7. ANNEXES

ω Characterization

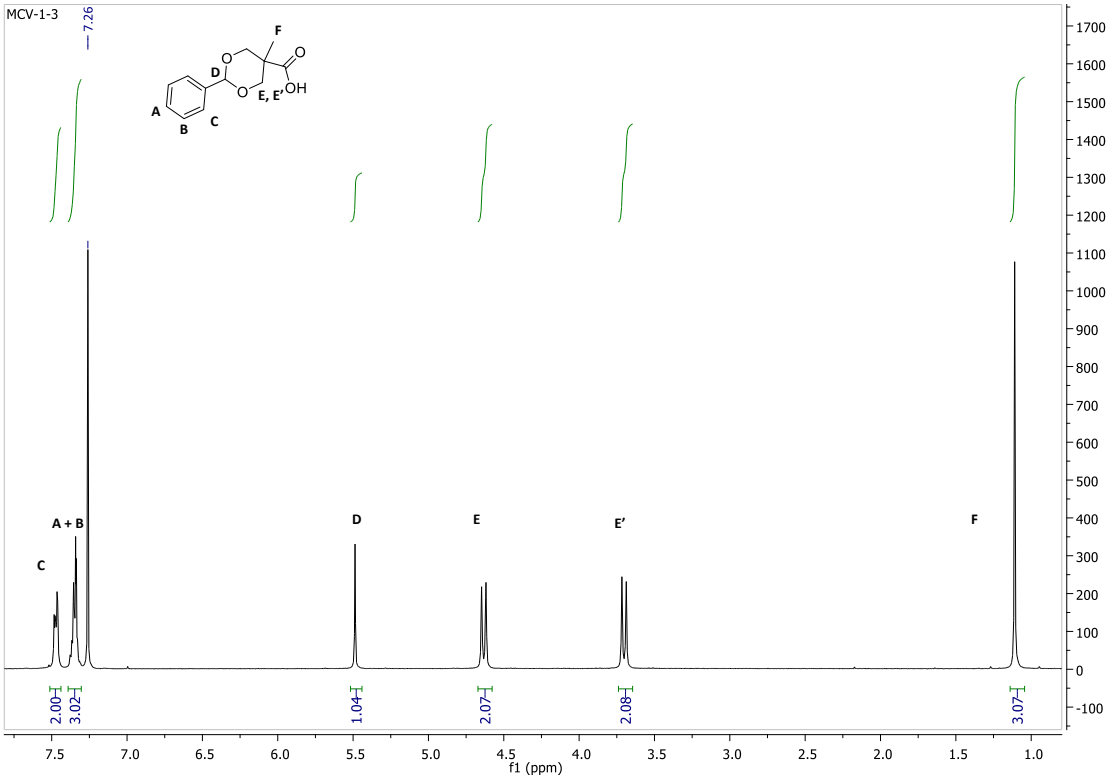

Figure I. [1]H-NMR spectrum of Benzylidene-2,2-bis(oxymethyl)propionic acid.

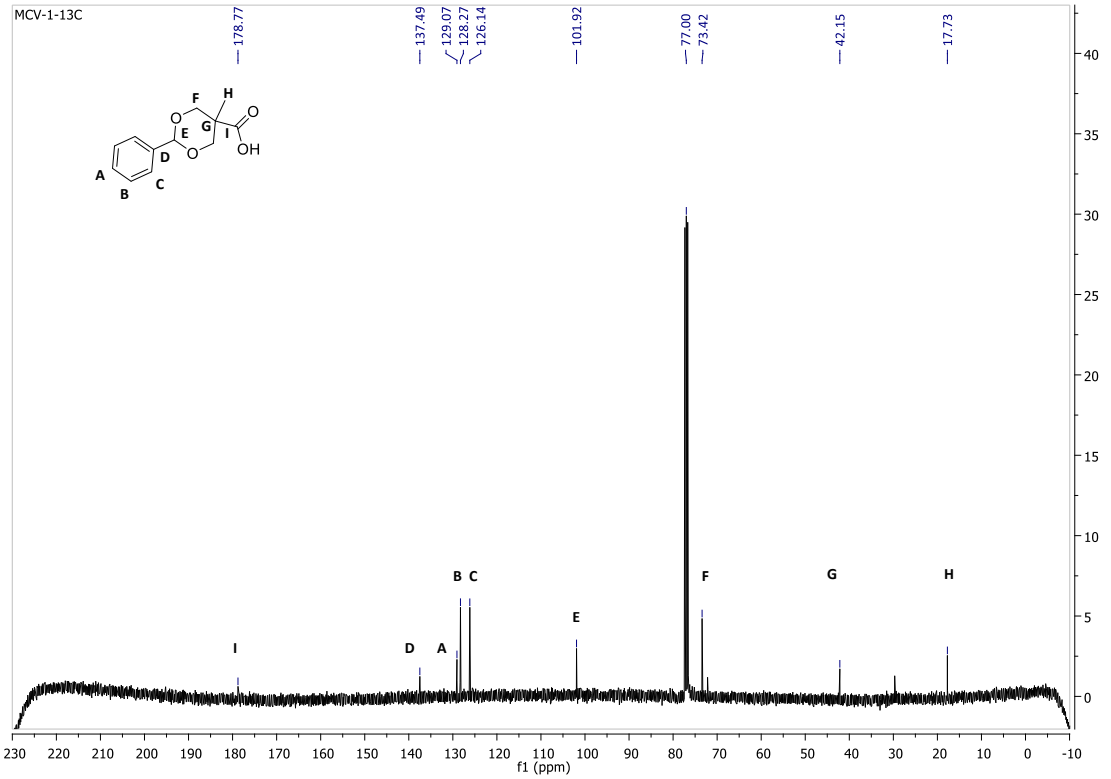

Figure II. [13]C-NMR spectrum of Benzylidene-2,2-bis(oxymethyl)propionic acid.

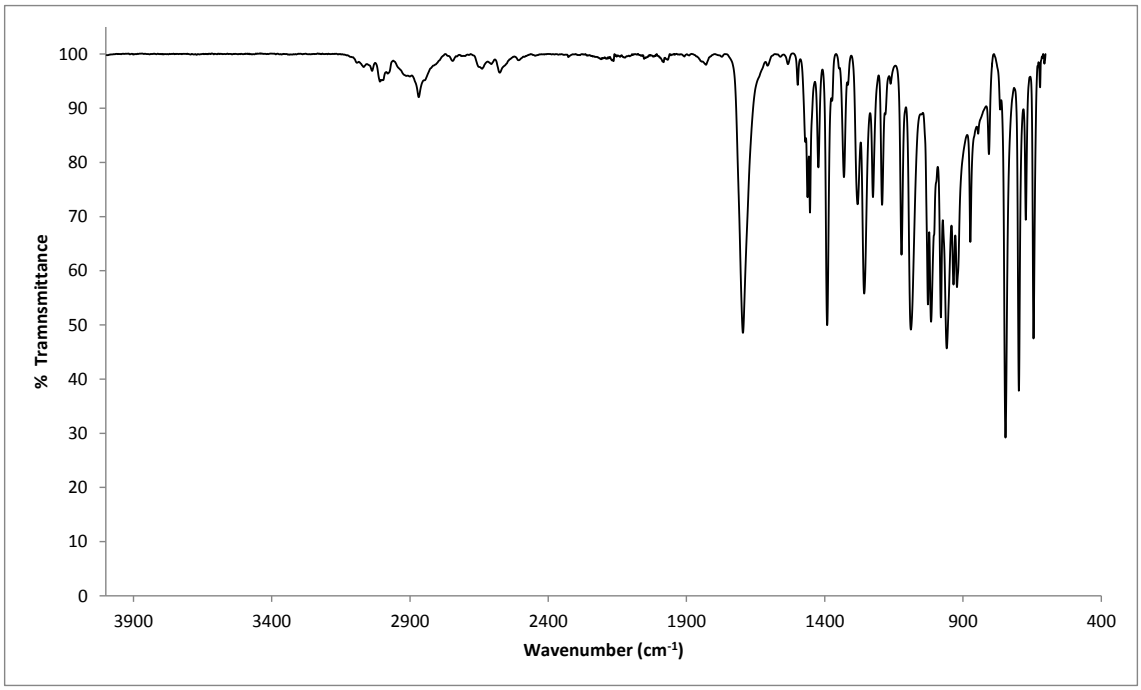

Figure III. FT-IR spectrum of Benzylidene-2,2-bis(oxymethyl)propionic acid.

