

## SHORT COMMUNICATIONS

## FULLERENES AS THE CARRIERS OF COMPOUNDS WITH AMIDE BOND

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1 Banacha St., 02-097 Warszawa, Poland<sup>2</sup> National Medicines Institute, 30/34 Chełmska St., 00-725 Warszawa, Poland**Keywords:** fullerene C<sub>60</sub>, endohedral fullerene, amide bond

Fullerenes are molecules created solely of carbon and have a highly unusual attributes. The smallest structure in the fullerene family is conformation C<sub>20</sub>, which consists of pentagonal rings only, resulting in its instability. The C<sub>60</sub> is the first fullerene containing 12 pentagonal and 20 hexagonal rings in which pentagons do not share an edge. It results in good stability of the carbon cage. The structure of C<sub>60</sub> is a truncated icosahedron, with two different bond lengths: the ones between two hexagons are shorter (bond C–C, length 1.44 Å), then between a hexagon and a pentagon (double-bond C=C, length 1.39 Å); average bond length is 1.4 Å (1–3). The

attributes like structure, geometry, size and shape of carbon cages yield the possibility to use them as vehicles to carry substances with pharmacological activity to molecular target.

The derivatives of fullerenes are classified into following categories according to their functionalization: endohedral fullerenes with active molecules residing inside the carbon cage, exohedral with wide variety of both inorganic and organic groups existing outside and connected to the fullerenes' exterior and heterofullerenes – when one or more carbon atoms that form the fullerene carbon cage are replaced by a non-carbon atom, i.e., a heteroatom.

## EXPERIMENTAL

The aim of this investigation was to analyze possibilities to use fullerenes as the potential carriers of compounds with peptide bond.

The N-aminofornamide (a), N-hydroxyformamide (b), N-methylformamide (c) and also their phosphorous analogues, where nitrogen atom from amide bond was replaced by phosphorus atom, were chosen for empirical studies (Fig. 2.). All these compounds were trapped into a carbon cage of fullerene C<sub>60</sub>.

The energies of inclusion, deformation and interaction as well as absolute energy of each examined compound and their complexes with fullerene C<sub>60</sub> were calculated with the use of the molecular modeling technique. The calculations for all the structures were carried out using DFT/B3LYP method at the level 6-31G\*\* implemented in

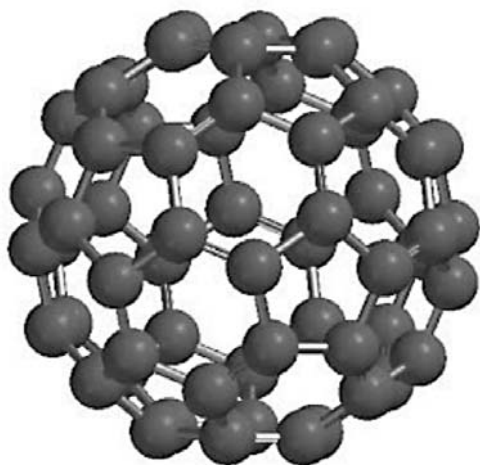


Figure 1. The structure of fullerene C<sub>60</sub>

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Spartan 08 and Gaussian 03 software (4, 5). The chosen basis set takes into consideration polarization functions on carbon and hydrogen atoms. The first calculation stage was geometry optimization, establishing the most stable conformation and the absolute energy (in energetic minimum) of each examined compound. After that, all the lowest energy conformers were placed into carbon cage of fullerene  $C_{60}$  and each complex geometry was optimized. The next step was delimitation of total energy of endohedral complexes ( $E_{cx}$ ). Finally, the energy values in geometry from endohedral complex – compound@ $C_{60}$  for N-aminoformamide, N-hydroxyformamide, N-methylformamide and their phosphorous analogues ( $E_{comp}$ ) and empty carbon cage ( $E_{cage}$ ) were calculated.

Stabilization energy ( $\Delta E_{stab}$ ) of the endohedral complexes was calculated as follows:

$$\Delta E_{stab} = E_{cx} - (E_{cage} + E_{comp})$$

where  $E_{cx}$  – complex energy,  $E_{cage}$  – fullerene  $C_{60}$  carbon cage energy,  $E_{comp}$  – every examined compound energy.

