Supporting Information

Supramolecular carbohydrate-based hydrogels from oxidative hydroxylation of amphiphilic β-*C*-glycosylbarbiturates and α-glucosidase-induced hydrogelation

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Table of Contents

1. Experimental section

1.1 Materials and instrumentation

All chemicals and amyloglucosidase from *Aspergillus niger* (72 units/mg solid) were obtained from Sigma-Aldrich (Saint Quentin Fallavier, France) and were used as received. The progress of the reactions was monitored by thin layer chromatography (TLC) using silica gel 60 F254 precoated plates (Merck). Spots were visualized using UV light or by charring with 3% H_2SO_4 in MeOH-water (1:1, v/v). Column chromatography was performed by manual flash chromatography (wet-packed silica, 40-50 μm). IR spectra were recorded using a PerkinElmer spectrometer from 400 to 4000 cm^{-1} (4 scans, resolution 2). Proton nuclear magnetic resonance spectra ^{1}H NMR and DEPT135

^{13}C NMR were recorded on a Bruker Advance DRX400 (400 MHz) and (100 MHz), respectively. Data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, qt = quartet, q = quintet, n = nonet, td = triplet of a doublet, dt = doublet of a triplet, b = broad, m = multiplet), and coupling constants in Hz. Chemical shifts are reported in ppm relative to the residual proton solvent peaks of CD_3OD (δ 3.31 and 49.15 ppm for ^{1}H and ^{13}C, respectively). High-resolution mass spectra (HRMS-ESI) were performed on a Waters Xevo® G2-S QTof.

1.2 Experimental procedure for tautomer forms determination using UV-Vis spectroscopy

The stock solution of **GlcB-1**, **GlcB(OH)-1-4**, and **MalB(OH)-1-4** (0.1 wt%) was prepared by dissolving 5.0 mg of each analyte in 5 mL water. A final concentration of 0.012 wt% were prepared by placing 0.24 mL of the stock solution into a test tube, and diluting the solution to 2 mL with water. UV spectra were recorded on a Cary 50 Bio UV/vis spectrophotometer between 200 and 400 nm.

1.3 Synthesis of series of GlcB(OH) and MalB(OH)

General procedure: To a solution of amphiphilic sodium salt of *N*-monosubstituted β-*C*-glycosylbarbiturates[1] (1 mmol) in water (10 mL), was added KH_2PO_4/H_2O (2 mmol, 1 M, 2.0 equiv.) and H_2O_2/H_2O (3 mmol, 30 wt.%, 3.0 equiv.). The mixture was stirred at 60 °C for about 2 h and the reaction was monitored by TLC (7/2/1 EtOAc/MeOH/Water; v/v/v). The crude product was purified by flash column chromatography on silica gel to afford the following carbohydrate derivatives.

GlcB(OH)-1

Yield: 58 %, white solid.

^{1}H NMR (CD_3OD, 400 MHz, ppm) δ 3.92-3.73 (m, 3H), 3.68-3.50 (m, 3H), 3.38 (t, J = 8.8, 1H), 3.29-3.17 (m, 2H), 1.62 (s, 2H), 1.34 (d, 22H), 0.93 (t, J = 6.6, 3H).

DEPT135 ^{13}C NMR (100 MHz, CD3OD) δ 171.7, 171.2, 170.8, 170.6, 152.1, 152.0, 82.9, 82.6, 82.4, 79.5, 78.3, 72.5, 71.3, 71.0, 63.0, 62.7, 43.1, 42.8, 33.2, 30.9, 30.8, 30.6, 30.5, 29.0, 28.1, 28.0, 23.9, 14.6.

HRMS(ESI): calcd for $[M-H]^-$, 501.28175; found, 501.28120.

FT-IR (cm^{-1}): 3381, 2921, 2852, 1682, 1442, 1408, 1361, 1182, 1084, 1051, 758.

GlcB(OH)-2

Yield: 64 %, white solid.

^{1}H NMR (CD_3OD, 400 MHz, ppm) δ 3.92-3.71 (m, 3H), 3.67-3.50 (m, 3H), 3.38 (q, 1H), 3.28-3.16 (m, 2H), 1.62 (s, 2H), 1.34 (d, 26H), 0.93 (t, J =6.9, 3H).

DEPT135 ^{13}C NMR (100 MHz, CD3OD) δ 171.8, 171.2, 171.0, 170.8, 152.2, 83.0, 82.9, 82.6, 82.4, 79.5, 78.3, 72.6, 72.5, 71.3, 71.0, 63.0, 62.7, 43.1, 42.8, 33.2, 30.9, 30.8, 30.6, 30.5, 29.0, 28.1, 28.0, 23.9, 14.6.

HRMS(ESI): calcd for $[M-H]^-$, 529.31305; found, 529.31213.

FT-IR (cm^{-1}): 3361, 2917, 2850, 1684, 1440, 1408, 1363, 1182, 1090, 1019, 761.

GlcB(OH)-3

Yield: 56 %, white solid.

^{1}H NMR (CD_3OD, 400 MHz, ppm) δ 3.92-3.71 (m, 3H), 3.68-3.50 (m, 3H), 3.38 (t, 1H), 3.28-3.16 (m, 2H), 1.63 (s, 2H), 1.34 (d, 30H), 0.93 (t, J =6.8, 3H).

DEPT135 ^{13}C NMR (100 MHz, CD_3OD) δ 171.7, 171.2, 170.8, 170.6, 152.1, 83.0, 82.9, 82.6, 82.4, 79.5, 78.3, 72.6, 72.5, 71.3, 71.0, 63.0, 62.7, 43.1, 42.8, 33.2, 30.9, 30.8, 30.6, 30.5, 29.0, 28.1, 28.0, 23.9, 14.6.

HRMS(ESI): calcd for $[M-H]^-$, 557.34435; found, 557.34303.

FT-IR (cm^{-1}): 3340, 2917, 2850, 1713, 1669, 1442, 1409, 1364, 1183, 1095, 1022, 763.

GlcB(OH)-4

Yield: 63 %, white solid.

^{1}H NMR (CD_3OD, 400 MHz, ppm) δ 5.41-5.36 (m, 2H), 3.92-3.74 (m, 3H), 3.68-3.50 (m, 3H), 3.39 (d, 1H), 3.29-3.17 (m, 2H), 2.07-2.01 (m, 3H), 1.63 (s, 2H), 1.34 (d, 22H), 0.93 (t, J =6.1, 3H).

DEPT135 ^{13}C NMR (100 MHz, CD_3OD) δ 171.7, 171.2, 170.8, 170.6, 152.1, 152.0, 131.7, 131.0, 82.9, 82.6, 82.4, 79.5, 78.3, 72.6, 72.5, 71.3, 71.0, 63.0, 62.7, 43.1, 42.8, 33.8, 33.2, 31.0, 30.9, 30.8 30.7, 30.6, 30.5, 30.3, 29.0, 28.3, 28.1, 28.0, 23.9, 14.6.

HRMS(ESI): calcd for $[M-H]^-$, 555.32870; found, 555.32739.

FT-IR (cm^{-1}): 3343, 2921, 2852, 1710, 1697, 1668, 1442, 1406, 1393, 1363, 1090, 1022, 771.

MalB(OH)-1

Yield: 72 %, white solid.

^{1}H NMR (CD_3OD, 400 MHz, ppm) δ 5.16 (q, 1H), 3.91-3.56 (m, 11H), 3.53-3.43 (m, 2H), 3.30-3.25 (m, 1H), 1.63 (s, 2H), 1.33 (d, 22H), 0.92 (t, J =6.9, 3H).

DEPT135 ^{13}C NMR (100 MHz, CD_3OD) δ 171.6, 171.2, 170.7, 170.5, 152.0, 103.0, 102.9, 82.9, 81.2, 81.0, 80.6, 80.3, 79.3, 78.3, 75.2, 74.9, 74.3, 72.1, 72.0, 71.6, 62.9, 62.4, 62.1, 43.2, 42.9, 33.2, 31.0, 30.9, 30.8, 30.6, 30.5, 29.0, 28.9, 28.1, 28.0, 23.9, 14.6.

HRMS(ESI): calcd for $[M-H]^-$, 663.33458; found, 663.33307.

FT-IR (cm^{-1}): 3340, 2921, 2852, 1720, 1688, 1441, 1408, 1367, 1022, 757.

MalB(OH)-2

Yield: 54 %, white solid.

^{1}H NMR (CD_3OD, 400 MHz, ppm) δ 5.16 (q, 1H), 3.91-3.56 (m, 11H), 3.53-3.43 (m, 2H), 3.30-3.25 (m, 1H), 1.62 (s, 2H), 1.33 (d, 26H), 0.92 (t, J =6.8, 3H).

DEPT135 ^{13}C NMR (100 MHz, CD_3OD) δ 171.6, 171.2, 170.7, 170.6, 152.1, 152.0, 103.0, 102.9, 82.9, 81.2, 81.0, 80.6, 80.2, 79.3 79.2, 78.3, 75.2, 74.9, 74.3, 72.1, 72.0, 71.6, 62.9, 62.8, 62.3, 62.1, 43.2, 42.9, 33.2, 30.9, 30.8, 30.6, 30.5, 29.0, 28.9, 28.1, 28.0, 23.9, 14.6.

HRMS (ESI): calcd for $[M-H]^-$, 691.36588; found, 691.36431.

FT-IR (cm^{-1}): 3326, 2921, 2853, 1720, 1688, 1441, 1408, 1367, 1022, 757.

MalB(OH)-3

Yield: 67 %, white solid.

^{1}H NMR (CD_3OD, 400 MHz, ppm) δ 5.17 (q, 1H), 3.92-3.56 (m, 11H), 3.53-3.43 (m, 2H), 3.30-3.25 (m, 1H), 1.63 (s, 2H), 1.34 (d, 30H), 0.93 (t, J =6.9, 3H).

