Supporting Information

Iminosugar-based glycopolypeptides: glycosidase inhibition with bioinspired glycoprotein analogue micellar self-assemblies

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Materials

All chemicals were purchased from Sigma-Aldrich and used as received unless otherwise noted. γ-Benzyl-L-glutamate and *DL*-propargylglycine were supplied by Bachem. Anhydrous DMF, DMSO, ethyl acetate, THF, and methanol were used directly from the bottle under an inert and dry atmosphere. γ-benzyl-*L*-glutamate NCA was synthesized following a literature procedure.[1] 2,3,4,6-Tetra-*O*-acetyl-β-*D*-galactopyranosylazide was synthesized following a literature procedure.[1] *N*-(6-Azidohexyl)-1,5-dideoxy-1,5-imino-*D*-glucitol was synthesized following a literature procedure.[2] The spectroscopic data of all the synthesized products were in agreement with literature data.

Methods

^{1}H NMR spectra were recorded at room temperature with a Bruker Avance 400 (400 MHz) and a Bruker Avance Ultrashield 600 (600 MHz). DMSO-d_6 and acetone-d_6 were used as solvents and signals were referred to the signals of residual protonated solvent signals. TMS was used as an internal standard for DMSO-d_6. Infrared measurements were performed on a Bruker Tensor 27 spectrometer using the attenuated total reflection (ATR) method. SEC analyses of glycosylated (γ-benzyl-L-glutamate–β–DL-propargylglycine) were performed in N,N,-dimethylacetamide (DMAc SEC) at 60 °C on a system equipped with a Waters 2695 separation module, a Waters 2414 refractive index detector (50 °C), a Waters 486 UV detector, and a PSS GRAM guard column followed by two PSS GRAM columns in series of 100 (10 mm particles) and 3000 (10 mm particles), respectively. DMAc was used as eluent at a flow rate of 1 mL/min. The molecular weights were calculated using polystyrene standards. Before SEC analyses were performed, the samples were filtered through a 0.2 μm PTFE filter (13 mm, PP housing, Alltech). Transmission Electron Microscopy (TEM) images were recorded on a Hitachi H7650 microscope working at 80 kV equipped with a GATAN Orius 11 Megapixel camera. Samples were prepared by spraying a 0.2 g/L solution of the block copolymer onto a copper grid (200 mesh coated with carbon) using a homemade spray tool and negatively stained with 1.2% uranyl acetate. Dynamic Light Scattering (DLS) was used to obtain the average size of the particles right after dialysis by using a Malvern ZetaSizer NanoZS instrument with 90° backscattering measurements at 25 °C. Atomic Force Microscopy (AFM) images were recorded in air with a Nanoscope III in dry Tapping mode. AFM measurements were performed at room temperature using a Veeco Dimension Icon AFM system equipped with a Nanoscope V controller. Topographic images of nanoparticles were obtained in

tapping mode using rectangular silicon cantilever (AC 160-TS, Atomic Force, Germany) with a spring constant of 42 N m^{-1}, a resonance frequency lying in the 290–320 kHz range, and a radius of curvature of less than 10 nm. The scan rates were in a range of 0.6 to 0.8 Hz. Samples were prepared by adsorption onto freshly cleaved HOPG (High Oriented Pyrolitic Graphite) from solutions at 1g.l^{-1} and allowed to dry overnight at room temperature.

Synthesis of propargylglycine NCA:
DL-propargylglycine (2.5 g, 22.1 mmol) and *α*-pinene (14.88 g, 109 mmol) were dissolved in 60 ml of anhydrous THF in a three-neck round-bottom flask. The reaction mixture was heated at 50 °C under nitrogen and then triphosgene (4.92 g, 16.6 mmol) in 20 mL of THF was added drop-wise over a period of one hour. The reaction was continued for 4 hours until the mixture became gradually clear. The mixture was concentrated under reduced pressure and the NCA precipitated by addition of 100 ml n-heptane. The mixture was then placed in a freezer overnight. After filtration, the crude product was dissolved in dry THF, and re-crystallized twice by addition of n-heptane. The obtained solid was washed with *n*-heptane, yielding white crystals in 75% yield. ^{1}H-NMR (400 MHz, acetone-d_6, δ, ppm): 2.62 (t, J = 2.5 Hz, 1H, ≡C**H**), 2.86 (dd, J = 4.5 and 2.5 Hz, 2H, -C**H$_2$**-C≡), 4.75 (t, J = 4.5 Hz, 1H, C**H**), 8.05 (s, 1H, N**H**). ^{13}C-NMR (400 MHz, acetone-d_6, δ, ppm): 22.26 (-**C**H_2-C≡), 57.30 (**C**H), 73.64 (≡**C**H), 78.23 (-**C**≡CH), 152.71 (-O(**C**O)NH-), 170.62 (-O(**C**O)CH). FTIR (neat, cm^{-1}): 3363, 3247, 1854, 1771, 1286, 1195, 1111, 1089, 934, 893, 777, 756, 723, 698, 668. Mp: 114 °C. For more characterizations or details regarding the preparation of propargylglycine NCA monomer, readers are encouraged to consult the reference 1 of this present supporting information.

