**Milo Malanga**

# Synthesis of novel, key cyclodextrin derivatives towards cyclodextrin-based nanocarriers

Ph.D. Thesis

Eötvös Loránd University Ph.D. School of Chemistry

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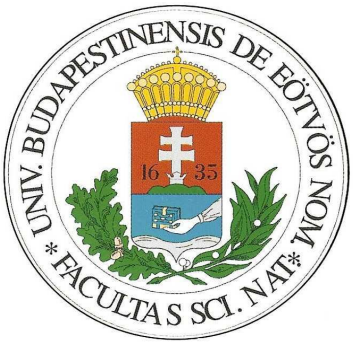


Eötvös Loránd University

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## Introduction and aims

Cyclodextrins (CDs) are a family of water soluble, biocompatible and biodegradable oligosaccharides known to improve the solubility, stability and bioavailability of many drugs.

Marie Curie Project CYCLON, composed of research groups from seven European countries, was assembled in order to develop, through **the training of young researchers** within a highly competent and multidisciplinary network, **a new generation of multifunctional drug nanocarriers** based on CDs.

These nano-platforms can help in accessing specific biological targets, in controlling the release of therapeutic compounds, in enhancing the *in vivo* efficiency of several drugs, in increasing the drug loading capacity and in providing multifunctionalization of a therapy.

CycloLab as the Hungarian participant in CYCLON project has almost 40-year experience in the preparation of various CD derivatives. As a PhD student I have developed new synthetic strategies and prepared novel derivatives based on this experience. Within the CYCLON project I had the possibility to work 3 and 4 months at the University of Almeria, Spain and at the University of Catania, Italy, where I took part in the research on biological targeting strategies as well as in photochemical studies, respectively.

During the three-years collaboration my role was to achieve the followings:

1) To synthesize novel biodegradable and biocompatible CD derivatives to be further applied for the elaboration of nanocarriers.

2) To create new CD derivatives labeled with fluorescent moieties for physico-chemical and *in-vitro* biological studies including photodynamic therapy applications.

3) To develop the syntheses of scaled up quantities of well characterized, industrially purified, key-intermediates in CD chemistry for further modifications.

4) To implement synthetic strategies in order to obtain amphiphilic and charged CDs and to build a library of compounds based on one-pot synthesis scheme.

## Experimental Part

The synthetic work was divided in two fundamental parts:

a) Scale-up of the common intermediates

The first part of my work consisted in the scaling-up of suitable key intermediates to be fluorescent tagged. In order to obtain CD scaffolds with different physico-chemical and functional properties (charge, solubility, complexing capacity, biocompatibility, etc.) the following versatile procedure was followed (Fig. 1).

The 6-monoazido βCD derivative is the key precursor of almost all the CD-scaffolds.

