



**HAL**  
open science

## Self-assemblies of azacitidine prodrugs: a promising strategy of treatment for myelodysplastic syndromes

Milad Baroud, Elise Lepeltier, Yolla El-Makhour, Sylvain Thépot, Olivier Duval

### ► To cite this version:

Milad Baroud, Elise Lepeltier, Yolla El-Makhour, Sylvain Thépot, Olivier Duval. Self-assemblies of azacitidine prodrugs: a promising strategy of treatment for myelodysplastic syndromes. 4th NANOMED EMJMD's workshop (Nanomed 2021), Jul 2021, Angers, France. hal-04127352

**HAL Id: hal-04127352**

**<https://hal.science/hal-04127352>**

Submitted on 14 Jun 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



# Self-assemblies of azacitidine prodrugs: a promising strategy of treatment for myelodysplastic syndromes

Baroud M<sup>1</sup>, Lepeltier E<sup>1</sup>, Thepot S<sup>2</sup>, El-Makhoor Y<sup>3</sup>, Duval O<sup>1\*</sup>

<sup>1</sup>Translational Micro and Nanomedecines (MINT, INSERM 1066 / CNRS 6021), Université d'Angers, Angers, France

<sup>2</sup>University Hospital of Angers, Hematology, 49933 Angers, France

<sup>3</sup>Laboratory of Cancer Biology and Molecular Immunology, Lebanese University, Faculty of Sciences, Hadath-Beirut, Lebanon

\*corresponding author : elise.lepeltier@univ-angers.fr

## Introduction

5-Azacitidine, a cytidine analogue and a hypomethylating agent, is a staple drug being in the treatment of myelodysplastic syndromes<sup>[1]</sup>. However, after administration, it exhibits several limitations including restricted diffusion and cellular internalization due to its hydrophilicity, and rapid enzymatic degradation by deaminases. **The aim of this research is to improve the drug diffusion and protect it from metabolic degradation via the synthesis of amphiphilic prodrugs and their potential self-assembly.** Azacitidine was conjugated to two different omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The carboxylic acid group of the omega-3 fatty acids was conjugated to the amine group of azacitidine, yielding an amphiphilic prodrug. Next, the nanoprecipitation of the obtained prodrugs was performed and self-assemblies were successfully obtained for both prodrugs with a diameter of 200 nm, a polydispersity index below 0.2 and a positive zeta potential. Both self-assemblies had an IC<sub>50</sub> close to azacitidine. AzaEPA showed a slow and gradual internalization. This strategy would allow protection while increasing azacitidine's specificity and bioavailability. This prodrug should be cleaved by cathepsin B [2], an enzyme overexpressed in cancerous cells [3], thus increasing the specificity of the drug.