Typical synthesis procedure of poly(γ-benzyl-*L*-glutamate–*b*–poly(*D*, *L* propargylglycine)1 :
The NCA monomer of γ-benzyl-L-glutamate (1.01 g, 3.82 mmol) was dissolved in 9 ml of DMF in a Schlenk tube. A solution of benzylamine (20.44 mg, 0.191 mmol) in 2 mL of dry DMF was added after NCA was dissolved. The reaction was stirred in a cold water bath at 0°C for 4 days under an inert atmosphere. After 4 days BLG-NCA had been completely consumed as monitored by FTIR and NMR and the PBLG macroinitiator was added to a solution of DL-propargylglycine NCA (267 mg, 1.9 mmol) in DMSO at room temperature. The reaction mixture was stirred for another five days. The reaction mixture was precipitated into an excess of diethylether, filtered and dried under vacuum obtaining a pale yellow solid (80%). ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 7.22(s broad, benzyl), 4.53-5.21(m broad, -C**H**-NH- + -O-C**H$_2$**-), 3.8-4.3 (s broad, -C**H**-NH-), 2.8 (s, C**H**CCH_2), 1.9-2.7 (m broad, -C**H$_2$**-CH_2-C(O)- + -CH_2-C**H$_2$**-C(O)- + CHCC**H$_2$**).

Typical synthesis procedure of poly(γ-benzyl-*L*-glutamate–*b*–poly(*D*,*L*-glycosylated propargylglycine)3: Poly(γ-benzyl-L-glutamate–*β*–*DL*-propargylglycine) **1** (50 mg), *N*-(6-azidohexyl)-1,5-dideoxy-1,5-imino-D-glucitol **2** (63mg, 1.2 equiv. to alkyne groups) and triethylamine (14.2 μL, 0.5 equiv) were dissolved in 8 mL of anhydrous DMSO in the Schlenk tube. The mixture was stirred and degassed by bubbling nitrogen for 30 min. $(PPh_3)_3$CuBr (19 mg, 0.1 equiv.) was then added and nitrogen was bubbled through the resulting solution for another 30 min. Then the Schlenk tube was placed in an oil bath at 30 °C for 72 h under nitrogen atmosphere. Then amberlite weak acid cation exchange resin (Sigma Aldrich, 150 mg) was added to remove traces of catalyst and the suspension gently stirred at ambient temperature overnight. After filtration and centrifugation, the polymer solution was precipitated in a 2:1 THF/diethyl ether mixture and washed with THF twice. The polymer was separated by centrifugation and dialyzed against distilled water for 3 days, then lyophilized giving an off-white polymer (yield 50%).

