Supporting Information

Multifunctional iron oxide magnetic nanoparticles: new insight about synthesis and application for Magnetic Resonance Imaging

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Materials and Methods

All commercially available reagents and solvents were purchased from Sigma-Aldrich (Milan, Italy) and used without further purification. β-CD was provided by Wacker Chemie (München, Germany). Reactions were carried out in a conventional oil bath and in a combined microwave/ultrasound (MW/US) system (Figure S1). This device was designed in our laboratory and was made by inserting a Pyrex sonic horn into a Microsynth (Milestone) microwave chamber. US irradiation at 20.2 kHz was performed using a Pyrex immersion horn made by Danacamerini s.a.s. (Italy). Thermogravimetric analyses were performed using a thermogravimetric analyzer TGA 4000 (PerkinElmer) at 10 °C min^{-1} operating with alumina crucibles that contained 10−20 mg of sample. The analyses were performed under an argon atmosphere at a starting temperature of 50 °C and an end temperature of 800 °C. Total mass loss was attributed to the functional groups that were covalently attached to the sidewalls. UV−vis absorption spectra were measured on a dual-beam spectrophotometer (Agilent Technologies Cary 60, G6860AA) equipped with a 1 cm path-length quartz cuvette. FT-IR analyses were recorded using a Shimadzu FT-IR 8001 spectrophotometer on pellets of the samples dispersed in KBr. An MCT detector was used at a resolution of 4 cm-2 with 32 scans being performed. NMR spectra were performed on a Bruker Avance (600 MHz and 75 or 125 MHz for 1H and 13C respectively). Chemical shifts were calibrated to the residual proton and carbon resonances of the solvent: DMSO-d6 (δH = 2.54, δC = 39.5), D2O (δH = 4.79), acetone (δH = 2.09, δC = 205.87). Chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. The hydrodynamic particle diameter, polydispersity index (PDI) and zeta potential of the colloidal iron oxide suspensions were measured via by Dynamic Light Scattering (DLS) (Malvern Instrument, Zetasizer nanoseries). Measurements were performed in triplicate. MNP size and morphology were analyzed using transmission electron microscopy (TEM JEOL JEM-3010, 300 kV, 0.17 nm of resolution, with Gatan camera US1000 CCD and OXFORD detector). Magnetization curves were recorded using a Lake Shore 7404 vibrating sample magnetometer at room temperature (25°C). The magnetic field was cycled between −20,000 and 20,000 Oe. Synthesis and characterization of products 1-3, βCD Citr-NH2 and carboxymethyl dextran

Synthesis of derivatives 1-3, *βCD* Citr-NH_2, carboxymethyl dextran

Synthesis of citric acid anhydride (3)

Citric acid (2.61 mmol, 0.50 mg) was added to a mixture of glacial acetic acid (0.232 mL) and acetic anhydride (0.486 mL). The solution was stirred at 38 °C for 24 h and finally dried under vacuum to obtain the product; transparent viscous oil (MW: 174.11 g/mol. 0.354 g. 78 % yield). (iPrOH/H_2O/EtOAc/NH_4OH 5:3:1:1). ^{1}H NMR (600 MHz, Acetone) δ 3.48 (d, J= 19.2 Hz 1H, H-a), 3.23 (d, J= 18 Hz 1H, H-c), 3.17 (d, J= 18 Hz 1H, H-d), 3.10 (d, J= 19.2 Hz 1H, H-b) ppm. By NMR we could observe that the product contain 15% of 6 term citric anhydride partially acylated and acetic acid

Synthesis of 6^I-O-*p*-toluenesulfonyl-βCD (1)

The reaction was performed on the basis of our previous experience [1, 2]

β-CD (1.14 mmol, 1.3 g) was dissolved in 90 mL of deionized water. The solution was sonicated and 1-(p-toluenesulfonyl)imidazole (1.01, g, 4.58 mmol) was added and the solution was sonicated for 10 min. A water solution of NaOH (2 mL, 560 mg, 14 mmol) was added dropwise and a precipitate was formed. The suspension was filtered and stirred for 30 min. NH_4Cl (1.67 g) was added and a precipitate was obtained. It was recovered by filtration under vacuum, washed with cold water and acetone and dried; white powder (1289.17 g/mol. 0.828 g. 55 % yield). Rf 0.44 (iPrOH/H_2O/EtOAc/NH_4OH 5:1:3:1). ^{1}H NMR (600 MHz, DMSO-d6): δ 7.71 (d, J = 8.3 Hz, 2H), 7.39 (d, J = 8.2 Hz, 2H), 5.6-5.8 (m, 14H), 4.79 (d, J = 7.5 Hz, 5H), 4.73 (d, J = 3.2 Hz, 2H), 4.51 – 4.39 (m, 5H), 4.37 – 4.25 (m, 1H), 3.69 – 3.38 (m, 28H), 2.39 (s, 3H).

Synthesis of 6^I-diethylentriamine-6^I-monodeoxy-βCD (2)

6^I-O-p-toluenesulfonyl-βCD (0.31 mmol, 0.40 mg) was added to 0.80 mL of diethylenetriamine. After complete solubilization, the mixture was stirred at 100 °C for 7 h. The product was precipitated via the addition of 2-propanol. The precipitate was filtered under vacuum, washed several times with 2-propanol and dried; white powder[3]. The product dissolved in the minimum amount of water and precipitated with 2-propanol, the solid was collected and dried, therefore the solid was precipitated with acetone to remove the 2-propanol included in the cavity. As described before the solid is dissolved in the minimum amount of water and precipitated with acetone. (MW: 1220.14 g/mol. 0.366 g. 96 % yield).TLC (iPrOH/H_2O/EtOAc/NH_4OH 5:3:1:1).

m. p. 220°C with degradation ^{1} H NMR (D_2O, 600 MHz): δ 4,039 (s, 7H, H-1), 3.88 – 3.79 (m, 26H, 7×H-3, 7×H-5, 12×H-6,), 3.61 – 3.55 (m, 13H, 7×H-2, 6×H-4), 3.41 (m, 1H, 1×H-4A), 3.03 (m, 1H, 1×H-6A), 2.78-2.76 (m, 3H, 2×H-2', 1×H-6B), 2.69-2.67 (m, 6H, 2×H-2', 2×H-4', 2×H-3') ^{13}C-NMR (150 MHz,D_2O, 25 °C): δ = 103,76 (C-1), 103,51 (C-1a), 85.49 (C-4A), 83.00, 82.84 (C-4), 75.044, 74.967, 74.905(C-3), 73.97, 73.77 (C-2. C-5), 72.25 (C-5A), 62.14 (C-6), 51.67, 51.45 (C-6AB, C-3'), 49,73 (C-2'),49.39 (C-1') 41,40 (C-4'). ppm. FT- IR 3286, 2921, 2840, 1589, 1481, 1312, 1021 $cm^{-1;}$ ESI-MS^+: m/z calc for $C_{46}H_{82}N_3O_{34}$ 1220,48; $[M+H]^+$ found 1221,00

Synthesis of *βCD Citr-NH_2*

Citric acid anhydride (60 mg) was dissolved in 1 mL of dry dimethylformamide (DMF). 200 mg of 6-diethylentriamine- βCD and 3 mL of pyridine were added and the solution was heated at 40 °C for 24 h. The product was precipitated via the addition of acetone. The precipitate was filtered under vacuum, washed several times with acetone and dried. The product was therefore dissolved in the minimum amount of water and precipitated with acetone, the solid was collected and dried to get a white powder (MW: 1394.26 g/mol. 0.082 g. 71 % yield).

