MULTIVALENT CARBOSILANE GLYCODENDRIMERS DESIGNED FOR BIOAPPLICATIONS

Monika Müllerová[1], Dina Maciel[3], Jan Malý[2], Dominika Wróbel[2], João Rodrigues[3], Tomáš Strašák[1]

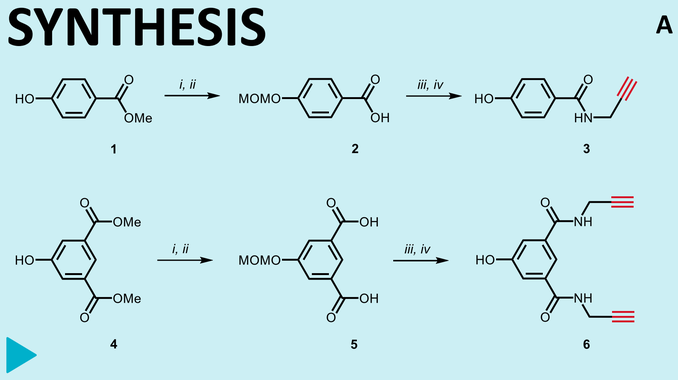
[1]Institute of Chemical Process Fundamentals, Academy of Sciences of the Czech Republic, Prague 6, Czech Republic
[2]Department of Biology, Faculty of Science, University of J.E. Purkinje, Usti N/L, Czech Republic
[3]CQM-Centro de Química da Madeira, Universidade da Madeira, 9000-390 Funchal, Portugal