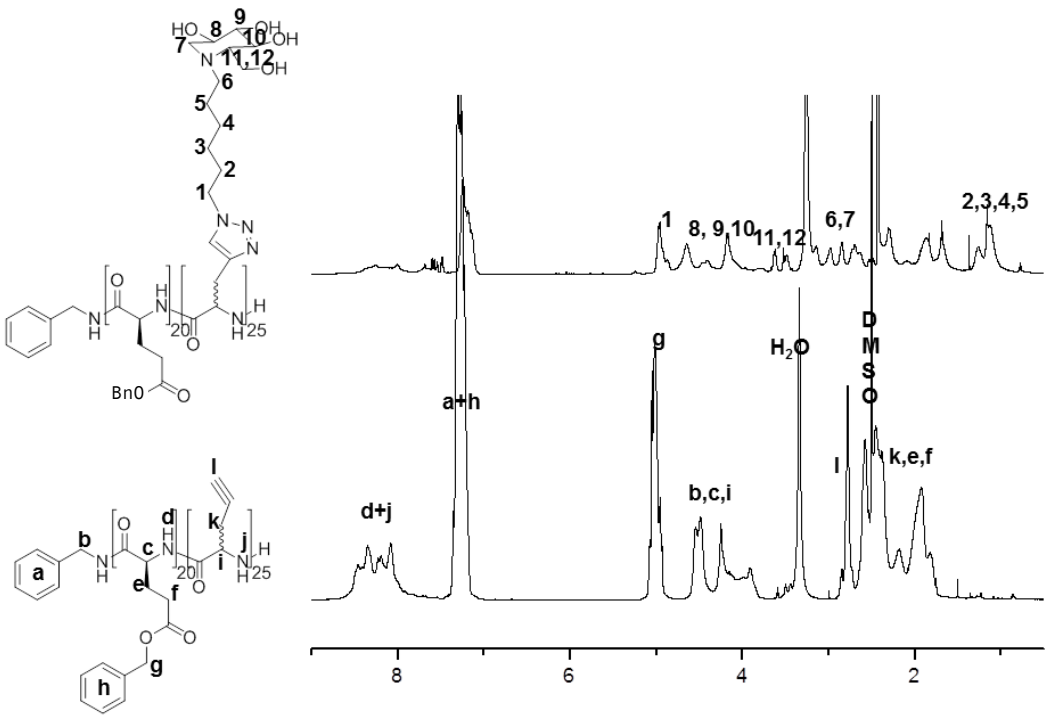


Figure S1 ^{1}HNMR of polymers before glycosylation (down) and after glycosylation (top) in DMSO-d_6

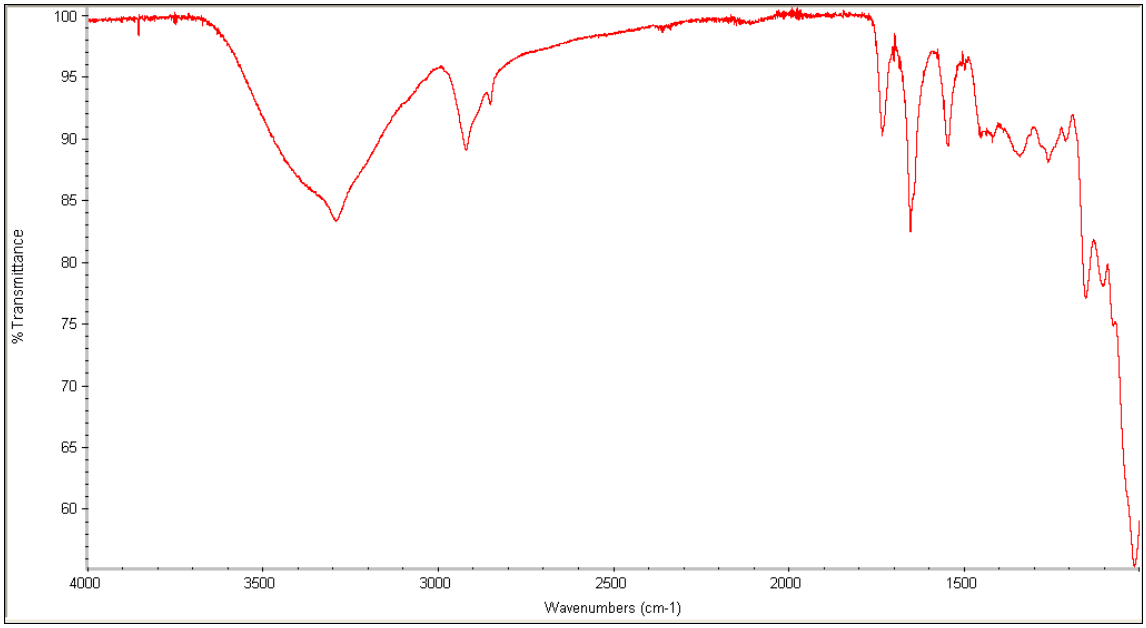


Figure S2 FTIR spectra of the polymer after glycosylation

	M_n before glycosylation (g/mol)	M_n after glycosylation (g/mol)	PDI after glycosylation
$PBLG_{20}$-*b*-PG_{25} (**3**)	5900	15000	1.4

Self-assembly of 3:
0.5 mL of block copolymer **3** solution in DMSO (5 mg/mL, filtered with 0.22 µm cellulose acetate membrane) was added instantaneously (1 s) into a glass vial containing 4.5 mL of ultrapure water under magnetic stirring (500 rpm) at 50 °C. The mixture was then dialyzed 24 h against water (Spectra/Por® MWCO 50kDa membrane) to remove the DMSO.