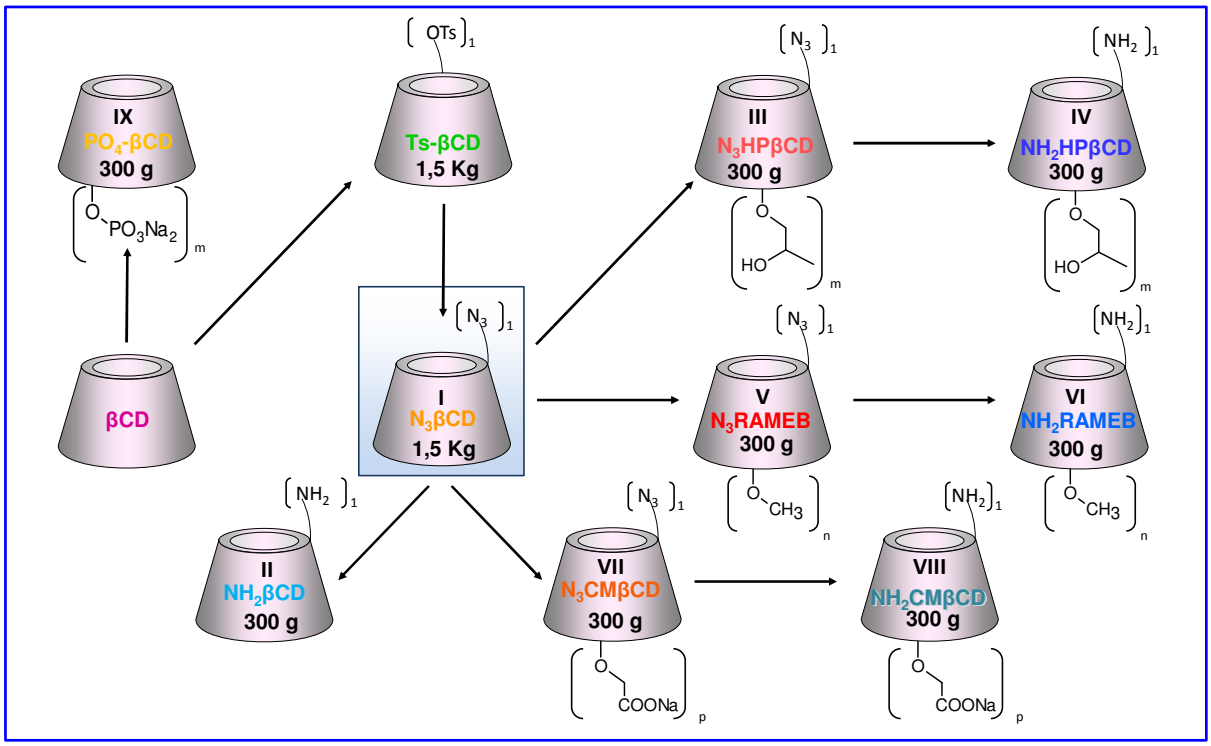


**Fig.1.** Scheme of the nine CD-scaffolds with the corresponding maximum batch size

I decided to randomly alkylate the starting 6-monoazido βCD in order to obtain the 2-hydroxypropyl, the methyl and the carboxymethyl 6-monoazido βCDs. The statistical alkylation of the 6-monoazido βCD yields products that can serve as a "library" for many different guests. This means that each of the obtained scaffolds is a mixture of structural isomers providing slightly different binding sites for the guest molecules, which can find, as in a library, the most suitable host molecule in this mixture.

b) Fluorescent labeling

Native CDs are UV/Vis spectroscopically inert, but they can be converted into spectroscopically active compounds by modification with a chromophore unit. Among the

chromophores the ones that guarantee the highest detection sensitivity and can ensure a very high resolution in the imaging process is the group of fluorophores.

The basic ideas for the fluorescent modification were the synthesis of CD intermediates possessing a single functional group to be exclusively dedicated for the labeling (azido-, amino group, see Fig. 1) and the choice of a synthetic strategy thus allowing the insertion of a single moiety of fluorophore per CD ring. At the same time, the "labeling-dedicated" functional group had to be versatile enough to allow further modifications of the CD scaffold by preserving the possibility of anchoring the fluorophore. According to this strategy, I synthesized, purified and characterized a series of rhodamine- and fluorescein-labeled CDs (Fig. 2).