(H_2O/1,4-dioxane/NH_4OH 1:1:0.1). m. p.= 280°C with degradation; ^{1}H NMR (D_2O, 600 MHz): δ 4,93 (s, 7H, H-1), 3.82 – 2.69 (m, 54H, 7×H-3, 7×H-5, 14×H-6, 7×H-2, 7×H-4, H-2′, H-3′, H-1′, H-4′, H-5′, H-6′) ^{13}C-NMR (150 MHz, D_2O, 25 °C) 175,09 (COO), 101.00, 101.97, 101.90 (C-1), 81.17 (C-4), 73.24, 73.13, 72,87, 72.05, 71.88, 71.73 (C-2, C-3, C-5, C-6' C-5') , 60.26 (C-6), 45,82, 45.008 (C-2', C-3'), 38.247(C-1', C-4').ppm. HR-MS m/z calc for $[M+H]^+$ $C_{52}H_{828}N_3O_{40}$ 1394,49386 found 1394,4986, 1376.4869 $[M-H_2O+H]^+$, 1332.4964 $[M-CO_2-H_2O+H]^+$, 1314.4864 $[M-CO_2-2H_2O+H]^+$ ^{13}C NMR was partially assigned on the basis of HMQC reported in the manuscript Fig1

Synthesis of carboxymethyl dextran

Dextran (6000 g/mol, 0.50 g, 3.09 mmol) was dissolved in 10.62 mL of tert-butanol. Subsequently, 1.8 mL of sodium hydroxide was added dropwise to the dextran solution at a concentration of 3.8 M. The solution was then stirred at room temperature for 1 h. Monobromoacetic acid (7.71 mmol) was added. The reaction mixture was stirred at 60 °C for 90 min. Subsequently, the mixture was cooled to room temperature and then neutralized with glacial acetic acid. Finally, the product was precipitated and washed twice with methanol, filtered under vacuum and dried. This process was repeated three times. After the carboxymethylation reaction, the modified dextrans were in the form of carboxylate sodium salts (–CH_2COONa). An acid wash was used to obtain the free acid forms (–CH_2COOH). For this, 1 g of CMDx was washed 10 min with 14 mL of a solution of methanol and chloridric acid 37 %v/v (20:1). The acid liquor was removed by vacuum filtration and the solid product was subsequently washed several times with ethanol to remove all traces of the acid reagent and dried under vacuum. Afterwards, the number of carboxylic (–COOH) groups per CMDx chain was determined by acidimetric titration. For the acidimetric titration, we prepared a solution of CMDx at 1 % w/v in a mixture of distilled water/acetone (1:1). NaOH 0.012 N was added dropwise and the solution was then titrated with hydrochloric acid 0.012 N, using phenolphthalein as the indicator. From the titration data, the equivalents of –COOH groups per gram of CMDx were calculated and a 30 % degree of substitution was obtained; white powder (MW: 179.84 g/mol, 75 % yield). FT-IR (KBr, cm^{-1}): 3318, 2938, 1737, 1635, 1384, 1231, 1157, 1020, 917.

Synthesis of MNPs: physical assembly

$$Fe^{2+} + 2Fe^{3+} + 8OH^- \rightarrow Fe(OH)_2 + 2Fe(OH)_3 \rightarrow Fe_3O_4\downarrow + 4H_2O$$

Scheme S1. Reaction scheme for the preparation of Fe_3O_4 NPs by co-precipitation method.

Bare MNPs

$FeCl_3 \cdot 6\ H_2O$ (8.64 mmol, 2.34 g) and $FeCl_2 \cdot 4\ H_2O$ (4.32 mmol) were dissolved in 40 mL of deionized water. The solution was degassed with nitrogen and heated up to 80 °C for 1 h, followed by the addition of 5 mL of 30% ammonia aqua. The heating was continued for 30 min. The mixture was cooled at room temperature and the magnetic nanoparticles were collected by an external magnet, rinsed with deionized water and dried under vacuum. When the reaction was performed under MW/US irradiation the degassed solution was irradiated by combined MW and US irradiation at 80 °C for 30 min (Ultrasound 20.2 KHz, Power: 30 W, Microwave Program: Pmax = 1000 W, 1 min to reach 80 °C, then T = 80° C for 30 min).

MNPs@StA

$FeCl_3 \cdot 6\ H_2O$ (0.31 mmol, 83 mg), $FeCl_2 \cdot 4\ H_2O$ (0.15 mmol) and stearic acid (0.15 mmol) were added in 20 mL of deionized water. 0.30 mL of 30% ammonia aqua was added dropwise. The solution was stirred at room temperature for 24 h. The magnetic NPs were collected by an external magnet, rinsed with deionized water and dried under vacuum. When the reaction was performed under MW/US irradiation the degassed solution was irradiated by combined MW and US irradiation at 65 °C for 30 min (Ultrasound 20.2 KHz, Power: 30 W, Microwave Program: Pmax = 1000 W, 1 min to reach 80 °C, then T = 80° C for 30 min)

MNPs@CMDx

CMDx (100 mg) was dissolved in 20 mL of water. Bare MNPs (100 mg) were added. The suspension was stirred for 1 h at 80 °C. After cooling at room temperature, MNPs were collected using an external magnet, rinsed with water and dried under vacuum. When the reaction was performed under MW/US irradiation the degassed solution was irradiated by combined MW and US irradiation at 80 °C for 30 min (Ultrasound 20.2 KHz, Power: 30 W, Microwave Program: Pmax = 1000 W, 1 min to reach 80 °C, then T = 80° C for 30 min)

MNPs@CA

Bare MNPs (100 mg) were dispersed in 20 mL of a citric acid solution (2.4 M). The suspension was stirred for 1 h at 80 °C. After cooling at room temperature, MNPs were collected using an external magnet, rinsed with ethanol and dried under vacuum. When the reaction was performed under MW/US irradiation the degassed solution was irradiated by combined MW and US irradiation at 80°C for 30 min (Ultrasound 20.2 KHz, Power: 30 W, Microwave Program: Pmax = 1000 W, 1 min to reach 80 °C, then T = 80° C for 30 min)

MNP@ CA-βCD

Citric Acid (100 mg) and βCD (200 mg) were dissolved in 20 mL of water. Bare MNPs (100 mg) were added. The suspension was stirred for 3 h at 80 °C. After cooling at room temperature, MNPs were collected using an external magnet, rinsed with water and dried under vacuum. When the reaction was performed under MW/US irradiation

the degassed solution was irradiated by combined MW and US irradiation at 80 °C for 45 min (Ultrasound 20.2 KHz, Power: 30 W, Microwave Program: Pmax = 1000 W, 1 min to reach 80 °C, then T = 80° C for 30 min)

Synthesis of MNPs formulations prepared through ligand exchange

MNPs@CMDx

MNPs@StA prepared under MW/US irradiation (100 mg) were finely dispersed in 50 mL of toluene. Carboxymethyl dextran (100 mg) was dissolved in 80 mL of basic deionized water (1 mL of KOH 1 N). The solutions were combined, and the mixture was refluxed 24 h. The organic layer was removed, MNPs were collected from the aqueous phase by an external magnet, rinsed with deionized water and acetone and dried under vacuum.

MNPs@CA

MNPs@StA prepared under MW/US irradiation (100 mg) were finely dispersed in 50 mL of toluene. Citric acid (100 mg) was dissolved in 80 mL of basic deionized water (1 mL of KOH 1 N). The solutions were combined, and the mixture was refluxed 12 h. The organic layer was removed, MNPs were collected from the aqueous phase by an external magnet, rinsed with deionized water and acetone and dried under vacuum.

MNPs@CA-βCD

MNPs@StA prepared under MW/US irradiation (100 mg) were finely dispersed in 50 mL of petrol ether. Citric acid (100 mg) and βCD (200 mg) were dissolved in 50 mL of deionized water. The solutions were combined and 0,5 mL of NaOH 1 N were added. The mixture was vigorously stirred at 50 °C for 12 h. The organic layer was removed, MNPs were collected from the aqueous phase by an external magnet, rinsed with deionized water and acetone and dried under vacuum.

