Ultra-Sub-Stoichiometric 'Dynamic' Bioconjugation Reduces Viscosity by Disrupting Immunoglobulin Oligomerization

Yuhui Gong, [‡,ǁ] Hoda Soleymani Abyaneh, [‡,¥] Nicole Drossis, [ζ] Andreas Niederquell, [§] Martin Kuentz,[§] Jean-Christophe Leroux, [ǁ] Hendrick de Haan, [ζ] and Marc A Gauthier [*,¥]

[ǁ] Swiss Federal Institute of Technology Zurich (ETHZ), Department of Chemistry and Applied Biosciences, Institute of Pharmaceutical Sciences, Vladimir-Prelog-Weg 3, 8093 Zurich, Switzerland

[¥] Institut National de la Recherche Scientifique (INRS), EMT Research Center, 1650 boul. Lionel-Boulet, Varennes, J3X 1S2, Canada

[ζ] University of Ontario Institute of Technology, Faculty of Science, Oshawa, Ontario, L1H 7K4, Canada

[§] University of Applied Sciences Northwestern Switzerland, School of Life Sciences, Institute of Pharma Technology, Hofackerstr. 30, 4132 Muttenz, Switzerland

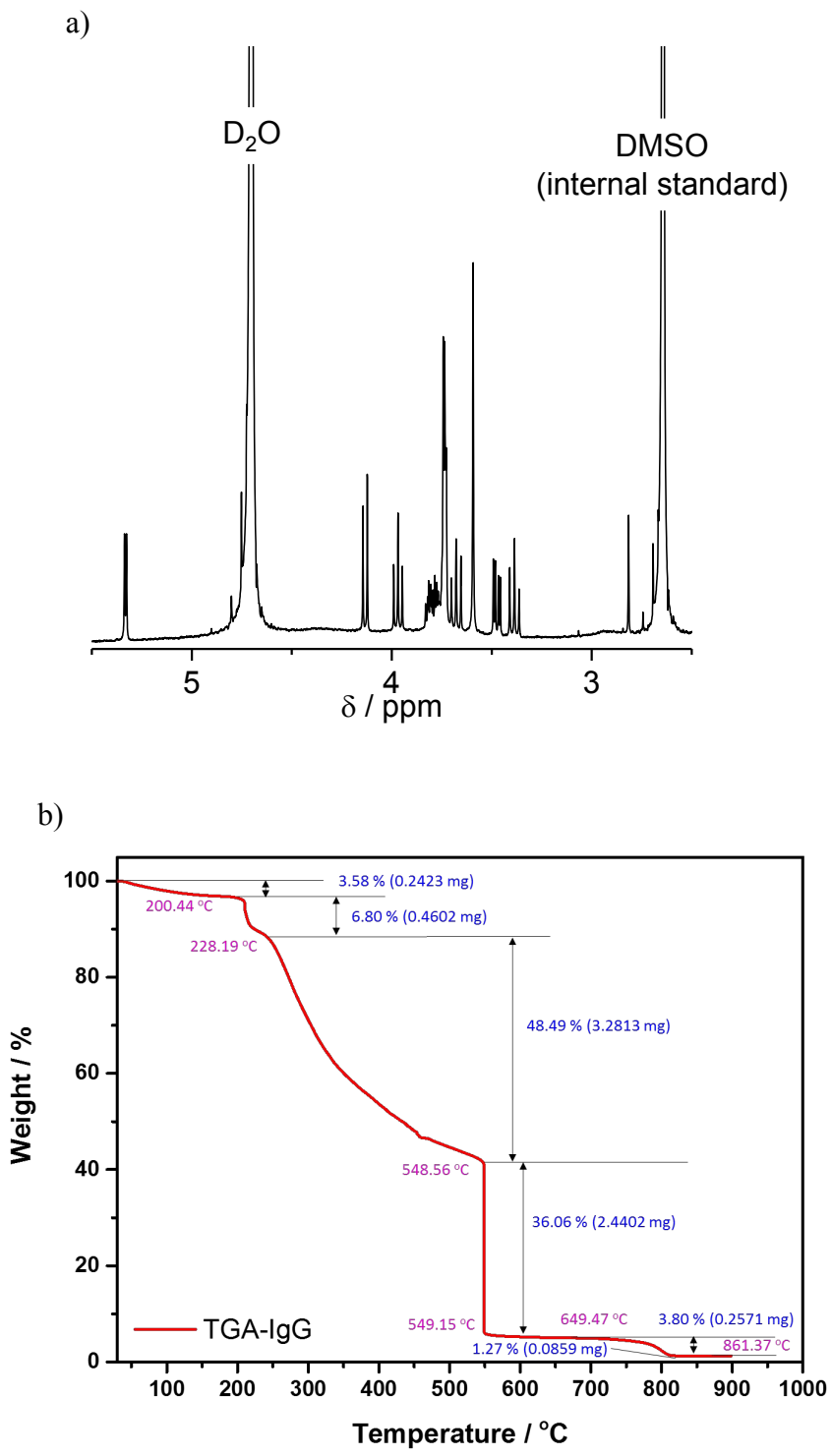
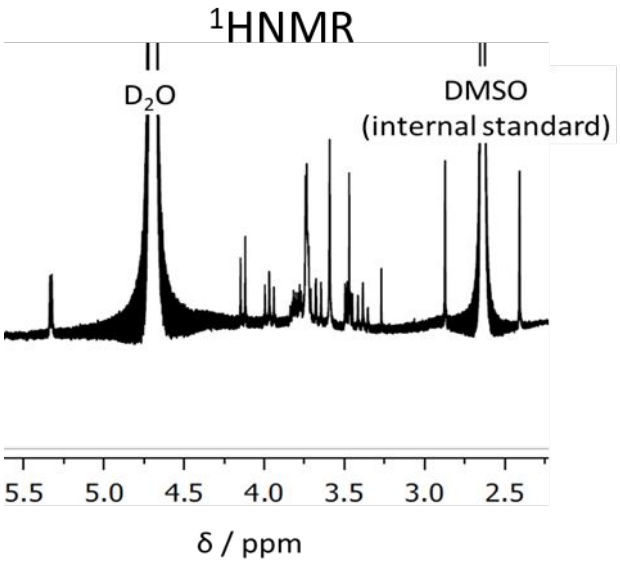