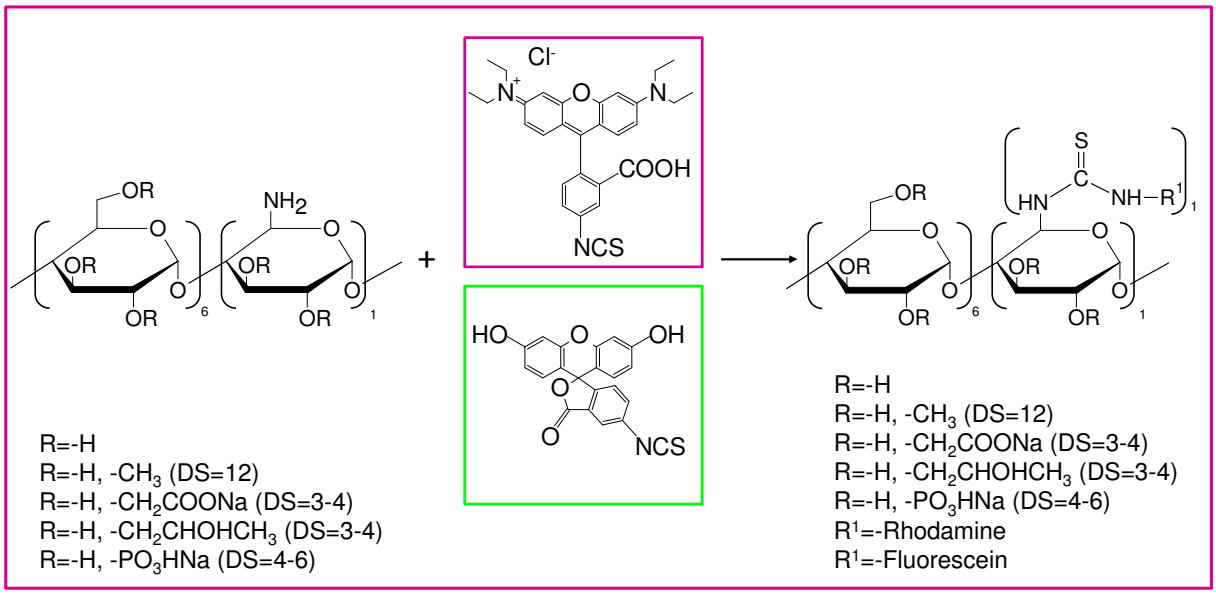


**Fig.2.** Scheme of rhodamine- and fluorescein-labeled CDs

The synthesis of the third series of fluorescent βCD derivatives, the nitrobenzofurazan-labeled CDs, was based on two different approaches. The first procedure relied on the nucleophilic substitution between 4-chloro-7-nitrobenzofurazan and the amino-βCD scaffolds (Fig. 3).

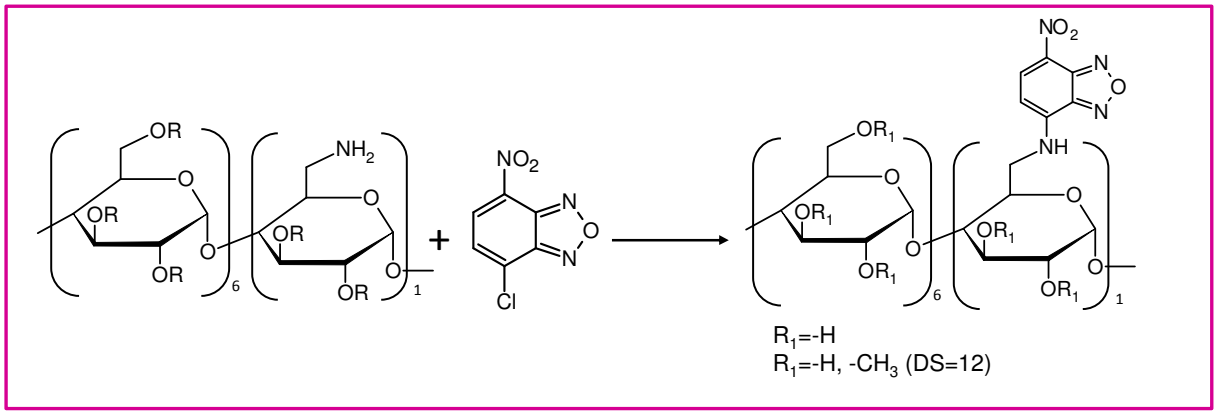


**Fig.3.** Scheme of nitrobenzofurazan-labeled CDs, first strategy

The second strategy dealt with azide-alkyne Huigens cycloaddition between the azido-βCD scaffolds and a propargylated derivative of the nitrobenzofurazan (Fig. 4).