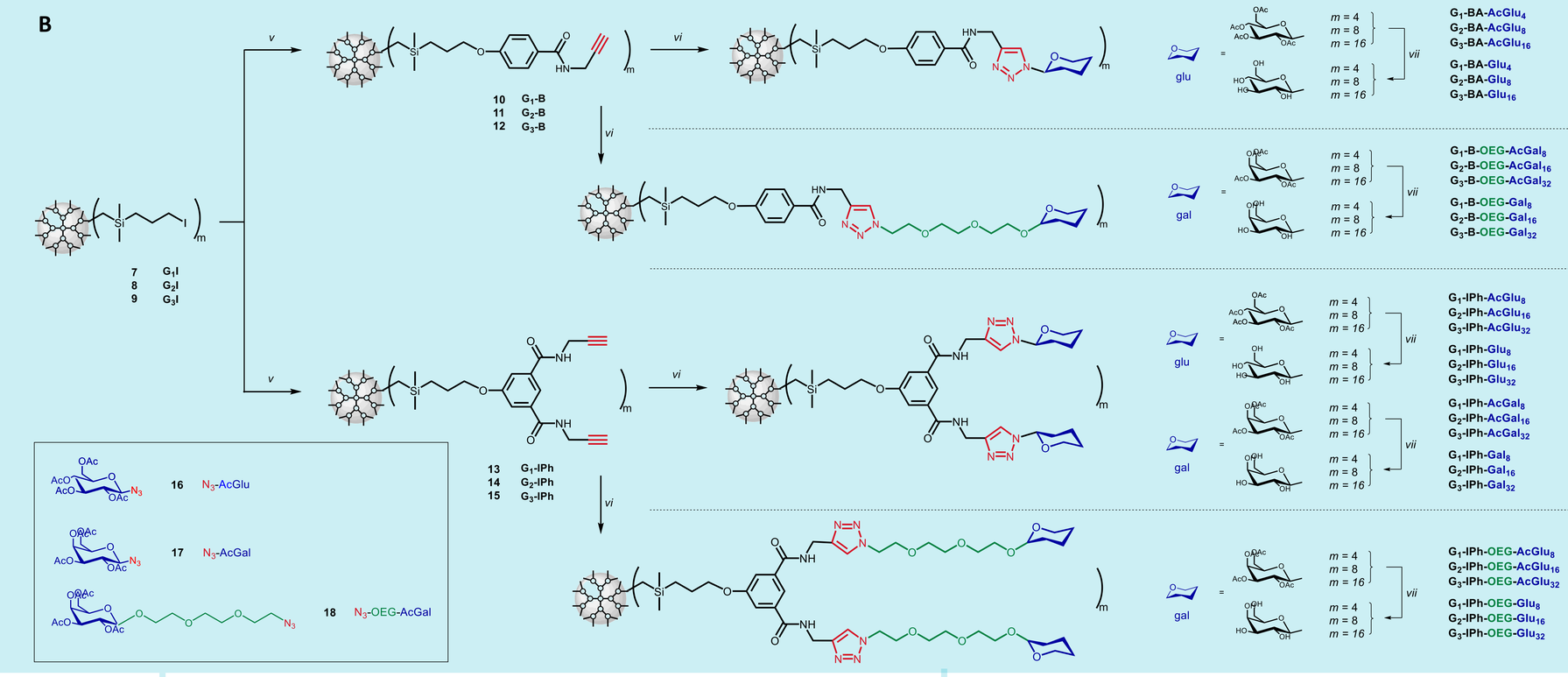
INTRODUCTION

An interplay of multiple interactions of the individual sugar units attached to the nano-scale molecular scaffolds governs ionic and H-bond interactions to promote crucial biological processes[1]. Moreover, to a large extent, it is responsible for the significantly reduced toxicity of the glycoconjugates compared to their molecular scaffolds[2,3]. To address the need for advanced synthetic tools, a robust and flexible route to conjugate carbohydrates to the periphery of carbosilane dendrimers (CS-DDMs) was developed. To boost multivalent presentation, the CS scaffolds was tailored with a derivative of 4-hydroxy isophthalic acid to double the amount of peripheral reactive sites. In this manner, three series of 1st – 3rd generation CS glyco-DDMs bearing gluco- and galacto- ligands conjugated to the molecule directly or via a short oligo ethylene glycol linker were synthesized to enhance biocompatibility and hydrosolubility of the compounds. The cytotoxicity evaluation of the glyco-DDMs against both non-cancer (BJ) and cancer (A2780 and MCF7) cells revealed their exceptional biocompatibility[4].

The anthracycline doxorubicin (DOX), one of the most powerful chemotherapeutics, still raises concerns regarding its toxicity towards non-targeted tissues[5]. To reveal the potential of the conjugates in drug delivery, molecules of DOX we encapsulated into the glyco-DDMs and an elevated anticancer activity of the resulting complexes was observed. The biochemical research was further broadened with hematotoxicity and drug release kinetics assays.

SYNTHESIS





Scheme 1: Synthetic route towards CS dendritic compounds for multivalent presentation of peripheral functionalities comprising alkyne-terminated CS-DDMs and CS glyco-DDMs. **(A)** Synthesis of the branching units **3** and **6**; (i) MOMBr, NaH, (ii) NaOH, MeOH, (iii) 2-propynyl amine, HOBt, DCC, NMM, DMF, (iv) DOWEX H^+, MeOH. **(B)** Alkyne-terminated CS-DDMs and CS glyco-DDMs were prepared by the sequence of reactions comprising alkylation of the iodine-propyl terminated CS-DDMs **10-15** with the branching units **3** and **6** and peripheral attachment of the glyco-units **16-18** via alkyne-azide (CuAAC) click reaction; (v) **3** or **6**, K_2CO_3, DMF, (vi) **16**, **17** or **18**, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, THF, water, (vii) Et_3N, MeOH, water, MW. The synthetic protocol allows flexible modification of the DDM periphery to prepare a broad range of glyco-DDMs.