Figure S1. Analysis of lot 1 of polyclonal IgG powder. (**a**) ^{1}H NMR spectrum in D_2O containing a known concentration of DMSO (internal concentration standard). Integration of peaks of sucrose relative to that of DMSO provides the concentration of sucrose in solution, which can then be related to the dry weight of the IgG formulation dissolved. (**b**) Thermogravimetric analysis (Q500, TA Instruments, Eschborn, Germany) of IgG formulation in air at a heating rate of 20 ºC·min^{-1}. Residual buffers salts account for 1.27% of the dry weight of the commercial IgG preparation.

a)



b)

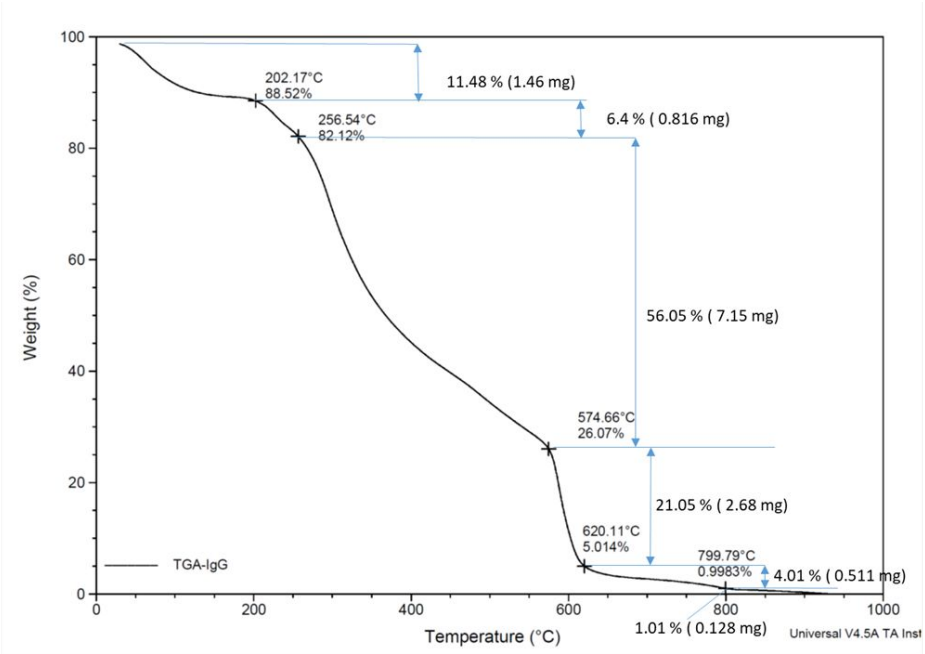


Figure S2. Analysis of lot 2 of polyclonal IgG powder. (**a**) ^{1}H NMR spectrum in D_2O containing a known concentration of DMSO (internal concentration standard). Integration of peaks of sucrose relative to that of DMSO provides the concentration of sucrose in solution, which can then be related to the dry weight of the IgG formulation dissolved. (**b**) Thermogravimetric analysis (Q500, TA Instruments, Eschborn, Germany) of IgG formulation in air at a heating rate of 20 ºC·min^{-1}. Residual buffers salts account for 1.01% of the dry weight of the commercial IgG preparation.