MNPs@CA-βCD-Citr-NH_2

MNPs@StA (100 mg) were finely dispersed in 50 mL of petrol ether. Citric acid (100 mg) and βCD citrate (βCD -Citr-NH_2) (244 mg) were dissolved in 50 mL of deionized water. The solutions were combined and 0,5 mL of NaOH 1 N were added. The mixture was vigorously stirred at 50 °C for 12 h. The organic layer was removed, MNPs were collected from the aqueous phase by an external magnet, rinsed with deionized water and acetone and dried under vacuum.

MNPs preparation protocols Figures and MW Power/Temperature profiles

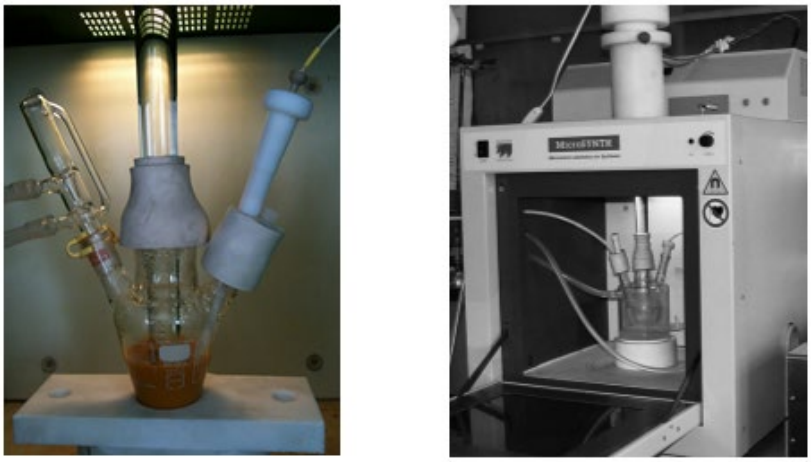

Figure S1. MW/US combined irradiation of iron salts solution under N_2 at 80°C, using a Microsynth Microwave Reactor and a pirex horn

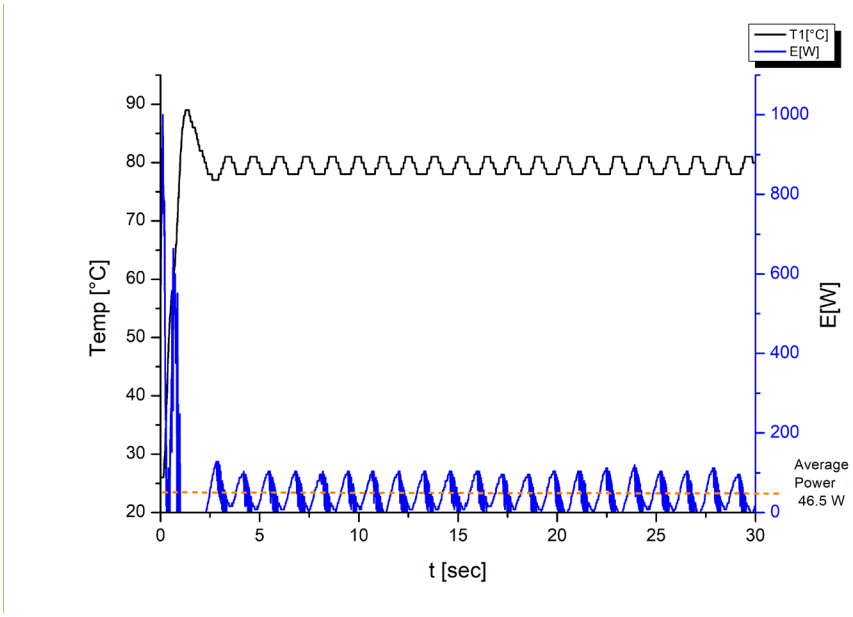

Figure S2. MW/US combined irradiation of iron salts solution under N_2 at 80°C, using a Microsynth Microwave Reactor and a pirex horn Temperature and power profile curves registered Program: Pmax = 1000 W, 1 min to reach 80 °C, then T = 80° C for 30 min

Figure S3. MNPs@StA dispersed in a water and toluene mixture.

MNP@CA- βCD preparation

Table S1 MNP@CA- βCD preparation: influence of MW/US on MNPs size, PDI, Z potential, coating w/w%

En	MNPs	Synthetic procedure	Reaction cond.	PDI[a]	Size [nm][b]	Coating [w/w %][c]	Zeta Potential [mV][d]
1	MNPs@CA-βCD	Physical assembly from Bare MNPs (entry 2, table 1)	oil bath (80 °C), 1 h	0.256	231 ± 12	22,8	-33.5 ± 0.1
2			MW/US (80 °C), 30 min	0.243	80 ± 0.5	25,9	-34 ± 0.6
3		Ligand exchange from MNPs@StA prepared by MW/US	Oil bath (50 °C), 12 h	0.228	86 ± 7 nm	33	-34 ± 1.5

[a] Polydispersity index of hexane suspensions acquired from the DLS analysis of coated MNPs. [b] Hydrodynamic diameter size acquired from the DLS analysis [c] Percentage of coating on MNP surfaces, measured using thermogravimetric analysis. [d] Zeta potential measured by DLS analysis.

Dispersion Stability

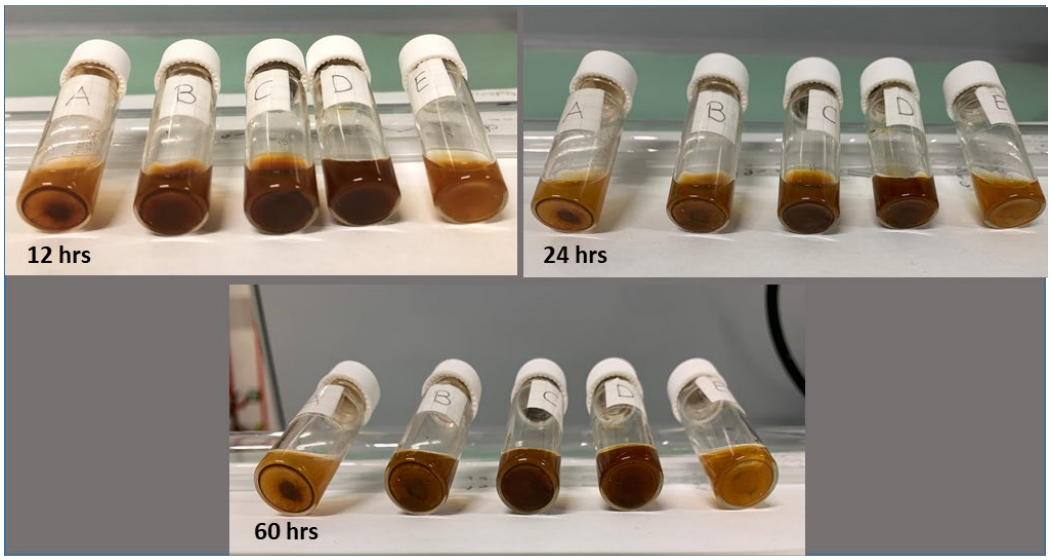

Figure S4. Dispersion stability of IONPs (4 mg/mL, sonicated for 3 min (3 times), stored for 12, 24 and 60 hrs: Sample A MNPs@CMDx physically assembly oil bath (Table 1, entry 8); Sample B MNPs@CMDx physically assembly MW/US (Table 1, entry 9); Sample C MNPs@CA physically assembly MW/US (Table 1, entry 5); Sample D MNPs@CA Ligand exchange (Table 1, entry 7); Sample E MNPs@CA-βCD Ligand exchange (Table 2, entry 3)

Figure S5. Dispersion stability of IONPs (4 mg/mL, sonicated for 3 min (3 times), stored for 12 hrs: Sample A MNPs@CA-βCD physically assembly oil bath (Table S1, entry 1); Sample B MNPs@CA-βCD physically assembly MW/US (Table S1, entry 2)