CYTOTOXICITY AND HEMATOTOXICITY EVALUATION, DRUG LOADING & RELEASE

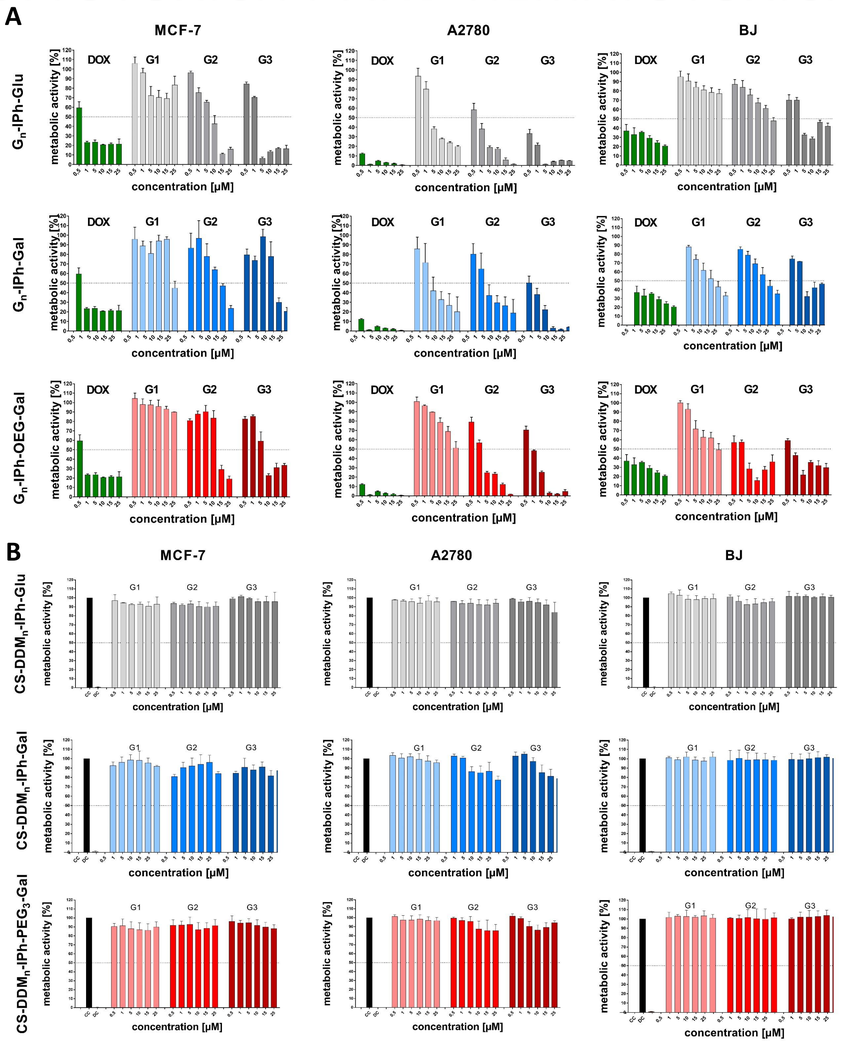


Figure 1: In vitro cell viability of cancer (MCF-7 and A2780) and non-cancer (BJ) cell lines after exposure to **(A)** G_1-G_3 DDMs of the series CS-DDM-IPh-Glu, CS-DDM-IPh-Gal, and CS-DDM-IPh-PEG3-Gal, and **(B)** to the corresponding glyco-DDM/DOX complexes revealed and exceptional biocompatibility of the glyco-DDMs and a promising anticancer activity of the glyco-DDM/DOX complexes especially against A2780 . Briefly, MCF-7, A2780 and BJ cells were distributed into 96-well plates at a density of 1.5×10^4 cells per well in the RPMI and/or DMEM medium. The next day, the medium was replaced with fresh medium containing 20 μL of the corresponding glyco-DDM, DOX, or glyco-DDM/DOX complex in 6 different concentrations (0.5 – 25 μM). After incubation at 37 °C for 48 h, the cell viability was quantified via the resazurin reduction assay.

