# Supporting Information

## Chemo-enzymatic synthesis of glycolipids, their polymerization and self-assembly

Dounia Arcens, Gaëlle Le Fer, Etienne Grau, Stéphane Grelier, Henri Cramail*, Frédéric Peruch*

Univ. Bordeaux, CNRS, Bordeaux INP, LCPO, UMR 5629, F-33600, Pessac, France

e-mail: peruch@enscbp.fr; cramail@enscbp.fr

### Material and method

### Material

12-hydroxystearic acid (12-HSA) was kindly provided by Iterg. Palladium diacetate and dibutyl tin dilaurate were purchased from TCI. Vinyl acetate (VA), isocyanoethyl methacrylate (IEMA), methyl methacrylate (MMA), benzyl mercaptan, 2-(2-cyano)-propyl benzodithiate and supported lipase from *Candida antarctica* B (CALB) were purchased from Sigma Aldrich. Glucose was purchased from Euromedex. *Azobisisobutyronitrile* (AIBN) was purchased from Acros Organics and recrystallized in ethanol prior to use. Toluene, THF and acetonitrile, Chromatosolv grade, were purchased from VWR Chemicals. All materials were used as received except acetonitrile which was dried prior to use. DMSO-$d_6$ was purchased from Euriso-top.

### Analyses

All proton nuclear magnetic resonance ($^1$H NMR) spectroscopy analyses were performed at 298 K on a Bruker Avance 400 spectrometer, operating at 400 MHz and equipped with a Bruker multinuclear z-gradient direct probe head capable of producing gradients in the z direction with 53.5 G.cm$^{-1}$ strength. DMSO-$d_6$ was used as solvent.

Fourier transform infrared (FTIR) was performed on a Bruker VERTEX Pike GladiATR plate (diamond crystal) for attenuated total reflectance (ATR) at room temperature and between 400 and 4000 cm$^{-1}$ (resolution 4 cm$^{-1}$, 64 scans).

Flash chromatography was performed on a Grace Reveleris apparatus, employing cartridges from Grace and gradient solvent (indicated in the method part for each case) equipped with ELSD and UV detectors at 254 and 280 nm. Elution solvents depend on the sample and are mentioned in the experimental parts.

Size Exclusion Chromatography (SEC) analyses were performed on a Thermoscientific Ultimate 3000 equipped with a multi-angle light scattering detector and a sensor of refractive index difference from Wyatt technology. The polymers were separated on three G2000, G3000 and G4000 TOSOH HXL gel columns with dimensions 300*7.8 mm (exclusion limits: from 1000 Da to 400,000 Da) using THF as eluent. Elution was carried out at a flow rate of 1 mL.min$^{-1}$ at 40°C. The dn/dC values (Table S1) of various glycopolymers in THF were calculated by plotting the RI signal area *versus* concentration (from 0.5 mg.mL$^{-1}$ to 10 mg.L$^{-1}$) and allowed to determine the absolute number-average molar masses $\bar{M}_n$ and the dispersities ($Đ$).

Differential Scanning Calorimetry (DSC) measurements were performed on a DSC Q100 (TA Instruments). The samples were first heated from -80°C to 100°C then cooled down to -80°C and reheated to 150°C at a heating rate of 10 °C.min$^{-1}$. Glass transition temperatures were measured from the second heating run.

Mono-angle dynamic light scattering (DLS) measurements were performed at 25°C on a Malvern Instruments Zetasizer Nano ZS equipped with a He-Ne laser source at the wavelength of 632.8 nm and a scattering angle of 90°. Samples were introduced into quartz cells (pathway: 10 mm) after filtration through 0.45 µm cellulose micro-filters.

The autocorrelation functions ($g_1(t)$) were analyzed in terms of relaxation time distribution ($\tau$) (Equation 1) and using the method of cumulants:

$$g_1(t) = \int A_{(\tau)} \exp\left(\frac{-t}{\tau}\right) d\tau \qquad \text{Equation 1}$$

Hydrodynamic radius ($R_h$) was determined from the Stokes-Einstein relation (Equation 2).

$$R_h = \frac{k_B T}{6\pi\eta_s D_0} \qquad \text{Equation 2}$$

where $D_0$ is the diffusion coefficient, $\eta_s$ is the viscosity of the solvent, T is the absolute temperature and $k_B$ the Boltzmann constant.