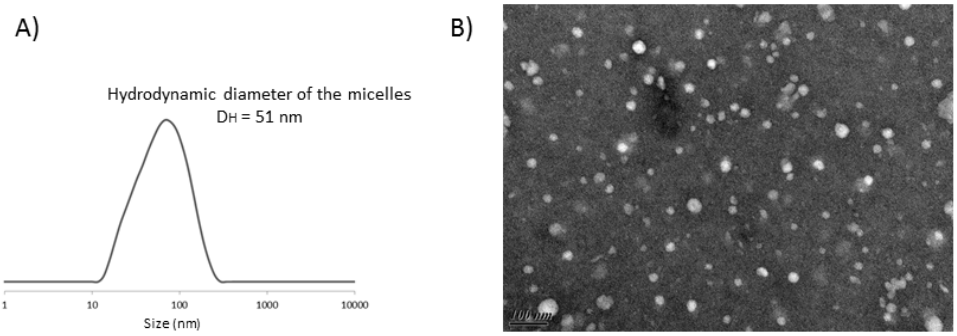
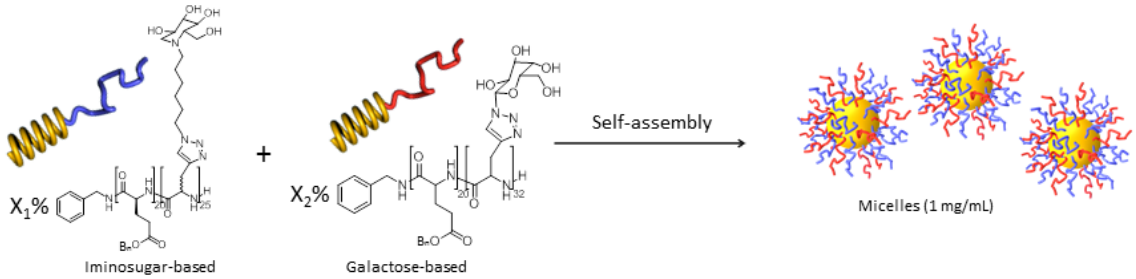


Figure S3 A) Intensity profile measured by dynamic light scattering of the nanoparticles; B) TEM imaging of the glycopolypeptide **3** after self-asembly.

Self-assembly of a mixture of 3 and poly(γ-benzyl-*L*-glutamate–*b*–poly(*D*, *L*-galactosylated propargylglycine):
0.5 mL of block copolymers binary solution containing various content of **3** (DMSO, 5 mg/mL, filtered with a 0.22 µm cellulose acetate membrane) was added instantaneously (1 s) into a glass vial containing 4.5 mL of ultrapure water under magnetic stirring (500 rpm) at 50 °C. The mixture was then dialyzed 24 h against water (Spectra/Por® MWCO 50kDa membrane) to remove the DMSO.



Sample	Copolymer concentration	X1 content	Iminosugar (NJ) concentration	Hydrodynamic diameter of the micelles	Polydispersity
1	1 mg/mL	0 %	0 mM	35 nm	0.24
2	1 mg/mL	10 %	0.18 mM	35 nm	0.22
3	1 mg/mL	33 %	0.56 mM	34 nm	0.24
4	1 mg/mL	50 %	0.86 mM	28 nm	0.22
5	1 mg/mL	66 %	1.12 mM	33 nm	0.25
6	1 mg/mL	100 %	1.70 mM	51 nm	0.25

Figure S4 Mixture of copolymers used to prepare micelles having different ratio of *N*-(6-azidohexyl)-1,5-dideoxy-1,5-imino-D-glucitol **2**.

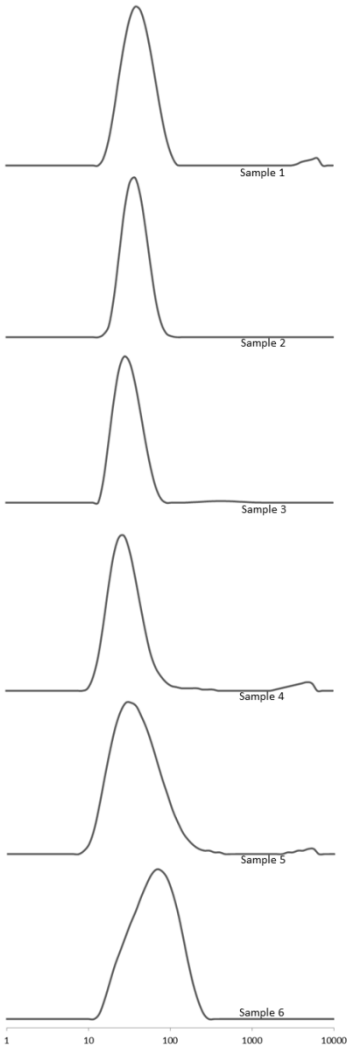


Figure S5 Dynamic light scattering analysis of samples 1-6 (scale in nm)