DEPT135 ^{13}C NMR (100 MHz, CD_3OD) δ 171.6, 171.2, 170.7, 170.5, 152.0, 151.9, 103.0, 102.9, 82.9, 81.2, 81.0, 80.6, 80.3, 79.3, 79.2, 78.3, 75.2, 74.9, 74.3, 72.1, 72.0, 71.6, 62.9, 62.4, 62.1, 43.2, 42.9, 33.2, 30.9, 30.8, 30.6, 30.5, 29.0, 28.9, 28.1, 28.0, 23.9, 14.6.

HRMS (ESI): calcd for $[M-H]^-$, 719.39718; found, 719.39544.

FT-IR (cm^{-1}): 3325, 2917, 2849, 1720, 1686, 1441, 1407,1367, 1025, 760.

MalB(OH)-4

Yield: 63 %, white solid.

^{1}H NMR (CD_3OD, 400 MHz, ppm) δ 5.42-5.36 (m, 1H), 5.17 (q, 1H), 3.85-3.56 (m, 11H), 3.54-3.44 (m, 2H), 3.31-3.26 (m, 1H), 2.07-2.01 (m, 3H), 1.63 (s, 2H), 1.34 (d, 22H), 0.93 (t, J =6.6, 3H).

DEPT135 ^{13}C NMR (100 MHz, CD_3OD) δ 171.6, 171.2, 170.7, 170.5, 152.0, 151.9, 131.7, 131.0, 103.0, 102.9, 82.9, 81.2, 81.0, 80.6, 80.3, 79.3, 78.3, 75.2, 74.9, 74.3, 72.1, 72.0, 71.6, 62.9, 62.4, 62.1, 43.2, 42.9, 33.8, 33.2, 31.0, 30.9, 30.8, 30.7, 30.6, 30.5, 29.0, 28.9, 28.3, 28.1, 28.0, 23.9, 14.6.

HRMS (ESI): calcd for $[M-H]^-$, 717.38153; found, 717.37992.

FT-IR (cm^{-1}): 3330, 2922, 2852, 1721, 1687, 1441, 1407, 1367, 1021, 757.

2. NMR copies of the carbohydrates derivatives

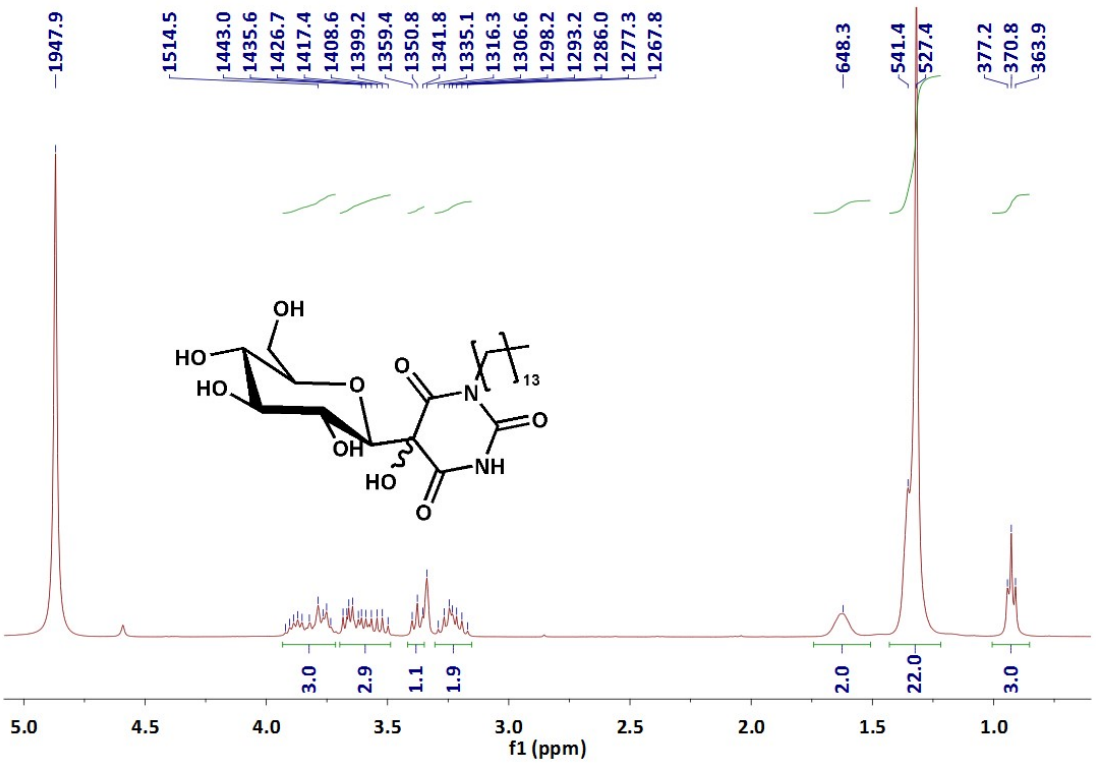


Fig. S1a ^{1}H NMR spectrum of **GlcB(OH)-1** in CD_3OD.

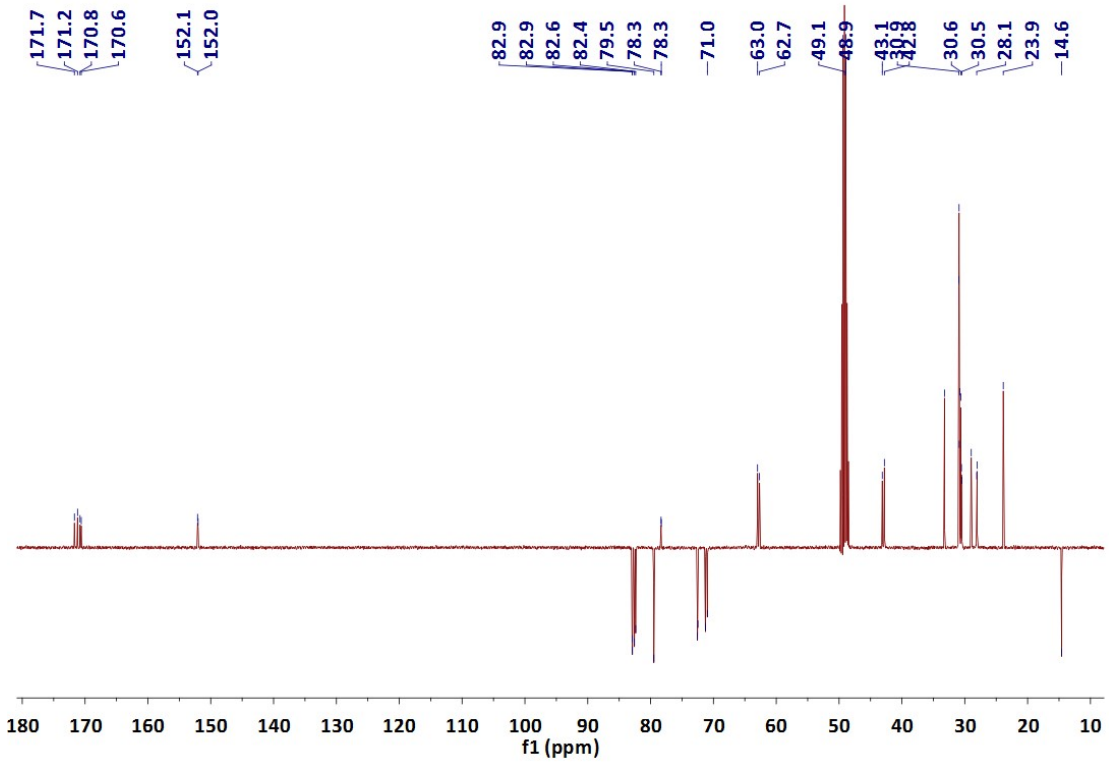


Fig. S1b DEPT-135 ^{13}C NMR spectrum of **GlcB(OH)-1** in CD_3OD.

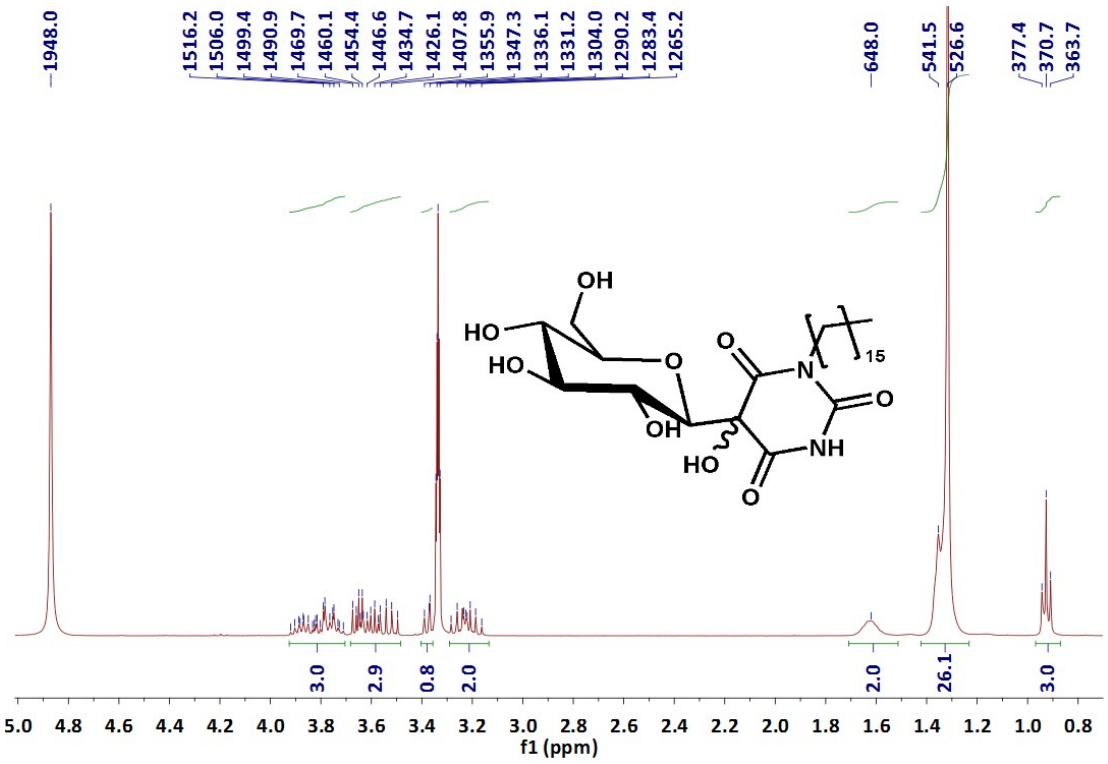


Fig. S2a ^{1}H NMR spectrum of **GlcB(OH)-2** in CD_3OD.

