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Catalytically active hybrid complex of fullerene C_{60} and vitamine B_{12}

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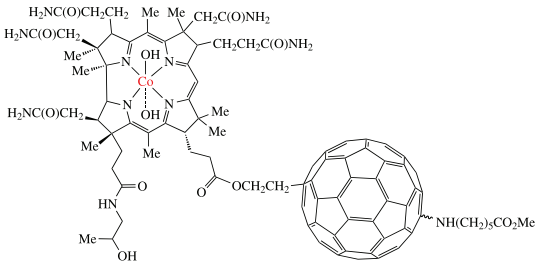
The reaction of hydroxycobinamide, a catalytically active derivative of vitamin B_{12}, with an amino acid derivative of fullerene C_{60} affords a hybrid complex. The catalytic activity of the complex is demonstrated by the autoxidation reaction of ascorbic acid.



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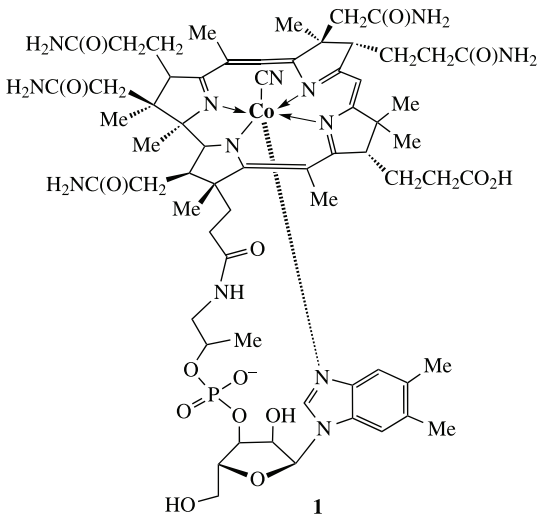
Transition metal complexes are active catalysts for many chemical reactions, similar to those occurring in living organisms under the action of metal enzymes.[1] Previously, it was assumed that catalysts for the release of reactive oxygen species (ROS), which are capable of selectively accumulating in tumors, may be active in suppressing the growth of tumor tissue.[2] At the same time, the native macrocyclic cobalt complexes, vitamin B_{12} and its derivatives (cobalamins, in particular, *e*-carboxylic acid of B_{12}: *e*-COOH–Cbl–CN **1**), are of most interest.

Early results,[2,3] in particular, the investigation of catalytic activity of these complexes in oxidation of natural substrates producing ROS in these reactions, oxidative cleavage of nucleic acids in the presence of these metal complexes and medico-biological examinations (investigations of antitumor activity of cobalt corrine complexes and their compositions with L-ascorbic acid) confirmed that catalytic system capable of generating ROS can be highly effective as antitumor and related agents.[4] Various methods have been developed for the synthesis of low-toxic amino acid and peptide derivatives of fullerene,[5,6] which have a wide spectrum of biological activity,[7] and the most effective derivatives with antitumor activity are those containing nitro groups or carnosine derivatives.[8,9] Indeed, fullerenes and their hybrid compounds turned out to be good photosensitizes for photodynamic therapy,[10,11] in the development of materials with biochemical and medical applications, as agents for the targeted drug delivery.[12] Moreover, the antitumor effect of drugs can be enhanced by the addition of active substances to fullerenes.[13,14]