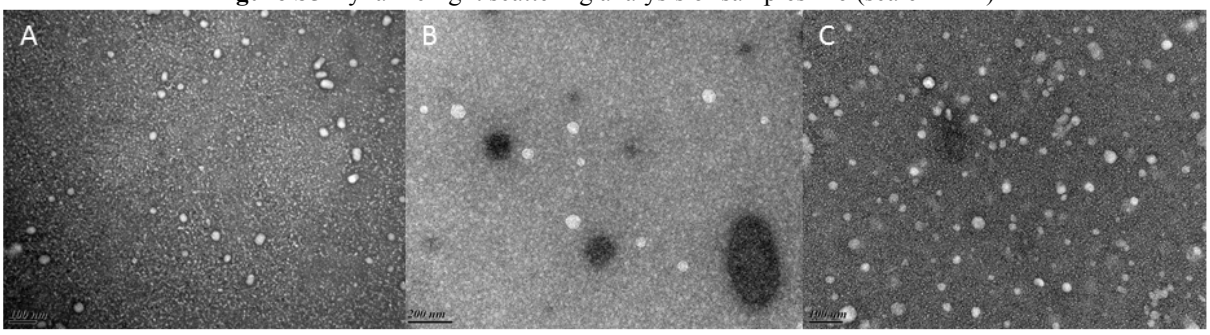


Figure S6 TEM imaging of the glycopolypeptides based micelles : A) sample **1**; B) sample **4**; C) sample **6**.

Glycosidase inhibition

General Procedures for Inhibition Assay. The glycosidases α-*Glc*ase (from yeast), isomaltase (from yeast), β-*Glc*ase (from almond), β-*Glc*ase (from bovine liver, cytosolic), naringinase (*Penicillium decumbes*), α-*Gal*ase (from green coffee beans), β-*Glc*ase (from *Escherichia coli*), α-*Man*ase (from jack bean) and β-*Man*ase (from *Helix pomatia*) were used in the inhibition studies, as well as the corresponding *o*- and *p*-nitrophenyl glycoside substrates, and were purchased from Sigma Chemical Co. Inhibitory potencies were determined spectrophotometrically measuring the residual hydrolytic activities of the glycosidases against the respective *o*- (for β-*Gal*ases from bovine liver and *Escherichia coli*) or *p*-nitrophenyl α- or β-D-glycopyranoside, in the presence of the corresponding iminosugar derivative. Each assay was performed in phosphate (at pH 7.3 or 6.8) or phosphate-citrate (for α- or β- *Man*ase at pH 5.5) buffer at the optimal pH for each enzyme. The K_m values for the different glycosidases used in the tests and the corresponding working pHs are listed herein: α-*Glc*ase (from yeast), K_m = 0.35 mM (pH 6.8); isomaltase (from yeast), K_m = 1.0 mM (pH 6.8); β- *Glc*ase (almond), K_m = 3.5 mM (pH 7.3); β-*Glc*ase (bovine liver), K_m = 1.0 mM (pH 7.3); naringinase (*Penicillium decumbes*), K_m = 2.7 mM (pH 6.8); α-*Gal*ase (coffee beans), K_m = 2.0 mM (pH 6.8); β-*Gal*ase (from *Escherichia coli*), K_m = 0.12 mM (pH 7.3); α-*Man*ase (jack bean), K_m = 2.0 mM (pH 5.5); β-*Man*ase (*Helix pomatia*), K_m = 0.6 mM (pH 5.5). The reactions were initiated by addition of enzyme to a solution of the substrate in the absence or presence of various concentrations of inhibitor. After the mixture was incubated for 10-30 min at 37 ºC, the reaction was quenched by addition of 1 M Na_2CO_3. The peak of absorbance of the resulting mixture was observed at 405 nm. The K_i value and enzyme inhibition mode were determined from the slope of Lineweaver-Burk plots and double reciprocal analysis using a Microsoft Office Excel 2007 program.