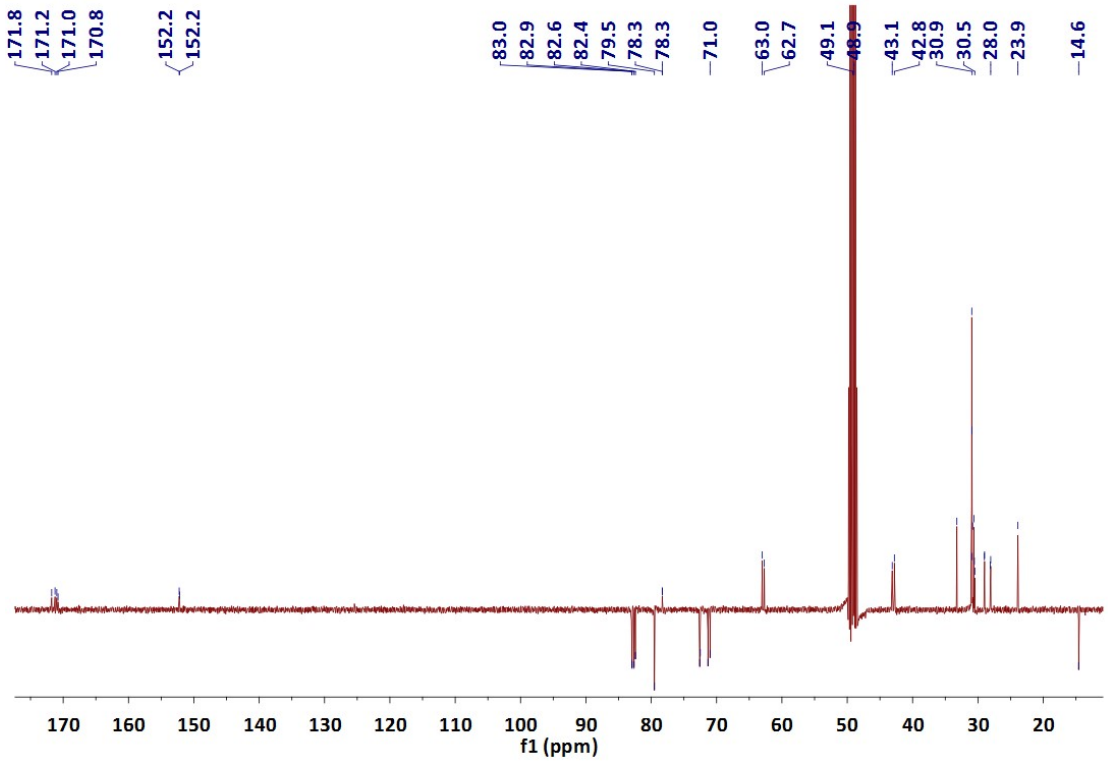


Fig. S2b DEPT135 ^{13}C NMR spectrum of **GlcB(OH)-2** in CD_3OD.

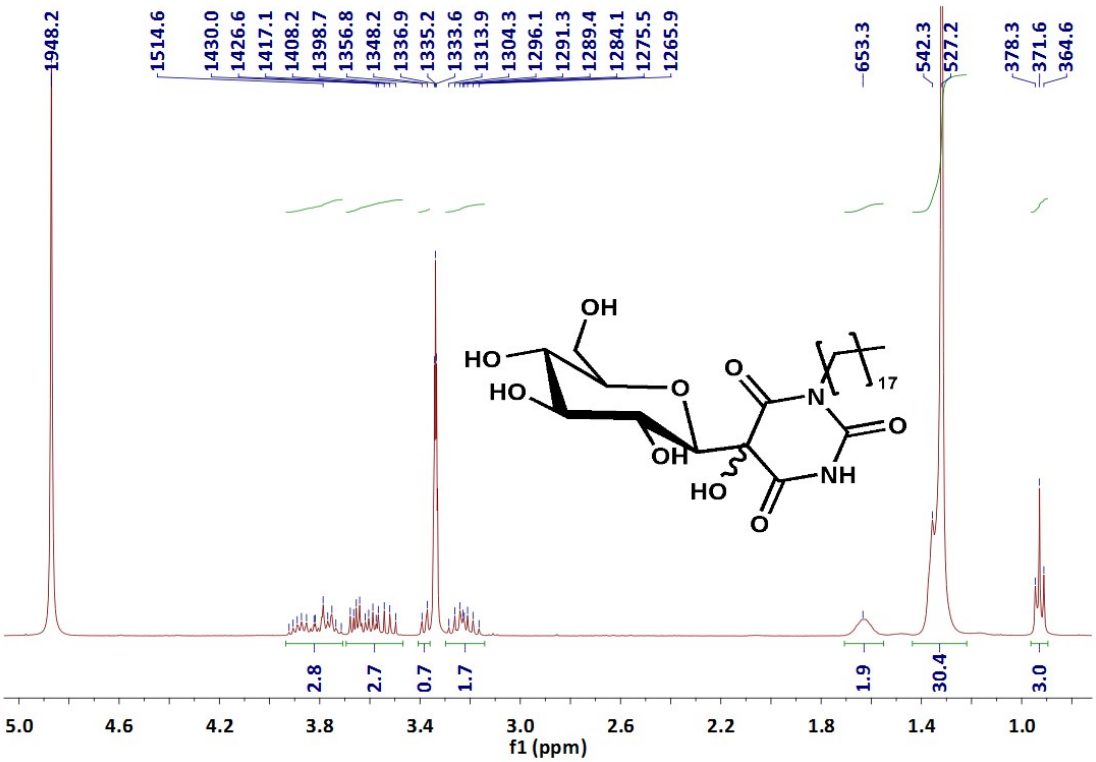


Fig. S3a ^{1}H NMR spectrum of **GlcB(OH)-3** in CD_3OD.

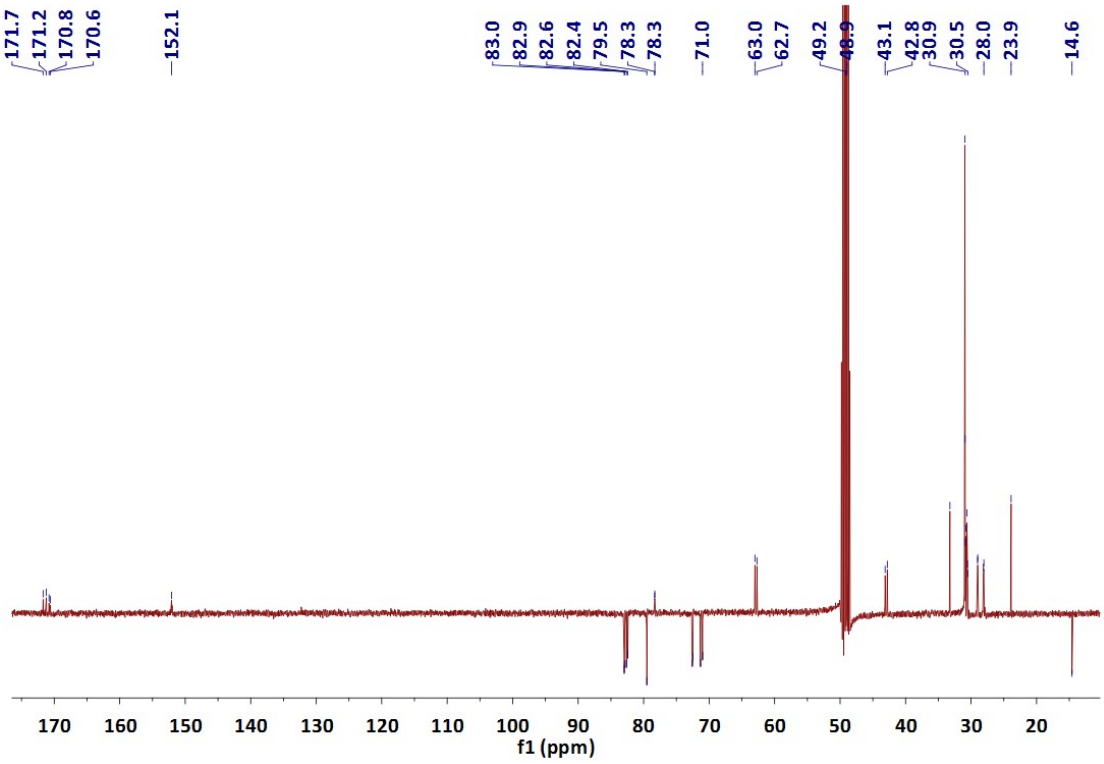


Fig. S3b DEPT135 ^{13}C NMR spectrum of **GlcB(OH)-3** in CD_3OD.

