Electronic Supplementary Material (ESI) for Polymer Chemistry.

ELECTRONIC SUPPORTING INFORMATION

Magnetic Glyconanoparticles for Selective Lectin Separation and Purification

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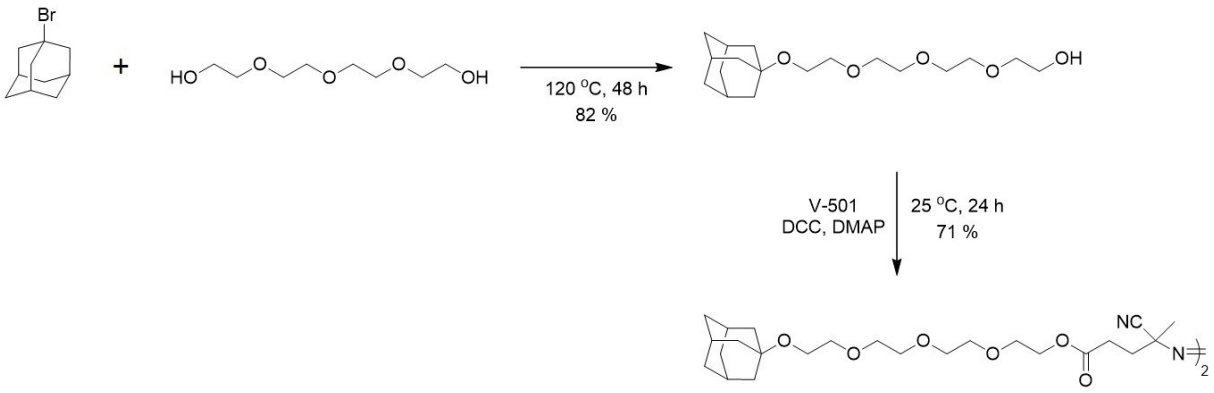


Scheme S1. Synthetic route of the adamantane modified azo-initiator (azobis-ADA).

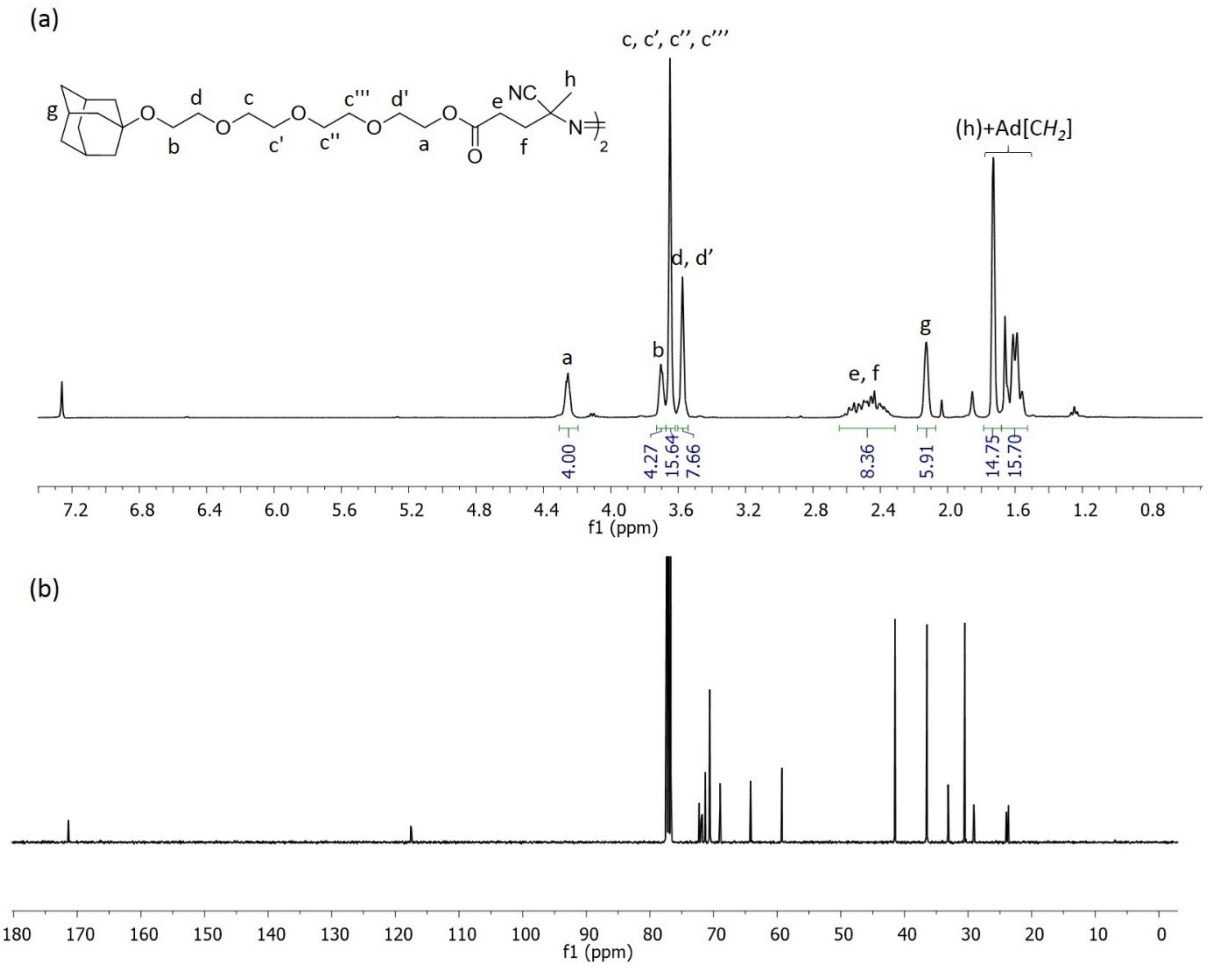


Figure S1. (a) ^{1}H-NMR and (b) ^{13}C-NMR spectrum of azobis-ADA initiator.