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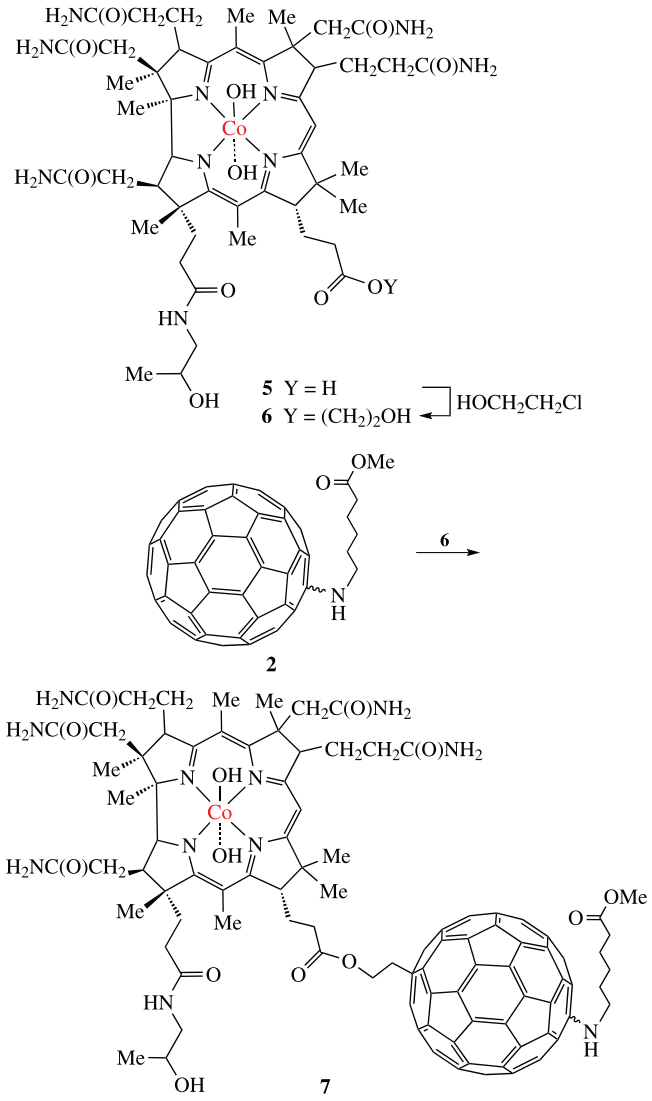
We assumed that the introduction of the active form of vitamin B_{12} into the molecule of a pharmacophore fullerene derivative would significantly increase the effectiveness of the antitumor action of the complexes.[15] As a result of the addition of the amino acid derivative of fullerene C_{60} **2** (ref. 16) to the carboxyl group of cobalamin derivative, new types of mixed biologically oriented cobalamin–fullerene complexes were obtained, namely, CN–Cbl–C(O)–C_{60}NH$(CH_2)_5CO_2$Me **3** and N–Cbl–C(O)OCH_2CH_2–C_{60}NH$(CH_2)_5CO_2$Me **4**. The structures of the obtained substances were established on the basis of elemental analysis data and the results of UV, IR, CD spectroscopic studies.[15]

At this stage of our research, the main task was to obtain and examine the catalytic properties of a hybrid structure based on the amino acid derivative of fullerene C_{60} **2** and another, catalytically active form of vitamin B_{12}, which was taken as *e*-(COOH)-dioxycobinamide [*e*-COOH–Cbi–$(OH)_2$] **5** (Scheme 1). The difficulty in the synthesis of such hybrid cobalamin fullerene complex in this case was the presence of several free hydroxy groups in compound **5**, which excluded the possibility of its addition directly to the fullerene framework of compound **2** by the chloroanhydride method. Therefore, we employed chloro ester **6** obtained by reacting compound **5** with ethylene chlorohydrin. A similar approach was used previously[15] to prepare the inactive form of the mixed complex CN–Cbl–C(O)OCH_2CH_2–C_{60}NH$(CH_2)_5CO_2$Me **4**. As a result, an aqueous solution of pure compound **7** was obtained (see Scheme 1 and Online Supplementary Materials for synthetic details).

Almost identical bands are present in the IR spectra of compounds **5** and **7** (see Online Supplementary Materials, Figure S1), with the exception of the 1227 cm^{-1} band that



Scheme 1

belongs to the free carboxy group of *e*-COOH-compound **5**, which is absent in spectrum of **7**. Also, the spectrum of **7** lacks a shoulder at 3420 cm^{-1}, which is characteristic of the stretching vibrations for the OH of the carboxy group of the starting compound **5**. Indeed, the following characteristic bands are observed in spectrum of **5**: intense wide bands of 3314 cm^{-1} with a shoulder of ~3420 and 3187 cm^{-1}, attributable to ν(OH) + ν(NH). These data confirm the attachment of **5** by the carboxy group to compound **2** *via* ethylene bridge.

The resulting data are also in good agreement with the electronic spectra of compounds **4**, **5** and **7** (*cf.* ref. 16, Figure S2). The UV-VIS spectra of compounds **4** and **5** containing corrine ligands show characteristic bands at 250, 360 and 540 nm, which correspond to the transitions of the corrine chromophore. So, the UV-VIS spectra of these compounds display all the bands that evidence the presence of the corrine ligand in both the starting and resulting compounds.