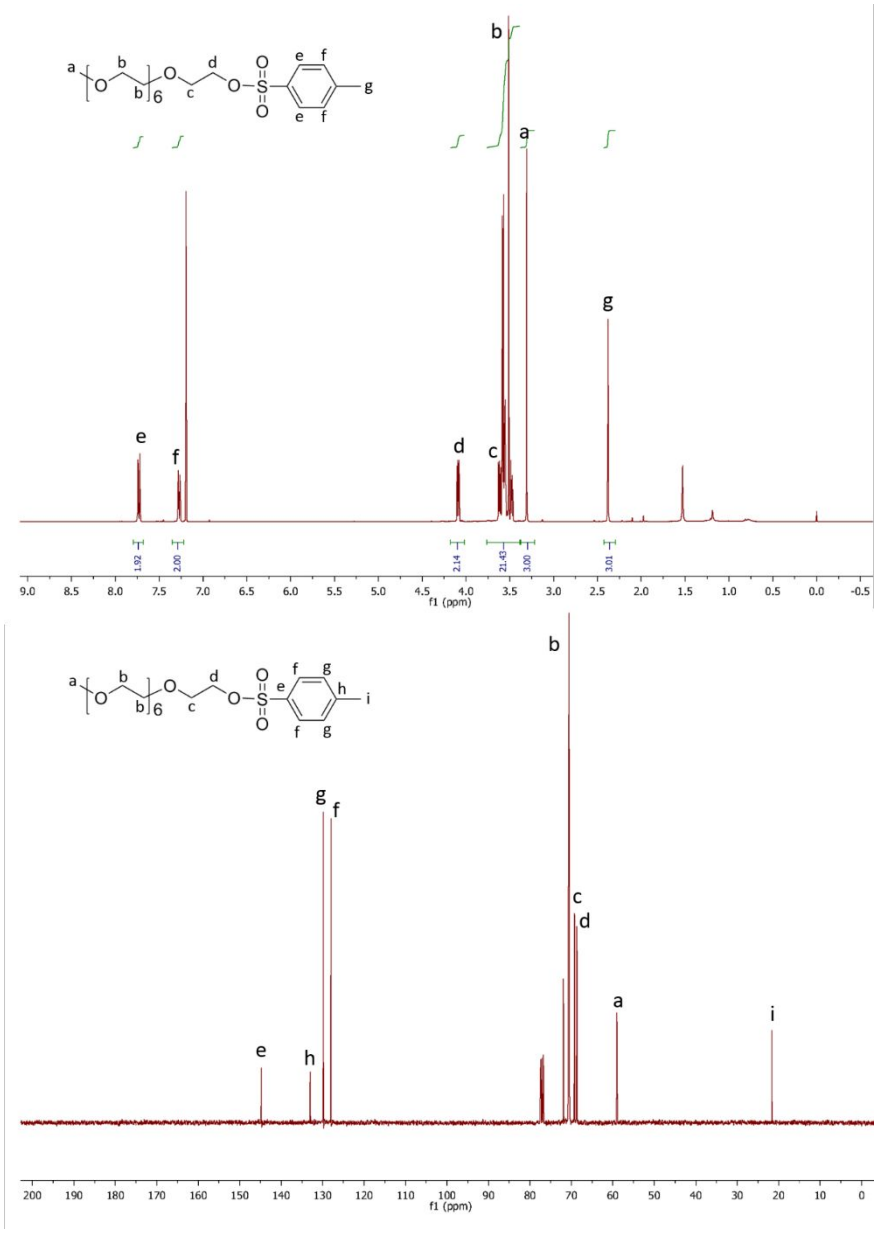


Figure S3. Assigned ^{1}H and ^{13}C NMR spectra of mPEG tosylate (0.35 kDa) in $CDCl_3$.