Enzymes	6	Monomer 4
α−glucosidase activity		
α−glucosidase (baker´s yeast)	**n.i.**	**395**
Isomaltase (baker yeast)	**n.i.**	**n.i.**
Amyloglucosidase (Asp. niger)	**4.8**	**2.8**
β-glucosidase activity		
β-glucosidase (bovine liver)	**n.i.**	**n.d.**
β−glucosidase (almonds pH 7.3)	**n.i.**	**15**
β−glucosidase (almonds pH 5.5)	**n.d.**	**38**
Naringinase (penicilium decumbens)	**n.i.**	**13.8**
β-galactosidase activity		
β-galactosidase (bovine liver)	**n.i.**	**138**
β-galactosidase (Escherichia coli)	**n.i.**	**n.i.**
α−galactosidase activity		
α−galactosidase (green coffee)	**n.i.**	**207**
α−mannosidase activity		
α−mannosidase (jack bean)	**0.15**	**516**
β−mannosidase activity		
β−mannosidase (helix pomatia)	**n.i.**	**n.i.**

Table S2. Activity of sample 6 (cf figure S4) against various glycosidases evidencing a significant potency for α-mannosidase inhibition.

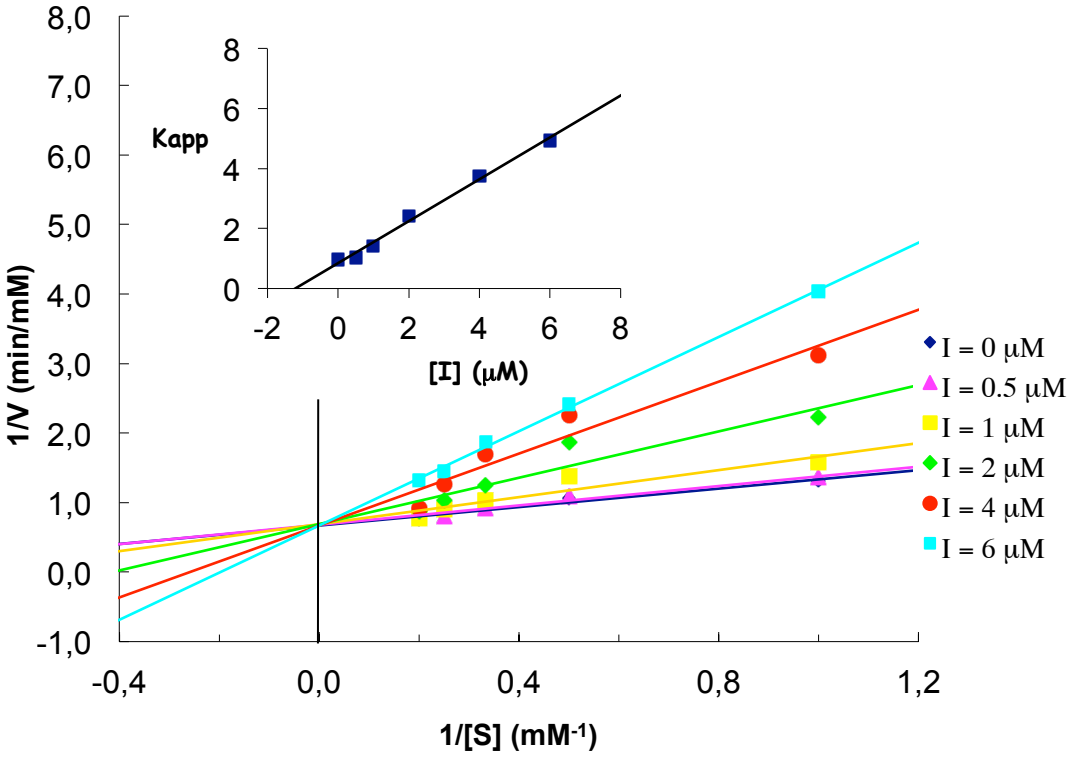


Figure S7. Lineweaver-Burk Plot for K_i determination (1.2 µM) of **sample 3** against α-*Man*ase (Jack beans; pH 5.5).