X-ray Diffraction Wide-angle X-ray scattering (WAXS) patterns were collected from a microfocus rotating anode C-ray source (Rigaku MicroMax-007 HF) combined with a performant multi-layers optics and a 3-pinholes collimation that provide an intense X-ray intensity on the sample. Samples were introduced in glass capillaries (diameter 1.5 mm) purchased from Lindemann. Silver behenate used as reference to calibrate the sample-detector distance. A 2-dimension detector Image plate from Mar research was used.

Transmission Electron Microscopy (TEM) micrographs were recorded at the Bordeaux Imaging Center, (BIC) (Bordeaux, France) on a Hitachi H7650 microscope working at 80 kV in high resolution mode and equipped with a GATAN Orius 10.5 Megapixel camera. A drop of

colloidal suspension was deposited on a copper grid (200 mesh coated with a carbon/formvar film) and the excess removed using a filter paper.

The confocal microscope observations were performed on a Leika instrument equipped with a HCXPL APO Lambda blue 63.0×1.40 OIL lens correlated to a scanner resonating at 8000 Hz. The samples were excited at a wavelength of 561 nm, using a DPSS 561 laser and the emitted light was collected using a photomultiplier between 570 and 623 nm.

**Synthetic Methods**

*Transvinylation of 12-hydroxystearic acid*

In a CEM Discover SP microwave reactor tube, 1 eq (3 g) of 12-hydroxystearic acid (12HSA) were dissolved into 16 eq. of vinyl acetate. 2 mol-% of palladium acetate and 4 mol-% of potassium hydroxide were added and the reaction medium was heated at 60°C under microwaves and magnetic stirring for 30 minutes. 2 mol-% of palladium acetate and 4 mol-% of potassium hydroxide were added again and the reaction was pursued at 60°C for 30 more minutes. Reaction was monitored by thin layer chromatography (TLC). When all 12-hydroxystearic acid was consumed, the reaction mixture was poured into dichloromethane and the solution was filtrated twice on a Büchner onto celite to eliminate the catalyst. The filtrate was concentrated under vacuum and vinyl 12-hydroxystearate (12HSV) was isolated as a yellowish waxy solid with a yield of 95%. **$^1$H NMR** (DMSO-$d_6$, 400 MHz, δ(ppm)): 0,88 (3H, t, $CH_3$), 1.19-1.39 (24H, massif, alkyl chain -$CH_2$-), 1.55 (2H, quintet, **C$H_2$**-$CH_2$-CO), 2.40 (2H, t, **C$H_2$**-CO), 3.35 (1H, quintet, **C**H-OH), 4,20 (1H, d, OH), 4.65 and 4.80 (2H, dd, **C$H_2$**=CH), 7.20 (1H, dd, $CH_2$=**CH**).

*Carbamation of vinyl 12-hydroxystearate*

1 eq (3 g) of vinyl 12-hydroxystearate (12HSV) was dissolved in toluene at 0.1 g.mL$^{-1}$. The mixture was heated to 40°C and then 1.2 eq of isocyanoethyl methacrylate (IEMA) were added dropwise. The reaction medium was then stirred for 24h at 40°C. The reaction was monitored by TLC. When vinyl 12-hydroxystearate was entirely consumed, the reaction medium was concentrated under vacuum and then purified by Flash chromatography using a cyclohexane/ethyl acetate mixture as eluent. A gradient from 3% to 10% of ethyl acetate was applied. The fraction containing pure urethane, methacrylated vinyl 12-hydroxystearate (MASV), was identified by $^1$H NMR and was concentrated under vacuum to give a white, waxy solid with a yield of 67%. **$^1$H NMR** (DMSO-$d_6$, 400 MHz, δ (ppm)): 0.88 (3H, t, $CH_3$), 1.19-1.39 (20H, multiplet, alkyl chain -$CH_2$-), 1.45 (4H, m, **(C$H_2$)$_2$**-CH-O), 1.55 (2H, quintet, **C$H_2$**-$CH_2$-CO), 1.80 (3H, s, **C$H_3$**-C(CO)=), 2.40 (2H, t, **C$H_2$**-CO), 3.25 (2H, q, **C$H_2$**-NH), 4.05