The energy of compound deformation ( $E_{def}$ ) was calculated as a difference between encapsulated optimized structure energy and total energy of the most stable conformation. The deformation energy

of fullerenes'  $C_{60}$  carbon cage was analogically estimated. The inclusion energy was calculated as a difference between complex energy in energetic minimum and sum of total energies of particular components. The interaction energy was calculated as the difference between complex energy in energetic minimum and the sum of total energies of particular components in the complex geometry (in single-point calculations – SP).

## RESULTS AND DISCUSSION

The results of the calculations are presented in Tables below.

Table 1 shows total energies of examined systems and energies of inclusion as well as interaction energy, respectively. The presented data reveal that complexes compound@ $C_{60}$  are thermodynamically unstable. It refers both to endohedral fullerenes, including compounds with nitrogen atom, and their phosphorous analogues. Nevertheless, nitrogen derivatives have higher stability. It results both from van der Waals volumes of trapped molecules and electron stability. The methyl group in both nitrogen compound and in its phosphorous analogue destabilizes the system the most, while hydroxy one – the

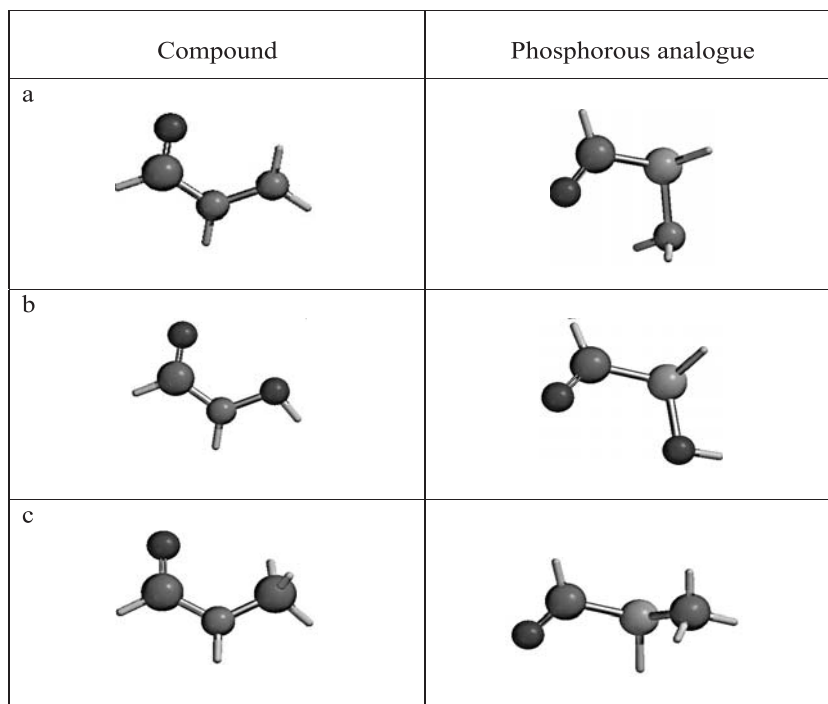


Figure 2. The structures of examined compounds

Table 1. Total energies of studied systems.

Compound	Complex energy ( $E_{cx}$ ) [hartree]	Inclusion energy ( $E_{inc}$ ) [kcal/mol]	Interaction energy ( $E_{int}$ ) [kcal/mol]
CHO-NH-NH <sub>2</sub>	-2511.165396	141.7	109.8
CHO-NH-OH	-2531.057702	106.7	86.0
CHO-NH-CH <sub>3</sub>	-2495.082799	188.8	139.8
CHO-PH-NH <sub>2</sub>	-2797.626637	204.0	148.0
CHO-PH-OH	-2817.557307	191.0	152.3
CHO-PH-CH <sub>3</sub>	-2781.489139	292.5	207.3

Table 2. The total energy and energy of deformation of studied compounds.

Compound	Total energy [hartree]	SP [hartree]	Energy of deformation ( $E_{def}$ ) [kcal/mol]
CHO-NH-NH <sub>2</sub>	-225.216830	-225.186915	18.8
CHO-NH-OH	-245.053341	-245.035131	11.4
CHO-NH-CH <sub>3</sub>	-209.209250	-209.162445	29.4
CHO-PH-NH <sub>2</sub>	-511.777230	-511.719734	36.1
CHO-PH-OH	-531.687195	-531.652959	21.5
CHO-PH-CH <sub>3</sub>	-495.780854	-495.684091	60.7

Table 3. The total energy and energy of deformation of fullerene C<sub>60</sub>.