## 1. Prodrug synthesis

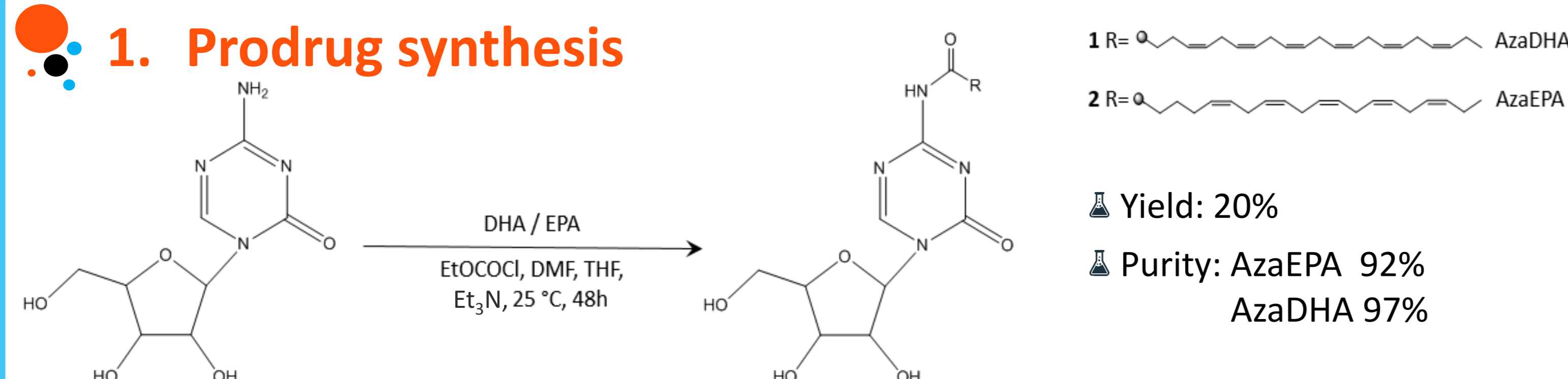


Figure: Synthesis of N<sup>4</sup>-azacitidine DHA (AzaDHA, 1) and N<sup>4</sup>-azacitidine EPA (AzaEPA, 2).

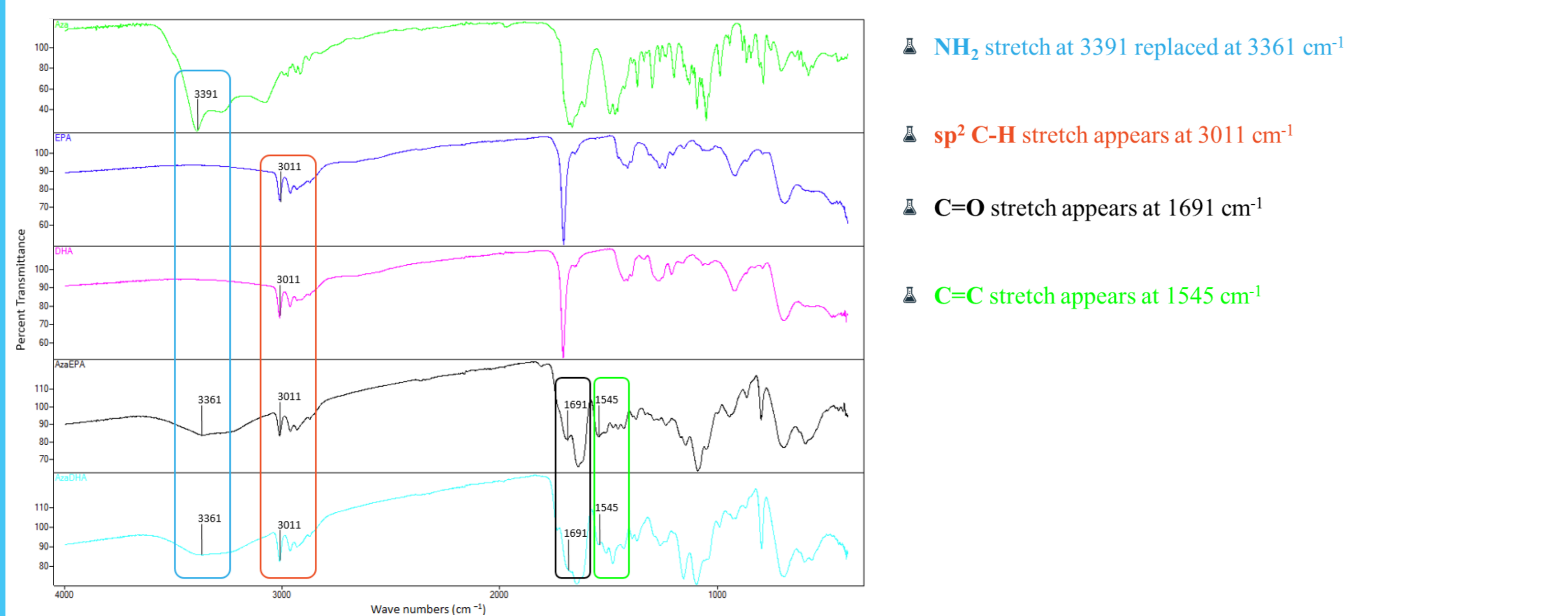


Figure: Fourier-Transform InfraRed spectroscopy curves and wave numbers of interest between 4000 cm<sup>-1</sup> and 600 cm<sup>-1</sup>