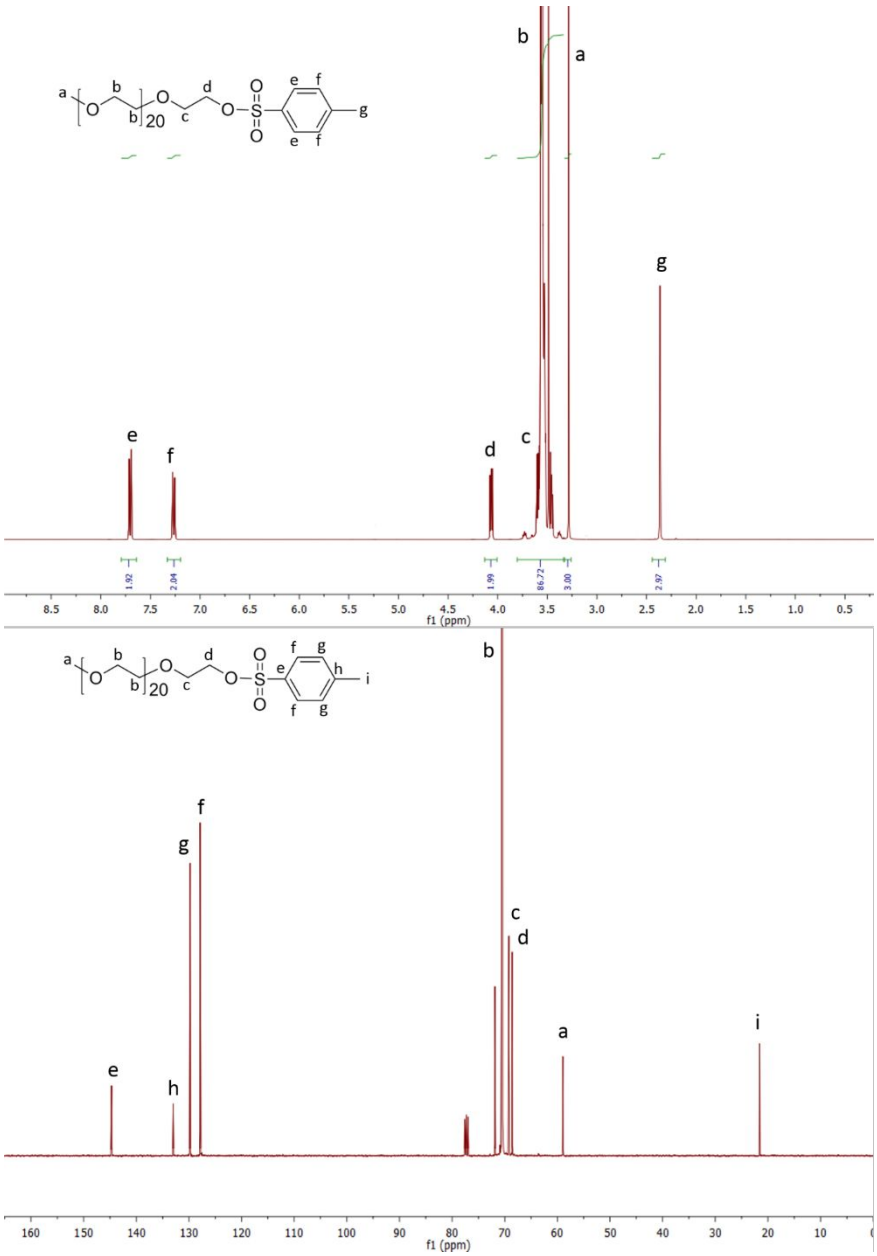


Figure S4. Assigned ^{1}H and ^{13}C NMR spectra of mPEG tosylate (1 kDa) in D_2O.