FT-IR spectra of MNPs@CA-βCD and MNPs@CA-βCD-Citr-NH_2

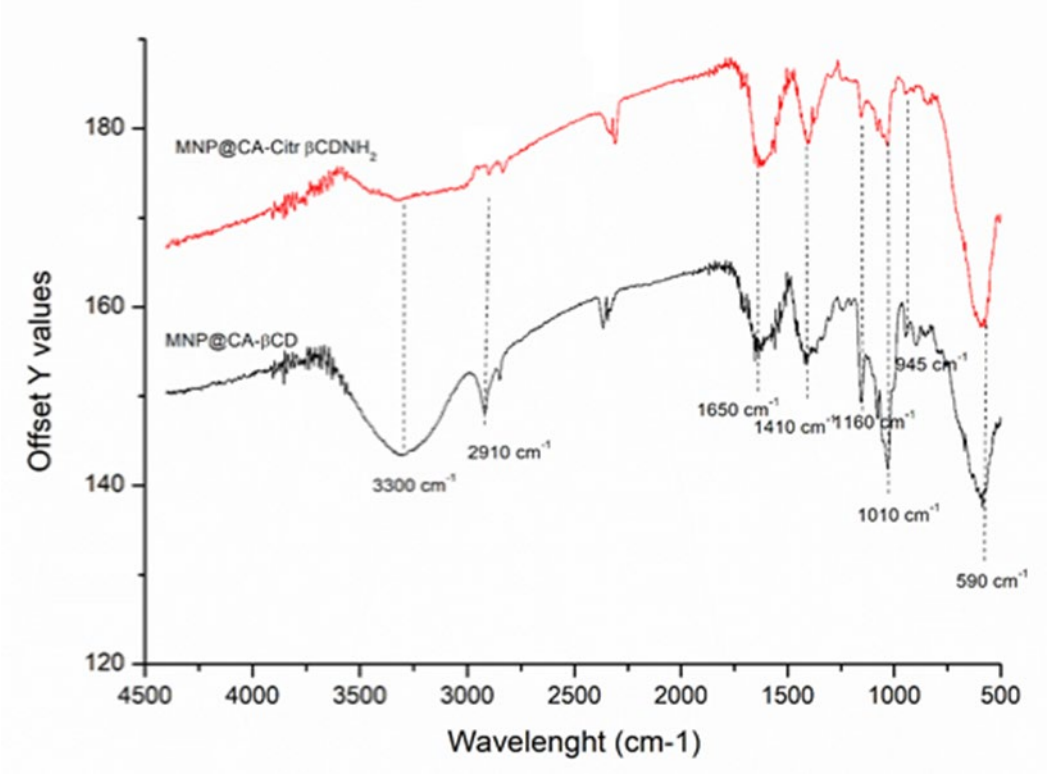

Figure S6. FT-IR spectra of MNPs@CA-βCD and MNPs@CA-βCD-Citr-NH_2

Transmission Electron Microscopy

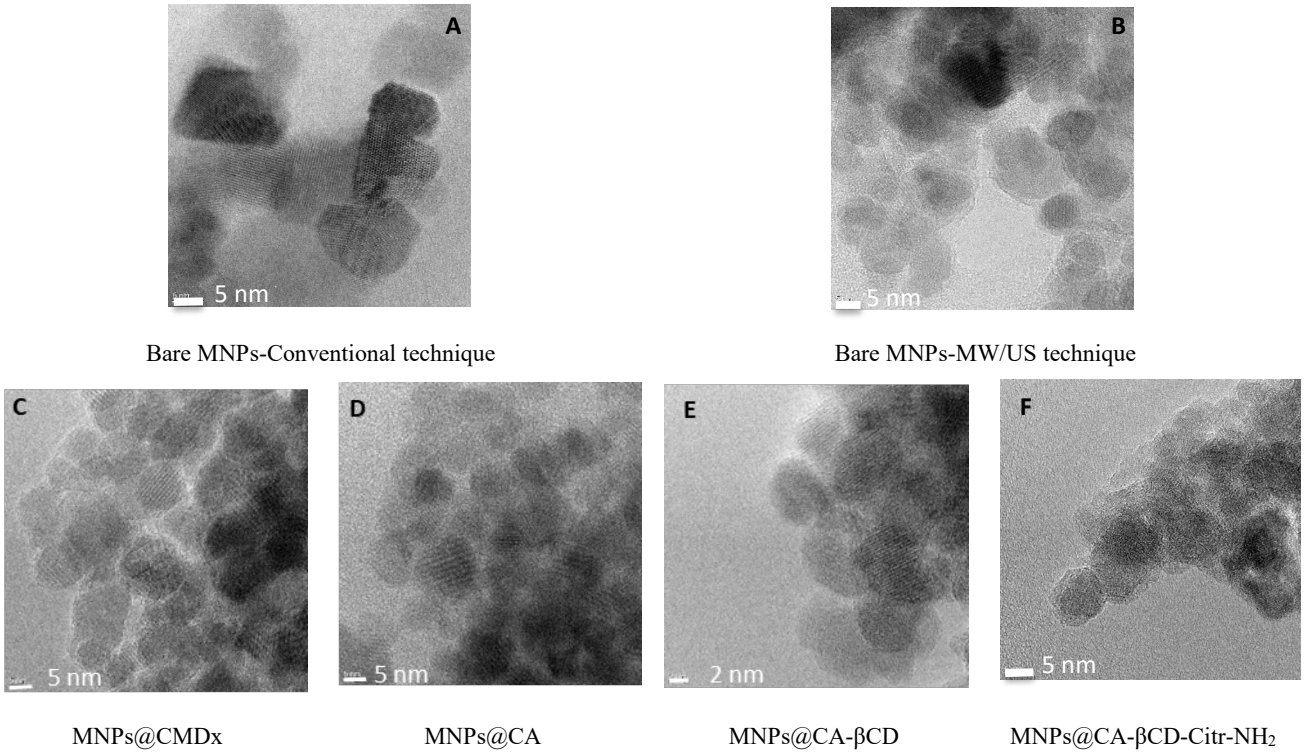

Figure S7. TEM images of MNPs, prepared through co-precipitation method (A) bare MNPs conventional heating (B) bare MNPs MW/US combined irradiation), (C) MNPs@CMDx, (D) MNPs@CA, (E) MNPs@CA-βCDA, (F) prepared by ligand exchange technique.

Sorption and Inclusion Capacity

Phenolphthalein calibration curve

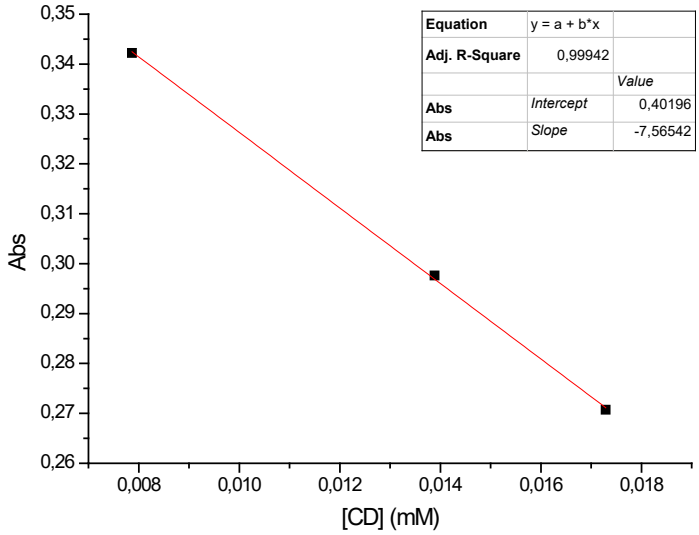

Figure S8. Calibration curve of Php-CD inclusion complex at 553 nm wavelength.