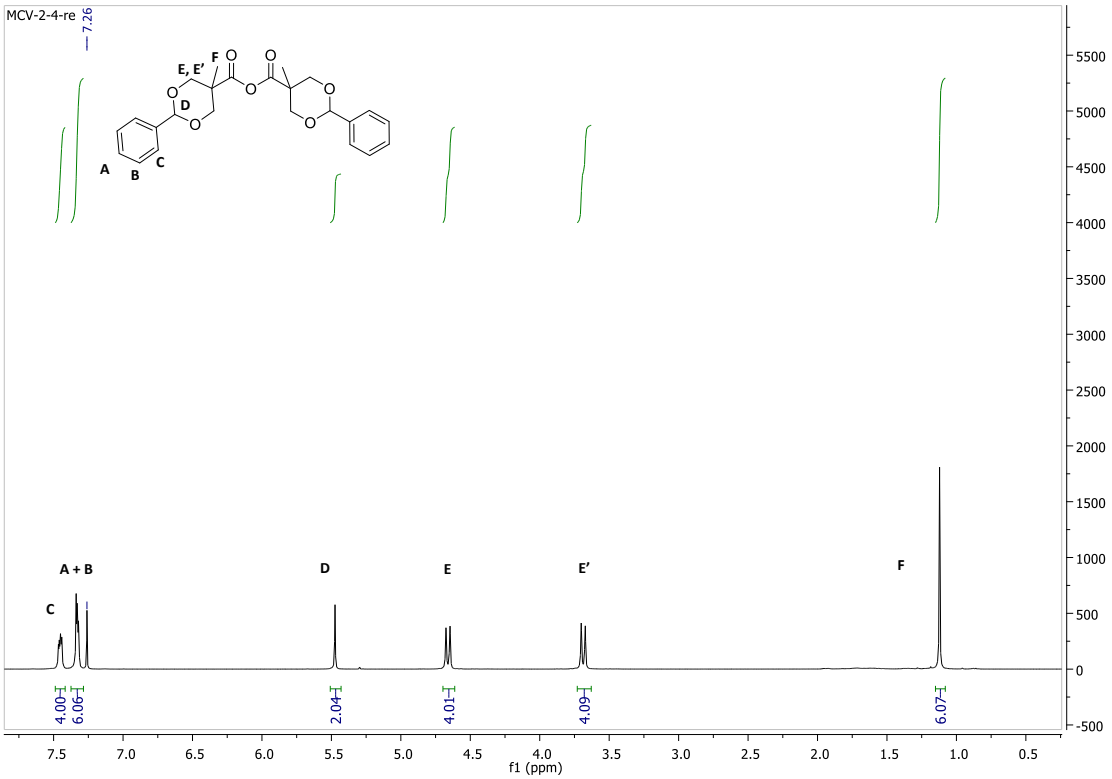

Figure IV. [1]H-NMR spectrum of Benzylidene-2,2-bis(oxymethyl)propionic anhydride.

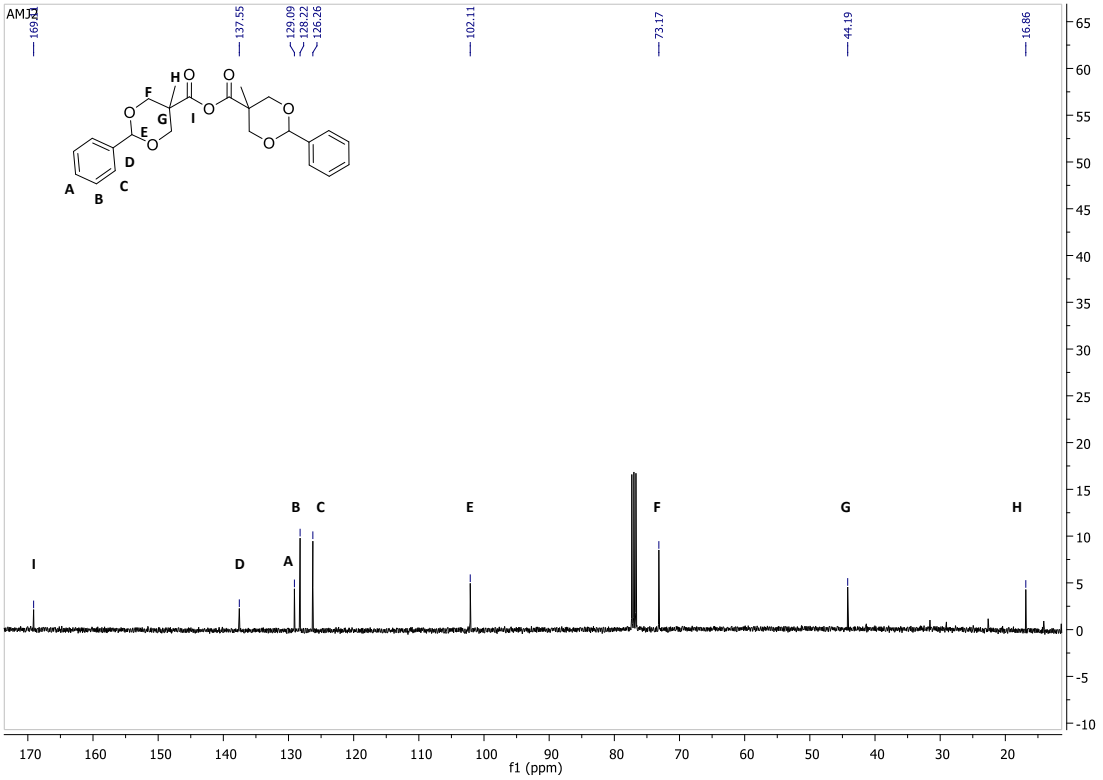

Figure V. ^{13}C-NMR spectrum of Benzylidene-2,2-bis(oxymethyl)propionic anhydride.

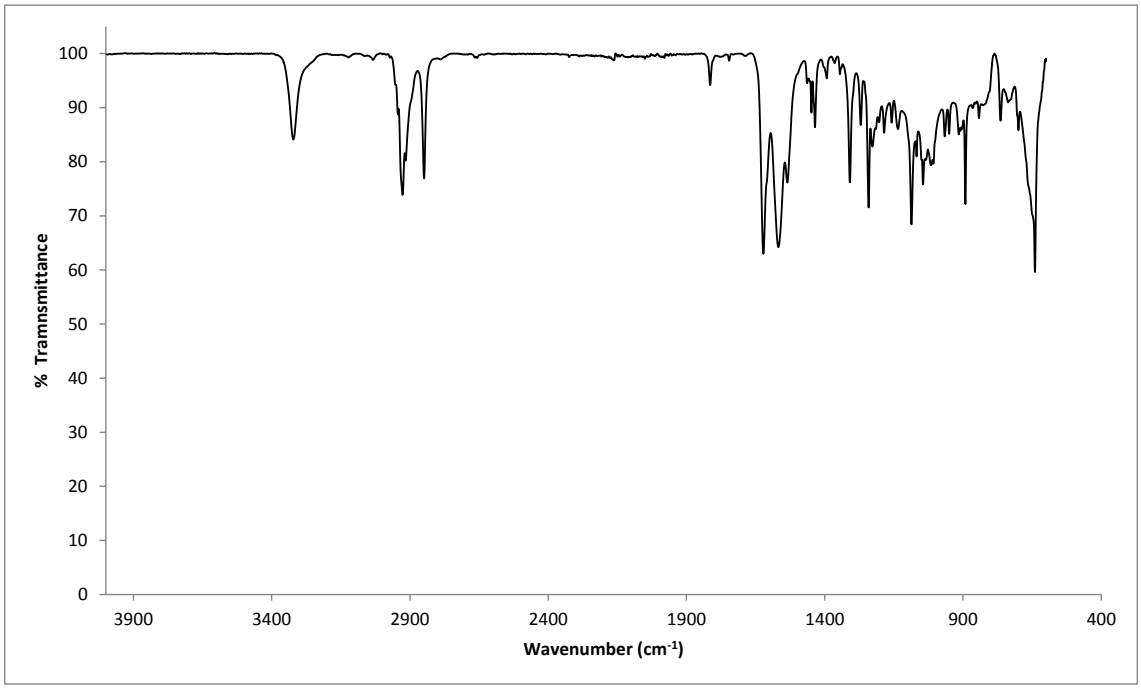

Figure VI. FT-IR spectrum of Benzylidene-2,2-bis(oxymethyl)propionic anhydride.