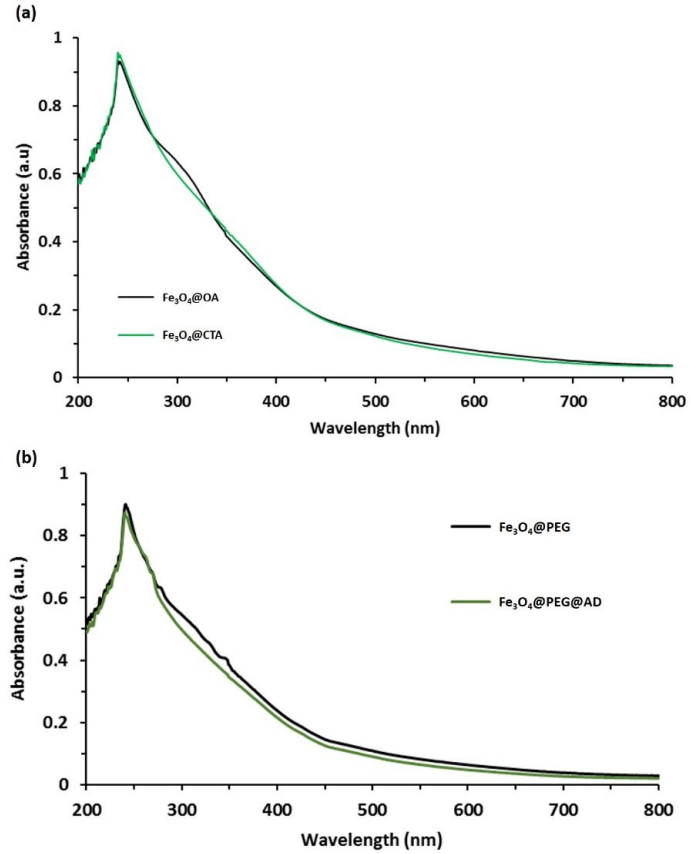


Figure S2. UV visible spectra of (a) Fe_3O_4@OA and Fe_3O_4@CTA, (b) Fe_3O_4@PEG and Fe_3O_4@PEG@AD.

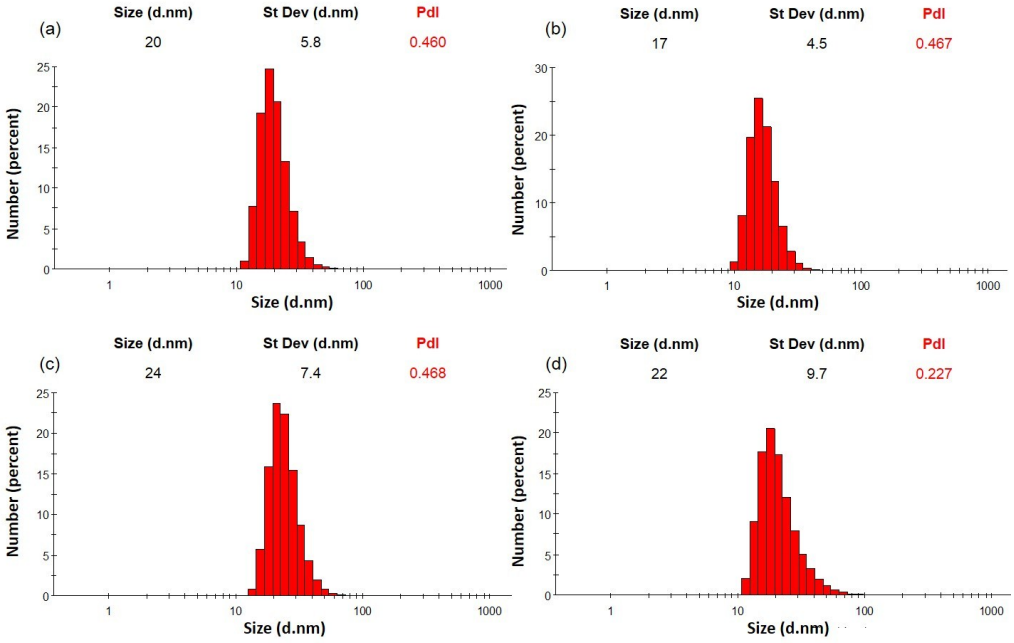
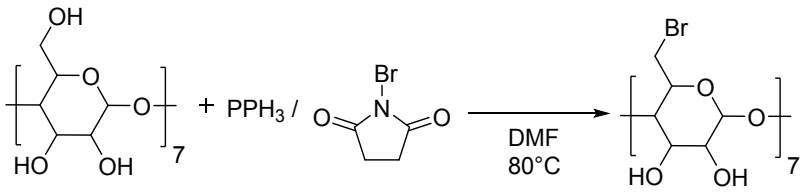


Figure S3. DLS measurements of magnetic NPs (a) Fe_3O_4@OA, (b) Fe_3O_4@CTA, (c) Fe_3O_4@PEG and (d) Fe_3O_4@PEG@AD NPs.

Synthesis of per-(6-deoxy-6-bromine)-β-cyclodextrin (β-CD-(Br)$_7$)



Scheme S2. Schematic representation of the synthetic approach to β-CD-(Br)$_7$.

Triphenylphosphine (Ph_3P, 36.72 g, 140 mmol) was dissolved in anhydrous DMF (150 mL) under stirring and cooled down to 0 °C. N-bromosuccinimide (NBS, 24.92 g, 140 mmol) was dissolved in anhydrous DMF (40 mL) and the solution was added dropwise to the Ph_3P solution under Ar atmosphere and then stirred at ambient temperature for 30 min. *β*-Cyclodextrin (*β*-CD, 11,35 g, 10 mmol) (previously recrystalized three times from water and dried in a vacuum oven at 50 °C for two days) was dissolved in anhydrous DMF (150 mL). The obtained Ph_3P/NBS solution was then added dropwise to the *β*-cyclodextrin solution at ambient temperature after which the solution temperature was increased to 80 °C. The mixed brown solution was stirred under Ar atmosphere overnight at 80 °C. Afterwards MeOH (40 mL) was added at ambient temperature and stirring was continued for 30 min. The reaction mixture was then cooled to 0 °C and the pH was adjusted to 9 by adding sodium methoxide, while further stirring for 1 h. The reaction mixture was then poured into stirred ice-water (4 L) resulting in a fine precipitate which was filtered and washed with MeOH. *Heptakis* (6-deoxy-6-bromo)-*β*-cyclodextrin was obtained as beige solids and dried under vacuum for 1 day. Yield: 11.32 g, 70%). ^{1}H NMR (400 MHz, DMSO-d_6, 298 K, ppm): δ =6.02 (*d*, 7 H, 6.7 Hz), 5.89 (*d*, 7 H, 1.9 Hz), 4.98 (*d*, 7 H, 3.4 Hz), 4.00 (*d*, 7 H, 9.8 Hz), 3.82 (*t*, 7 H, 9.3 Hz), 3.65 (*m*, 14 H), 3.38 (*m*, 14 H, overlap with H_2O). MALDI-TOF MS m/z: calculated for $C_{42}H_{63}Br_7O_{28}K^+$: 1614.73; found, 1614.74.

