene diamine core was added to, and the metal catalyst bis (cyclopentadienyl) hafnium (IV) (C₁₀H₁₀Cl₂Hf 175 mg). The resulting mixture was allowed to reflux with stirring continued for 24 h.

Chromatographic analysis highlights the existence of two spots, one being unreacted fullerene and the second being functionalized dendrimers, which indicates that there is only one product functionalized. For UV-VIS analysis to prepare a solution at 1 mg / ml in toluene, which was measured at a dilution of 10⁻², the maximum values seen in the two drawings: from 283 to 334 nm, respectively.

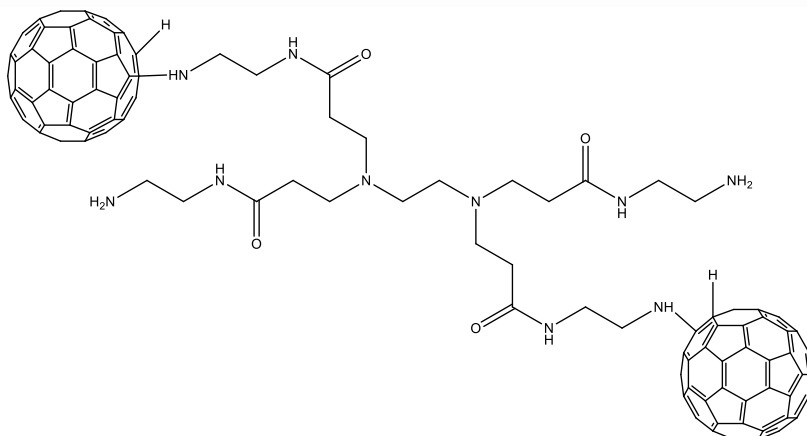




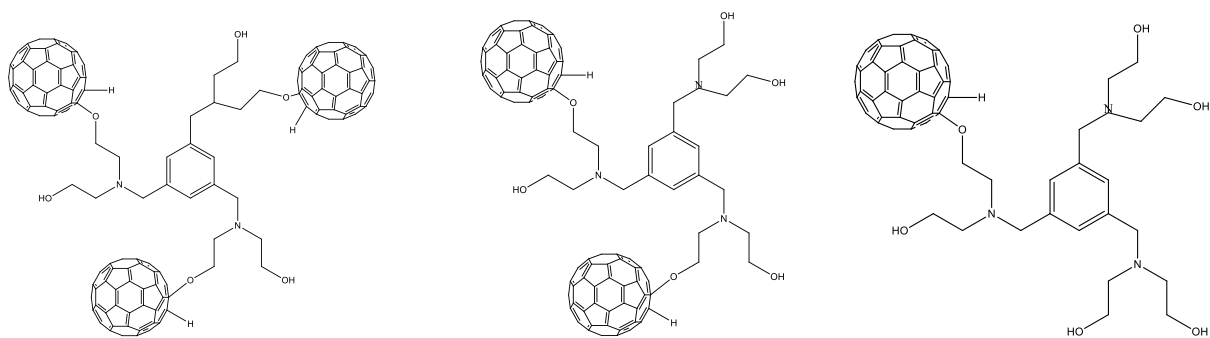
1 H-NMR spectrum and 13 C-NMR fullerene functionalized dendrimers earth metal.

The chromatographic analysis was conducted on a sample concentration of 1 mg / ml using a column Cosmosil thermostat to 25°C, eluent toluene (100%), pressure column 59 bars at 3 different wavelengths: 285.8, 312.16 respectively 325.16 nm yielding two peaks one being unreacted fullerene, and the other reaction products.

From the analysis of IR, NMR, MS mass spectra results that the reaction product has the following structure:



7) Functionalization of dendrimers with tri-bromo ethylbenzene core under catalyst chloride biscyclopentadienyl hafnium and titanium in a microwave field, was achieved by the following method: a tube capacity of 7 ml used in the reaction in the microwave field, fullerene C₆₀ is dissolved in toluene (C₇H₈ 5 ml). Add dendrimer generation 0 5: 1 and the metal catalyst bis (cyclopentadienyl) hafnium (IV). The resulting mixture is left under stirring for 6 hours at 150°C in a microwave field (EMF reactor). The reaction mixture is filtered washed and dried, resulting in a dark-colored compound. Data analysis IR, NMR and MS results in structures with surface functionalized dendrimers by ether linkage. The chromatographic analysis was conducted on a sample concentration of 1 mg / ml using a Cosmosil column thermostat to 25°C, eluent toluene (100%), 59 bar pressure column 3 different wavelengths: 285.8, 312.16 325.16 nm respectively, obtainandu- the four peaks at retention times of 8.83 min (fullerene); 9.20 (mono-substituted compound), 10.64 (di-substituted compound), and 12.98 (trisubstituted). 3 thus obtained functionalized structures:



Due to the steric impediment caused by the size of the molecule may not be functionalized by fullerenes all hydroxyl groups on the surface of fullerene from this generation.