Fullerene C <sub>60</sub> „guest” molecule	Total energy [hartree]	SP [hartree]	Energy of deformation [kcal/mol]
CHO-NH-NH <sub>2</sub>	-2286.174447	-2286.153464	13.2
CHO-NH-OH	-2286.174447	-2286.159638	9.3
CHO-NH-CH <sub>3</sub>	-2286.174447	-2286.143197	19.6
CHO-PH-NH <sub>2</sub>	-2286.174447	-2286.142816	19.8
CHO-PH-OH	-2286.174447	-2286.147069	17.2
CHO-PH-CH <sub>3</sub>	-2286.174447	-2286.135481	24.4

least, probably due to the size of functional groups in examined compounds. The inclusion energies range from 106.7 to 292.5 kcal/mol. Therefore, it could be concluded that these systems can be created at higher temperature (e.g., plasma) and their disintegration time will depend, in the first approximation, on the energetic barrier defined by inclusion energy.

Tables 2 and 3 show the total energies of compounds and fullerenes C<sub>60</sub> after their geometry optimization and their complex geometry energy values, as well as their deformation energies. They reveal the level of deformation energy of disturbed structures, which are molecules of fullerenes C<sub>60</sub> and the

examined compounds, as the result of complex formation.

The phosphorous analogues are more destabilized except for compounds with hydroxy group – its energy of deformation value is smaller than for N-methylformamide. It probably results from the tendency for intermolecular hydrogen bond formation. The nitrogen derivative with hydroxy group of high possibility to form this type of bond (size of molecule, distance between atoms) is relatively less destabilized.

Tables 4 and 5 contain bond lengths of examined compounds after their geometry optimization –

before ( $G_B$ ) and after ( $G_A$ ) inside fullerenes' cage placement. In the case of derivatives with hydroxy group, when the tendency for intermolecular hydrogen bond formation is high, the angle values between atoms were also analyzed. The  $\alpha$  is the angle between atoms: oxygen (O') and hydrogen of hydroxy group and oxygen of formyl group. The  $\beta$  angle is defined as between carbon and oxygen of formyl group and hydrogen of hydroxy group. The distances between the atoms: hydrogen and nitrogen (N') as well as hydrogen and carbon (C') in amine and methyl groups, respectively, were expressed as the average value of those bond lengths.

The lengths in amide bond atoms are succumb to disturbances, the angle alteration is observed and

total geometry of encapsulated molecules undergoes deformation. The most significant changes are observed between the atom lengths in CHO-PH-CH<sub>3</sub> (phosphorous analogue of N-methylformamide) molecule, the least in CHO-NH-OH (N-hydroxyformamide) based on difference between lengths of particular bonds in investigated compounds. It is not surprising if the molecule sizes are taken into consideration. The placement of compounds with hydroxy group inside of carbon cage of fullerene C<sub>60</sub> elicited significant shortening of bond lengths between hydrogen atoms of hydroxy group and oxygen atom of formyl group. In contrast, the probability of intermolecular hydrogen bond formation increased molecule deformation. Together with O—

Table 4. The comparison of particular bond lengths [Å] and angles of nitrogen compounds.

Bond	CHO-NH-N <sup>'</sup> H <sub>2</sub>		CHO-NH-O <sup>'</sup> H		CHO-NH-C <sup>'</sup> H <sub>3</sub>	
	G <sub>B</sub>	G <sub>A</sub>	G <sub>B</sub>	G <sub>A</sub>	G <sub>B</sub>	G <sub>A</sub>
C=O	1.219	1.213	1.226	1.224	1.219	1.209
C-N	1.362	1.306	1.353	1.308	1.362	1.302
N-N'	1.409	1.361				
N-O'			1.402	1.351		
N-C'					1.453	1.392
O'-H			0.981	1.002		
N'-H (śr.)	1.021	1.011				
C'-H (śr.)					1.093	1.051
O—H			2.023	1.495		
$\alpha$			119.09°	128.83°		
$\beta$			81.41°	88.84°		

Table 5. The comparison of bond lengths [Å] and angles of phosphorous analogues.