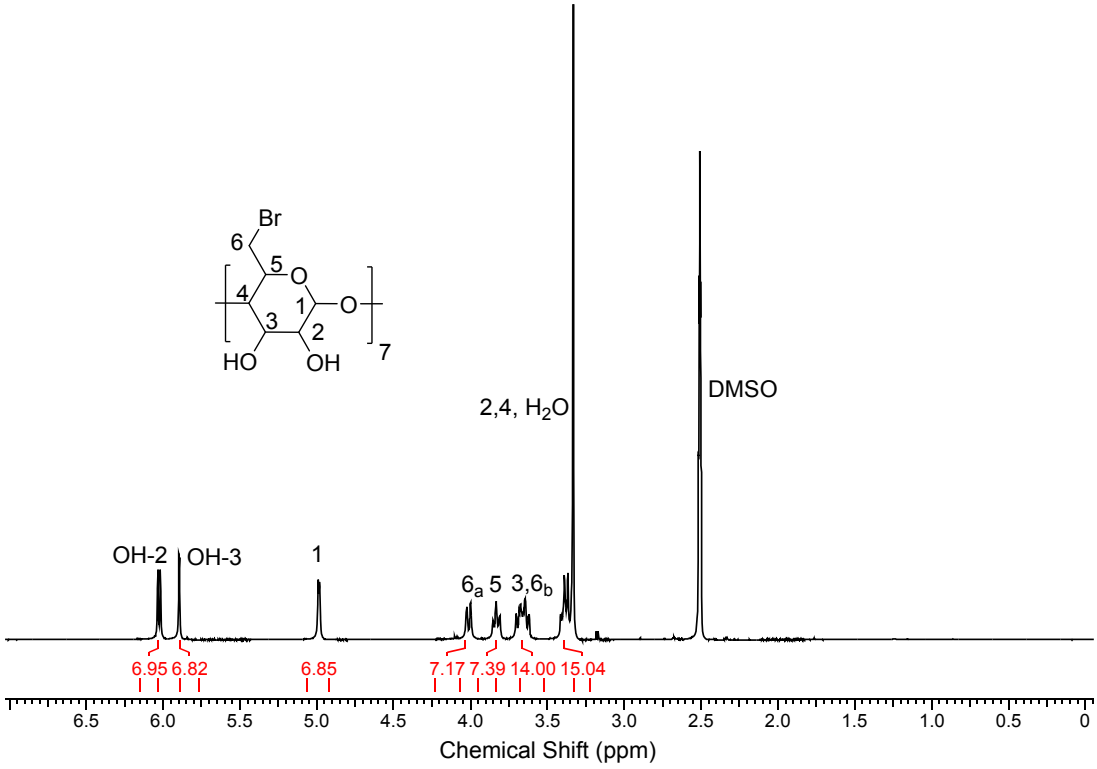
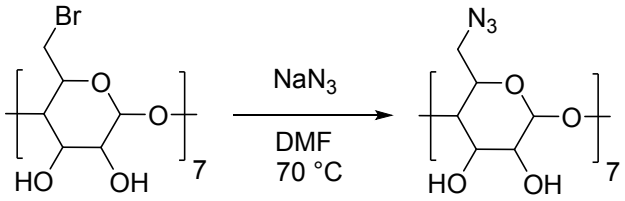


Figure S4. ^{1}H-NMR spectrum spectrum of Heptakis (6-deoxy-6-bromo)-β-cyclodextrin.

Synthesis of per-(6-deoxy-6-azido)-β-cyclodextrin (β-CD-$(N_3)_7$)



Scheme S3. Schematic representation of the synthetic approach to β-CD-$(N_3)_7$.

Heptakis (6-deoxy-6-bromo)-*β*-cyclodextrin (10 g, 6.3 mmol) was dissolved in anhydrous DMF (80 mL) and NaN_3 (5.78 g, 88.8 mmol). The resulting suspension was stirred at 70 °C under Ar for 36 h. The suspension was then allowed to cool down and precipitated in 2 L of stirred ice-water. The precipitate was filtered, washed with water and redissolved in DMF (20 mL) and precipitated in 1L of stirred ice-water. The precipitate was filtered and washed with water and with little acetone. The resulting product was a white solid (yield: 7.2 g, 86.5 %) and was dried under vacuum overnight. ^{1}H NMR (400 MHz, DMSO-d_6, 298 K, ppm): δ =5.90 (*d*, 7 H, 6.8 Hz), 5.75 (*d*, 7 H, 2 Hz), 4.91(*d*, 7 H, 3,4 Hz), 3.74 (*m*, 14 H), 3.59 (*m*, 14 H),

3.36 (*m*, 14 H, overlap with H_2O). MALDI-TOF MS m/z: calculated for $C_{42}H_{63}N_{21}O_{28}K^+$: 1348.37; found, 1348.27.

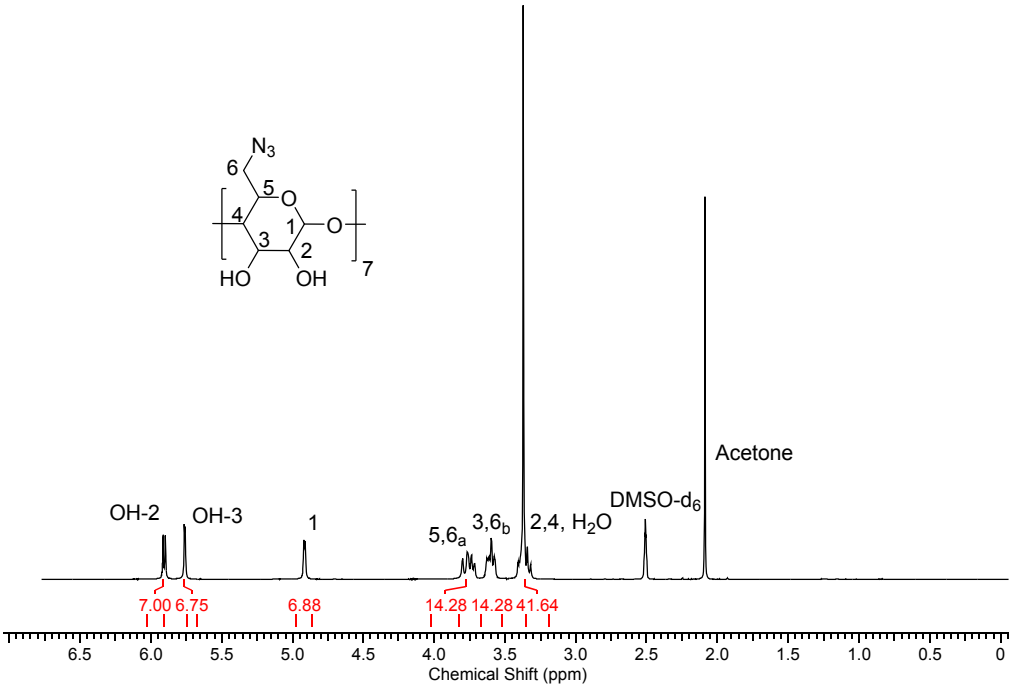
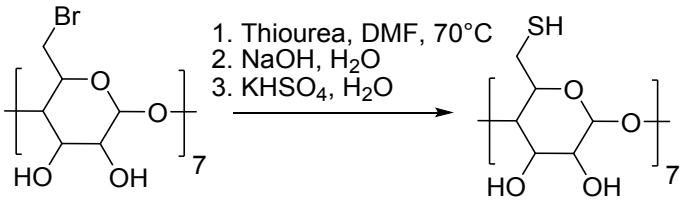


Figure S5. ^{1}H-NMR spectrum of *heptakis* (6-deoxy-6-azido)-β-cyclodextrin.