Adamantane amine calibration curve

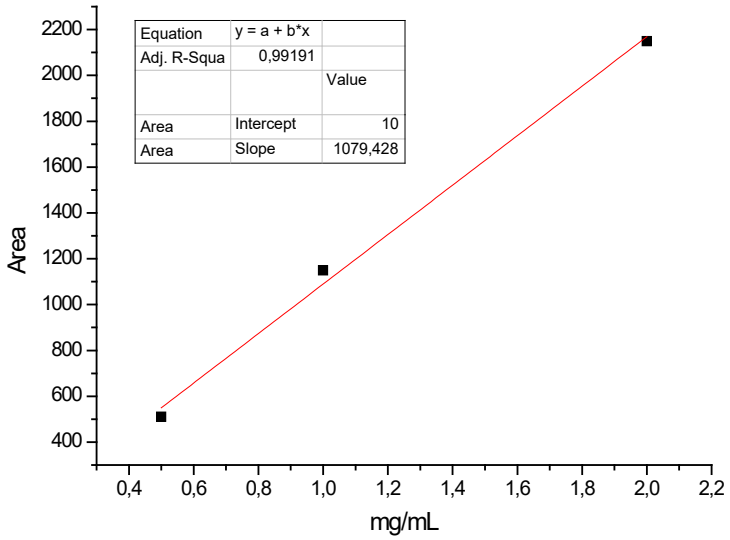

Figure S9 Calibration curve of adamantane amine measured using GC-FID.

Relaxometric properties: R_2/R_1 ratio

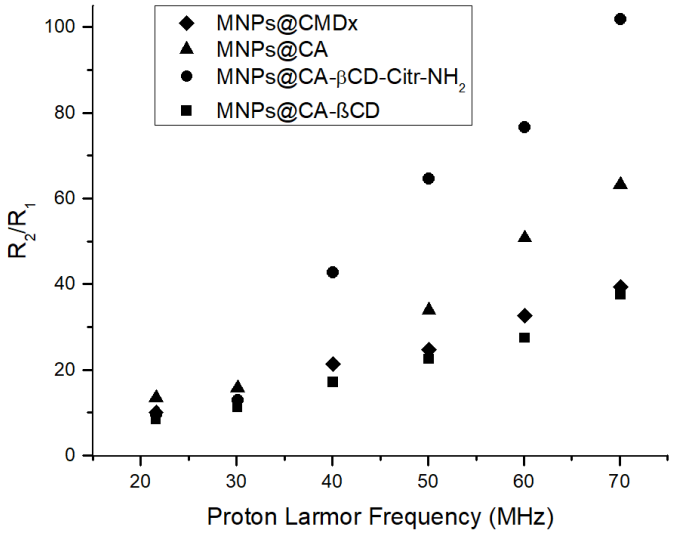

Figure S10. R_2/R_1 ratio of MNPs@CMDx, MNPs@CA, MNPs@CA-βCD and MNPs@CA-βCD-Citr-NH_2.

Characterization of synthesized derivatives 1-3 , βCD Citr-NH_2

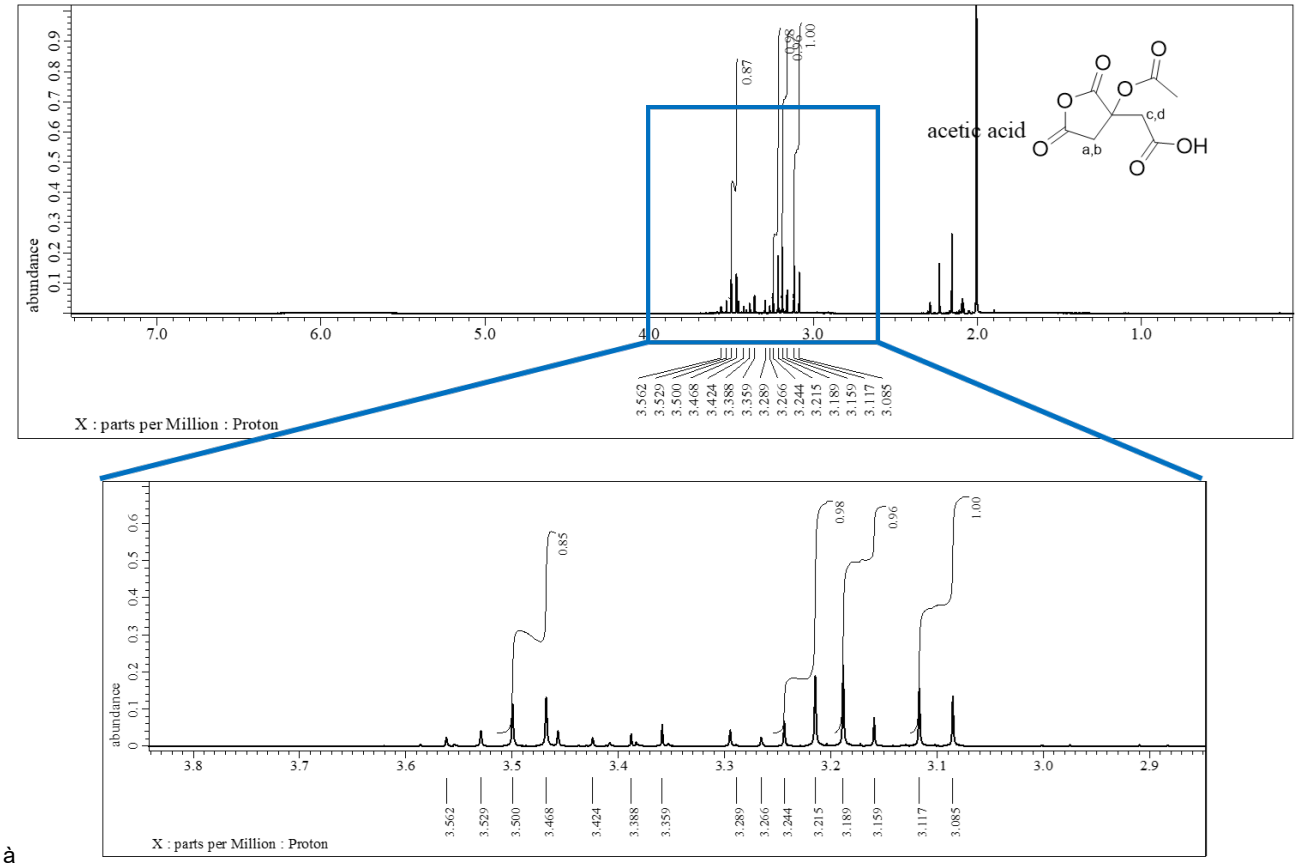

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Figure S11. ^{1}H NMR of citric acid anhydride (**3**)

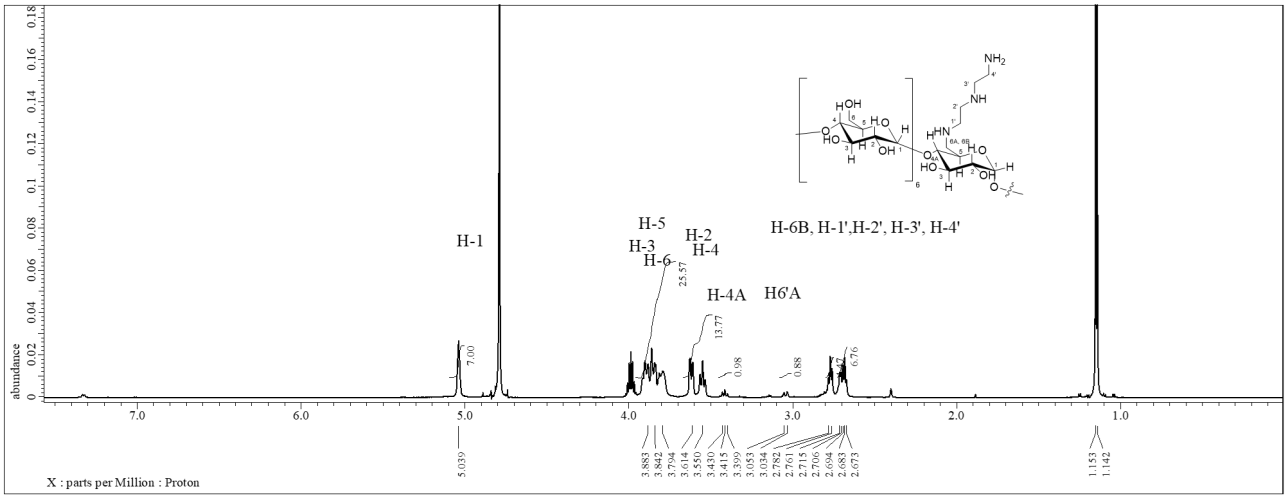

Figure S12. ^{1}H NMR (D_2O) of 6^{I}-diethylentriamine-6^{I}-monodeoxy-βCD (**2**) the product includes iPrOH