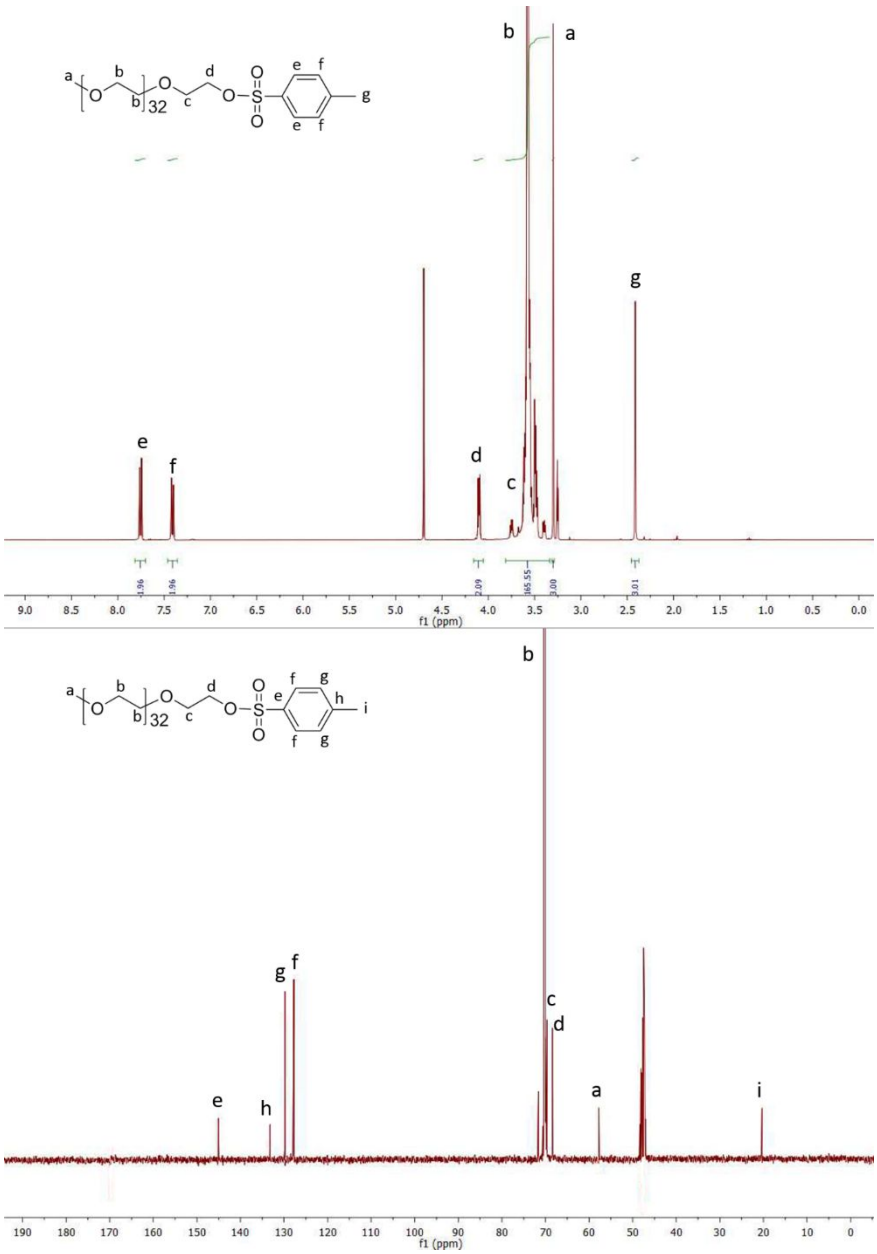


Figure S5. Assigned ^{1}H and ^{13}C NMR spectra of mPEG tosylate (2 kDa) in D_2O.