(2H, t, $CH_2$-**$CH_2$**-OCO), 4.60 (1H, quintet, **CH**-O-CO), 4.65 et 4.80 (2H, dd, **$CH_2$**=CH), 5.70 et 6.1 (2H, 2 dd, **$CH_2$**=C(CO)-$CH_3$), 7.15 (1H, t, NH), 7.20 (1H, dd, $CH_2$=**CH**).

*Glycolipid synthesis*

In an oven-dried Schlenk under argon flux, 1 eq (3 g) of MASV was dissolved in anhydrous acetonitrile at 90 $mmol.L^{-1}$. 2 $mg.mL^{-1}$ of supported CALB were added to the reaction mixture. The reaction was started by adding 2 eqs of glucose and the mixture was stirred under static argon atmosphere at 45°C for 72h. The reaction was monitored by $^1H$ NMR. When the reaction was complete, acetonitrile was evaporated under vacuum. Dichloromethane was then added and the mixture was filtrated on a Büchner to eliminate the lipase and the remaining glucose. The filtrate was concentrated under vacuum and then purified by flash chromatography using an eluent composed of a dichloromethane/ethyl acetate/methanol mixture, with a gradient from 44.5/44.5/1 to 35/35/30. The pure glycolipid fraction was concentrated under vacuum and methacrylated 12-hydroxystearate glucose (MASG) was isolated as a white solid with a yield of 65%. $^1H$ **NMR** (DMSO-$d_6$, 400 MHz, δ(ppm)): 0.88 (3H, t, $CH_3$), 1.19-1.39 (20H, multiplet, alkyl chain -$CH_2$-), 1.45 (4H, m, **($CH_2$)$_2$**-CH-O), 1.55 (2H, quintet, **$CH_2$**-$CH_2$-CO), 1.80 (3H, s, **$CH_3$**-C(CO)=), 2.26 (2H, t, **$CH_2$**-CO), 3.05 (1H, m, H4), 3.15 (1H, m, H2), 3,25 (2H, dt, **$CH_2$**-NH), ), 3.40 (1H, m, H3), 3.76 (1H, m, H5), 3.95 (1H, m, H6a), 4.05 (2H, t, $CH_2$-**$CH_2$**-OCO), 4.28 (1H, m, H6b), 4.51 (1H, d, OH3),4,60 (1H, quintet, **CH**-O-CO), 4.70 (1H, d, OH2), 4.90 (1H, t, H1), 5.02 et 5.08 (1H, 2 d, OH4a et OH4b), 5.70 and 6.10 (2H, **$CH_2$**=C(CO)-$CH_3$), 6.30 and 6.65 (1H, 2 d, OH1a and OH1b), 7.15 (1H, t, NH).

*Glycopolymer synthesis*

In an oven-dried Schlenk under argon flux, different ratios of freshly distilled MMA to MASG monomers (MMA:MASG from 100:0 to 0:100) were dissolved in anhydrous THF, the total monomer concentration being fixed to 1.5 $mol.L^{-1}$. For benzyl mercaptan mediated free radical polymerization (BM) and reversible addition-fragmentation chain transfer radical polymerization (RAFT), 2 mol-% of benzyl mercaptan or 2 mol-% of 2-cyano-2-propyl benzothioate were added respectively. 0.8 mol-% of AIBN were then added. The solution was degassed by three freeze-pump-thaw cycles and the reaction medium was heated to 70°C for 16h under magnetic stirring and static vacuum. When the polymerization was complete, the reaction medium was dried under dynamic vacuum for 24h to evaporate both THF and residual MMA monomer. The resulting poly(methyl methacrylate-*co*-12-hydroxystearate glucose methacrylate) (p($MMA_{(1-p)}$-$MASG_p$)) copolymers were obtained as a white powder for regular free radical polymerization (Non-Controlled, NC) without and with BM, and as a pink powder