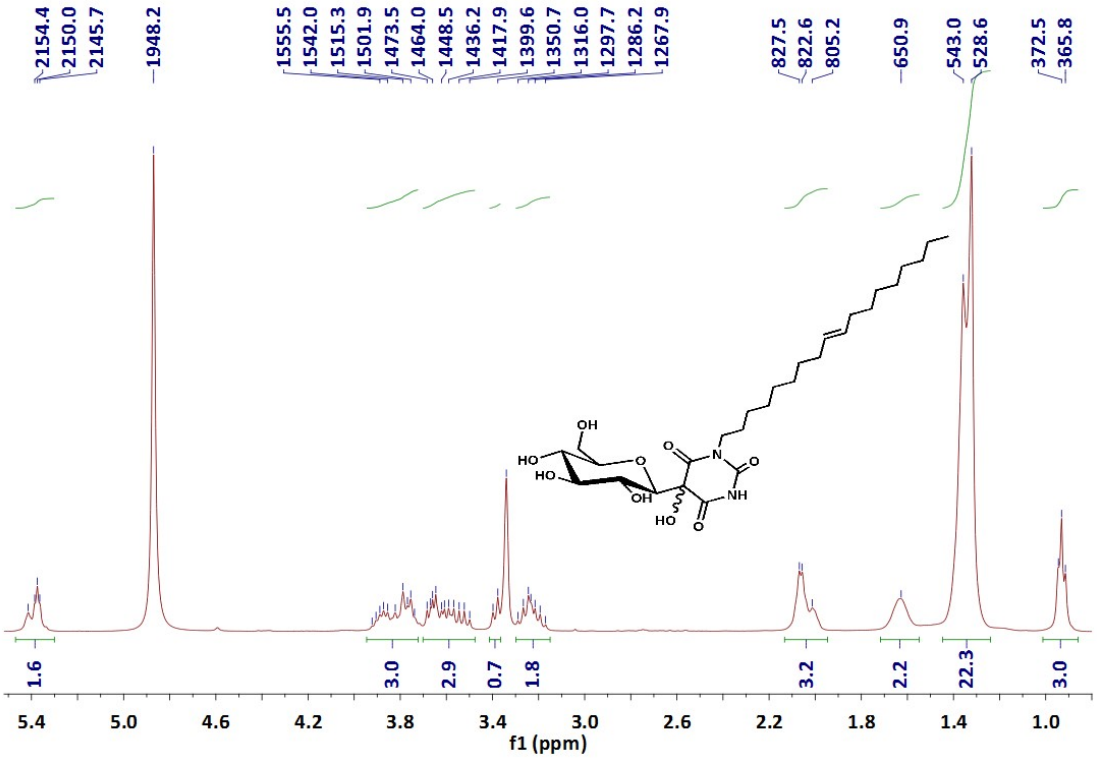


Fig. S4a ^{1}H NMR spectrum of **GlcB(OH)-4** in CD_3OD.

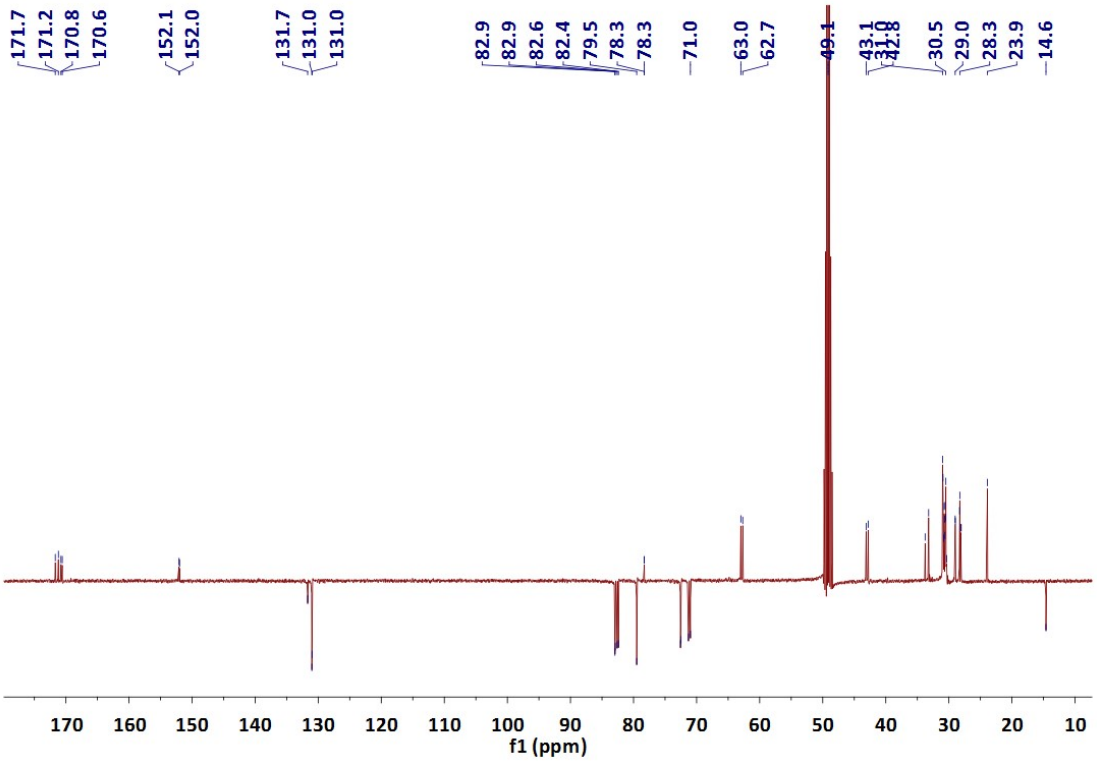


Fig. S4b DEPT135 ^{13}C NMR spectrum of **GlcB(OH)-4** in CD_3OD.

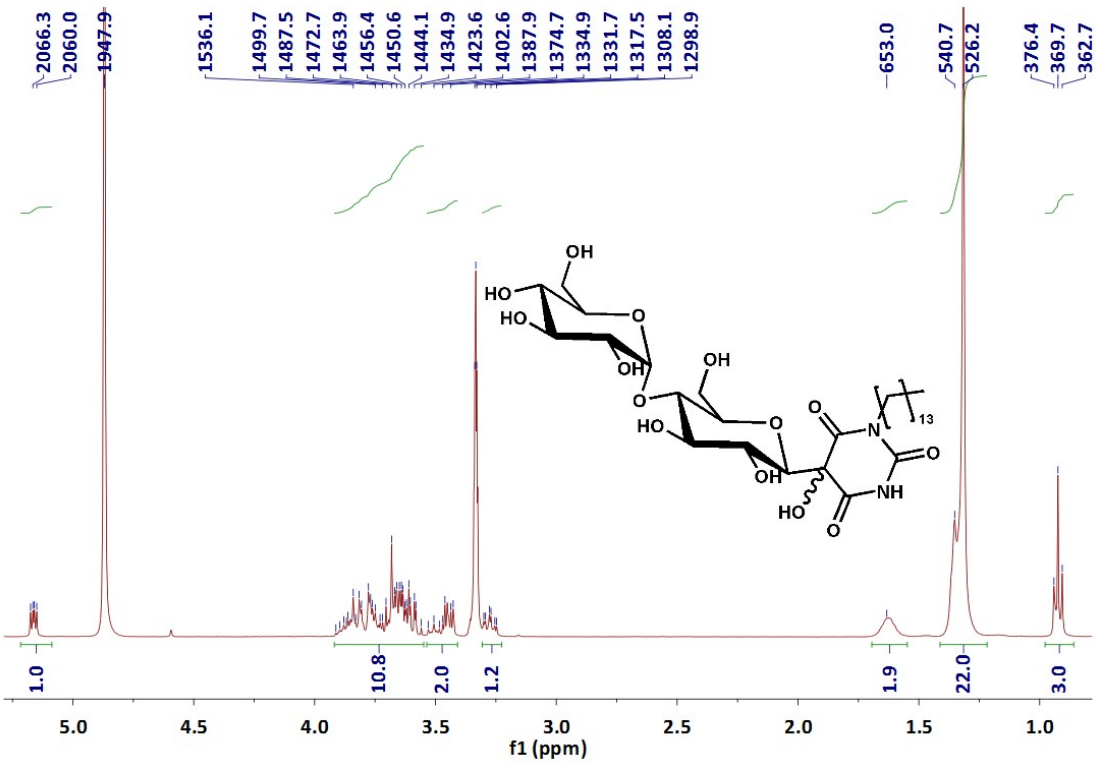


Fig. S5a ^{1}H NMR spectrum of **MalB(OH)-1** in CD_3OD.

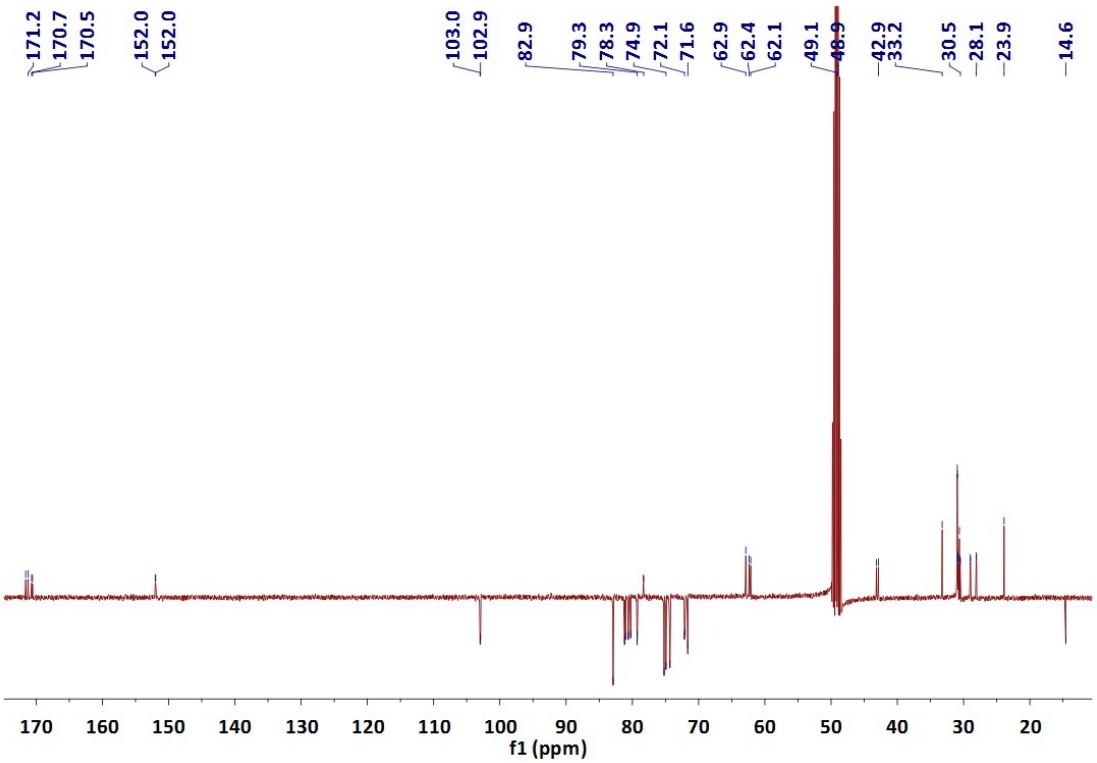


Fig. S5b DEPT135 ^{13}C NMR spectrum of **MalB(OH)-1** in CD_3OD.