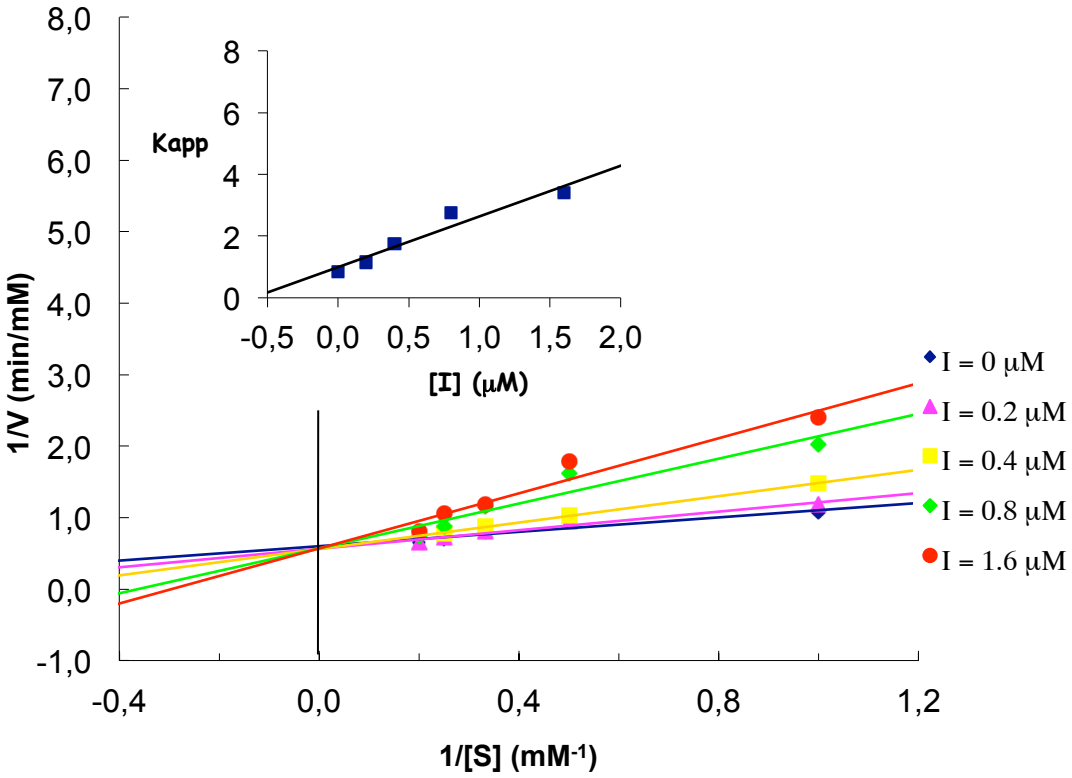


Figure S8. Lineweaver-Burk Plot for K_i determination (0.60 µM) of **sample 4** against α-*Man*ase (Jack beans; pH 5.5).