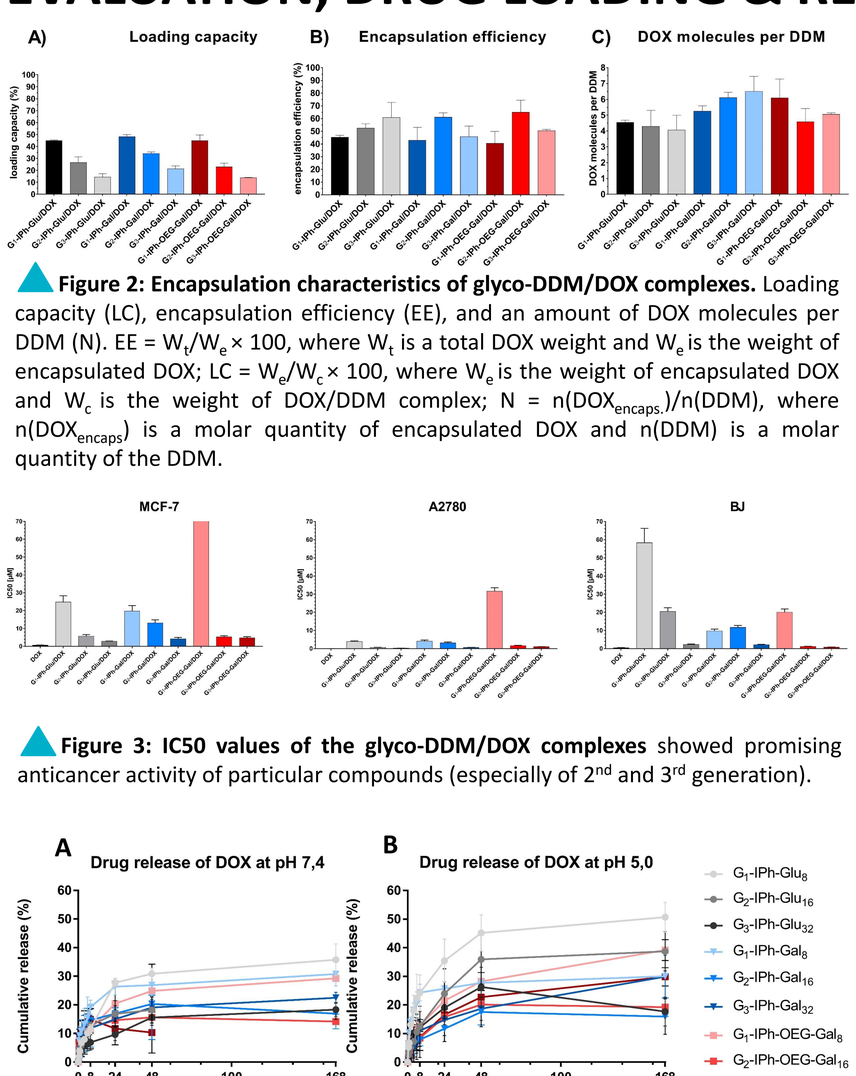


Figure 2: Encapsulation characteristics of glyco-DDM/DOX complexes. Loading capacity (LC), encapsulation efficiency (EE), and an amount of DOX molecules per DDM (N). EE = $W_t/W_e \times 100$, where W_t is a total DOX weight and W_e is the weight of encapsulated DOX; LC = $W_e/W_c \times 100$, where W_e is the weight of encapsulated DOX and W_c is the weight of DOX/DDM complex; N = $n(DOX_{encaps.})/n(DDM)$, where $n(DOX_{encaps})$ is a molar quantity of encapsulated DOX and n(DDM) is a molar quantity of the DDM.

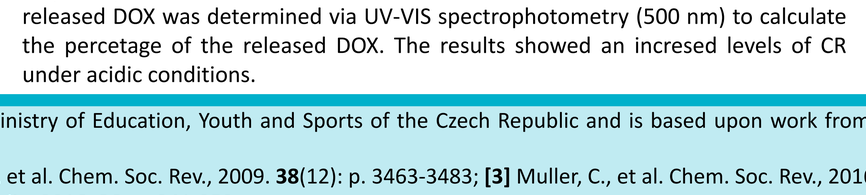


Figure 3: IC50 values of the glyco-DDM/DOX complexes showed promising anticancer activity of particular compounds (especially of 2nd and 3rd generation).

Figure 4: In vitro cumulative release (CR) of DOX from glyco-DDM/DOX complexes in PBS solutions at pH of a) 7.4 a b) 5.0 using a dialysis method. Briefly, each sample was weighted into dialysis vial and dissolved in distilled water. Then, the vial was incubated in PBS medium at 37°C. At different time intervals, the aliquote portions of the PBS solution were withdrawn and the concentration of released DOX was determined via UV-VIS spectrophotometry (500 nm) to calculate the percetage of the released DOX. The results showed an incresed levels of CR under acidic conditions.