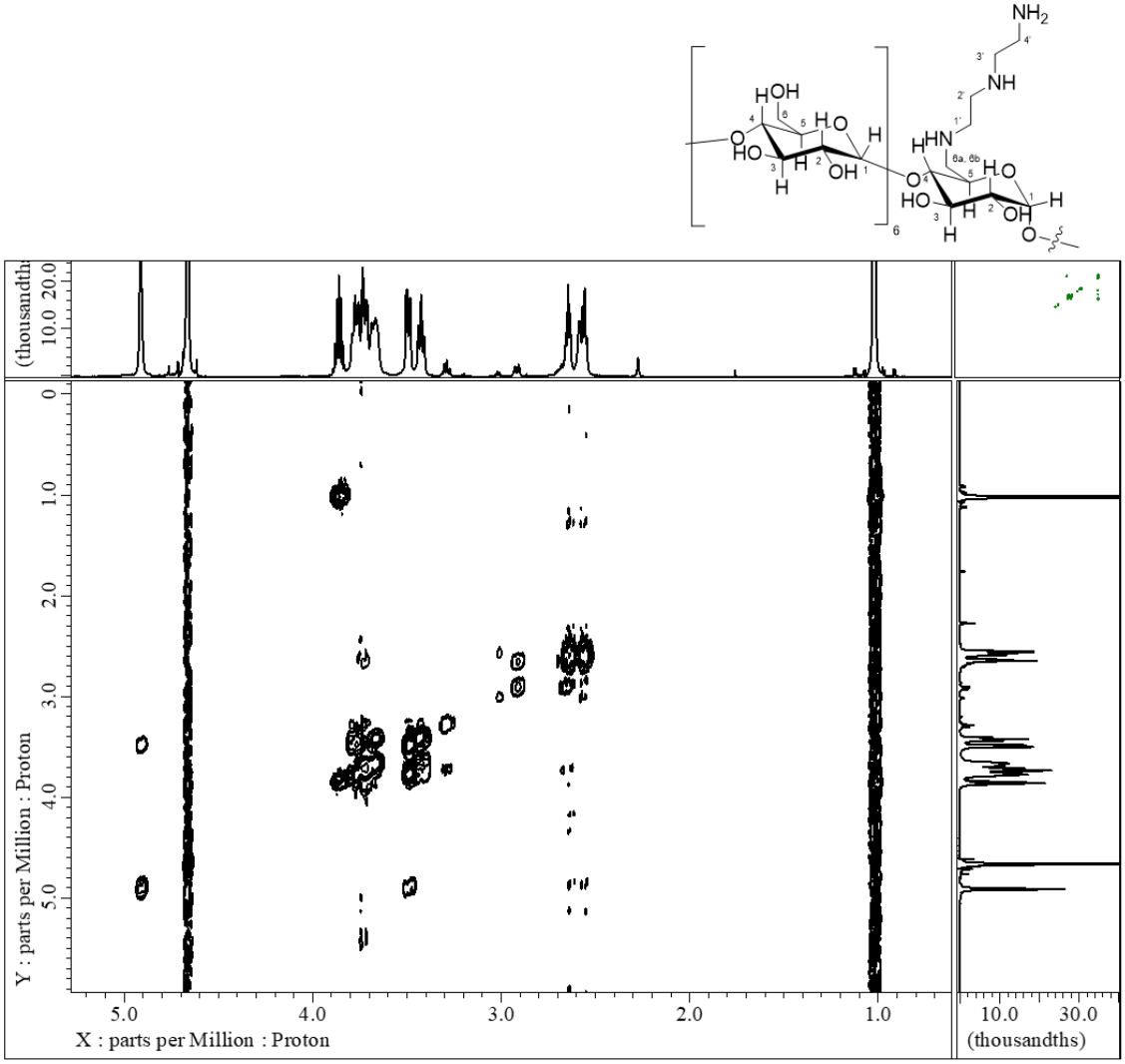

Figure S13. COSY (D_2O) of 6^{I}-diethylentriamine-6^{I}-monodeoxy-βCD (**2**) the product includes iPrOH

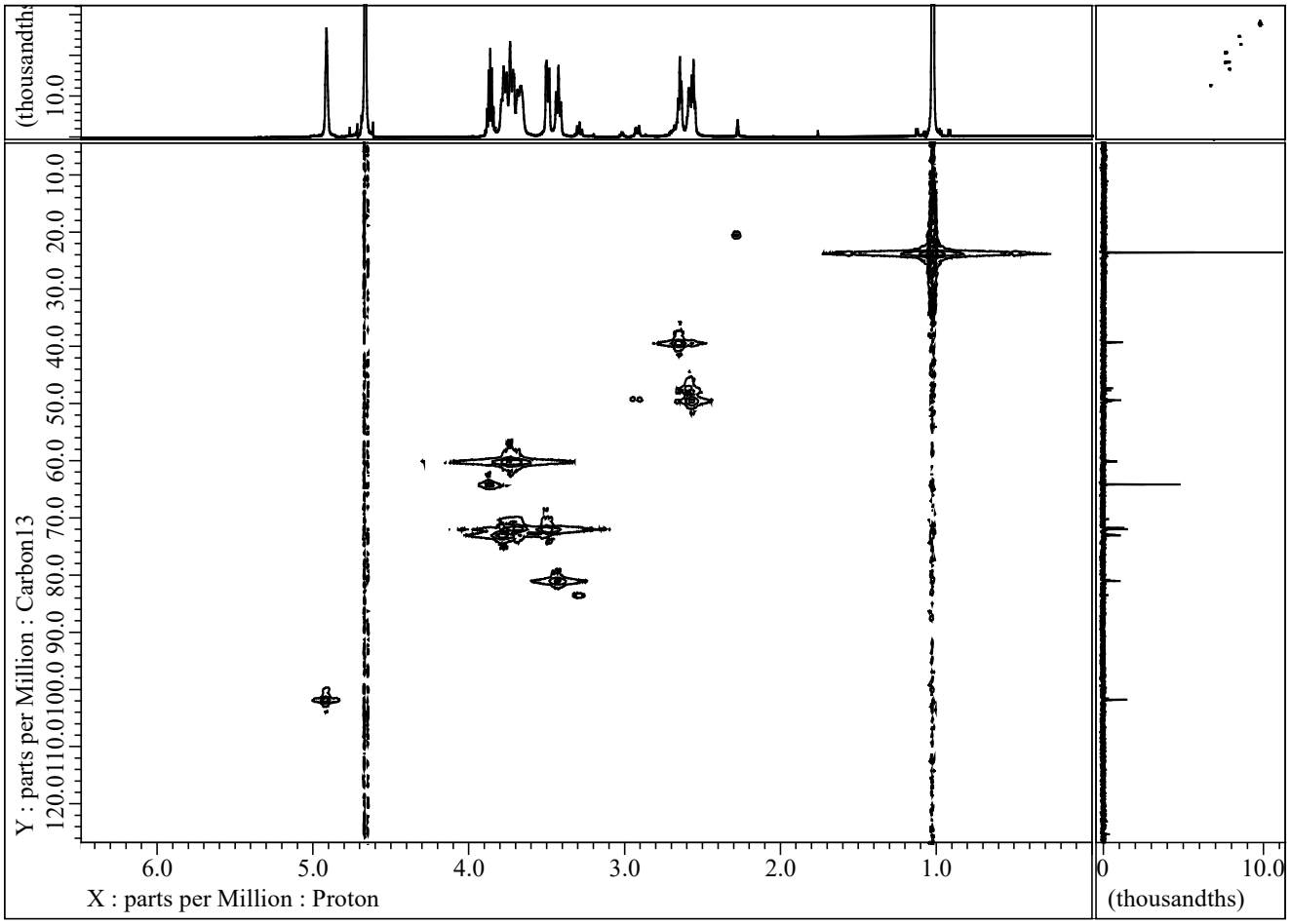

Figure S14. HMQC (D_2O) of 6^I-diethylentriamine-6^I-monodeoxy-βCD (**2**) the product includes iPrOH