| Sample | Formula   | Calculated % |      |      | Found% |      |      |
|--------|---|--------------|------|------|--------|------|------|
|        |   | C            | H    | N    | C      | H    | N    |
| AzaEPA | C <sub>28</sub> H <sub>40</sub> N <sub>4</sub> O <sub>6</sub> | 63.62        | 7.63 | 10.6 | 61.66  | 7.63 | 9.67 |
| AzaDHA | C <sub>30</sub> H <sub>42</sub> N <sub>4</sub> O <sub>6</sub> | 64.96        | 7.63 | 10.1 | 63.2   | 7.71 | 9.31 |

Table: Elemental analysis of AzaDHA and AzaEPA

## 2. Critical aggregation concentration & nanoprecipitation

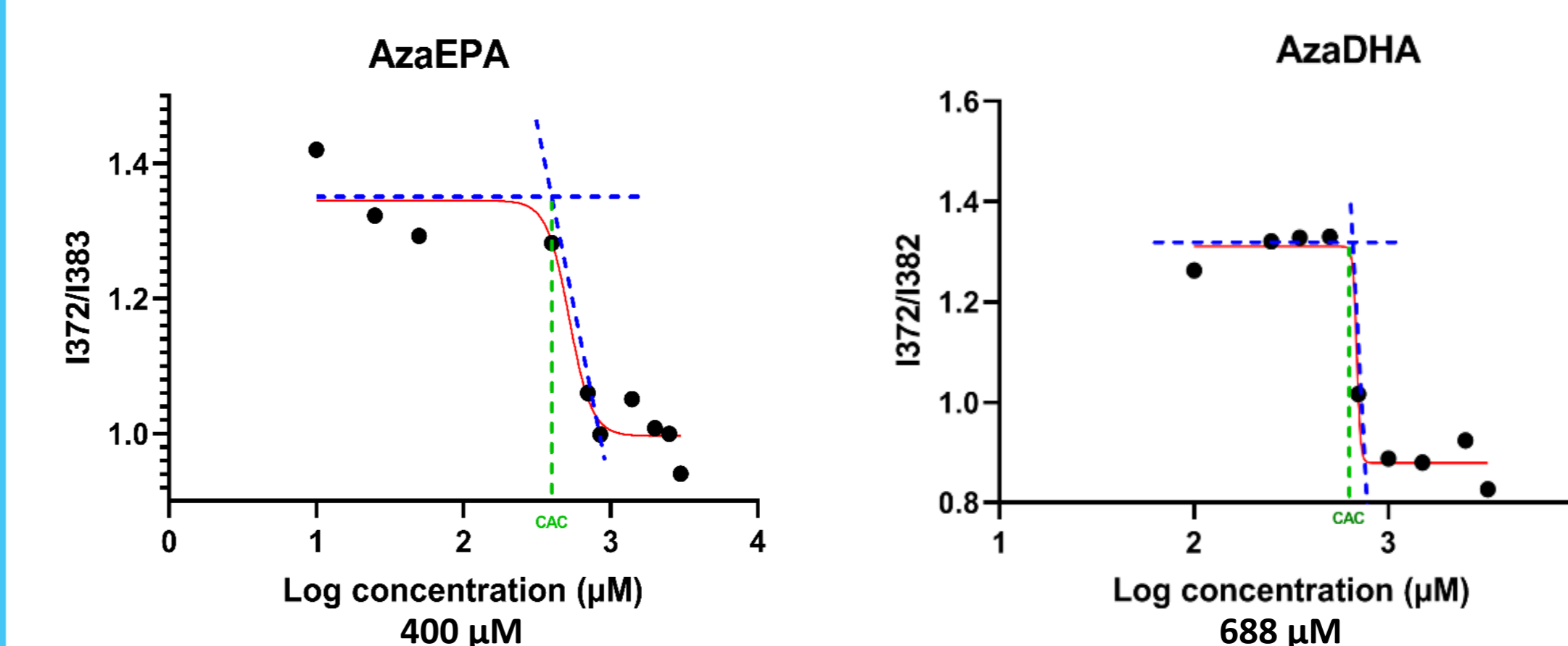


Figure: Boltzmann-type sigmoid obtained for the AzaEPA and AzaDHA suspensions in water showing the CAC value, corresponding to the first sharp decrease point.