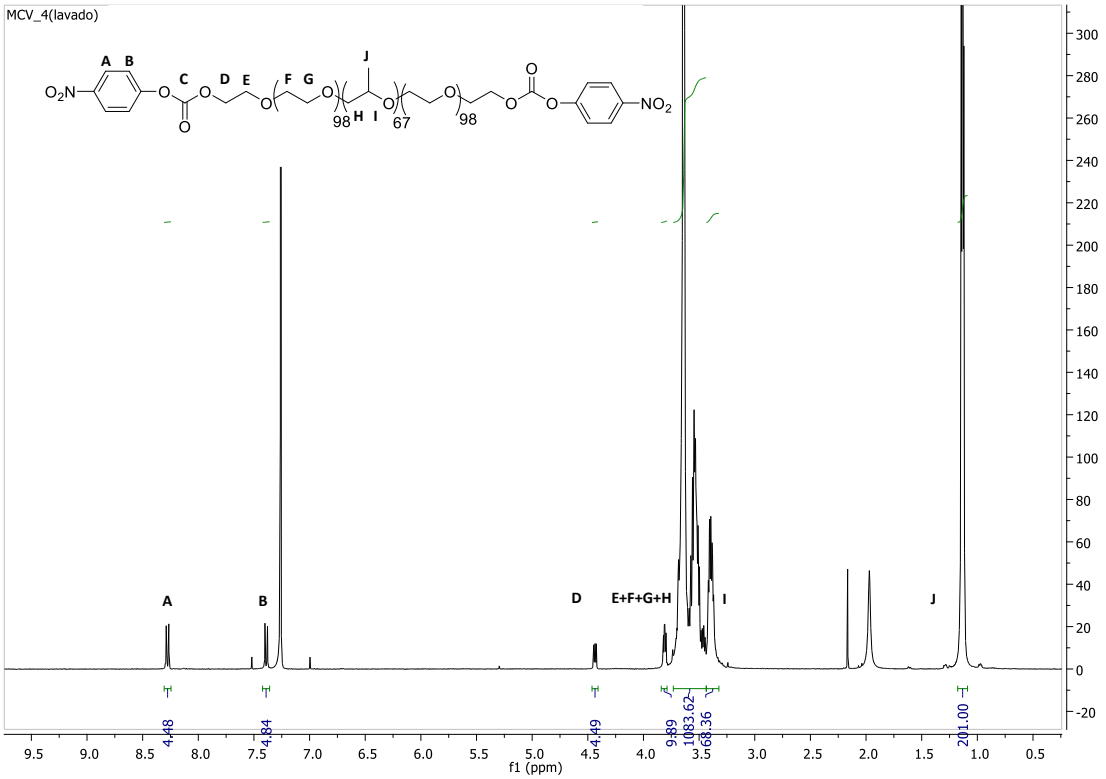

*Figure VII. [1]H-NMR spectrum of **F127-NP-2**.*

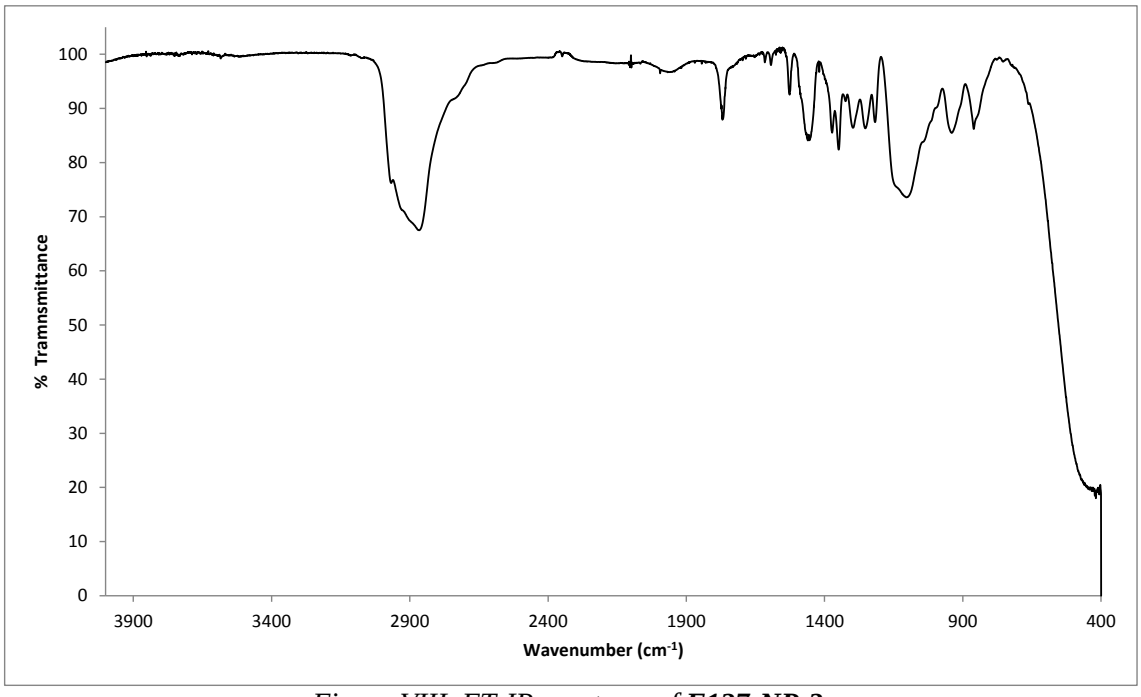

*Figure VIII. FT-IR spectrum of **F127-NP-2**.*

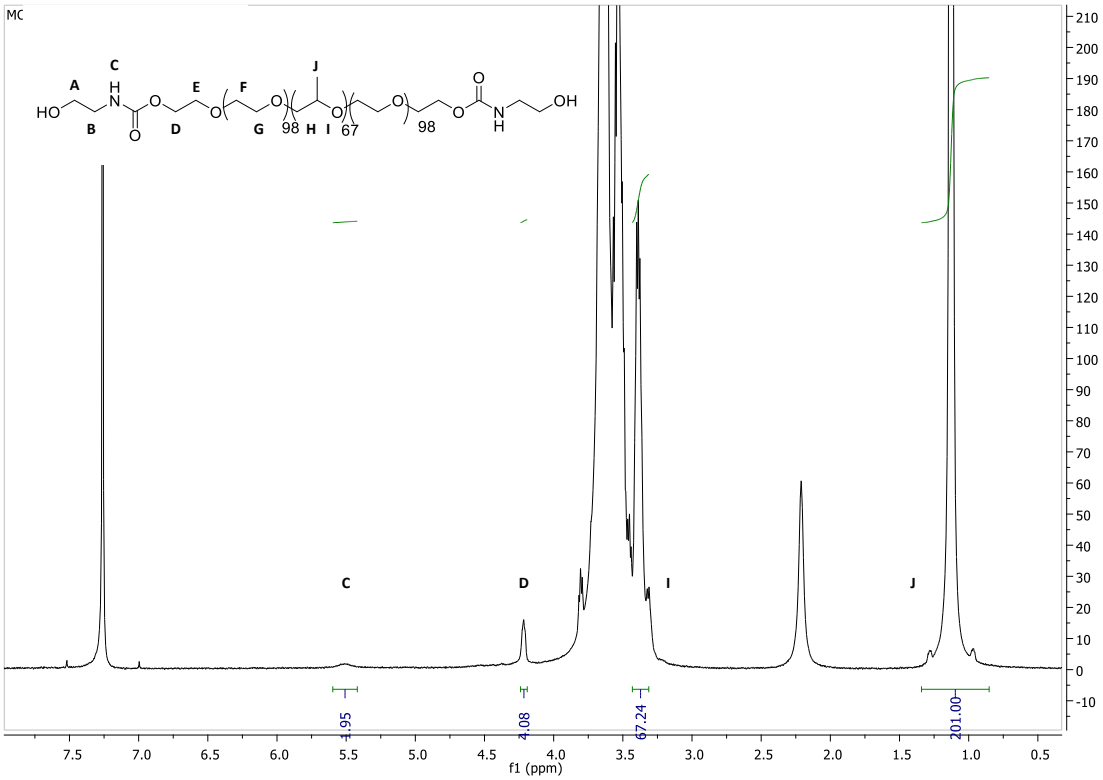

*Figure IX. ^{1}H-NMR spectrum of **F127-C-2**.*

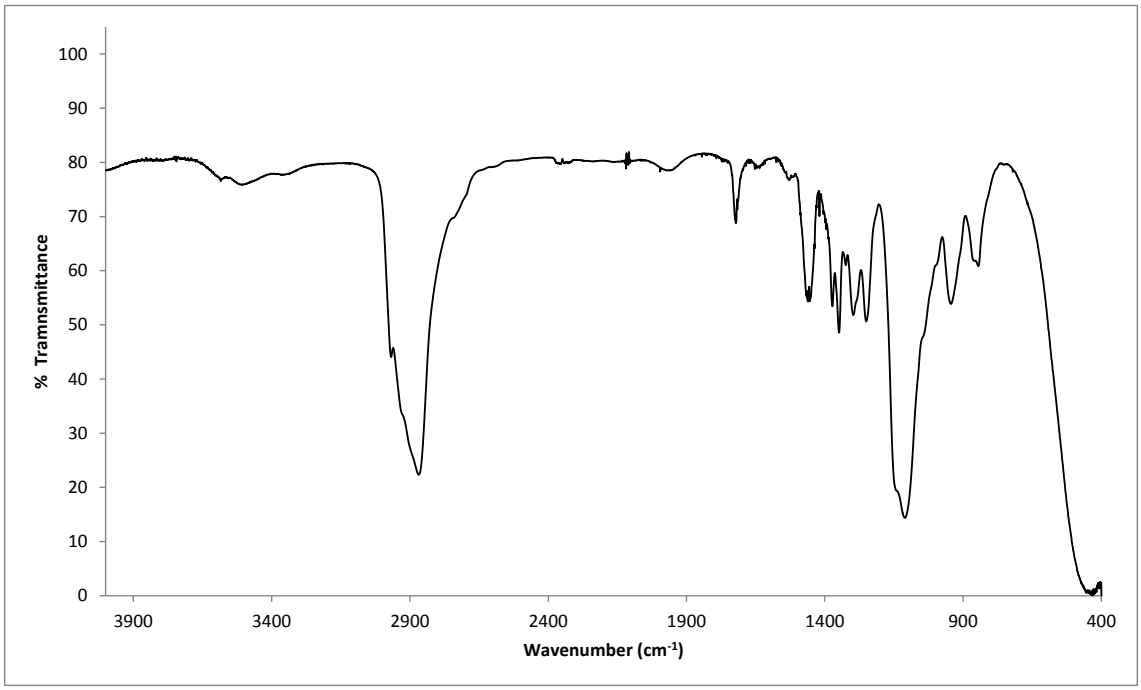