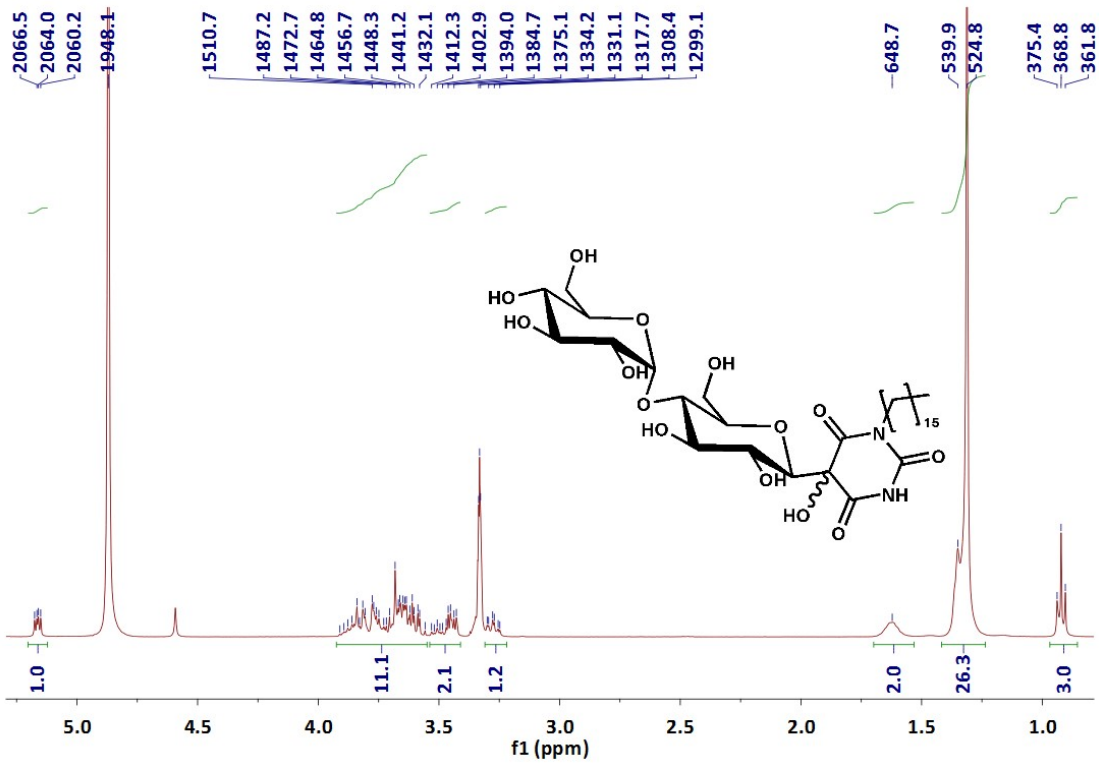


Fig. S6a ^{1}H NMR spectrum of **MalB(OH)-2** in CD_3OD.

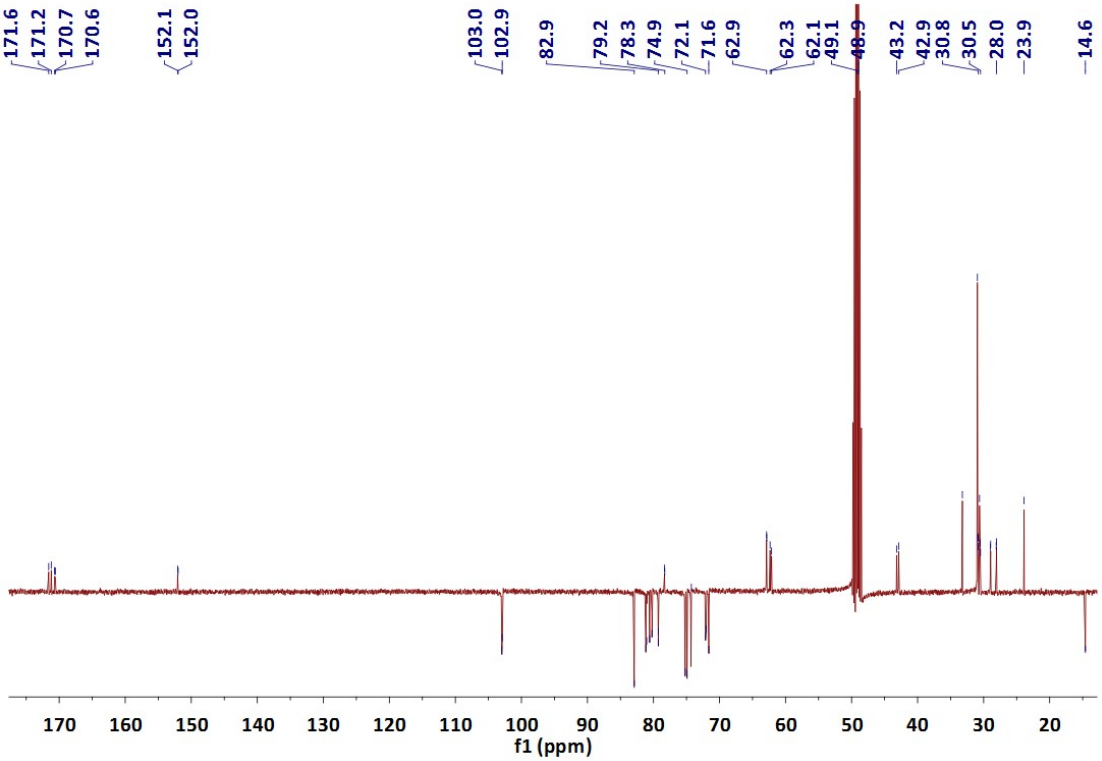


Fig. S6b DEPT135 ^{13}C NMR spectrum of **MalB(OH)-2** in CD_3OD.

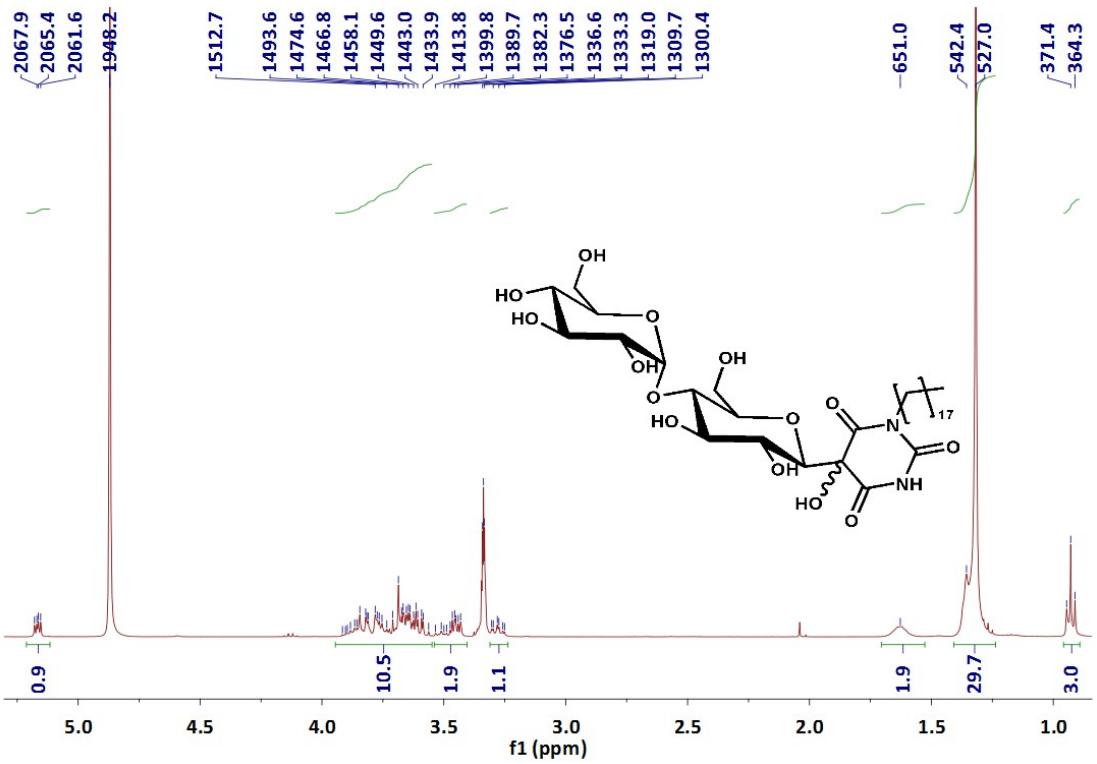


Fig. S7a ^{1}H NMR spectrum of **MalB(OH)-3** in CD_3OD.