Synthesis of per-6-thio-β-cyclodextrin (β-CD-(SH)$_7$)



Scheme S4. Schematic representation of the synthetic approach to β-CD-(SH)$_7$.

β-CD-(Br)$_7$ (5 g, 3.17 mmol) and thiourea (2.5 g, 33.3 mmol) were dissolved in DMF (50 mL) and the mixture was heated to 70 °C under argon atmosphere. After 24 h, DMF was removed under reduced pressure and the obtained brown oil was dissolved in water (200 mL). Sodium hydroxide (2.22 g, 55.5 mmol) was then added and the reaction mixture was heated to a gentle reflux under nitrogen atmosphere. After 1 h, the resulting suspension was acidified with aqueous $KHSO_4$ forming a white precipitate which was then filtered and

washed thoroughly with water and dried under vacuum. Compound β-CD-SH was recovered as white powder (yield: 3.2 g, 81%). ^{1}H NMR (400 MHz, DMSO-d_6, 298 K, ppm): δ =5.91 (*d*, 7 H, 6.8 Hz), 5.81 (*d*, 7 H, 2 Hz), 4.93(*d*, 7 H, 3.3 Hz), 3.68 (*t*, 7 H, 8.5 Hz), 3.61 (*t*, 7 H, 9.2 Hz), 3.29-3.40 (*m*, 14 H, overlap with H_2O), 3.19 (*m*, 7H), 2.75 (*m*, 7 H), 2.13 (*t*, 7 H, 8.3 Hz).

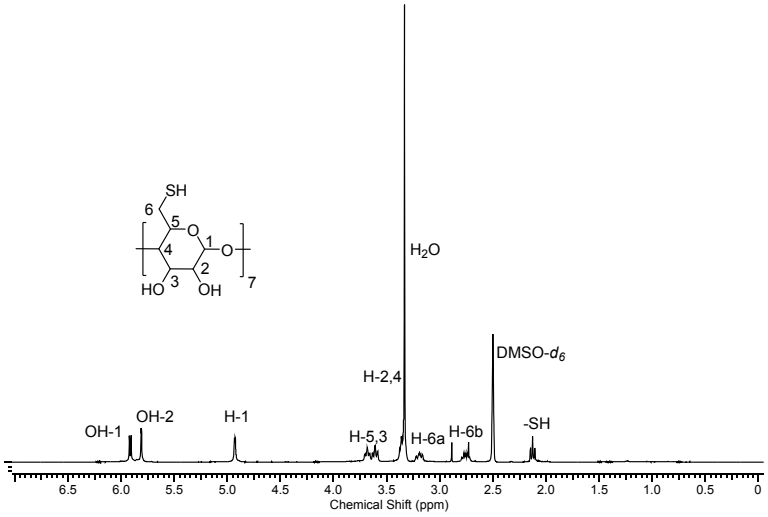
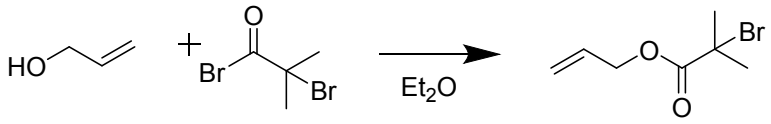


Figure S6. ^{1}H-NMR spectrum of per-6-thio-β-cyclodextrin (β-CD-$(SH)_7$) in DMSO-d_6.

Synthesis of allyl 2-bromoisobutyrate



Scheme S5. Schematic representation of the synthetic approach to allyl 2-bromoisobutyrate.

Allyl alcohol (16.2 mL, 16.42 g, 282 mmol) and triethylamine (47.3 mL, 34.33 g, 339 mmol) were dissolved in diethyl ether (150 mL) and cooled down to 0 °C in an ice-water bath. A solution of α-bromoisobutyryl bromide (BIBB) (27 mL, 50 g, 217 mmol) in 20 mL diethyl ether was added dropwise over a period of 20 min. The mixture was allowed to stir for 1 h at 0 °C after which it was allowed to reach room temperature and stirring was continued overnight. The solution was washed 3 x 50 mL 10% HCl solution, 3 x 50 mL 5% NaOH solution, 3 x 50 mL water and subsequently dried over $MgSO_4$. After evaporating the solvent via rotary evaporation, the product was purified by flash chromatography on silica gel using chloroform as an eluent affording a colorless oil. (yield: 76% , 23 g). ^{1}H NMR (400 MHz,

$CDCl_3$, 298 K, ppm): δ = 5.9 (*ddt*, 1 H, 5.5 Hz, 10.6 Hz, 17.2 Hz), 5.35 (*dq*, 1 H, 1.5 Hz, 17.2 Hz), 5.24 (*dq*, 1 H, 1.3 Hz, 10.6 Hz), 4.63 (*dt*, 2 H, 1.4 Hz, 5.6 Hz), 1.91 (*s*, 6 H).

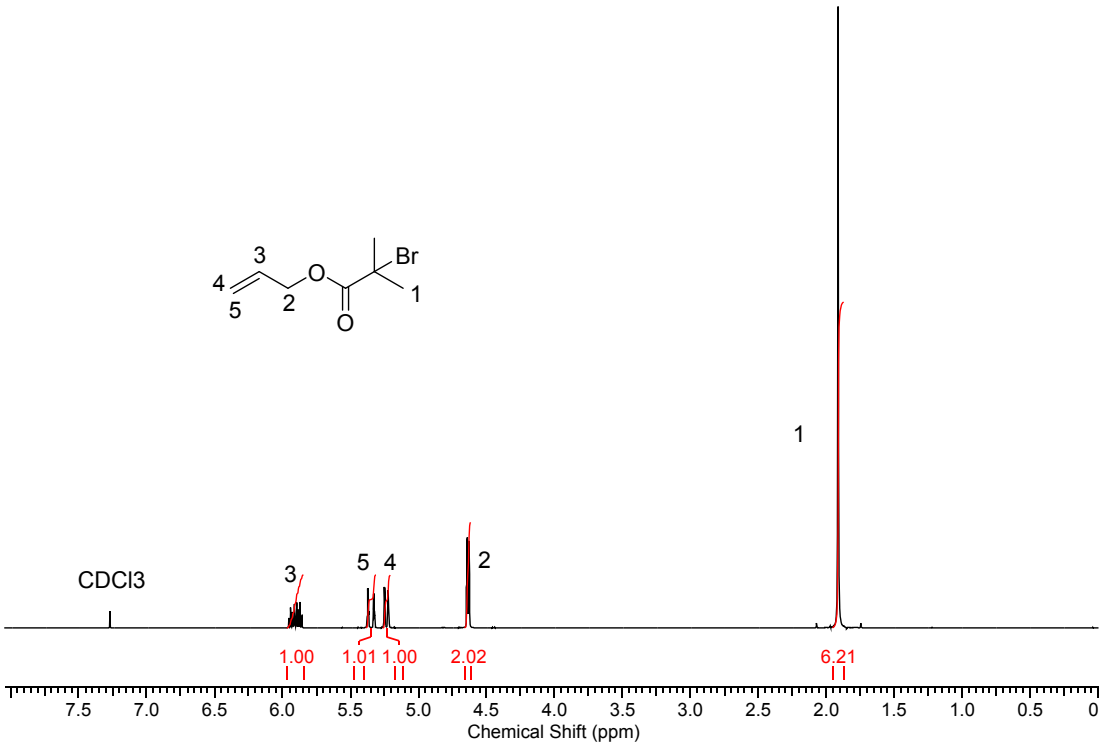
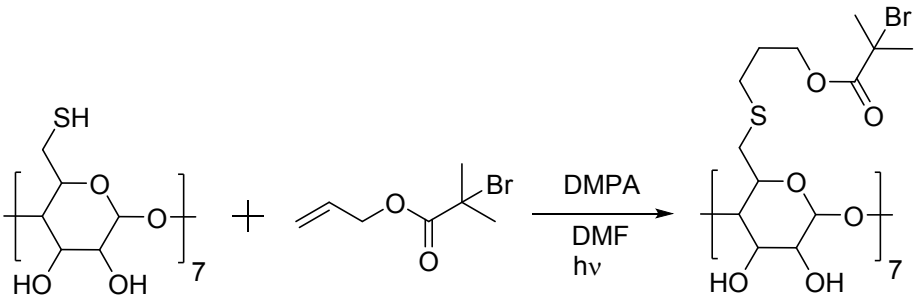