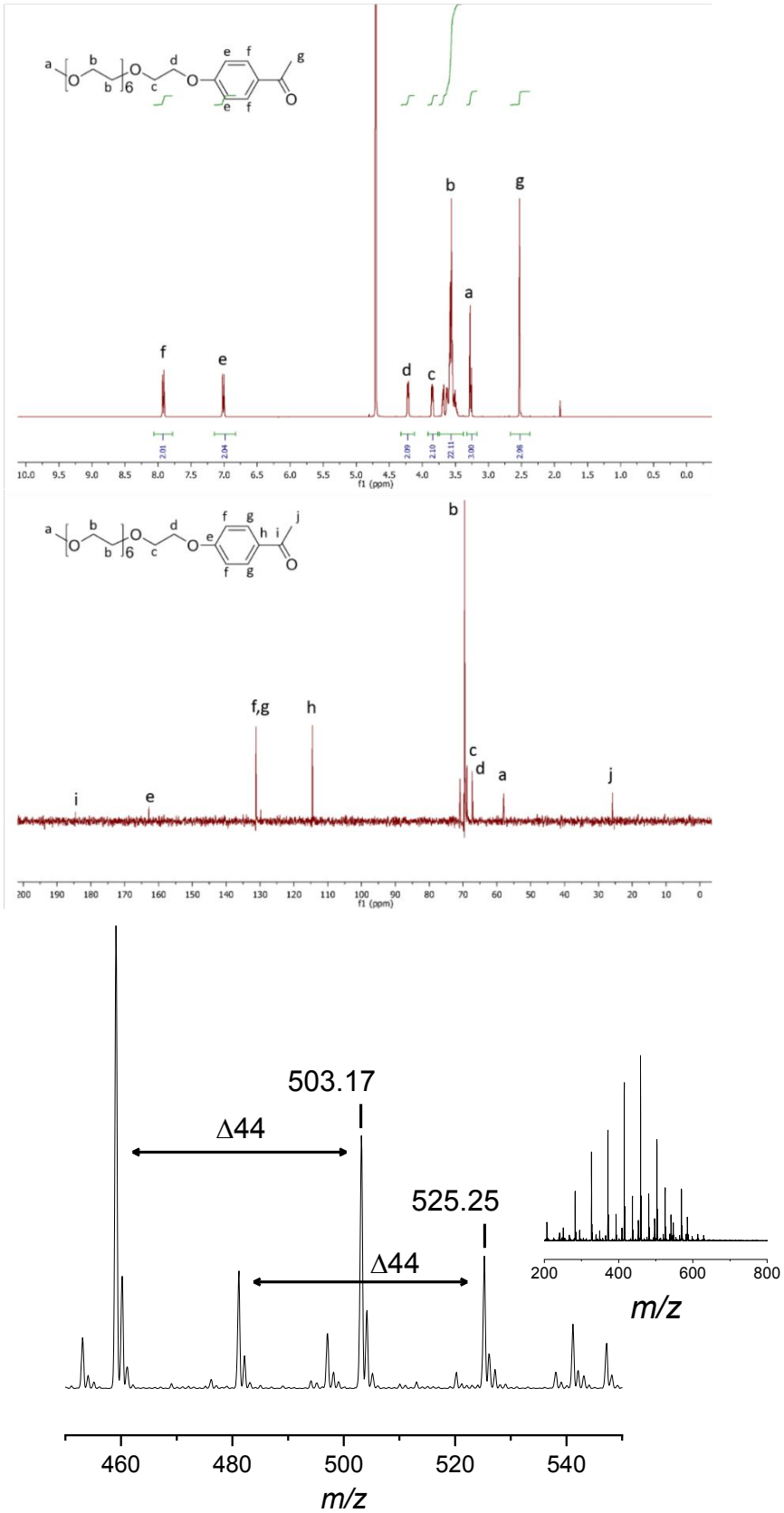


Figure S6. Assigned ^{1}H / ^{13}C NMR (D_2O) and mass spectra of compound **2** (0.35 kDa). HRMS (ESI-ion trap) m/z: Parent ions: $[M + H]^+$ Calcd for $C_{25}H_{43}O_{10}$ 503.29 (Found 503.17); $[M + Na]^+$ Calcd for $C_{25}H_{42}NaO_{10}$ 525.27 (Found 525.25).