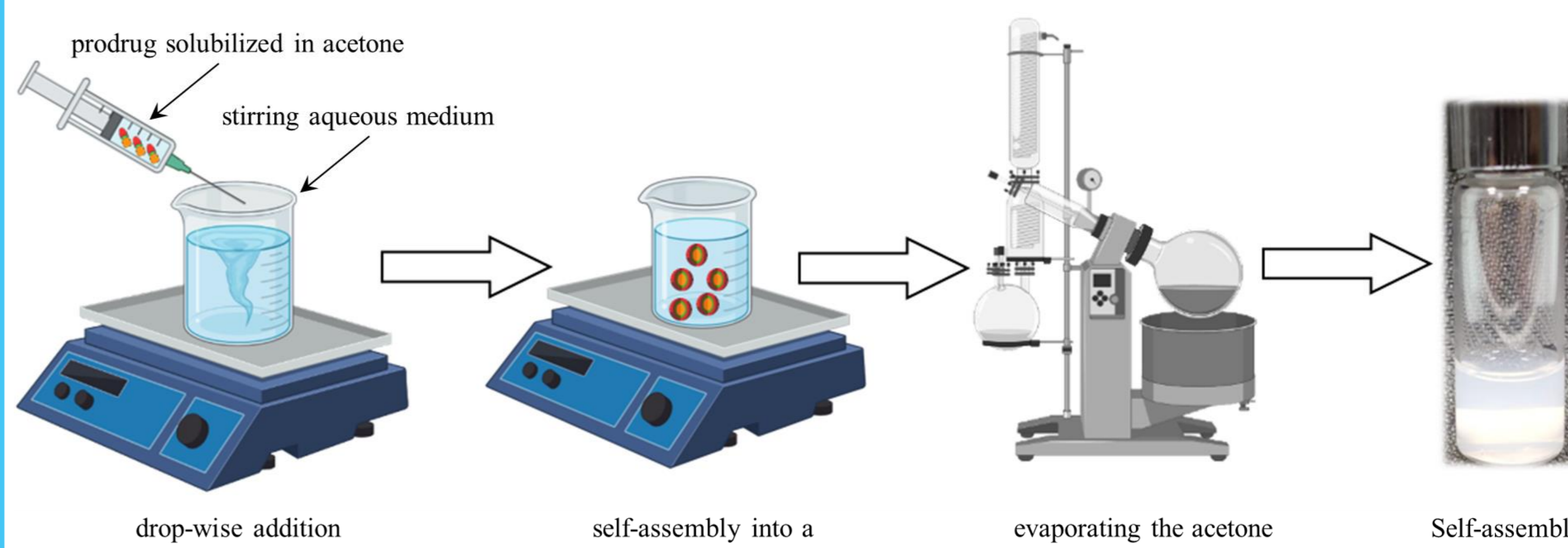


Figure: Formulation of self-assemblies via nanoprecipitation. The conjugates are dissolved in an organic solvent, then added drop-wise to a stirring aqueous medium allowing for the spontaneous formation of self-assemblies. The organic solvent is then evaporated using a rotary evaporator, thus yielding an opalescent aqueous suspension.