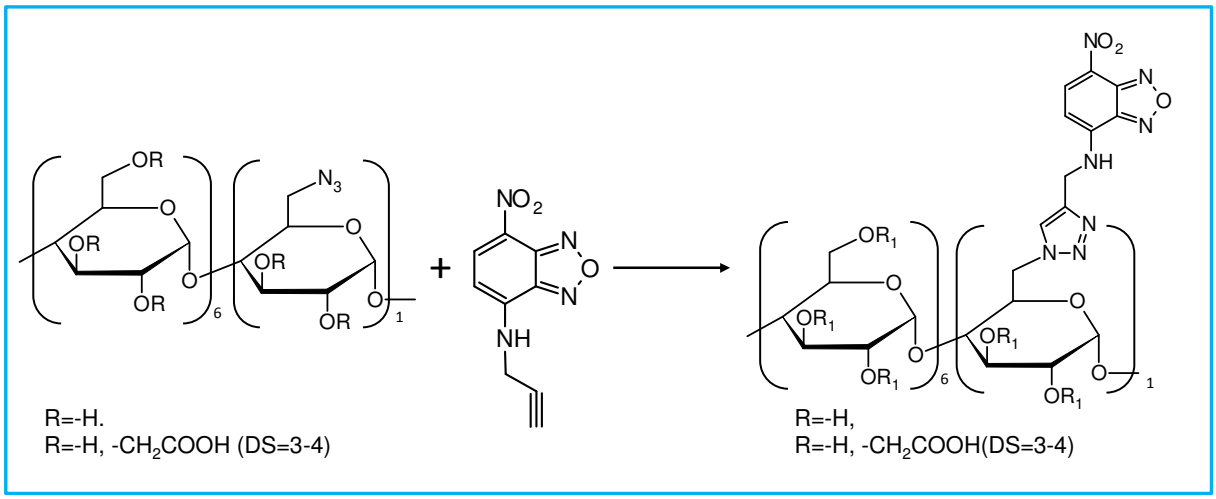


**Fig.4.** Scheme of nitrobenzofurazan-labeled CDs, second strategy

The introduction into fluorescent CD derivatives of phosphate groups which are ionized at physiological pH, can modify (increase) the water solubility of these compounds by preserving their biocompatibility.

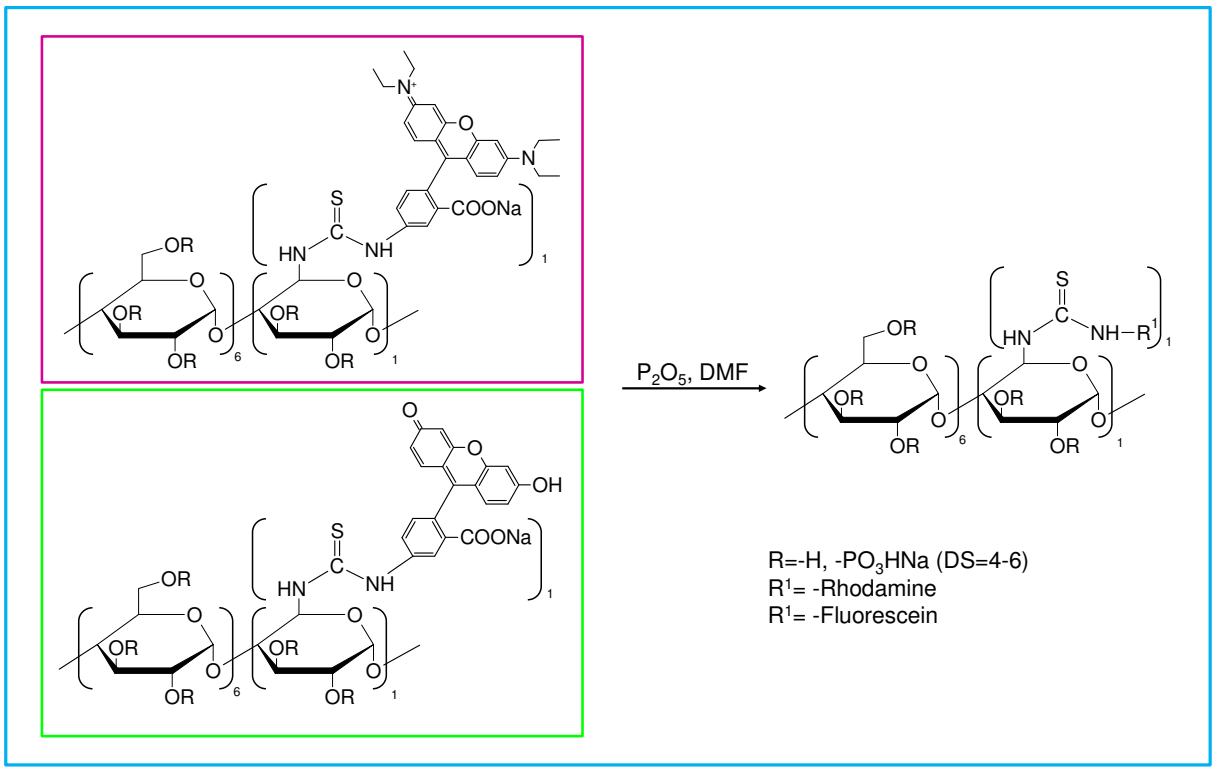


**Fig.5.** Scheme of phosphorylation for fluorescent βCDs

The strategy for the phosphorylation of the fluorescent CD derivatives was based on a one step reaction (Fig. 5). The advantages of this approach are that the introduction of phosphate

groups can be accomplished in a one (terminal) step and that the reaction can be easily reproduced and scaled-up.