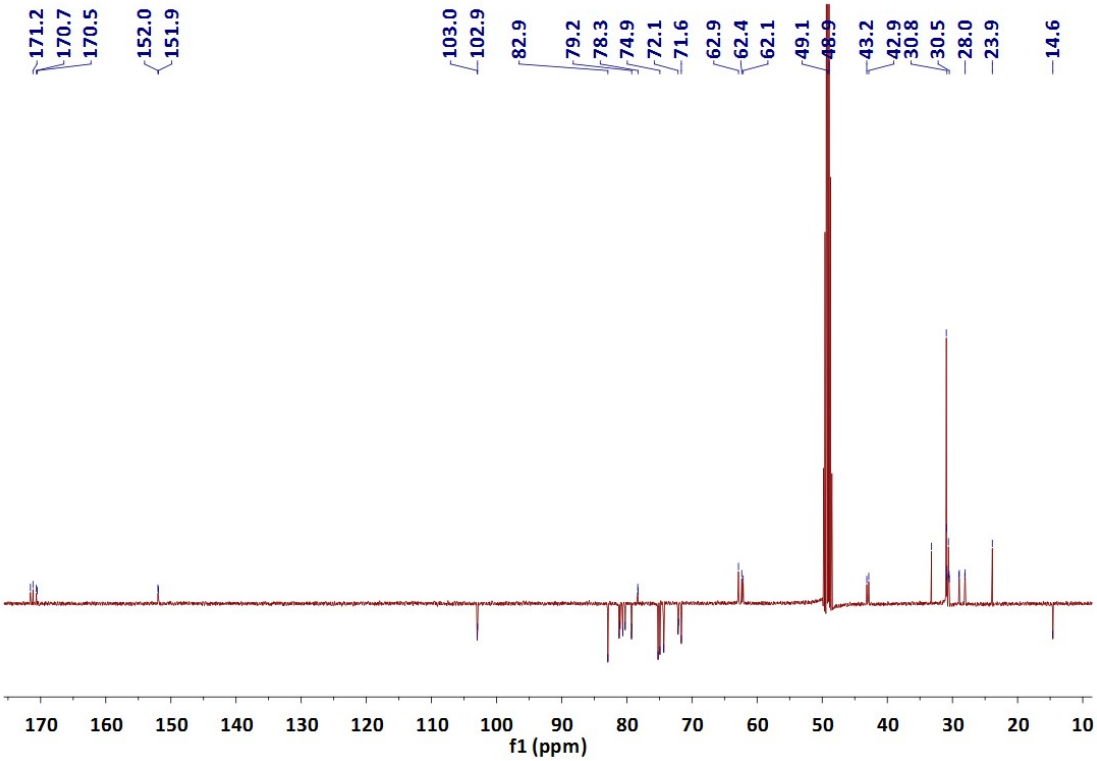


Fig. S7b DEPT135 ^{13}C NMR spectrum of **MalB(OH)-3** in CD_3OD.

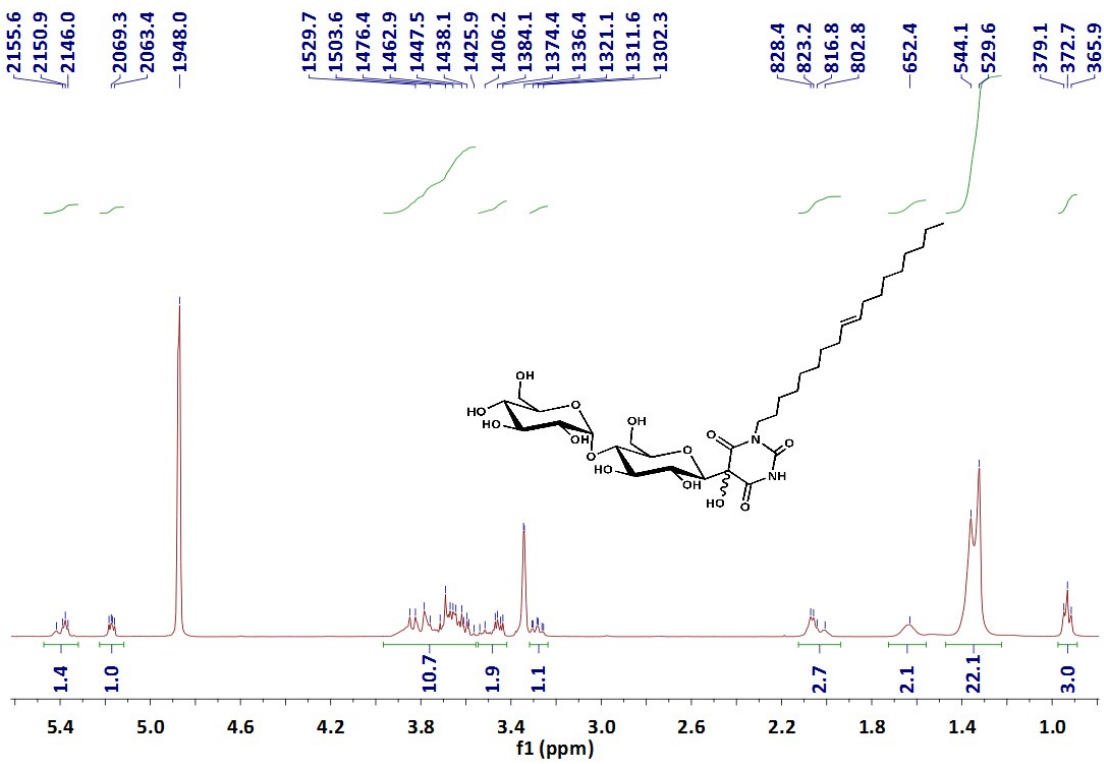


Fig. S8a ^{1}H NMR spectrum of **MalB(OH)-4** in CD_3OD.

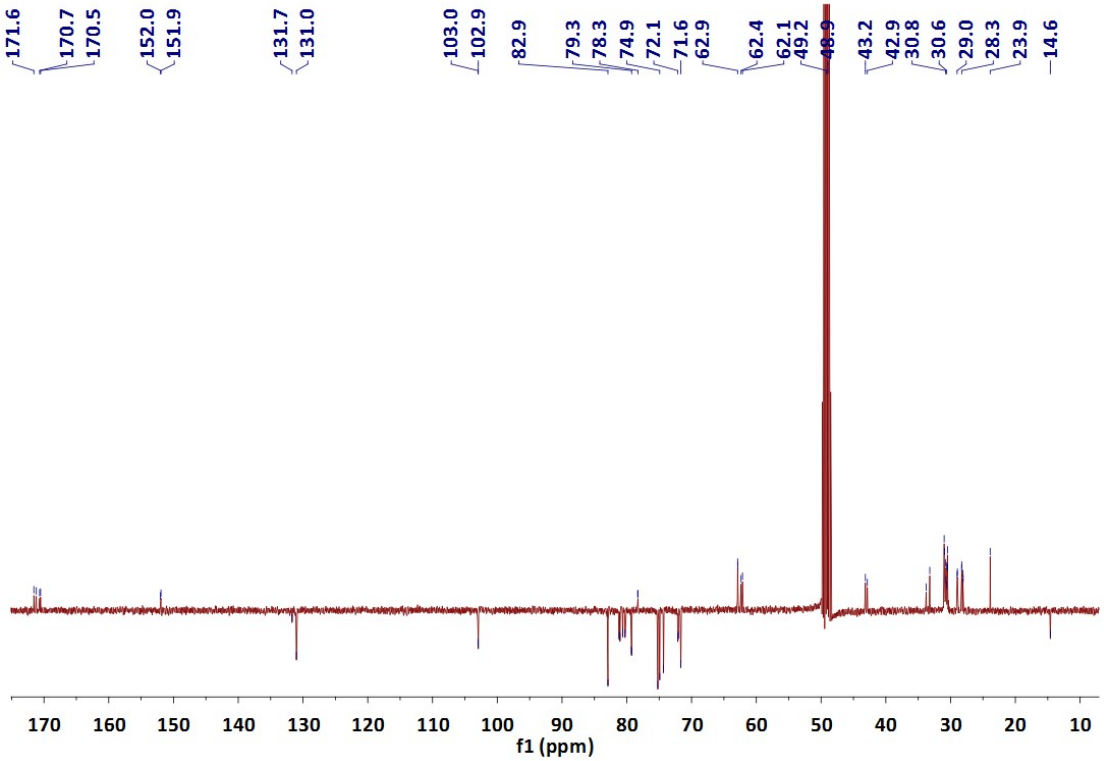
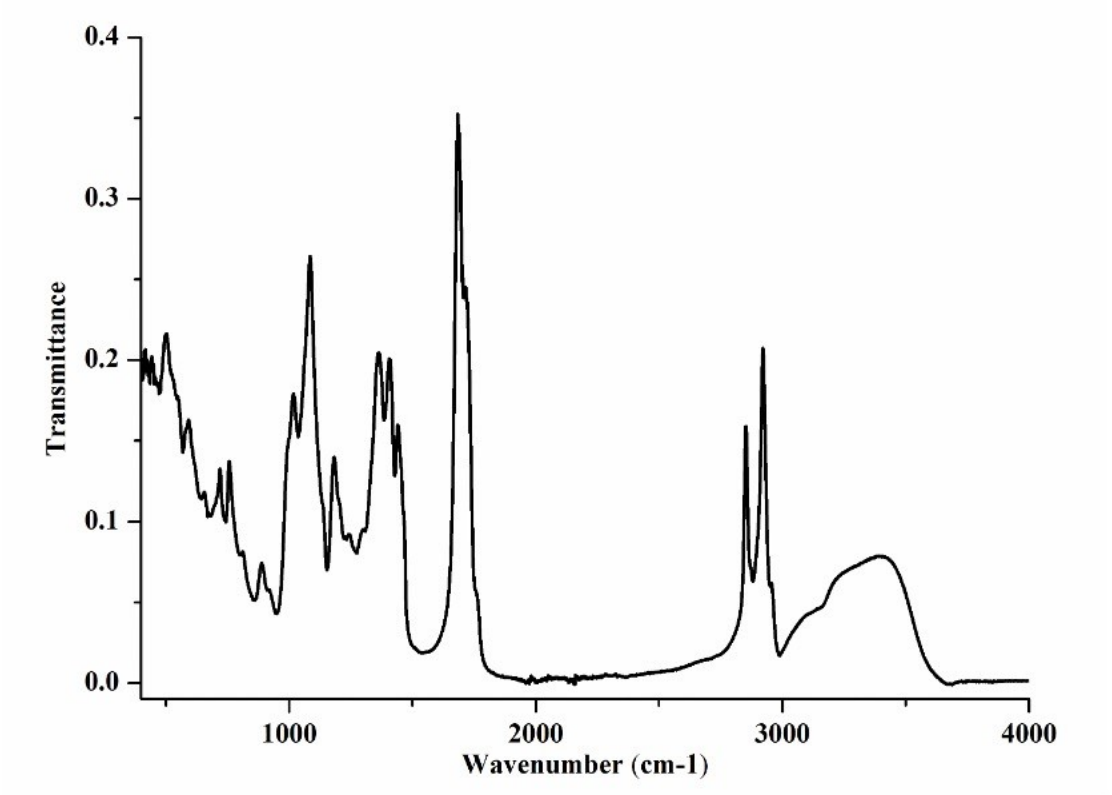