for RAFT polymerization. **$^1$H NMR for p= 0.2 as an example:** (DMSO-$d_6$, 400 MHz, δ(ppm)) 0.60-0.86 (180H, two multiplets, $CH_3$), 1.07-1.38 (220H, massif, $CH_2$ alkyl chain), 1.38-1.57 (60H, multiplet, **$(CH_2)_2$**-CH-O et **$CH_2$**-$CH_2$-CO), 1.63-1.96 (100H, multiplet, -$CH_2$- polymer backbone), 2.27 (20H, m, $CH_2$-CO), 3.03 (10H, m, H4), 3.13 (10H, m, H2), 3.24 (20H, massif, O- $CH_2$-**$CH_2$**-NH), 3.43 (10H, m, H3), 3.54 (120H, m, $CH_3$-O-CO), 3.76 (10H, m, H5), 3.88 (20H, massif, O-**$CH_2$**-$CH_2$-NH), 3.98 (10H, m, H6a), 4.28 (10H, m, H6b), 4.51 (10H, massif, OH3), 4.63 (10H, massif, CH-O) 4.74 (10H, massif, OH2), 4.89 (10H, massif, H1), 5.02 et 5.08 (10H, 2 d, OH4b et OH4a), 6.33 et 6.63 (10H, 2 d, OH1b et OH1a), 6.94-7.19 (10H, m, NH)

**Nanoprecipitation**

The glycopolymers were dissolved in THF at a concentration of 5 mg.mL$^{-1}$ and the solutions were stirred at 200 rpm at ambient temperature. A syringe pump was used for the drop by drop injection of 4.5 mL of previously filtered pure water into 0.5 mL of polymer solution. The influence of the speed of water addition was studied by using various flow rates from 0.05 mL.min$^{-1}$ to 0.5 mL.min$^{-1}$. THF was left evaporated during 16h under the fumehood. The final concentration of the suspensions obtained was 0.55 mg.mL$^{-1}$.

**MMA and MASG conversion calculations**

Respective MMA and MASG monomer conversions were calculated from the $^1$H NMR spectrum of the reaction medium after 16 hours at 70°C by integration of the methacrylate protons peaks from each monomer, at 5.7 and 6.0 ppm, noted $I_{MMA}$ and $I_{MASG}$ on Figure S1. The peak at 2.26 ppm corresponding to -$CH_2$-$^b$ was used as a reference. Its integration $I_{REF}$ was set to 2.0 protons.

MMA and MASG conversion calculated from Equations (1) and (2):