## 3. Cryo-TEM and MTT studies

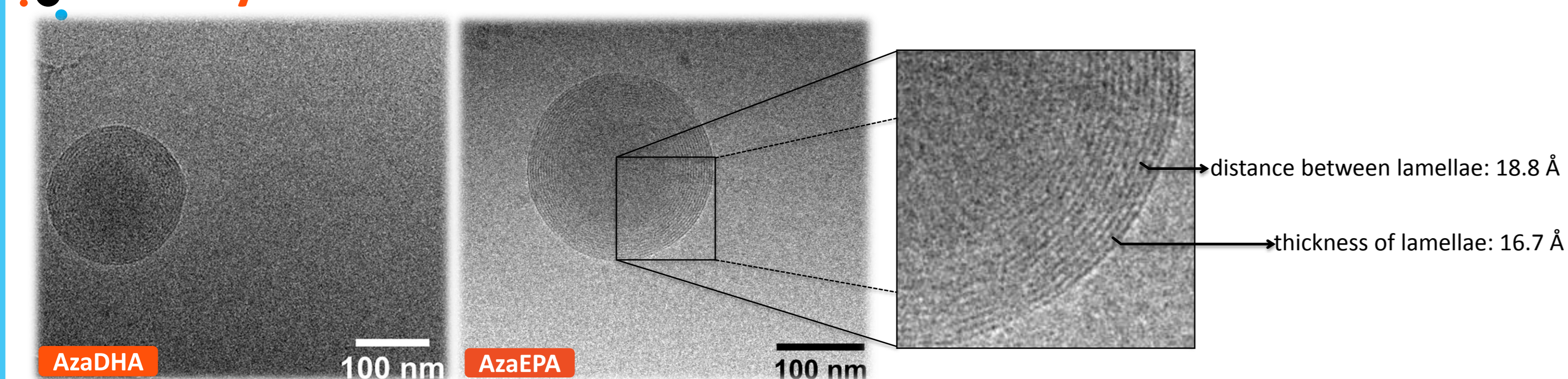


Figure: Cryo-TEM images of the prodrugs self-assemblies; forming multilamellar vesicles (MLVs).

| IC <sub>50</sub> | Azacitidine | DHA      | EPA      | AzaDHA  | AzaEPA  |
|------------------|-------------|----------|----------|---------|---------|
| 6 hours          | 6.5 μM      | 100.2 μM | 196.7 μM | 27.5 μM | 33.7 μM |
| 24 hours         | 1.0 μM      | 92.8 μM  | 115.9 μM | 11.3 μM | 16.6 μM |
| 48 hours         | 1.4 μM      | 44.5 μM  | 103.8 μM | 10.2 μM | 13.7 μM |

Table: The half maximal inhibitory concentration determined by a MTT assay on HL-60 cells, after treatment by azacitidine, fatty acids and self-assemblies at different time points.

## 4. Perspectives

- Release study of azacitidine under the action of cathepsin B
- Protection from deamination study
- DOSY and SAXS experiments to further verify the obtained MLVs
- Stability of azacitidine vs. the self-assemblies in water
- Pharmacokinetics of drug release from MLVs
- Determining the internalization pathway
- Cell cycle studies
- Apoptosis studies
- Post-treatment cell morphology studies using Mgg coloration
- LINE-1 global methylation studies

## Conclusion

The synthesis of two amphiphilic conjugates was successfully performed and verified. Their self-assembly in water was successful and confirmed using the fluorescent probe pyrene method to determine the critical aggregation concentration. The formed nanoassemblies were then characterized presenting a hydrodynamic diameter of 190 nm and a positive surface charge. The self-assemblies attain a multilamellar vesicle supramolecular structure. The obtained formulations had a low IC<sub>50</sub> comparable to that of free azacitidine, allowing them to achieve a close cytotoxicity. Additional in vitro studies on the HL-60 cell line (human acute myeloid leukemia) will be conducted to compare the efficacy of the self-assemblies compared to the parent drug.

## References:

[1] Issa, J.-P. J. The myelodysplastic syndrome as a prototypical epigenetic disease. *Blood* 121, 3811–3817 (2013)

[2] Dheer, D., Nicolas, J. & Shankar, R. Cathepsin-sensitive nanoscale drug delivery systems for cancer therapy and other diseases. *Advanced Drug Delivery Reviews* (2019)

[3] Alatrash, G. et al. Cathepsin G is broadly expressed in acute myeloid leukemia and is an effective immunotherapeutic target. *Leukemia* 31, 234 (2016)