Fig. S8b DEPT135 ^{13}C NMR spectrum of **MalB(OH)-4** in CD_3OD.

3. Representative IR spectrum with GlcB(OH)-1



4. Stacked ^{1}H NMR of GlcB-2 and GlcB(OH)-2

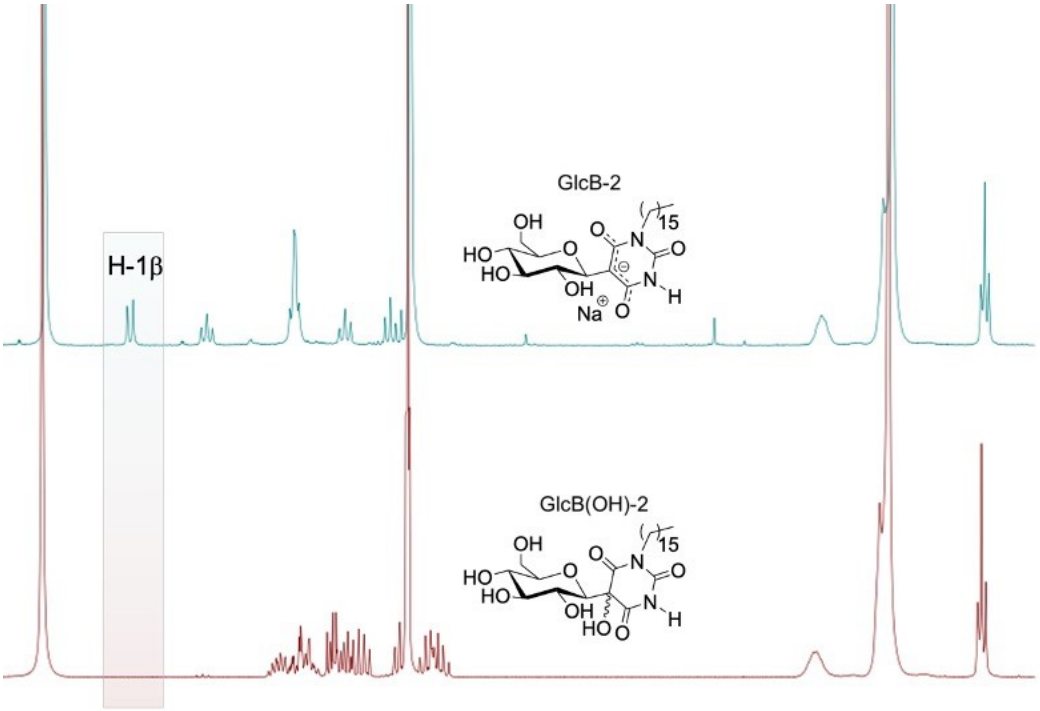


Fig. S9 Stacked ^{1}H NMR of **GlcB-2** and **GlcB(OH)-2**

4. Preparation of the Gel

In a septum-capped vial, a weighed amount of glycoamphiphiles (10 mg for GlcB(OH)-2, 15 mg for GlcB(OH)-3 and GlcB(OH)-4, 20 mg for MalB(OH)-3 and 25 mg for MalB(OH)-4) was suspended in 500 μL of deionized water. The suspension was sonicated to help dissolution, then heated in an oil bath (80 °C) to form a clear solution. Next, the solution was allowed to cool-down to room temperature. Hydrogelation was confirmed whether the sample doesn't flow by inverting the vial.

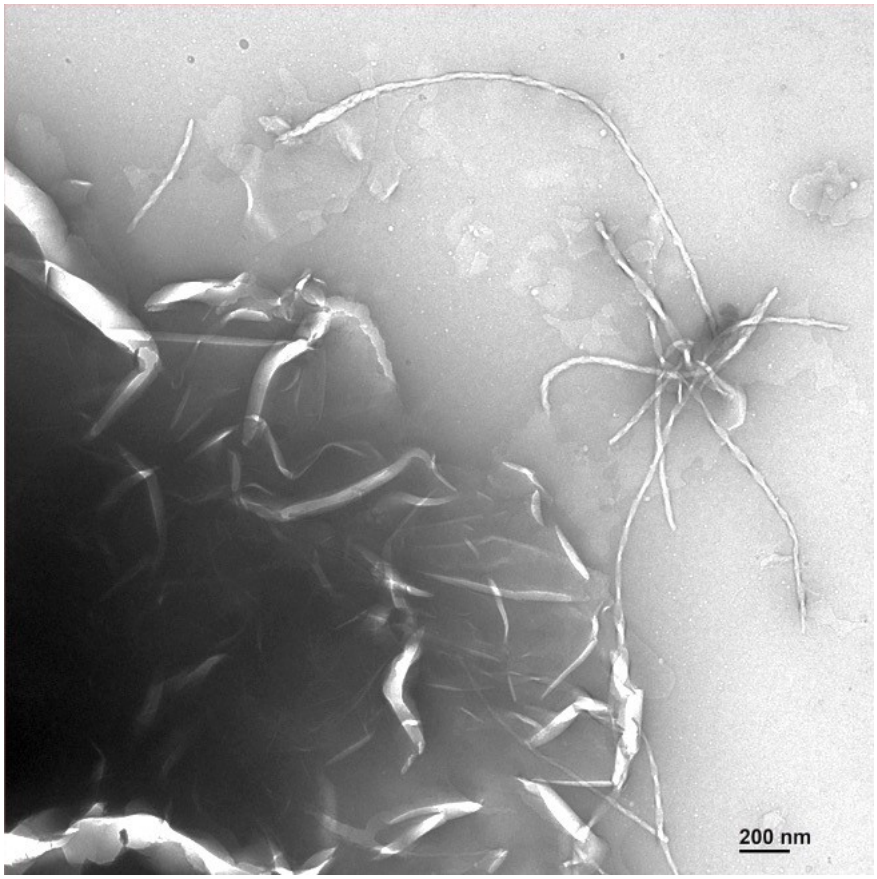
5. Differential Scanning Calorimetry (DSC)

Multicell differential scanning calorimetric instrument from the TA Instruments DSC Q200, equipped with a RCS 90 cooling unit, was used to examine the gel-to-sol transition temperature (T_{gel}) of the hydrogel at the minimal gelation weight concentration. The gel was placed in the sample cell and water solvent was added in the reference cell. Pans were closed with their lids and the heat change was recorded in 1 s intervals. DSC scans started with an isotherm at 10 °C for 5 min, followed by a 10 °C.min^{-1} ramp up to 95 °C under a nitrogen atmosphere.

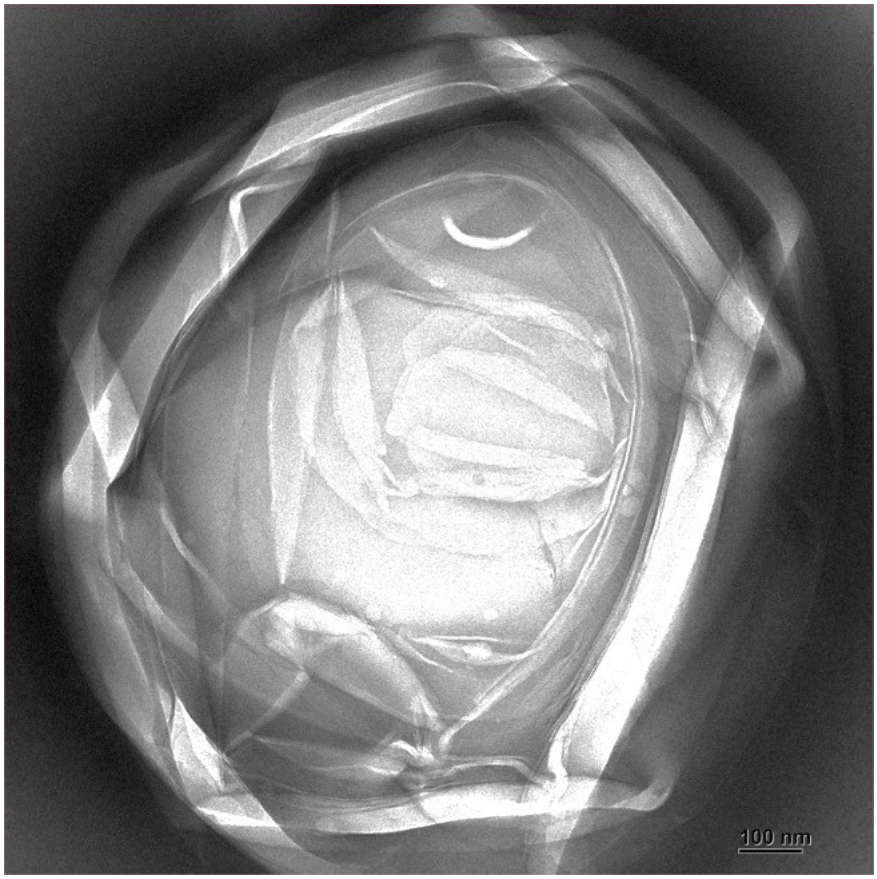
6. Preparation of TEM samples and images

Since the gels could not be directly observed by TEM, small fragments were taken, redispersed in water, and homogenized by vortexing or mild sonication to obtain non-viscous solutions or smaller gel microfragments. Droplets of the suspensions were deposited onto freshly glow-discharged carbon-coated copper grids. The liquid in excess was blotted with filter paper and the preparations were negatively stained with 2 wt% uranyl acetate. The specimens were observed with a JEOL JEM-2100 Plus microscope operating at 200 kV and images were recorded with a Gatan Rio 16 digital camera.