*Figure X. FT-IR spectrum of **F127-C-2**.*

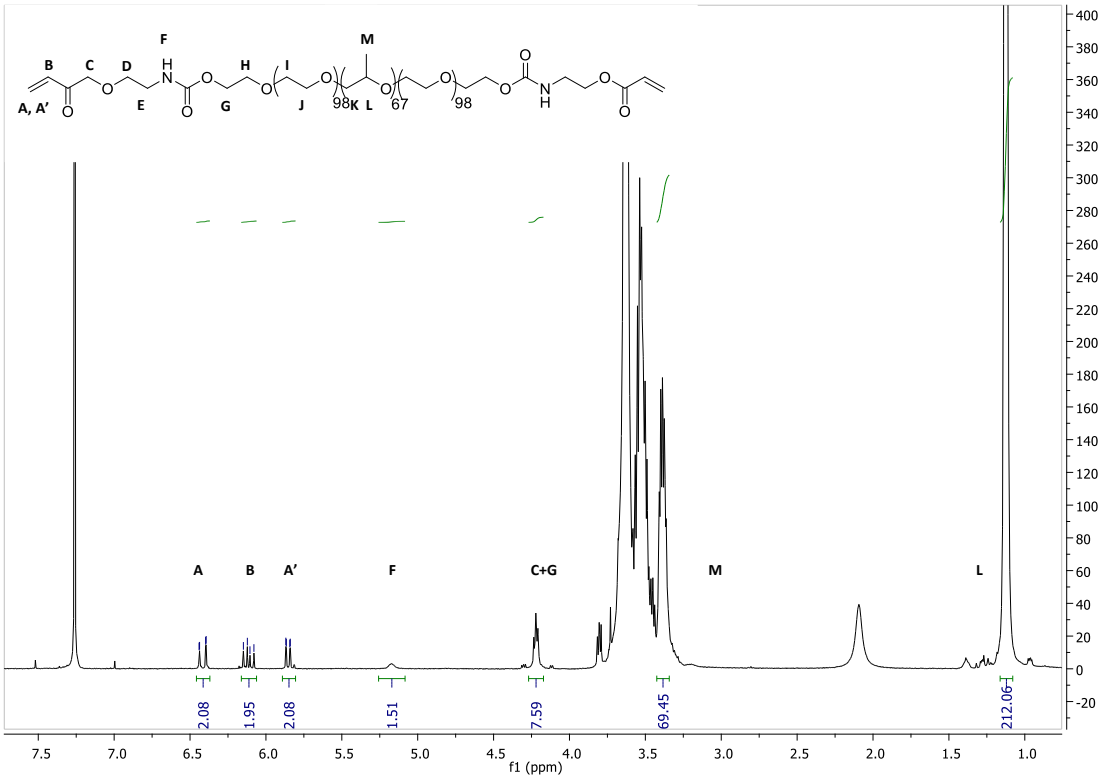

*Figure XI. [1]H-NMR spectrum of **F127-A-2**.*

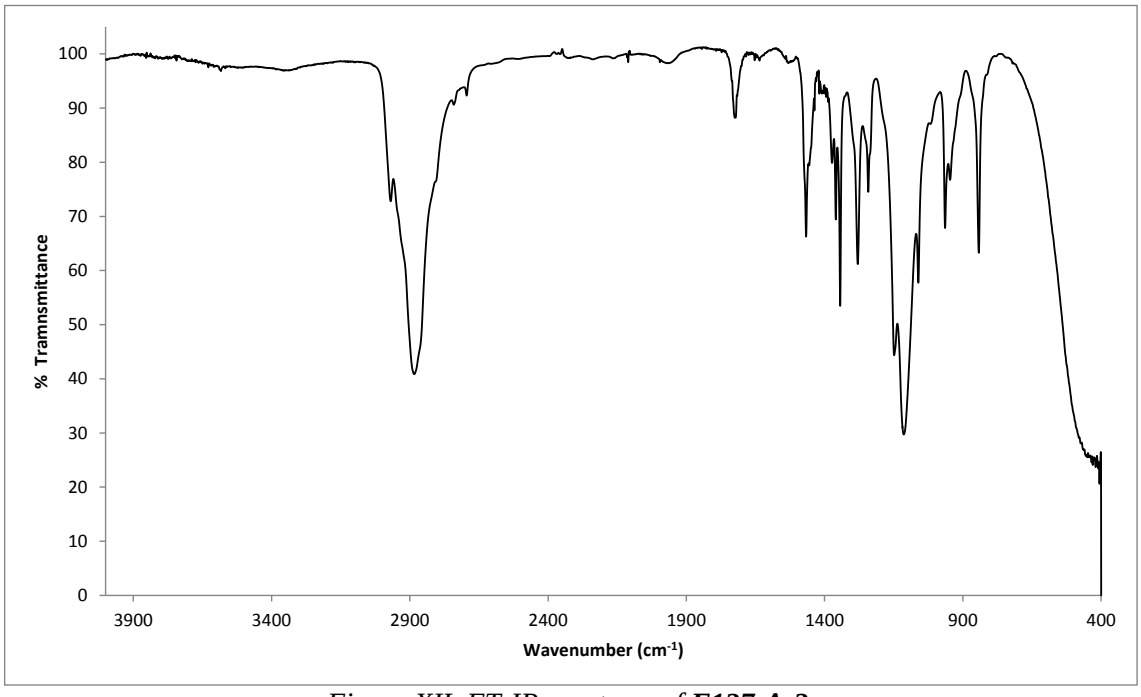

*Figure XII. FT-IR spectrum of **F127-A-2**.*

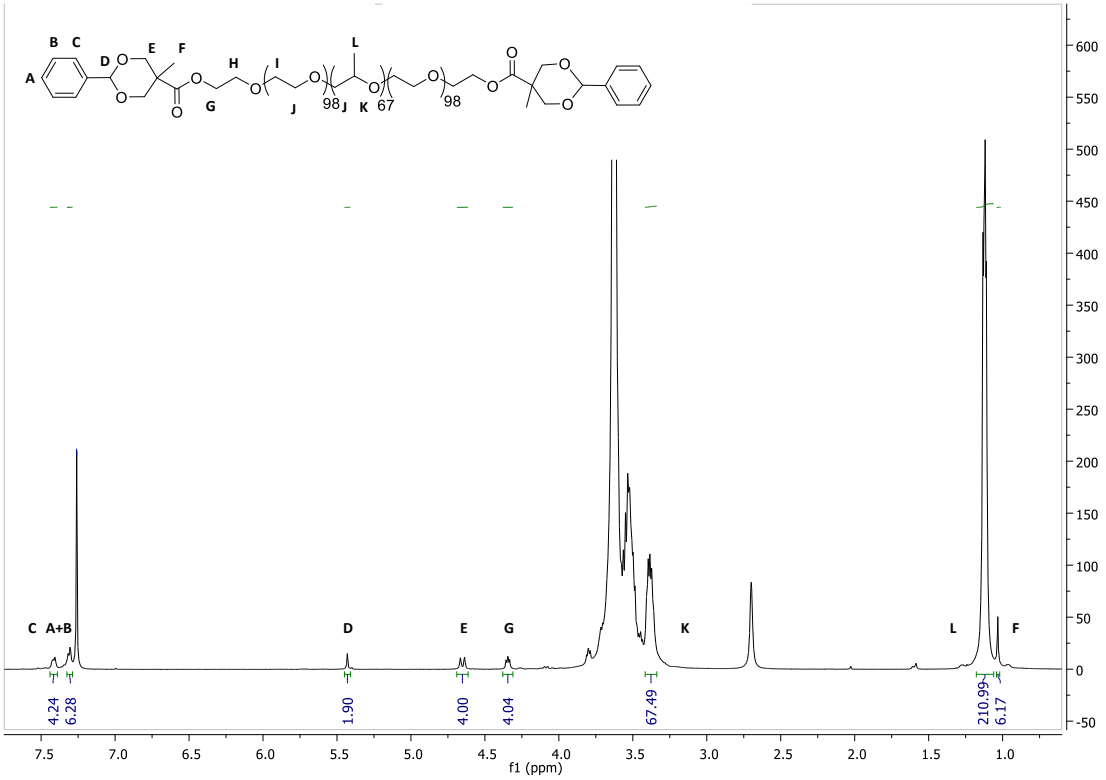

*Figure XIII. ^{1}H-NMR spectrum of **F127-Bn-2**.*