Figure S7. ^{1}H-NMR spectrum of allyl 2-bromoisobutyrate in $CDCl_3$.

Synthesis of per-6-deoxy-6-(thiopropyl-2-bromo-2-methylpropanoate)-β-cyclodextrin

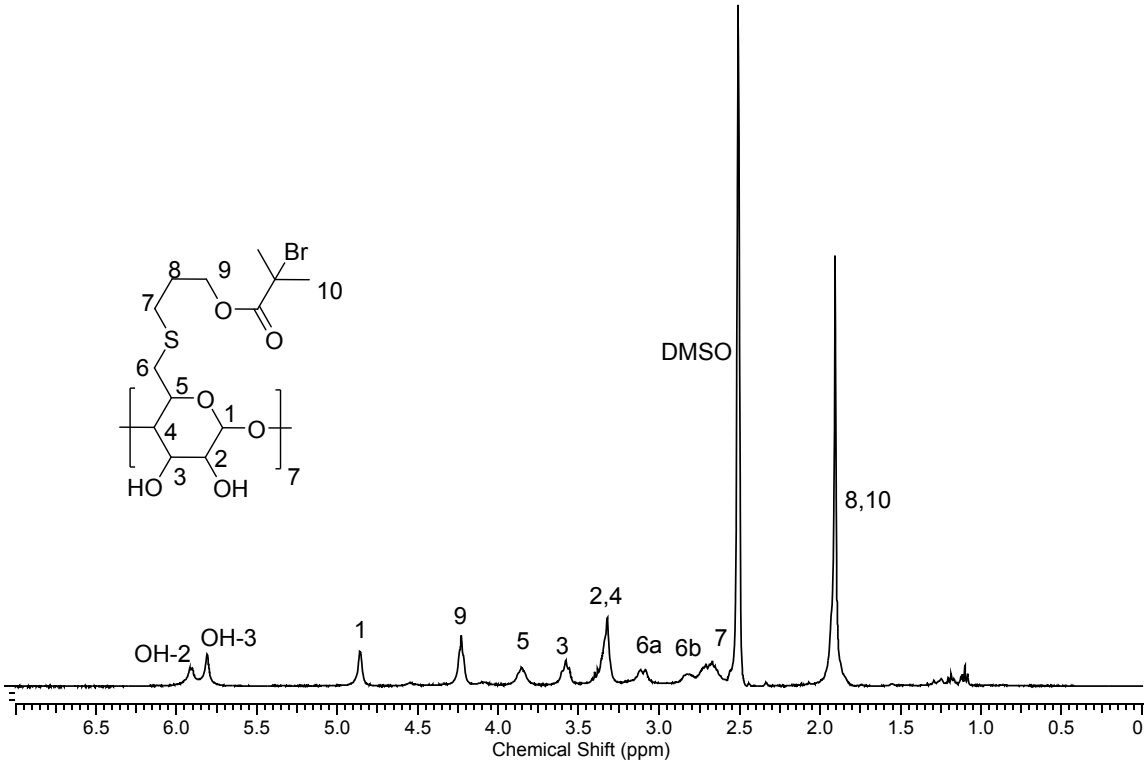


Scheme S6. Schematic representation of the synthetic approach to per-6-deoxy-6-(thiopropyl-2-bromo-2-methylpropanoate)-β-cyclodextrin (β-CD initiator).

Per-6-thio-β-cyclodextrin (2.5 g, 2 mmol), and dithiotreitol (DTT, 618 mg, 4 mmol) were dissolved in 40 mL anhydrous DMF under Ar and heated to 60 °C. After 60 h the reaction mixture was allowed to cool down to room temperature and allyl 2-bromoisobutyrate (14.53

g, 70 mmol), 2,2-dimethoxy-2-phenylacetophenone (DMPA, 179 mg, 7 mmol) were added to the reaction mixture and stirring was continued for 5 h under UV irradiation (365 nm).

The solution was precipitated in 500 mL of methyl tert-butyl ether (MTBE) divided in ten 50 mL centrifuge tubes and centrifuged at 8000 rpm for 5 min. The solvent was decanted and all precipitated fractions collected in two 50 mL centrifuge tubes and fresh MTBE was added, mixed and centrifuged again. This procedure was repeated 4 times in order to remove DMF and allyl 2-bromoisobutyrate. Subsequently the product was dried under vacuum, yielding a fine beige solid. (3.7 g, yield: 68%). ^{1}H NMR (400 MHz, DMSO-d_6, 298 K, ppm): δ = 5.90 (*d*, 7 H, 5.6 Hz), 5.8 (*m*, 7 H), 4.85 (m, 7 H), 4.22 (*t*, 14 H, 5.2 Hz), 3.85 (*m*, 7 H), 3.57 (*m*, 7 H), 3.33 (*m*, 14 H), 3.09 (*d*, 7 H, 10.6 Hz), 2.82 (*m*, 7 H), 2.69 (*m*, 14 H), 1.90 (*s*, 56 H). MALDI-TOF MS m/z: calculated for $C_{91}H_{147}Br_7O_{42}S_7K^+$:2733.12; found: 2733.36.