GlcB(OH)-2



GlcB(OH)-3



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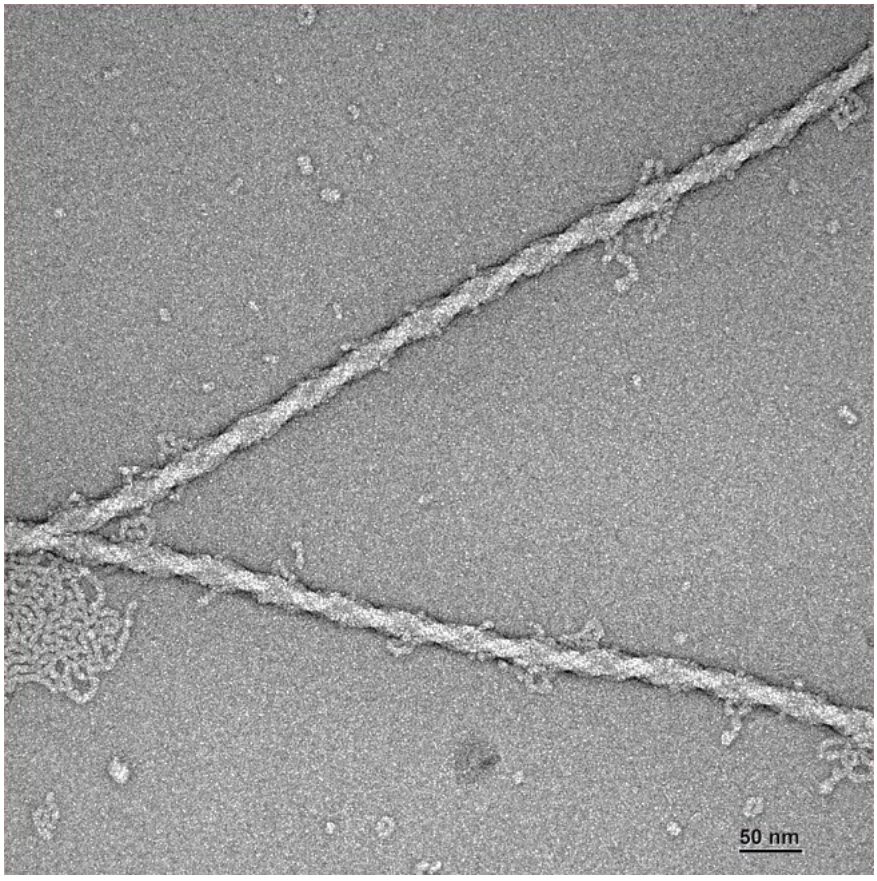

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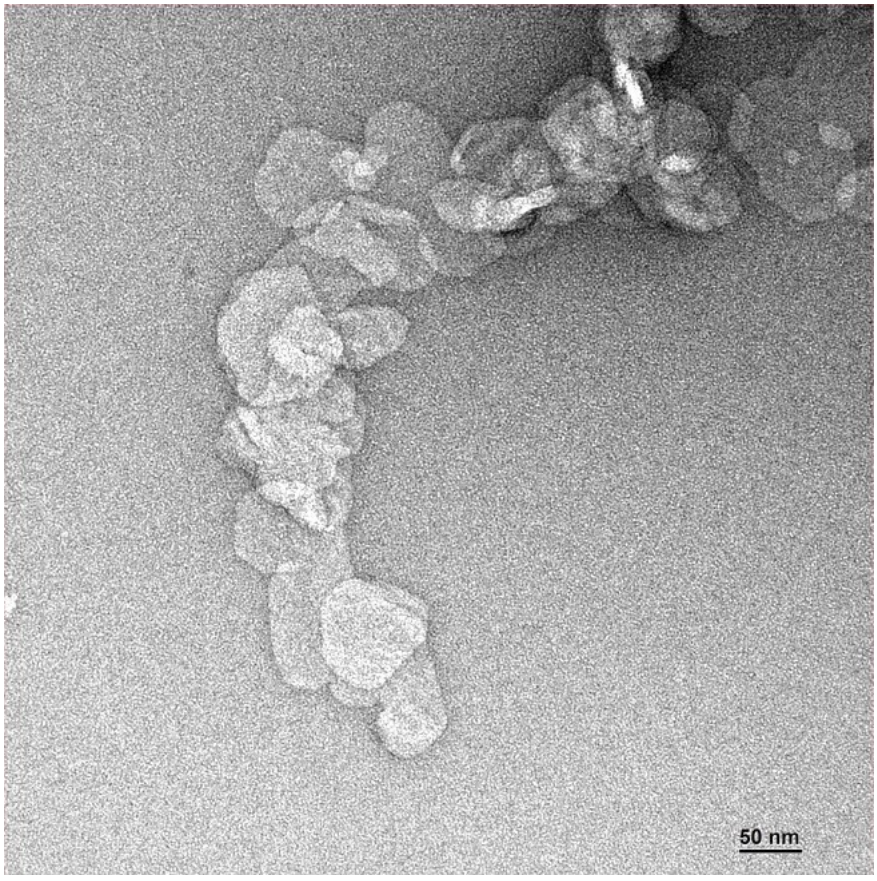

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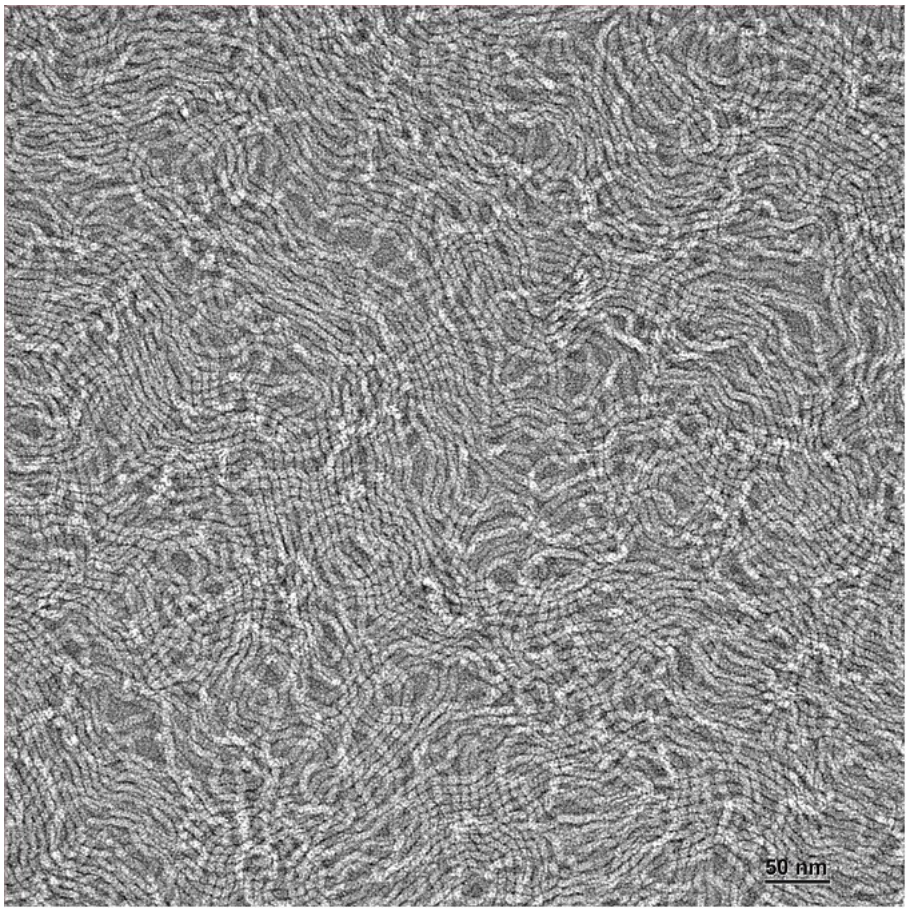

7. Enzyme-triggered hydrogel formation

MalB(OH)-2 solutions were prepared at a final concentration of 30 or 6 mg.mL^{-1} in citrate buffer (pH 4.8, 50 mM). 70 U.mL^{-1} of amyloglucosidase from *Aspergillus niger* was then added to the solution and incubated at 45°C to achieve non-reducing end glucose hydrolysis affording **GlcB(OH)-2** and to promote hydrogelation. For both experiments, an opaque gel was formed after overnight.

8. References

1- S. Yao, R. Brahmi, F. Portier, J.-L. Putaux, J. Chen and S. Halila, *Chem. Eur. J.*, 2021, **27**, 16716–16721.