## The new findings of my research

1) I have built-up a library made of fourteen of fluorescent βCD derivatives. All the compounds were characterized by suitable chromatographic and spectroscopic techniques (TLC, HPLC, NMR) and their purity was checked by capillary electrophoresis. I synthesized original fluorescent molecules using fluoresceinyl, rhodaminyl, nitrobenzofurazanyl functions built-up on various CD scaffolds, such as hydroxypropyl, randomly methyl, carboxymethyl βCDs, thus creating fluorescent amphiphilic and charged CDs that can be exciting tools for basic and applied research as well as multifunctional nanocarriers in photodynamic therapy.
2) I have worked out general synthetic strategies useful for the preparation of various monofunctionalized CD derivatives based on the randomly alkylation of 6-monosubstituted CD derivatives.
3) I have synthesized and characterized phosphorylated-βCD derivatives, together with their fluorescent analogues. These derivatives are able to interact in a controlled way with cationic architectures such as nano-metal-organic framework (MOF). The promising results that could be achieved resulted in the joined patent CNRS/CycloLab.
4) Joining the research group at the University of Catania I have participated in the development of nanocarriers useful for photoactivated nitric oxide releasing compounds.
5) The rhodamine-labeled CD derivatives prepared by me can indicate the presence of the anticancer drug topotecan due to the energy transfer between the two molecules.
7) The fluorescein-labeled CD derivatives are excellent tools for biological imaging.
8) The nitrobenzofurazan-labeled CD derivatives can act as nanocarriers for new photosensitizers.

## Conclusions

I have synthesized novel CD derivatives to be further applied for the elaboration of nanocarriers.

I developed the scaled-up synthesis of the following intermediates: 6-monotosyl-βCD, 6-monoazido-βCD, -HPβCD, -RAMEB, -CMβCD and 6-monoamino- βCD. -HPβCD, -RAMEB, -CMβCD in 10-300 g scale.

I have worked out the synthesis and prepared new CD derivatives labeled with fluorescent moieties by appending the fluorophore via a stable thioureido group. The applications of these materials span from the biological field and material science to the pure physico-chemical investigations.

By implementing the synthetic strategies I have built a library of compounds. The library of the fluorescent CD derivatives synthesized in my PhD work consists of 5 CD scaffolds with 3 fluorescent moieties. The strategy I worked out for the synthesis was summarized for the rhodaminyl derivatives in publication No. 4. This strategy can be easily adapted for further CD scaffolds including α- and γ-CD derivatives, also their polymers, with various functionalities (the same or different applied in my work) as well as further fluorescent moieties.

The fluorescent CD derivatives are now on the fine chemicals list of CycloLab creating a new product line for the company.

## Publications:

1. **M. Malanga**: *Fluorescent Cyclodextrin Derivatives: Major Recent Advances*, Cyclodextrin News, 25, 2, 1-9, 2011.

2 É. Fenyvesi**, M. Malanga**: *Cyclodextrins in the photodynamic therapy*, Cyclodextrin News, 27, 4, 1-4, 2013.

3 **M. Malanga**: *Symmetric cationic γCD as extracellular inhibitor for bacteria*, Cyclodextrin News, 24, 8-9, 1-4, 2013.

4 **M. Malanga**, L. Jicsinszky, É. Fenyvesi: *Rhodamine-labeled cyclodextrin derivatives*, J. Drug Del. Sci. Tech., 22, 260-265, 2012. (IF: **1.088**).