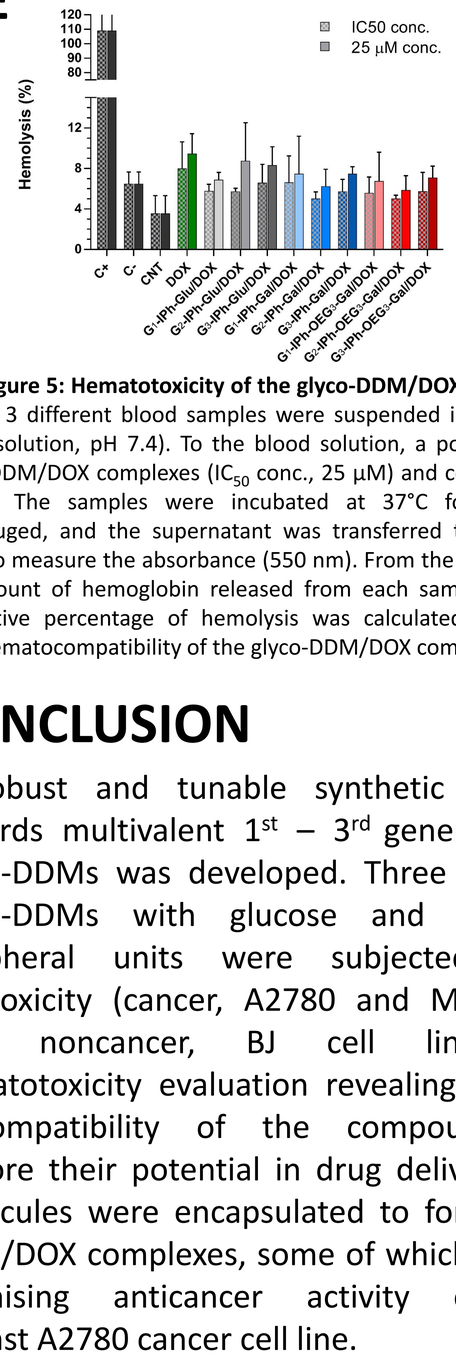


Figure 5: Hematotoxicity of the glyco-DDM/DOX complexes. Briefly, 3 different blood samples were suspended in PBS (10 % blood solution, pH 7.4). To the blood solution, a portion of the glyco-DDM/DOX complexes (IC_{50} conc., 25 μM) and controls were added. The samples were incubated at 37°C for 3 hours, centrifuged, and the supernatant was transferred to a 96-well plate to measure the absorbance (550 nm). From the absorbance, an amount of hemoglobin released from each sample and the respective percentage of hemolysis was calculated[6], revealing high hematocompatibility of the glyco-DDM/DOX complexes.

CONCLUSION

A robust and tunable synthetic protocol towards multivalent 1st – 3rd generation CS glyco-DDMs was developed. Three series of glyco-DDMs with glucose and galactose peripheral units were subjected to a cytotoxicity (cancer, A2780 and MCF-7 cell line; noncancer, BJ cell line) and hematotoxicity evaluation revealing superior biocompatibility of the compounds. To explore their potential in drug delivery, DOX molecules were encapsulated to form glyco-DDM/DOX complexes, some of which showed promising anticancer activity especially against A2780 cancer cell line.

Acknowledgements: This work was supported by project COST LTC19049 supported by the Ministry of Education, Youth and Sports of the Czech Republic and is based upon work from COST Action "Nano2Clinic. Cancer Nanomedicine – from the bench to the bedside" CA17140 supported by COST (European Cooperation in Science and Technology).

References: [1] Liegertova, M., et al. Nanotoxicology, 2018, **12**(8): p. 797-818; **[2]** Jayaraman, N., et al. Chem. Soc. Rev., 2009. **38**(12): p. 3463-3483; **[3]** Muller, C., et al. Chem. Soc. Rev., 2016, **45**(11): p. 3275-3302; **[4]** Mullerova, M., et al. Publication in preparation. **[5]** Tacar, O., et al. J. Pharm. Pharmacol., 2013. **65**(2): p. 157-70. **[6]** Hu, W., et al. Acta Biomaterialia, 2016. **36**: p. 241-253.