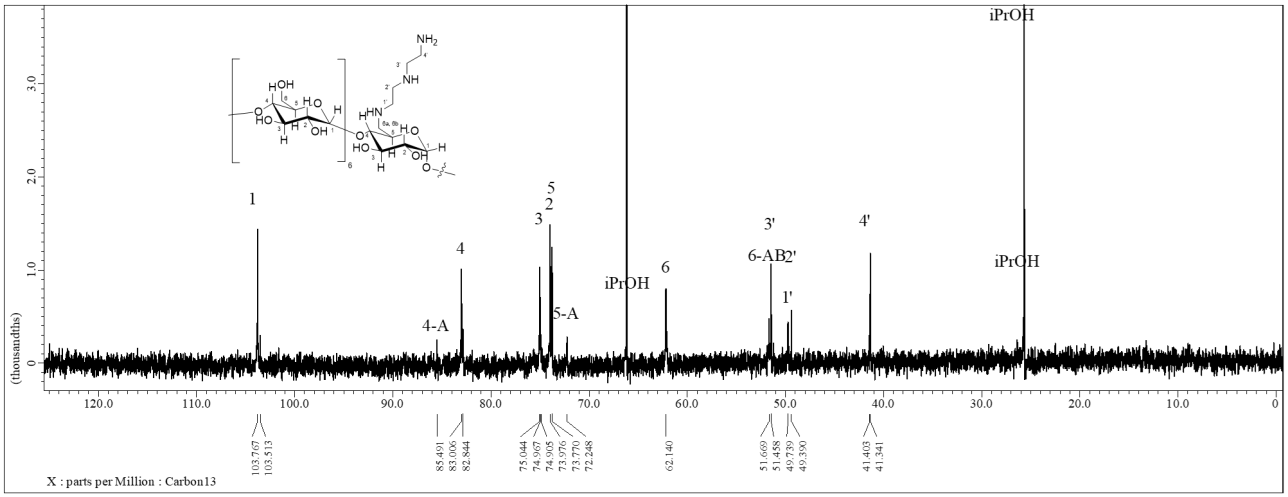

Figure S15. ^{13}C NMR of 6^I-diethylentriamine-6^I-monodeoxy-βCD (**2**) the product includes iPrOH

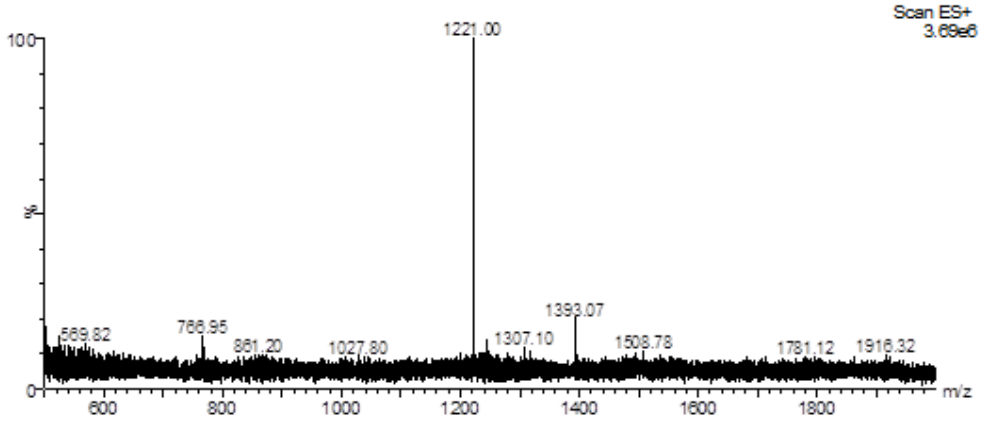

Figure S16. ESI-MS^{+} spectrum of 6^{I}-diethylentriamine-6^{I}-monodeoxy-βCD (**2**)

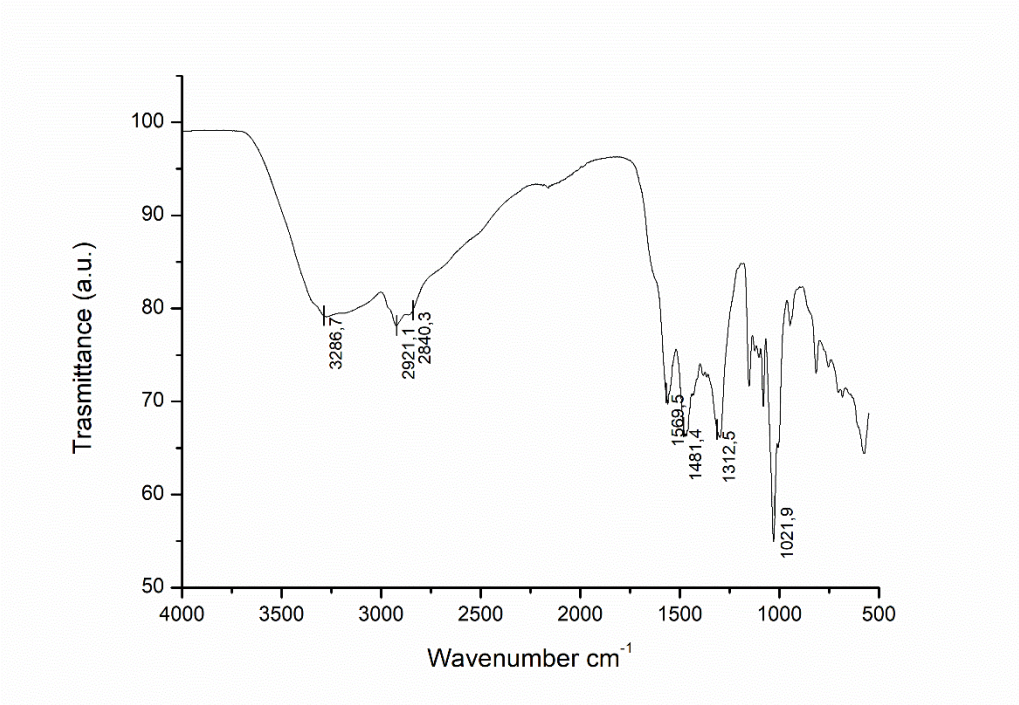

Figure S17. FT-IR spectra of 6^{I}-diethylentriamine-6^{I}-monodeoxy-βCD (2)