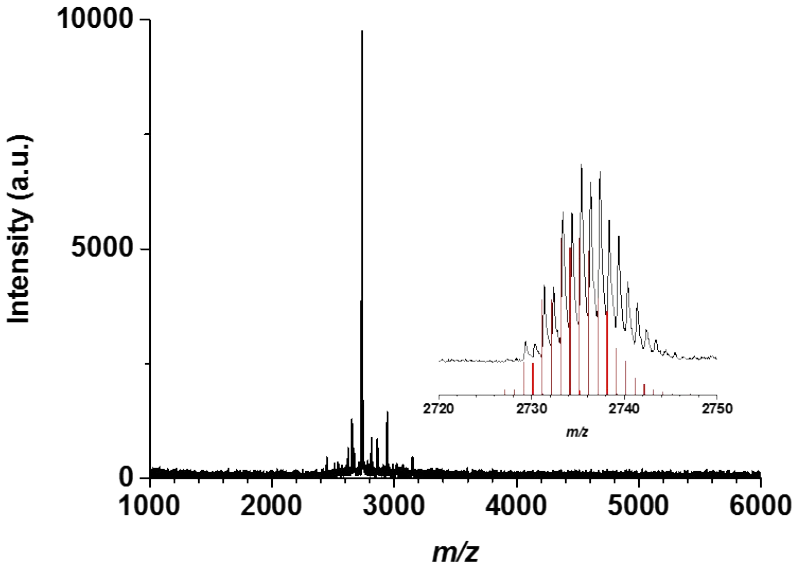


Figure S8. ^{1}H-NMR spectrum (in DMSO-d_6) (top) and MALDI-TOF spectrum with detail of the peak of interest (bottom) of per-6-deoxy-6-(thiopropyl-2-bromo-2-methylpropanoate)- β-cyclodextrin.

Synthesis of 1-(2'-Propargyl) D-Mannose

1-(2'-Propargyl) D-Mannose was prepared in according to the procedure reported by Mukhopadhyay et al.[1] A suspension solution of D-mannose (10 g, 55.5 mmol), propargyl alcohol (16.2 mL, 277.8 mmol) and H2SO4-silica (300 mg) was stirred at 65 °C for overnight. After cooling to ambient temperature, the reaction mixture was transferred to a silica gel column and eluted with CHCl3-MeOH (8:1) to remove the excess propargyl alcohol. 1-(2'-Propargyl) D-mannose was obtained as white solid after drying under vacuum (Yield: 39%). FT-IR ν (cm^{-1}): 3347 (OH), 3285 (C≡C-H), 2118 (C≡C). ESI-MS *m/z*: calcd for C9H14O6 (M^+Na^+), 241.1; found, 241.1.

Synthesis of 1-(2'-Propargyl) D-Galactose

1-(2'-Propargyl) D-Galactose was prepared *via* the procedure described above. A suspension of D-galactose (10 g, 55.5 mmol), propargyl alcohol (16.2 mL, 277.8 mmol) and H2SO4-silica (300 mg) was stirred at 65 °C for overnight. After cooling to ambient temperature, the reaction mixture was transferred to a silica gel column and eluted with CHCl3-MeOH (8:1) to remove excess propargyl alcohol. 1-(2'-Propargyl) D-galactose was obtained as white solid

after drying under vacuum (Yield: 33%). FT-IR ν (cm^{-1}): 3347 (OH), 3285 (C≡C-H), 2118 (C≡C). ESI-MS *m/z*: calcd for C9H14O6 (M^+Na^+), 241.1; found, 241.2.

Synthesis of D-Mannose glycomonomer

1-(2'-propargyl) D-mannose (2.46 g, 12.6 mmol) and 3-azidopropyl acrylate (2.85 g, 11.8 mmol) were dissolved in MeOH/H_2O (2:1 vol/vol, 60 mL), aqueous solution of $CuSO_4 \cdot 5H_2O$ (246 mg, 0.9 mmol) and (+)-sodium L-ascorbate (284 mg, 1.2 mmol) were added into the reaction solution. The reaction mixture was stirred at ambient temperature for 24 h and then the methanol was removed under vacuum and residue mixture was freeze dried to remove water. The purification of the obtained product was done by silica gel column chromatography using dichloromethane-MeOH (8:1) as eluent. After the removing of solvent, the product was obtained as white (1.62 g, yield: 58.2%).

^{1}H NMR (D_2O, 298 K, 400 MHz): δ =8.07, 8.06 (s, overlaped, 1 H, NC*H*=C), 6.37 (dd, J=1.8, 15.5 Hz), 6.36 (dd, J=1.6, 15.7 Hz) (anomeric 1 H, CH_2=C), 6.14 (dd, J=10.4, 6.9 Hz), 6.13(dd, J=10.4, 7.0 Hz) (anomeric, 1 H, CH_2=C*H*C=O), 5.89 (dd, 1 H, J=1.5, 8.9 Hz, CH_2=C), 4.70-5.05 (m, CH_2-OH, H-1 of mannose , overlap with H_2O), 4.64 (d, 1 H, J=12.3 Hz, CH_2-OH), 4.55 (t, 2 H, J=6.9 Hz, CH_2-N), 4.19 (t, 2 H, J=6.0 Hz, C=O-O-CH_2), 3.40-3.92 (m, H residues of mannose), 2.30 (m, 2H, CH_2-CH_2-CH_2) ppm. ^{13}C NMR (D_2O, 298 K, 400 MHz): δ =146.4 (*C*=O), 145.4 (N-CH=*C*), 131.9 (*C*H_2=C), 129.2 (CH_2=*C*), 125.6 (N-*C*H=C), 100.8 (β anomeric, C 1 of mannose), 100.7 (α anomeric, C 1 of mannose), 78.4,75.2, 75.0, 72.5, 72.3, 72.0, 68.6, 68.4 (carbons of anomeric mannose), 63.0(*C*H_2-OH), 62.6 (C=O-O-*C*H_2), 60.7 (C-*C*H_2-O), 48.5 (CH_2-*C*H_2-N), 28.5(CH_2-*C*H_2-CH_2) ppm.

Synthesis of D-Galactose glycomonomer

1-(2'-propargyl) D-galactose (2.21 g, 11.4 mmol) and 3-azidopropyl acrylate (2.57 g, 10.6 mmol) were dissolved in MeOH/H_2O (2:1 vol/vol, 60 mL), aqueous solution of $CuSO_4 \cdot 5H_2O$ (222 mg, 0.81 mmol) and (+)-sodium L-ascorbate (256 mg, 1.08 mmol) were added into the reaction solution. The reaction mixture was stirred at ambient temperature for 24 h and then the methanol was removed under vacuum and residue mixture was freeze dried to remove water. The purification of the obtained product was done by silica gel column chromatography using dichloromethane-MeOH (8:1) as eluent. After the removing of solvent, the product was obtained as white (1.12 g, yield: 46.7%).