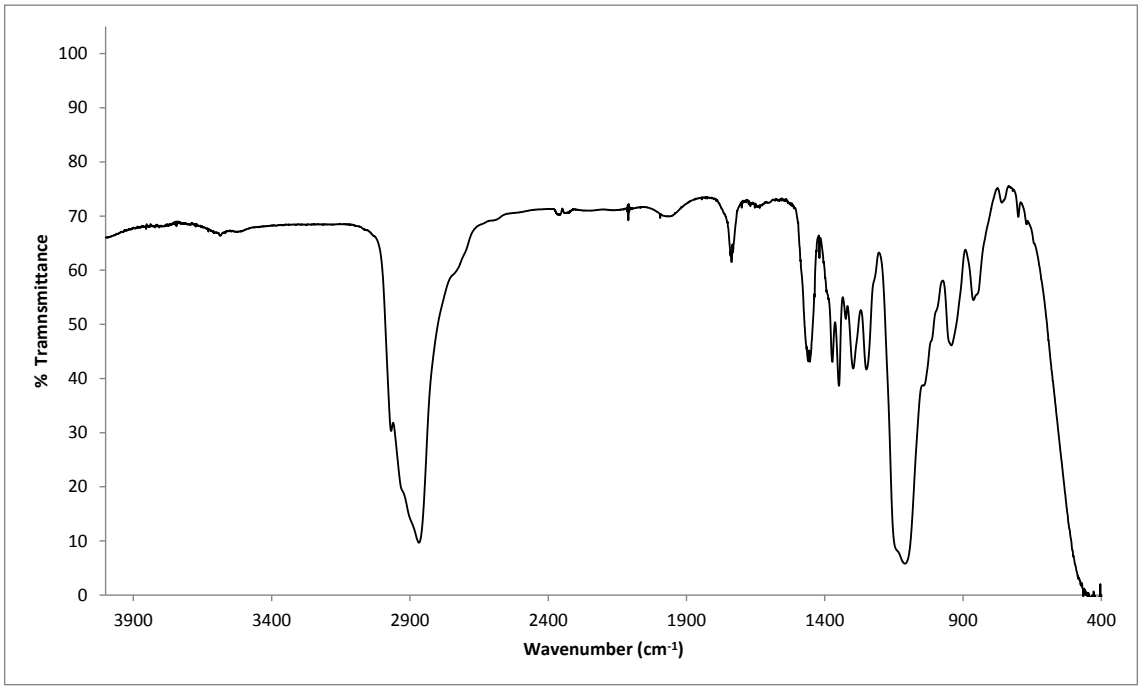

*Figure XIV. FT-IR spectrum of **F127-Bn-2**.*

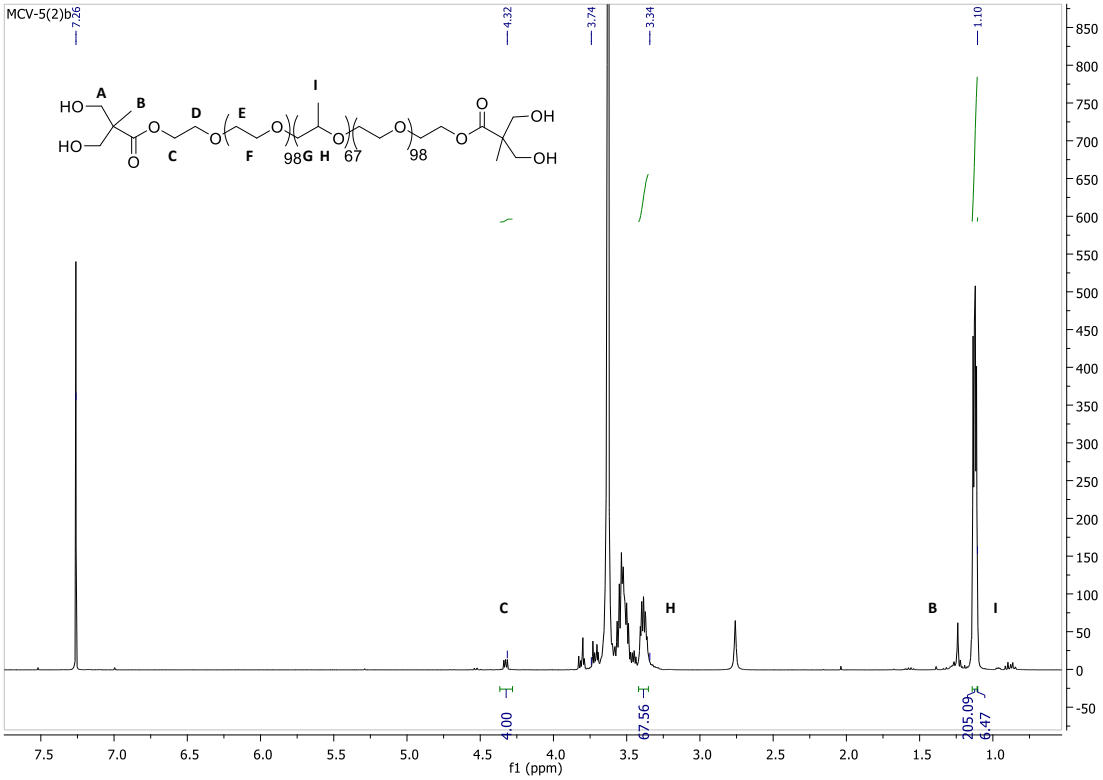

*Figure XV. ^{1}H-NMR spectrum of **F127-OH-4***

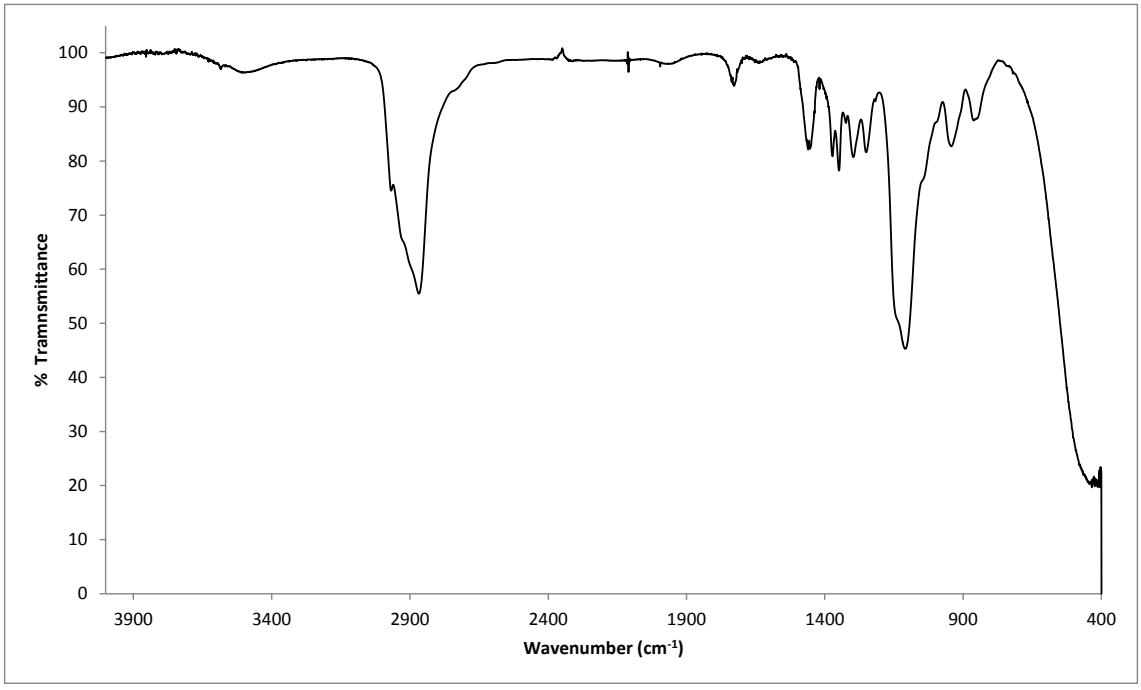

*Figure XVI. FT-IR spectrum of **F127-OH-4**.*

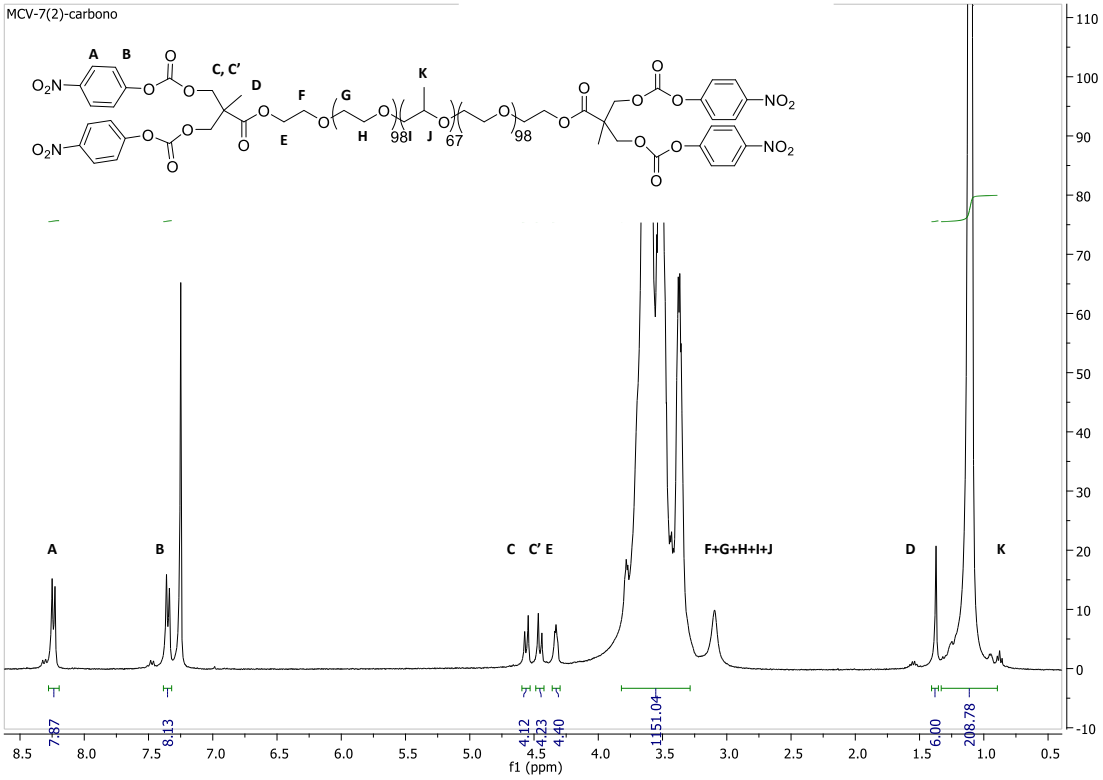