As shown earlier, the molecules of fullerene derivatives are found in water as associates, and the degree of their association depends on many parameters. Methyl esters of amino acid derivatives of fullerene are insoluble in aqueous media. However, the final product, mixed complex **7** containing a fragment of the B_{12} derivative, is already soluble in water. The dissolution mechanisms of the derivatives for the active (**7**) and inactive (**3**, **4**) forms of B_{12}, according to the light scattering data (see Online Supplementary Materials, Figures S3–S5), most likely differ from each other. Data on the dissolution of the inactive form of complexes with a direct bond with the fullerene framework **3** (Figure S3) or through an ethylene bridge **4** (Figure S4) show that a mixed dissolution mechanism would occur. In this case, low molecular weight fractions are obtained due to the appearance of hydrogen bonds of water molecules with hydrophilic fragments of vitamin B_{12}, and a high molecular weight fraction appears due to the twisting of hydrophobic parts of the molecule and it dissolves due to the hydrophilicity of the B_{12} fragment.

A different picture is observed in the compound with the active form B_{12} **7**. The B_{12} fragment is much smaller in size than in the previous complexes, but the number of hydroxy groups increases, which opens up additional opportunities for the formation of hydrogen bonds between water molecules and hydroxy groups. Therefore, we observe the formation of only low molecular weight associates (see Figure S5). These data are in good agreement with previous studies on the mechanism of dissolution of amino acid methyl esters derivatives of fullerene with a maleimide fragment.[17]

The different mechanism of dissolution is also confirmed by the CD spectra of concentrated and dilute aqueous solutions of the initial hydroxycobinamide **5**. The differences in these spectra can be explained by the fact that at a high concentration of compound **5**, the dissolution occurs due to the hydrophilicity of the entire hydroxycobinamide molecule (Figure S6). Meanwhile, in the case of dilution of the solution by a factor of 10 (Figure S7), the presence of a sufficient number of hydroxy groups results in the formation of a significant number of hydrogen bonds with water molecules, which ultimately leads to a change in the CD spectrum.

The CD spectra of previously synthesized compounds **3** (Figure S8) and **4** (Figure S9) containing an inactive form of vitamin B_{12}, as well as the initial dihydroxycobinamide **5** and mixed complex **7** (Figure S10) with a catalytically active form of cobalamin, obtained in this work, show the presence of a peak characteristic of cobalamins at 425 nm, which is in good agreement with our previously published data.[15]

We suggested that the addition of a fullerene fragment to *e*-(COOH)-dioxycobinamide **5**, without significantly changing the catalytic activity of the initial complex, would improve its delivery into the cell. We studied the autooxidation of ascorbic acid (AH_2), and the effect on this process of the initial *e*-(COOH)-dioxycobinamide *e*-COOH–Cbi–$(OH)_2$ **5** and $(HO)_2$–Cbi–*e*-C(O)OCH_2CH_2–$C_{60}NH(CH_2)_5CO_2Me$ **7**. Autooxidation of AH_2 in the presence of cobalamins can be represented by equations (1) and (1′) (Scheme 2).

$$AH_2 + O_2 \xrightarrow{[M]} A + H_2O_2 \quad (1)$$

$$2AH_2 + O_2 \xrightarrow{[M]} 2A + 2H_2O \quad (1')$$

Scheme 2

Our previous results[2] show that under these conditions, one of the most effective catalysts was the synthetic complex *e*-COOH–Cbi–$(OH)_2$ **5**. The data obtained now are given in Table 1. As can be seen, the catalytic system generating reactive oxygen species based on the compound $(HO)_2$–Cbi–*e*-C(O)

Table 1 The study of ascorbic acid autooxidation in the presence of complexes **5** and **7**.

Entry	System	α ($\alpha = \Delta D/D \times 100$) (%)[a]
1	AH_2	8.1
2	AH_2 + (**5**)	27.2
3	AH_2 + (**7**)	15.2

[a] α – relative transformation of AH_2.

OCH_2CH_2–$C_{60}NH(CH_2)_5CO_2Me$ **7** (entry 3) has about two times less activity than the starting complex **5** (entry 2). This can be explained by the quenching of the excited singlet state due to the transfer or excitation of an electron to the fullerene nucleus. These data fit well the results obtained for similar structures, but based on pure chlorin and its fullerene derivative.[18]

Thus, the addition of this catalytic system to fullerene derivatives, although it somewhat decreases its catalytic activity, allows them to be transported directly into the cell.[17] To summarize, a synthesis of potential anticancer drugs of binary structure based on fullerene C_{60} and vitamin B_{12} has been developed. New derivatives based on fullerene C_{60} and vitamin B_{12} thus obtained possess antitumor properties. It can be assumed that compounds of this structure may have enhanced antimetastatic and antitumor effects.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.11.025.

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