The results have materialized in these ISI articles and international conferences:

1. Szefler, M.V. Diudea, Polybenzene multitori, *Cent. Eur. J. Chem.*, 10(6), 1779-1785, **2012**.
2. Mircea V. Diudea, B. Szefler, Nanotube junctions and the genus of multi-tori, *Phys. Chem. Chem. Phys.*, 14, 8111–8115, **2012**
3. M.V. Diudea, Centric connectivity index by shell matrices, *Iranian Journal of Mathematical Sciences and Informatics* 7, 67-74, **2012**.
4. M.E. Füstös , M.V. Diudea, G. Katona, Functionalization of multi-walled carbon nanotubes with diamino-alkyl moieties, *Studia Universitatis Babeş-Bolyai Chimia*, **2012**, accepted.
5. Iranmanesha, M.V. Diudea, Cluj-Tehran Index, *MATCH Commun. Math. Comput. Chem.*, 69, 121-130, **2013**.
6. R. Pop, M. Medeleanu, M.V. Diudea, B. Szefler, J. Cioslowski, Fullerenes Patched by Flowers with Octagonal Core, *Central European Journal Of Chemistry*, **2013**, accepted.
7. M.A. Naghia, A. Varga, M. Füstös, G. Katona, V. Zaharia, Heterocycles 35: CaL-B mediated synthesis of optically pure (*R*)- and (*S*)- ethyl 3-hydroxy-3-(2-aryl-thiazol-4-yl)propanoates, *Tetrahedron Asymmetry*, **2013**, accepted.
8. G. Katona, M.V. Diudea, Chemical Graph Theory and Nanoscience in Mathematical Chemistry Monographs, Ed. University of Kragujevac and Faculty of Science Kragujevac, Vol. 16, pp. 179-224, **2014**
9. M.E. Füstös , N.A. Dima, G. Katona, Functionalization of C₆₀-fullerenes, XVIII International Conference on Chemistry, 22-25 november **2012**, Baile Felix.
10. N.Csaba, N. Katalin, M.V. Diudea, G. Katona, Theoretical study of high symmetry multi-terminal armchair carbon nanotube junctions, International Meeting on Atomic and Molecular Physics and Chemistry, 12-14 septembrie **2012**, Pisa, Italy.
11. C. Nagy, K. Nagy, Phosphorus-doped tetrahedral heterofullerenes and corresponding tetrapodal junctions, 22,23 martie **2012**, Rennes, Franta.
12. M.V. Diudea, Quasicrystals, from spongy to full space filling, Graph- and group-theoretical approach in crystallography and crystal chemistry, 11-15 decembrie **2012**, Dresden Germania.
13. M.V. Diudea, Diamond d5 and related nanostructures, 1st International Conference on Nanostructures and Nanomaterials: Science and Applications, 7-9 februarie, **2012**, Masjedsoleyman, Iran.
14. M.V. Diudea, Centric connectivity index by shell matrices, 5th Conference and workshop on Mathematical Chemistry, 15-17 februarie **2012**, Yazd, Iran.
15. M.E. Füstös , N.A. Dima, G. Katona, Synthesis and characterisation of trimethylene benzene core dendrimers, XIX International Conference on Chemistry, 21-24 november **2013**, Baia Mare.
16. M.V. Diudea, B. Szefler, Diamond D5 allotropes, From molecule to material chemistry for the future, 27-31 mai, **2013**, Wroclaw, Polonia

17. M.V. Diudea. Exotic nanostructures, IV Grebenshchikov, 10-14 septembrie, **2013**, St. Petersburg, Rusia.
18. M.V. Diudea, C. Nagy, Novel carbon nanostructures, 58th Workshop: Carbon Topology, 19-23 iunie, **2013**, Erice, Sicilia, Italia.