5 N. Kandoth, **M. Malanga**, A. Fraix, L. Jicsinszky, É. Fenyvesi, T. Parisi, I. Colao, M. T. Sciortino, S. Sortino: *A Host–Guest Supramolecular Complex with Photoregulated Delivery of Nitric Oxide and Fluorescence Imaging Capacity in Cancer Cells*, Chem. Asian J., 7, 2888-2894, 2012. (IF:**4.572).**

6 I. Puskás, M. Schrott, **M. Malanga**, L. Szente: *Characterization and control of the aggregation behavior of cyclodextrins,* J. Incl. Phenom. Macrocycl. Chem., 75, 269-276, 2013. (IF:**1.399).**

7 I. Puskás, A. Szemjonova, É. Fenyvesi, **M. Malanga**, L. Szente: *Aspects of determining the molecular weight of cyclodextrin polymers and oligomers by static light scattering,* Carb, Polym., 94, 124-128, 2013. (IF:**3.479**).

8 R. Anand, **M. Malanga**, I. Manet, F. Manoli, K. Tuza, A. Aykaç, C. Ladavière, É. Fenyvesi, A. Vargas-Berenguel, R. Gref, S. Monti: *Citric acid–γ-cyclodextrin crosslinked oligomers as carriers for doxorubicin delivery,* Photochem. Photobiol. Sci., 12, 1841-1854, 2013. (IF:**2.923**).

9 F. Fenyvesi, K. Szászné Réti Nagy, Z. Bacsó, Z. Gutay-Tóth, **M. Malanga**, É. Fenyvesi, L. Szente, J. Váradi, I. Bácskay, Z. Ujhelyi, P. Fehér, G. Szabó, M. Vecsernyés: *Fluorescent randomly methylated-beta-cyclodextrin enters the intestinal epithelial Caco-2 cells by endocytosis*, Manuscript Submitted, PLOS ONE, 2013.(IF:**3.730**).

10 V. Agostoni, P. Horcajada, M. Noiray, **M. Malanga,** A. Aykac, L. Jicsinszky, A. Vargas Berenguel, N. Semiramoth, V. Nicolas, C. Martineau, F. Taulelle, J. Vigneron, A. Etcheberry, C. Serre, R. Gref.: *A "green" Lego-type strategy to construct stable and bioactive coatings on porous nanoMOFs*, manuscript submitted.

11 A. Aykaç, **M. Malanga**, V. Agostini, É. Fenyvesi, R. Gref, A. Vargas-Berenguel: *Surface functionalization of metal–organic frameworks (MOFs) for the construction of imaging and targeted drug delivery systems*, manuscript submitted.

12 É. Fenyvesi, J. Szemán, K. Csabai, **M. Malanga**, L. Szente: *Modulation of Solubilizing Efficiency of Methyl Beta-Cyclodextrins*, manuscript submitted.

13 R. Anand, F. Manoli, I. Manet, M. P. Donzello, E. Viola, **M. Malanga**, L. Jicsinszky, É. Fenyvesi, S. Monti: *Study of the association of a water soluble ZnII pyrazinoporphyrazine octacation to fluorescent cyclodextrins by spectroscopic techniques*, manuscript submitted.

## Poster presentations:

1. **M. Malanga**, J. Szemán, K. Csabai, L. Jicsinszky, É. Fenyvesi, G.Tárkányi, O. Tőke, L. Tölgyesi, F. Fenyvesi, G. Gyémánt J. Kerékgyártó, L. Szente: *Are all Commercial Hydroxypropyl-beta-Cyclodextrins the same?*, 15th International Cyclodextrin Symposium, May 9-12, 2010, Vienna.

2. **M. Malanga**, L. Jicsinszky, É. Fenyvesi, N. Kandoth, S. Sortino: *Synthesis and Characterization of Fluorescent Cyclodextrin Derivatives,* Convegno Nazionale di Fotochimica, June 10-12, 2011, Messina.

3. **M. Malanga**, L. Jicsinszky, K. Tuza, É. Fenyvesi: *Synthesis and Characterization of Fluorescent Cyclodextrin Derivatives as Tool for Monomolecular Visualization*, 4th European Conference on Chemistry for Life Sciences, September 13, 2011, Budapest.

4 Y. Wang, B. Cohen, J. A. Organero, **M. Malanga**, L. Jicsinszky, A. Douhal: *Femto-Microsencond Studies of a Porphyrin in Chemical and Biological Nanocavities*, The SJ Nano2011 Toledo. I Bilateral Spanish-Japanese School/Workshop on Nanotechnology and New Materials with Environmental Challenges, September 13-16, 2011, Toledo.