^{1}H NMR (D_2O, 298 K, 400 MHz): δ =8.07, 8.06 (s, overlaped, 1 H, NC*H*=C), 6.38 (dd, 1 H, J=1.6, 15.6 Hz, CH_2=C), 6.16 (dd, 1 H, J=10.4, 6.9 Hz, CH_2=C*H*C=O), 5.92 (dd, 1 H, J=1.5, 8.9 Hz, CH_2=C), 4.91 (d, J=12.6 Hz, CH_2-OH, β anomeric), 4.86 (d, 1 H, J=3.7 Hz, H-1 of galactose, α anomeric), 4.67-4.88 (m, C-CH_2-O, C=O-O-CH_2 β anomeric, 4.56 (t, 2 H, J=6.9 Hz, CH_2-N), 4.28 (d, J=7.7 Hz, H-1 of galactose, β anomeric), 4.12-4.24 (m, 2 H, C=O-O-CH_2), 3.15-4.00 (m, H residues of galactose), 2.30 (m, 2H, CH_2-CH_2-CH_2) ppm.

^{13}C NMR (D_2O, 298 K, 400 MHz):δ =166.9 (*C*=O), 145.6 (N-CH=*C*), 132.3 (*C*H_2=C), 129.5 (CH_2=*C*), 125.7 (N-*C*H=C), 101.2 (β anomeric, C 1 of galactose), 98.4 (α anomeric, C 1 of galactose), 78.2,76.6, 75.3, 73.9, 73.2, 71.7, 71.4 (carbons of anomeric galactose), 62.6(*C*H_2-OH), 62.4 (C=O-O-*C*H_2), 61.1 (C-*C*H_2-O), 49.5 (CH_2-*C*H_2-N), 30.2(CH_2-*C*H_2-CH_2) ppm.

Synthesis of Poly(galactose) Conjugated Beta-cyclodextrin (β-CD-p(Gal)$_{280}$)

A Schlenk tube was charged with galactose acrylate monomer (520 mg, 280 eq), pre-activated Cu(0) wire (5 cm), $CuBr_2$ (0.32 mg, 0.28 eq) and Me_6TREN (1.53 mg, 1.33 eq) in DMSO (2 ml) and then the mixture was degassed by gentle bubbling of argon gas for 30 min. Pre-degassed CD initiator in 1 ml DMSO (13.5 mg, 1 eq) was then added *via* gas tight syringe sequentially. The Schlenk tube was sealed and the reaction mixture was allowed to polymerize at 25°C. Sampling was carried out using a degassed syringe to check the conversion of monomer. After it reached to full conversion, the polymerization solution was directly dialyzed against water for 3 days. The CD initiated glycopolymer was recovered as a powder by freeze drying.

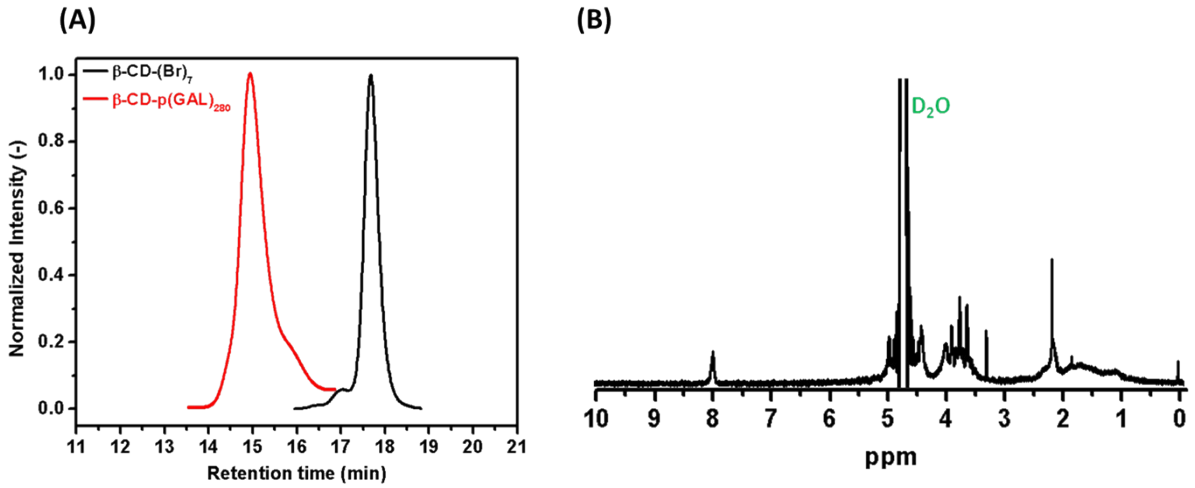


Figure S9. SEC traces of the synthesized β-CD-$(Br)_7$ initiator and CD-based galactose glycopolymer

(A), and ^{1}H NMR spectra of β-CD-p(Gal)$_{280}$ glycopolymer in D_2O **(B)**.

Table S1. Summary of number average molar masses (M_n) and molar mass distributions ($Đ$) of the synthesized mannose-containing β-cyclodextrin constructs.

Code	$M_{n,Theo}$ (g.mol^{-1})	$M_{n,SEC}$ (g.mol^{-1})	$Đ$
β-CD-$(N_3)_7$	1350	3400	1.02
β-CD-$(Man)_7$	2875	5800	1.05
β-CD-$(Br)_7$	2730	4600	1.04
β-CD-p$(Man)_{280}$	107200	32300	1.20
β-CD-p$(Gal)_{280}$	107200	34800	1.22

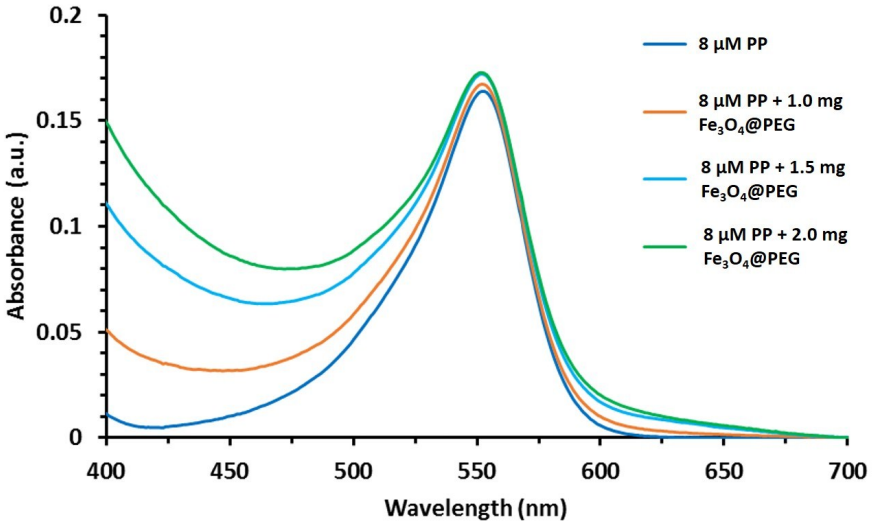


Figure S10. UV-vis spectra of 8 µM PP solutions at pH=10.2 after addition of varying amounts of Fe_3O_4@PEG MNPs.