*Figure XVII. ^{1}H-NMR spectrum of **F127-NP-4.***

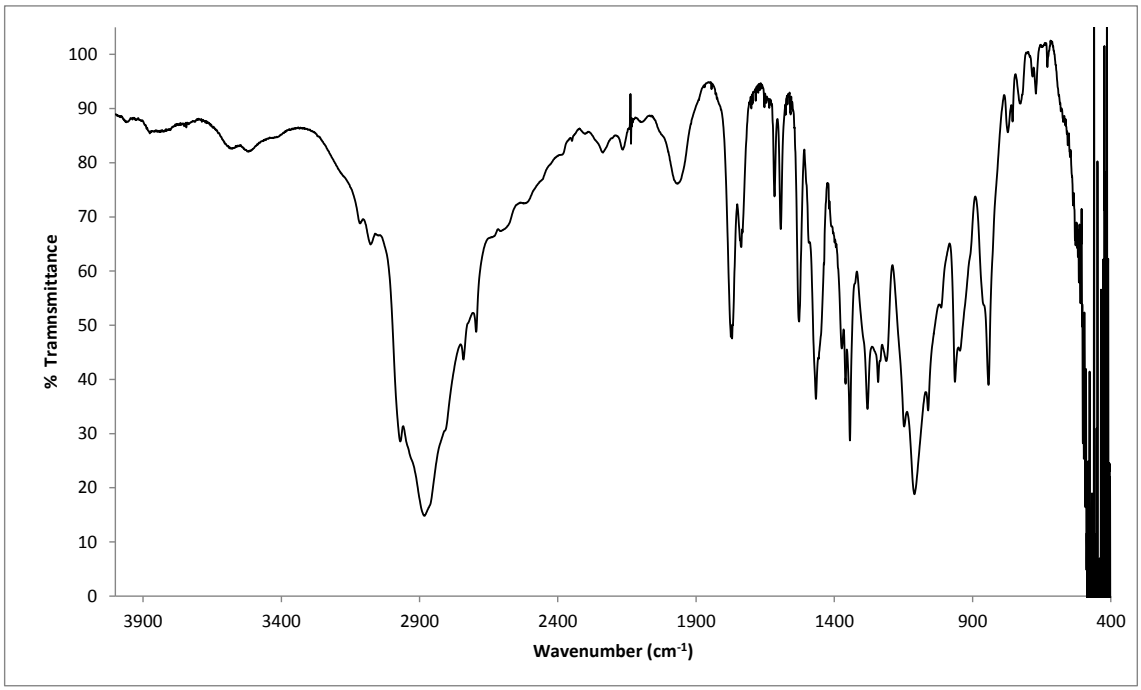

*Figure XVIII. FT-IR spectrum of **F127-NP-4**.*

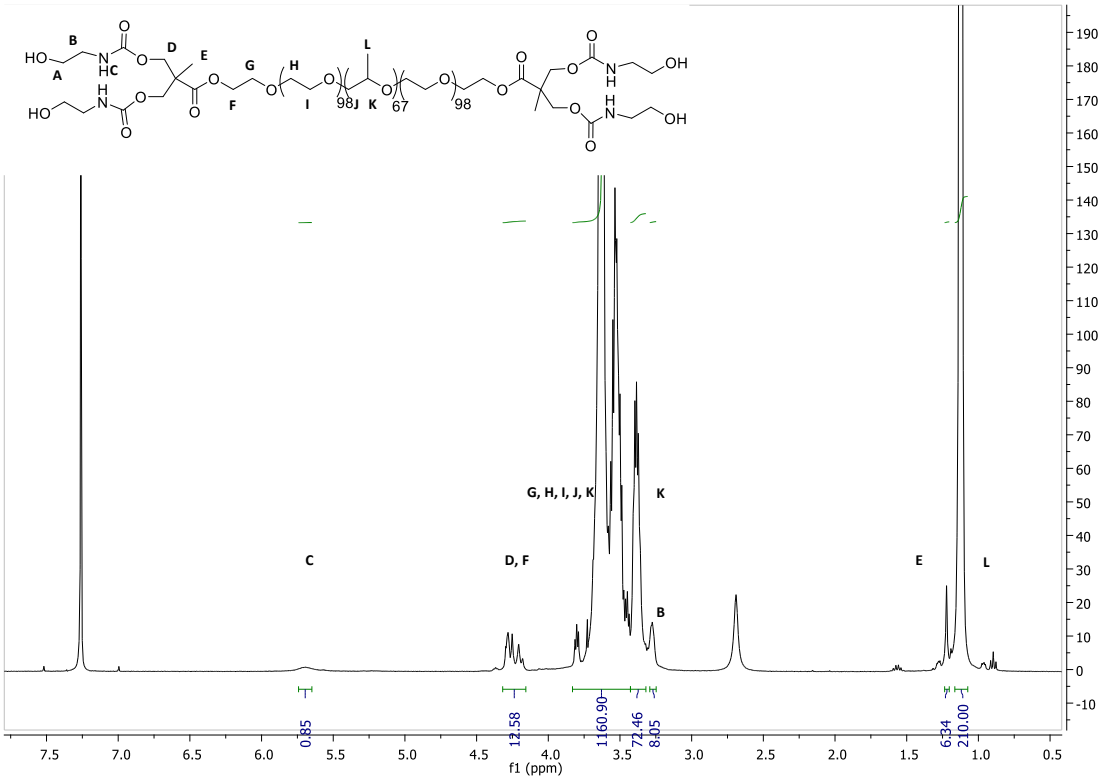

*Figure XIX. ^{1}H-NMR spectrum of **F127-C-4.***

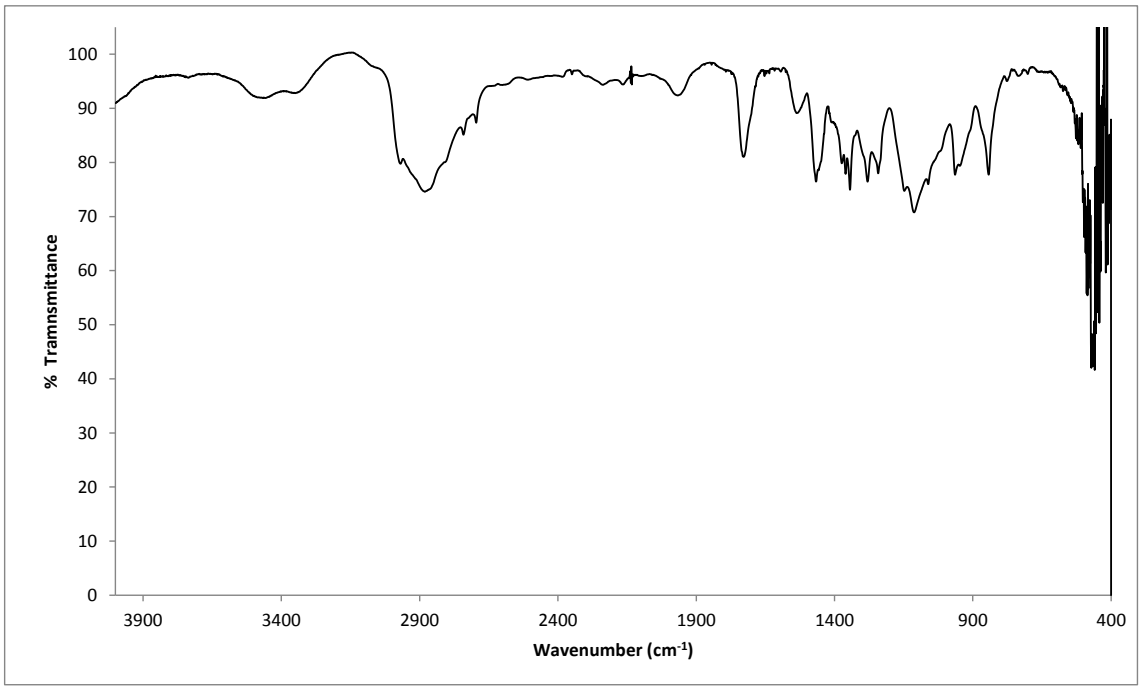

*Figure XX. FT-IR spectrum of **F127-C-4**.*