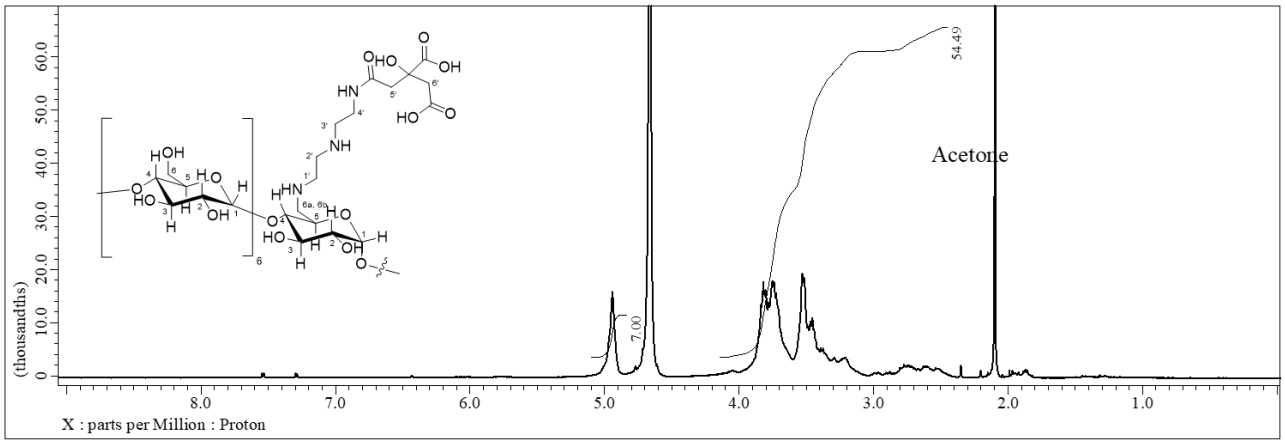

Figure S18. ^{1}H NMR D_2O of **βCD Citr-NH₂** the product includes acetone

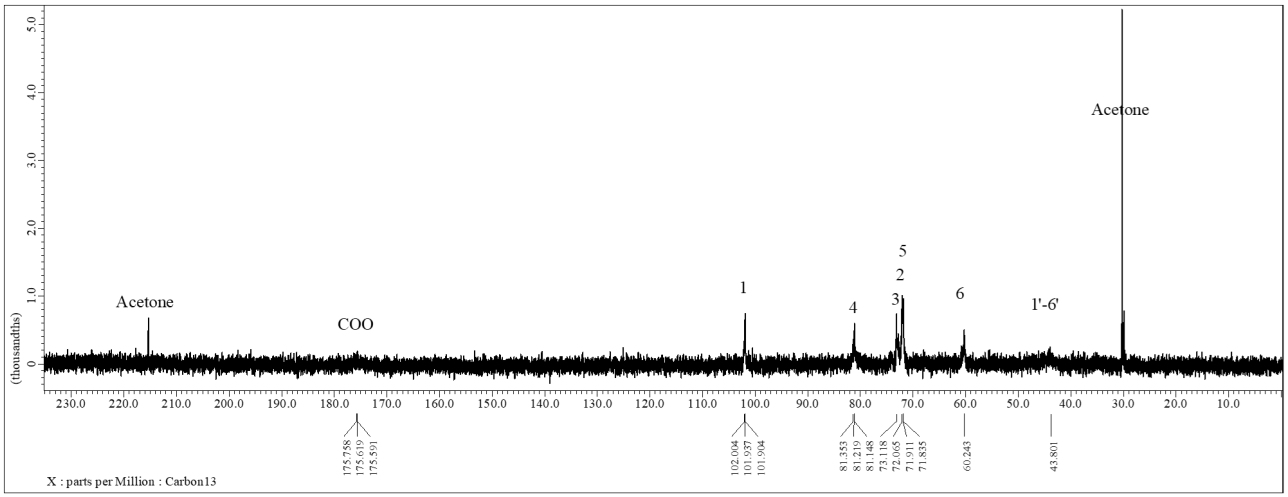

Figure S19 ^{13}C NMR (D_2O) of **βCD Citr-NH₂** the product includes acetone

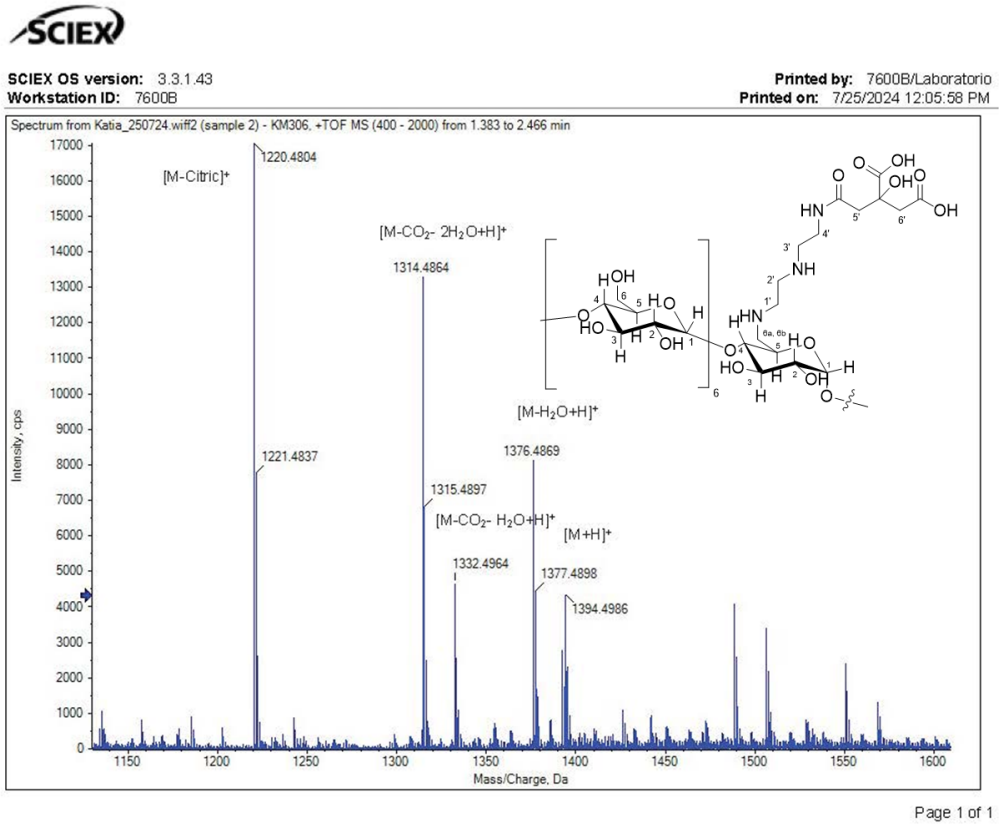

Figure S20. HR-MS^{+} **βCD-Citr-NH$_2$**

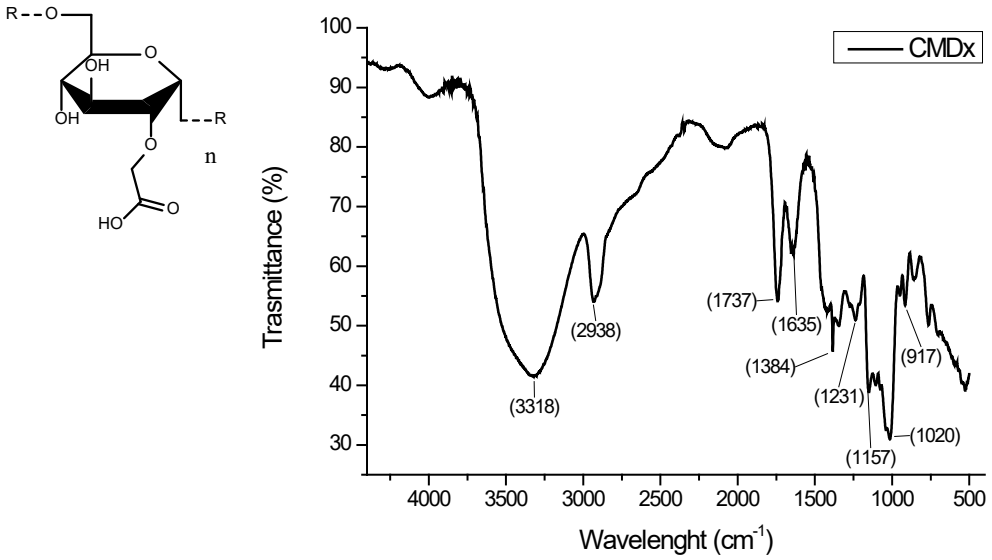

Figure S21 FT-IR of carboxymethyl dextran (**5**)

References

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