Bond	CHO-PH-NH <sub>2</sub>		CHO-PH-O <sup>'</sup> H		CHO-PH-C <sup>'</sup> H <sub>3</sub>	
	G <sub>B</sub>	G <sub>A</sub>	G <sub>B</sub>	G <sub>A</sub>	G <sub>B</sub>	G <sub>A</sub>
C=O	1.210	1.252	1.209	1.215	1.212	1.199
C-P	1.872	1.803	1.873	1.705	1.868	1.702
P-N	1.715	1.756				
P-O'			1.665	1.578		
P-C'					1.861	1.760
O'-H			0.966	1.006		
N-H (aver.)	1.011	1.005				
C'-H (aver.)					1.092	1.050
O—H			3.493	1.410		
$\alpha$			53.60°	136.92°		
$\beta$			73.05°	95.64°		

Table 6. The bond lengths [ $\text{\AA}$ ] changes [ $G_B - G_A$ ] in compounds studied. (+) depicts elongation of bond, (-) depicts shortening of bond (based on Tables 4 and 5).

Bond	CHO-NH-N <sup>+</sup> H <sub>2</sub>	CHO-NH-O <sup>-</sup> H	CHO-NH-C <sup>+</sup> H <sub>3</sub>	Bond	CHO-PH-NH <sub>2</sub>	CHO-PH-O <sup>-</sup> H	CHO-PH-C <sup>+</sup> H <sub>3</sub>
C=O	(-) 0.006	(-) 0.002	(-) 0.010	C=O	(+) 0.052	(+) 0.006	(-) 0.013
C-N	(-) 0.056	(-) 0.045	(-) 0.060	C-P	(-) 0.069	(-) 0.168	(-) 0.166
N-N <sup>+</sup>	(-) 0.048			P-N	(+) 0.041		
N-O <sup>-</sup>		(-) 0.051		P-O <sup>-</sup>		(-) 0.087	
N-C <sup>+</sup>			(-) 0.061	P-C <sup>+</sup>		(+) 0.040	(-) 0.101
O <sup>-</sup> -H		(+) 0.021		O <sup>-</sup> -H			
N <sup>+</sup> -H (aver.)	(-) 0.010			N-H (aver.)	(-) 0.005		
C <sup>+</sup> -H (aver.)			(-) 0.042	C <sup>+</sup> -H (aver.)			(-) 0.042
O $\rightarrow$ -H		(-) 0.528		O $\rightarrow$ -H		(-) 2.083	

-H distance shortening the  $\alpha$  and  $\beta$  angles increase, as well as the distance between the hydroxy group atoms. The biggest changes are observed in CHO-PH-OH (phosphorous analogue of N-hydroxyformamide).

## CONCLUSIONS

The results reveal that examined systems consisting of compounds with amide bond and their phosphorous analogues build into carbon cage of fullerene C<sub>60</sub> are thermodynamically unstable. However, calculation with the use of DFT method suggests that those systems could exist despite positive energies of inclusion values. The energy related barrier of fullerene breaking results in retention of the compound inside the carbon cage in spite of angles and bond lengths deformation in molecules of examined compounds.

The values of deformation, inclusion and interaction energies are higher in case of phosphorous analogues, which can be, in the first approximation, explained by the phosphorous atom size compared with the replaced nitrogen atom and consequently larger so-called van der Waals volume of entire molecules.

Comparing the results obtained for formamide incorporated into spherical cap of fullerene C<sub>60</sub> by H. Dodziuk et al. (6) the analogies are apparent. Energy of stabilization value, calculated with the use of molecular mechanics (MM) (INSIGHT program package (version 98.0) with CVFF and ESFF force fields), was 62.7 kcal/mol, which is an evidence of complex formamide@fullerene C<sub>60</sub> instability. The stability of complexes with fullerenes C<sub>70</sub>, C<sub>80</sub>, C<sub>76</sub>, C<sub>82</sub> was also studied. It turned out that the system CHONH<sub>2</sub>@C<sub>82</sub> has achieved the highest stability, in which energy of stabilization was -24.5 kcal/mol. According to the authors of „Molecular mechanics study of endohedral fullerene complexes with small molecules” (6) fullerene energy of stabilization depends on many weak interactions between „host” and „guest”, respectively, fullerenes carbon cage and molecules „guest” sizes as well as symmetry of fullerene.

The results of analysis performed indicate the possible use of fullerenes as the carriers of compounds with peptide bond. The study on model structures: N-aminofornamide, N-hydroxyformamide, N-methylformamide and their phosphorous analogues suggest that fullerene C<sub>60</sub> could serve as a carrier for peptide nature substances not provoking danger of amide bond structure disturbance. The geometry deformation of structures trapped in car-

bon cage could only have influence on properties and reactivity during their transport to molecular target.

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