5 **M. Malanga**, L. Jicsinszky, N. Kandoth, S. Sortino, V. Agostoni, R. Gref, V. Kirejev, M. Ericson, É. Fenyvesi: *Fluorescent Cyclodextrin: Aid in the Development of Novel Anticancer Therapy*, 2nd European Conference on Cyclodextrin, October 2-4, 2011, Asti.

6a. **M. Malanga**, A. Aykaç, L. Jicsinszky, É. Fenyvesi, R. Gref, V. Kirejev, M. Ericson, A. Antonio Vargas-Berenguel: *Cyclodextrin-based polymer for visualization and selective targeting of cancer cells*, The 16th International Cyclodextrin Symposium (ICS16) Nankai University, May 6-10, 2012, Tianjin.

6b. **M. Malanga**, A. Aykaç, L. Jicsinszky, É. Fenyvesi, R. Gref, V. Kirejev, M. Ericson, A. Antonio Vargas-Berenguel: *Cyclodextrin-based polymer for imaging and selective targeting of cancer cells*, The 26th International Carbohydrate Symposium, July 22-27, 2012, Madrid.

7 R. Anand, F. Manoli, I. Manet, S. Monti, M. P. Donzello, E. Viola, **M. Malanga**, É. Fenyvesi: *Study of the association of a water soluble ZnII porphyrazine octacation to fluorescent cyclodextrin derivatives by spectroscopic techniques*, Applications of Nanodrugs in Photodynamic Therapy, April 11-12, 2013, Gothenburg.

8 G. Benkovics, Z. Tosner, K. Tuza, **M. Malanga**, I. Puskas, J. Jindrich: *Formation of supramolecular oligomers based on cinnamyl modified α- and β-cyclodextrins,* The 3rd European Conference on Cyclodextrin, October 2-4, 2013, Antalya.

9 R. Anand, I. Manet, F. Manoli, S. Monti, **M. Malanga,** E. Fenyvesi, A. Maksimenko, R. Gref, A. Aykac, A. Vargas-Berenguel: *Cyclodextrin polymers as carriers for anthracycline drug delivery,* The 4th National Conference CD.TE.C, May 8-9, 2013, Messina.

## Oral presentations:

1. **M. Malanga**: *They are with you, but you don't know*, Marie Curie Conference, July 1-2, 2010, Turin.

2. **M. Malanga**, L. Jicsinszky, K. Tuza, É. Fenyvesi: *Fluorescent Cyclodextrin Derivatives Aid in the Development of Novel Anticancer Strategies And in Visualizing Biological Barrier Crossing*, 16th European Carbohydrate Symposium, July 3-7, 2011, Sorrento.

3 I. Puskás, M. Schrott, É. Fenyvesi, **M. Malanga**, L. Szente: *Self-assembly of Cyclodextrins and their Derivatives,* The 16th International Cyclodextrin Symposium (ICS16) Nankai University, May 6-10, 2012, Tianjin.

4 I. Puskás, M. Schrott, É. Fenyvesi, **M. Malanga**, L. Szente: *Self-assembly of Cyclodextrins and their Derivatives,* Workshop on Carbohydrate, Nucleotide and Antibiotics Chemistry, Hungarian Academy of Sciences, May 31-June 1, Debrecen, 2012.

5 **M. Malanga**, L. Jicsinszky, K. Tuza. É. Fenyvesi: *Fluorescent Labelling of Cyclodextrin-based Nanoconstucts*, Applications of Nanodrugs in Photodynamic Therapy, April 11-12, 2013, Gothenburg.

6 **M. Malanga**, L. Jicsinszky, É. Fenyvesi: *Synthetic approaches toward fluorescent cyclodextrin derivatives*, Annual Meeting of the Hungarian Carbohydrate Society, May 22-24, 2013, Matrafured.

## Patent:

R. Gref, V. Agostoni, S. Daoud-Mahammed, V. Rodriguez-Ruiz, **M. Malanga**, L. Jicsinszky, P. Horcajada, C. Serre: *Solide hybride organique inorganique amélioré à surface externe modifiée*, May 31st, 2012, French Patent Application, N°12/55065.