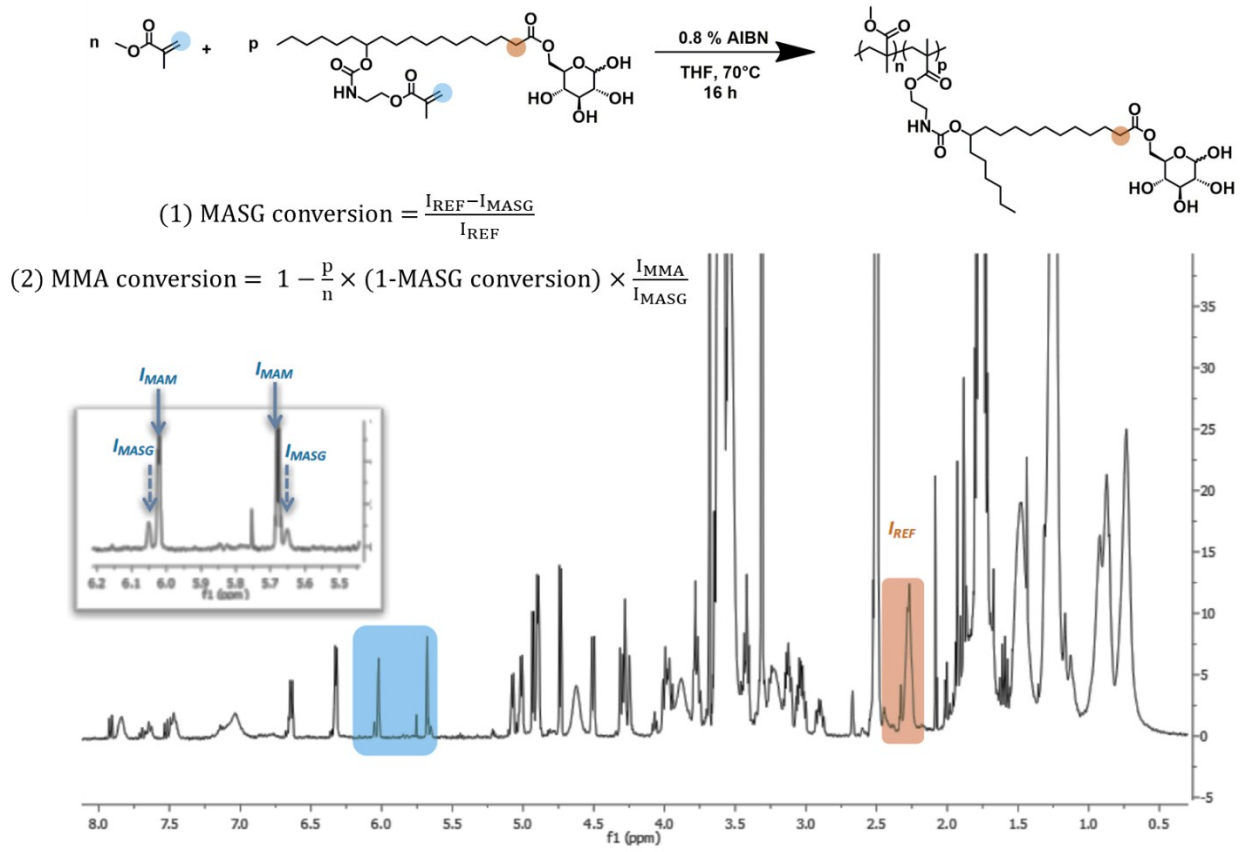


**Figure S1. Respective final MMA and MASG conversion calculations by $^1$H NMR from crude reaction medium.**

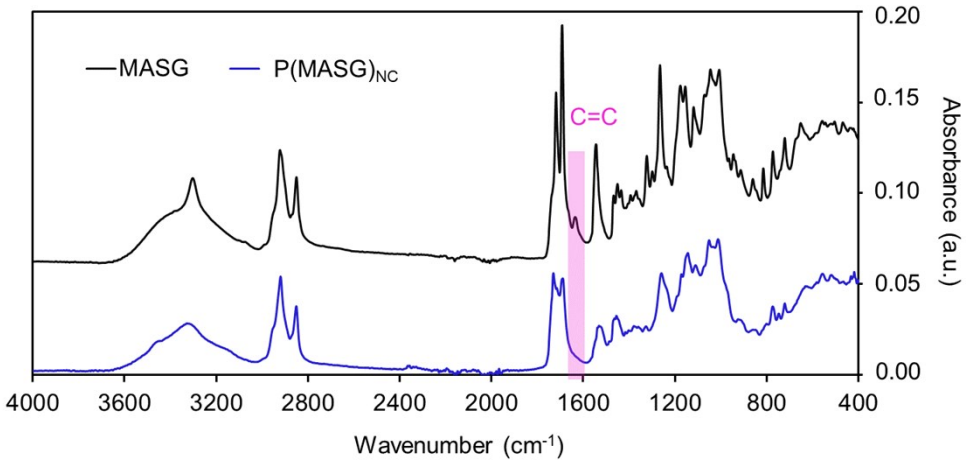


**Figure S2. FTIR spectra of MASG monomer and p(MASG)$_{NC}$ attesting the disappearance of the band from the elongated vibration of C=C bonds of methacrylate functions.**