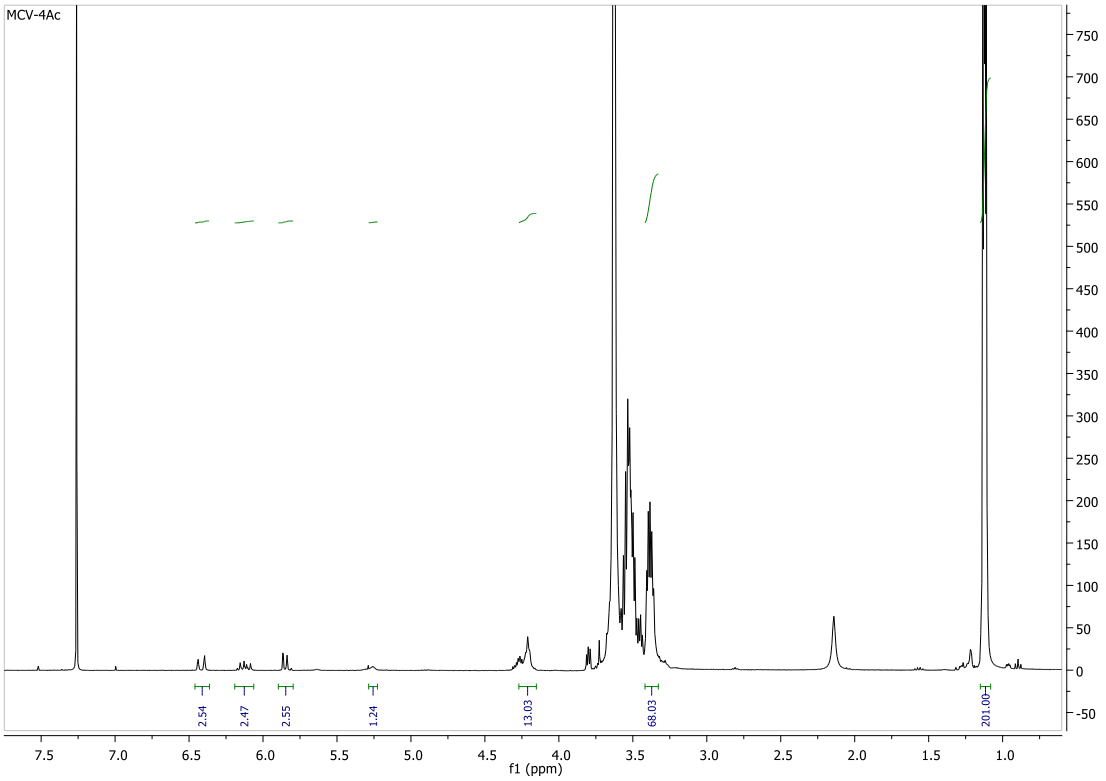

*Figure XXI. ^{1}H-NMR spectrum of partially functionalized **F127-A-4.***

ω Photopolymerization system

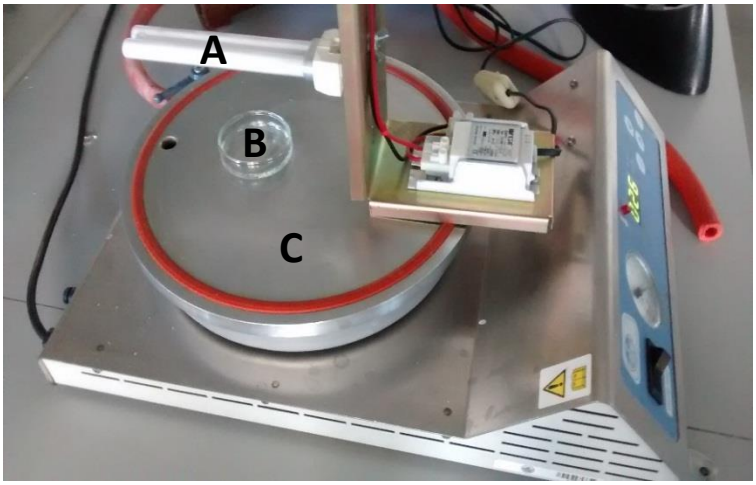

Figure XXII. Photopolymerization system used for the preparation of the CLPMs. A: UV lamp, B: cylindrical glass vessel, C: hotplate.