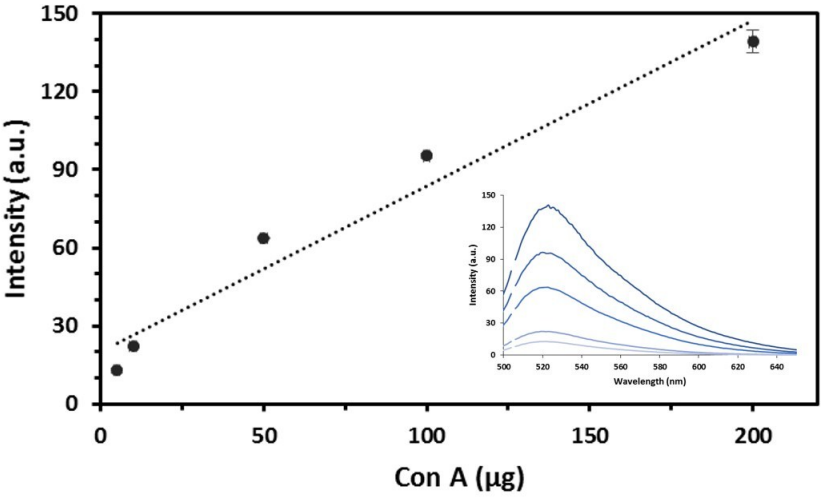


Figure S11. Calibration curve of FITC-labelled ConA using fluorescence spectroscopy.

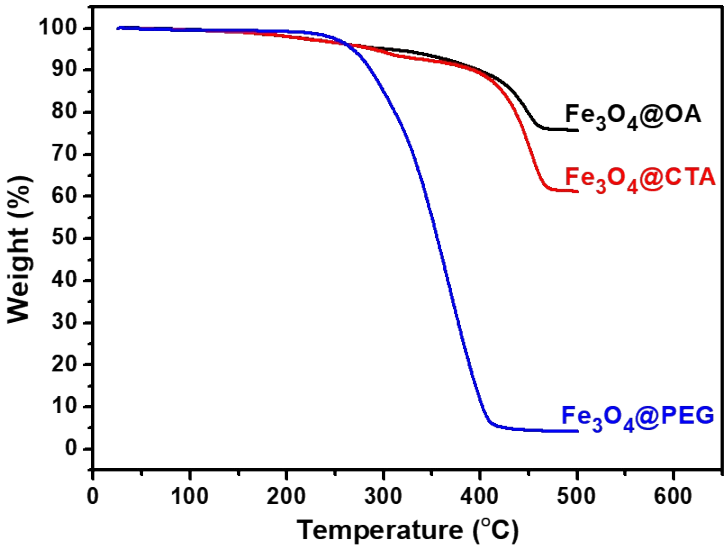


Figure S12. TGA measuremants of OA, CTA and PEG polymer coated MNPs.

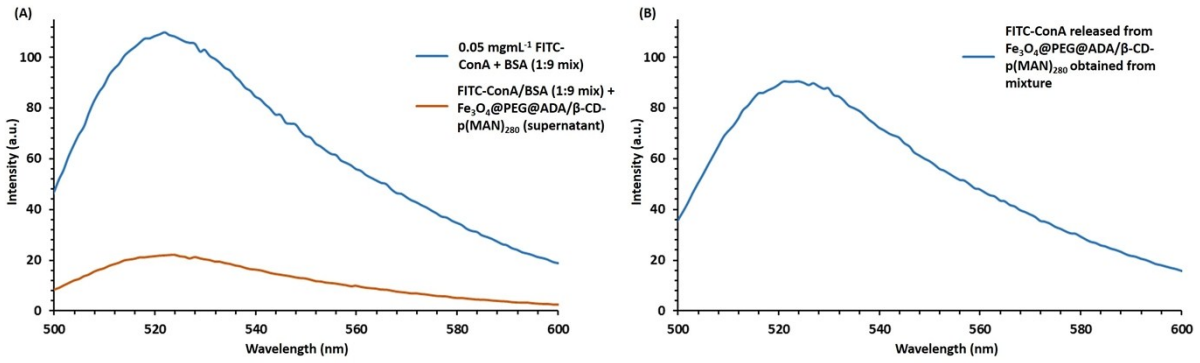


Figure S13. Fluorescence spectra of (A) FITC-ConA/BSA mixture solution and supernatant after treatment with Fe_3O_4@PEG@ADA/β-CD-p$(MAN)_{280}$ MNPs, and (B) released ConA.

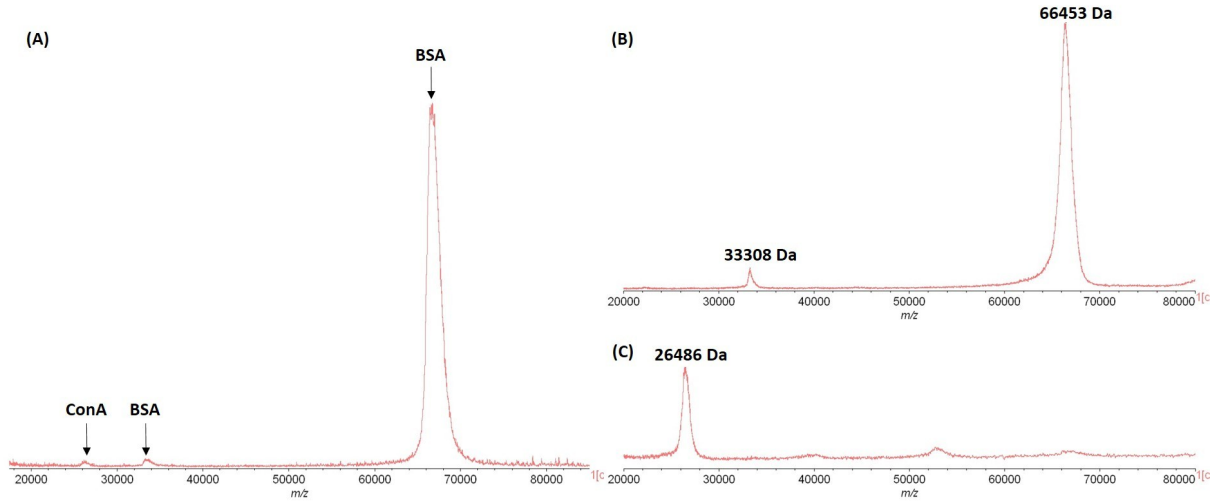


Figure S14. MALDI-TOF mass spectra of (A) the BSA/ConA mixture (9:1); (B) residual BSA in the supernatant after magnetic separation and (C) ConA recovered from MNPs ConA-BSA mixture.

1) B. Roy and B. Mukhopadhyay, Tetrahedron Lett. 2007, 48, 3783-3787.