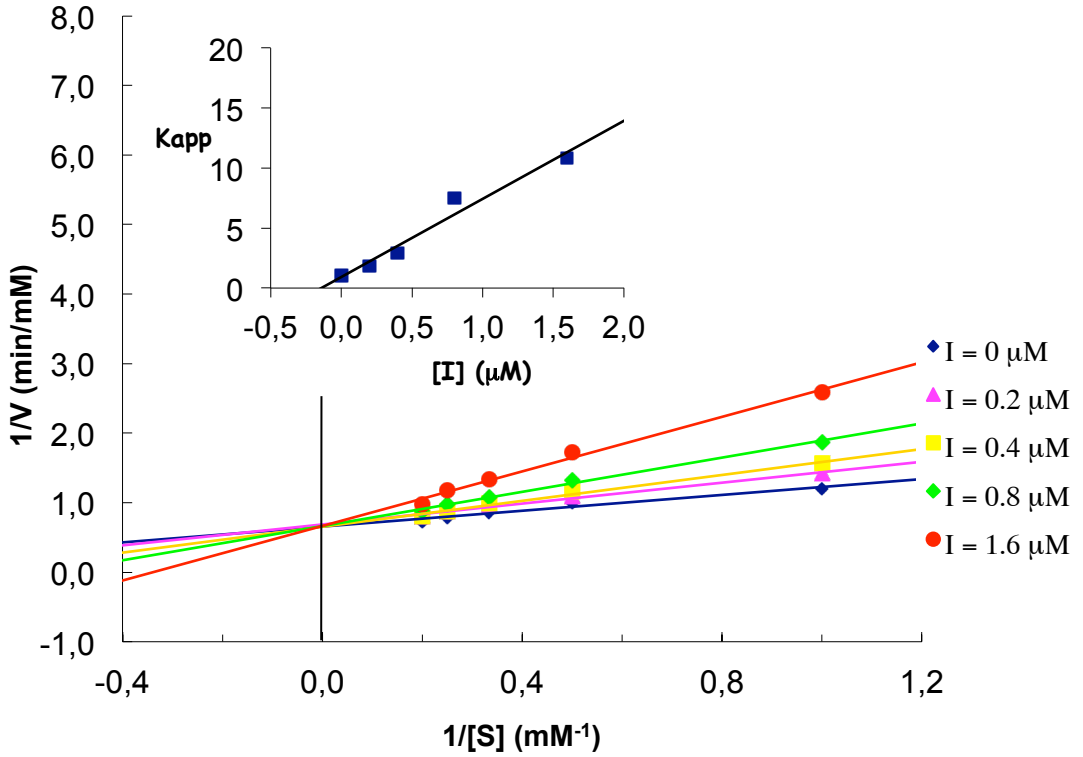


Figure S9. Lineweaver-Burk Plot for K_i determination (0.15 µM) of **sample 5** against α-*Man*ase (Jack beans; pH 5.5).

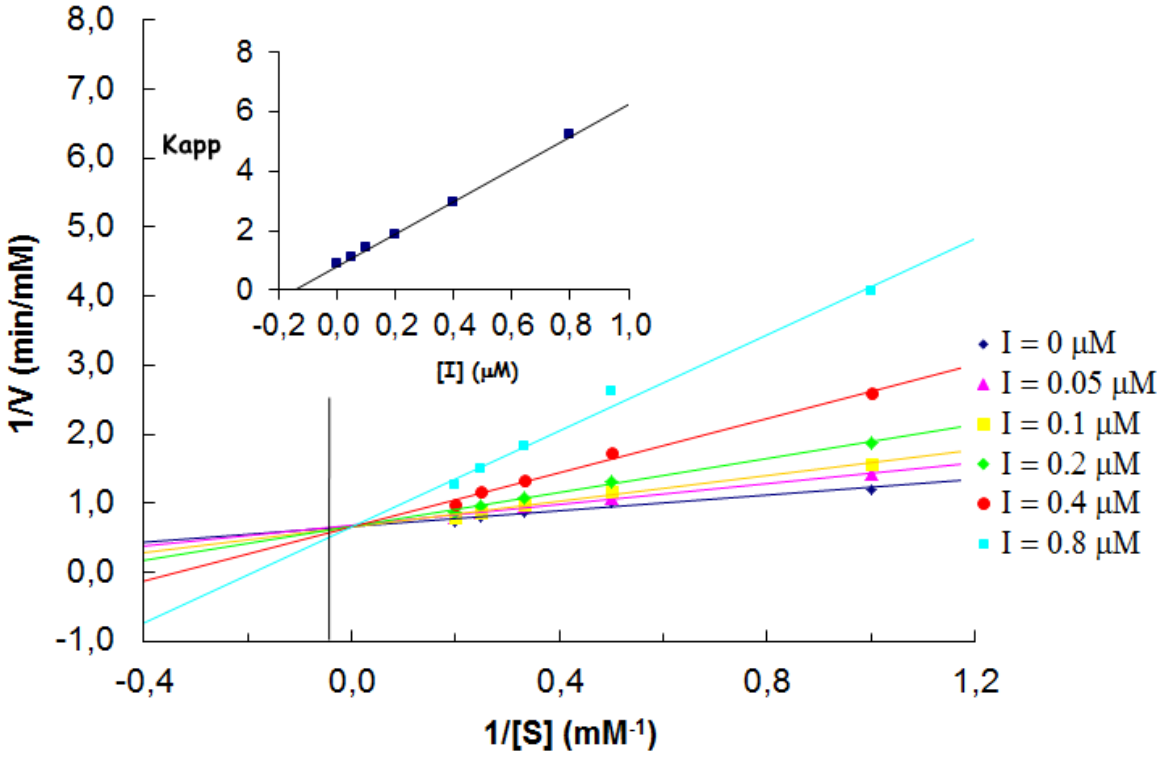


Figure S10.Lineweaver-Burk Plot for K_i determination (0.15 µM) of **sample 6** against α-*Man*ase (Jack beans; pH 5.5).

1. J. Huang, C. Bonduelle, J. Thevenot, S. Lecommandoux and A. Heise, *J. Am. Chem. Soc.*, 2012, **134**, 119-122.
2. P. Compain, C. Decroocq, J. Iehl, M. Holler, D. Hazelard, T. Mena Barragán, C. Ortiz Mellet and J. F. Nierengarten, *Angew. Chem. Int. Ed.*, 2010, **49**, 5753-5756.