ω Camptothecin and Chloroquine encapsulation

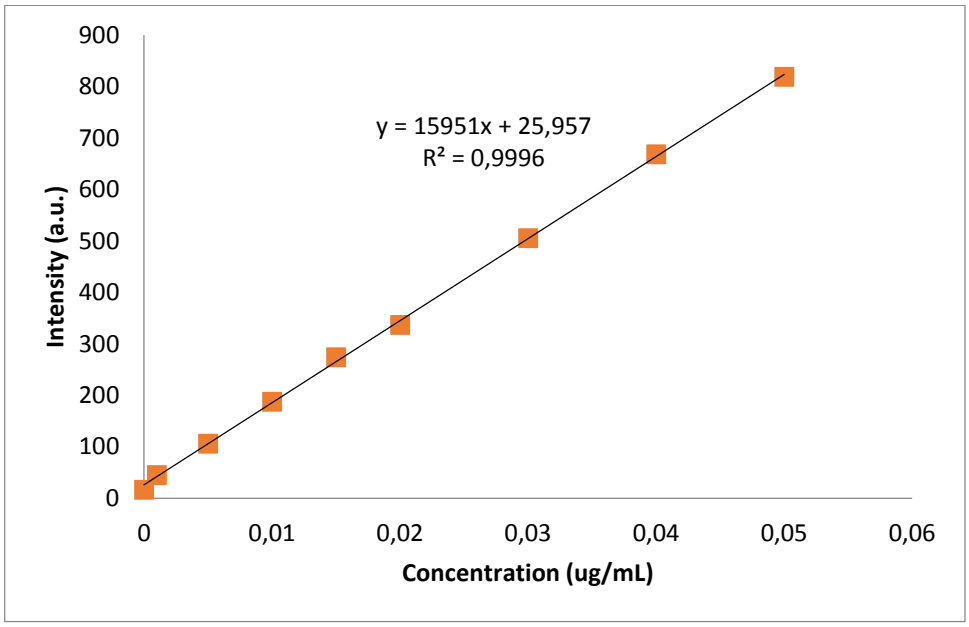

Figure XXIII. Fluorescence calibration curve for CPT in DMSO.

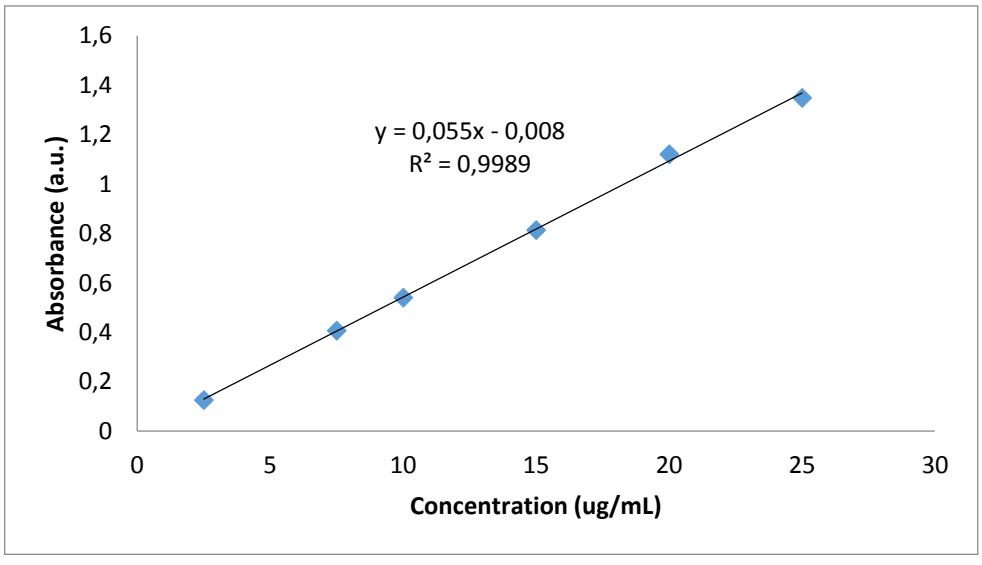

Figure XXIV. UV-vis calibration curve for CQ in water.

ω Materials and methods

Materials:

All the products were purchased from Sigma-Aldrich and Acros Organics.

The dialysis membrane was purchased from Spectrum.

General methods:

- ^{1}H-NMR spectra were recorded on a Bruker AV-400 operating at 400 MHz. $CDCl_3$ was used as solvent, chemical shifts are given ppm relative to TMS, and the solvent residual peak was used as internal standard.
- The infrared spectra of all the compounds were obtained with a Perkin Elmer 1600FT in Transmission mode in neat on NaCl. The vibrations are expressed in wavenumbers (cm^{-1}).
- SEM analyses were performed with a SEM Inspect F50 with gold or platinum coated samples, at the Laboratory of Advanced Microscopy (LMA) of the INA (Nanosciences Institute of Aragon).
- TEM measurements were performed using a TECNAI G20 (FEI COMPANY), 200 kV, at the Laboratory of Advanced Microscopy (LMA) of the INA (Nanosciences Institute of Aragon). Samples were prepared on holey carbon film 300 Mesh Cu (50) from Agar Scientific.
- DLS measurements were performed using a Malvern Instrument Nano ZS that uses a He-Ne laser, 633 nm, and a detection angle of 173°.
- Fluorescence measurements were performed in a Perkin Elmer LS 55 fluorescence spectrophotometer.
- UV-vis measurements were performed in a Cary Bio 100 UV-visible spectrophotometer.

ω In-vitro anti-HCV studies

Cells and replicon system

The highly permissive cell clone Huh 7-Lunet, as well as Huh 7 cells containing subgenomic hepatitis C virus (HCV) replicons I389luc-ubi-neo/NS3-3'/5.1 (Huh 5-2), I377NS3-3'/wt (Huh 9-13) or I389/hygro-ubi-NS3-3/5.1 (a kind gift from Dr. V. Lohmann and Dr. R. Bartenschlager) have been described recently. Briefly, this system allowed the efficient propagation of genetically modified HCV RNAs (replicons) in a human hepatoma cell line (Huh). The amount of the RNA that has been transcribed and translated is determined through the quantification of a reporter contained in the replicon system (luciferase). The amount of luminescence detected (after adding the substrate specific for this enzyme) is proportional to the virus replication rate. Cells were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco, Belgium) supplemented with 10% heat-inactivated fetal bovine serum (PAN-Biotech GmbH, Germany), 1X non-essential amino acids (Gibco), 100 IU/mL penicillin (Gibco), 100 µg/mL streptomycin (Gibco), and 250 µg/mL geneticin (G418; Gibco).

Antiviral assay with Huh 5-2 Cells

Antiviral assays for assessing the efficacy of the **F127-A-2-077-CPT** system were performed as described in literature. Briefly, Huh 5-2 cells were seeded at a density of $7x10^3$ cells per well in a tissue culture-treated white 96-well view plate (Techno Plastic Products AG, Switzerland) in complete DMEM supplemented with 250 µg/mL G418. After incubation for 24 hours at 37°C medium was removed and 2-fold serial Dilutions up to 0.3 µM in complete DMEM (with G418) of the **F127-A-2-077** were added in a total volume of 100 µL. After 3 days of incubation at 37°C luciferase activity was determined using the Bright-GloTM Luciferase Assay System (Promega Corporation, The Netherlands). The luciferase signal was measured using a Synergy HT Multimode Reader (BioTek Instruments Inc, USA).

Cytostatic assay

Cytostatic assays for assessing the cell viability of **F127-A-2-077** were performed as described in literature. Briefly, Huh 5-2 cell line was seeded at a density of $7x10^3$ cells per well of a 96-well plate in complete DMEM (with the appropriate concentrations of G418). Serial dilutions of the test compounds up to 0.3 µM in complete DMEM (with G418) were added 24 hours after seeding. Cells were allowed to proliferate for 3 days at 37°C, after which the cell number was determined by CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega Corporation). All the experiments were carried out in triplicate and each experiment was repeated three different days.