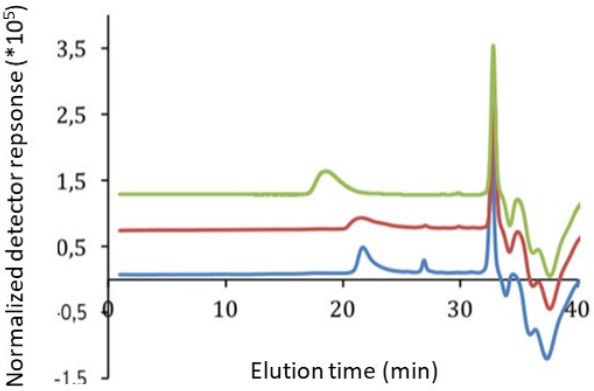


**Figure S3. SEC chromatograms of: Green = p(MMA$_{0.8}$-MASG$_{0.2}$)$_{NC}$, Red = p(MMA$_{0.8}$-MASG$_{0.2}$)$_{BM}$, Blue = p(MMA$_{0.8}$-MASG$_{0.2}$)$_{RAFT}$**

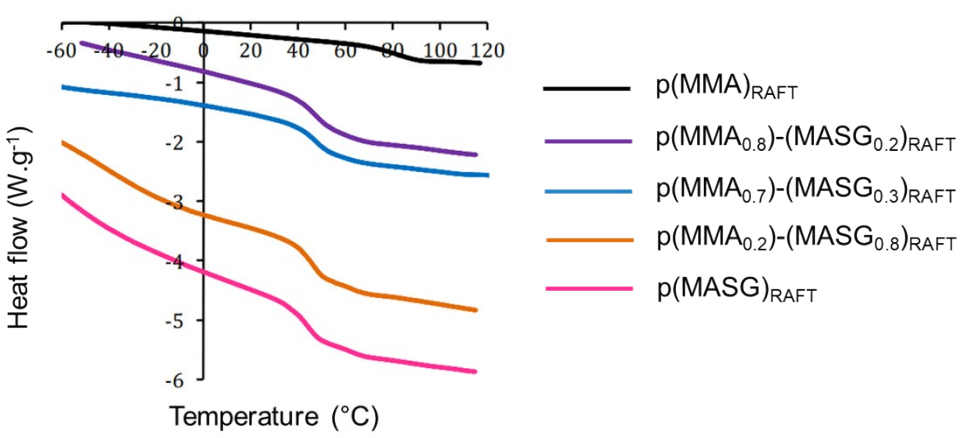


**Figure S4. Differential scanning calorimetry (DSC) traces for p(MMA)$_{RAFT}$ and the various p(MMA$_n$-MASG$_p$) copolymers.**

**Table S1. dn/dC values obtained by SEC with a RI detection for 3 polymers of each series.**

| MMA:MASG ratio | NC platform | BM platform | RAFT platform |
|---|---|---|---|
| **100:0** | 0.0626 | 0.0618 | 0.0618 |
| **80:20** | 0.0637 | 0.0568 | 0.0757 |
| **70:30** | Not soluble | 0.0667 | 0.0735 |

**Table S2. Characteristics of glycopolymer nanoparticles determined by DLS.**

| Glycopolymer | Water addition flow rate (mL.min$^{-1}$) | $D_h$ - PDI |
|---|---|---|
| P(MMA$_{0.8}$-MASG$_{0.2}$) | 0.05 | 640 nm - 0.14 |
| | 0.5 | 300 nm - 0.10 |

| | | |
|---|---|---|
| | 2.0 | 200 nm - 0.08 |
| | 5.0 | 160 nm - 0.04 |
| $P(MMA_{0.8}\text{-}MASG_{0.2})_{BM}$ | 0.5 | 460 nm - 0.14 |
| $P(MMA_{0.8}\text{-}MASG_{0.2})_{RAFT}$ | 0.5 | 460 nm - 0.10 |
| $P(MMA_{0.7}\text{-}MASG_{0.3})_{RAFT}$ | | 350 nm - 0.05 |
| $P(MMA_{0.4}\text{-}MASG_{0.6})_{RAFT}$ | | 380 nm - 0.04 |
| $P(MMA_{0.2}\text{-}MASG_{0.8})_{RAFT}$ | | 610 nm - 0.07 |
| $P(MASG)_{RAFT}$ | | 830 nm - 0.27 |