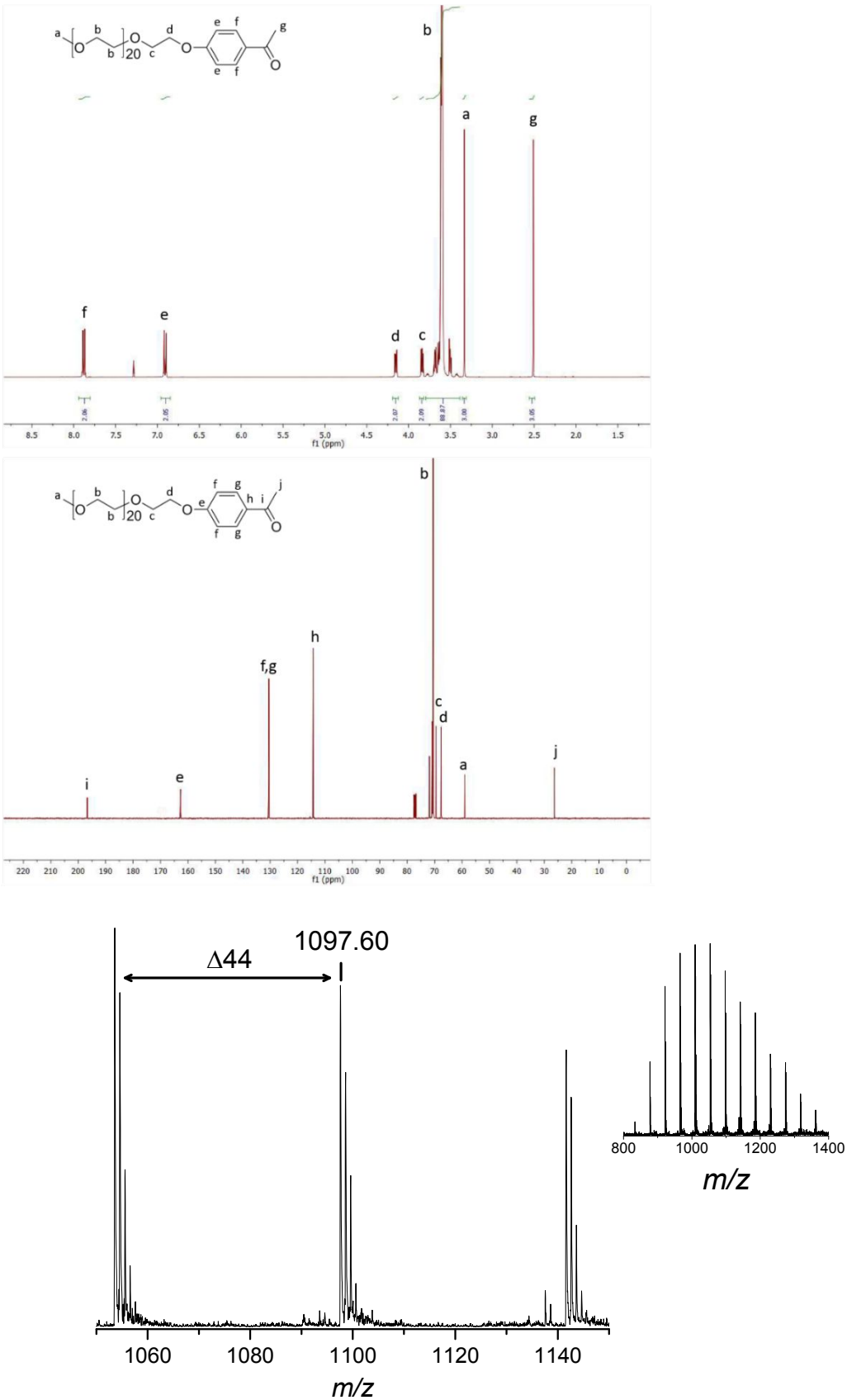


Figure S7. Assigned ^{1}H / ^{13}C NMR ($CDCl_3$) and mass spectra of compound **2** (1 kDa). HRMS (MALDTI-TOF) m/z: Parent ions: $[M + Na]^+$ Calcd for $C_{51}H_{94}NaO_{23}$ 1097.61 (Found 1097.60).

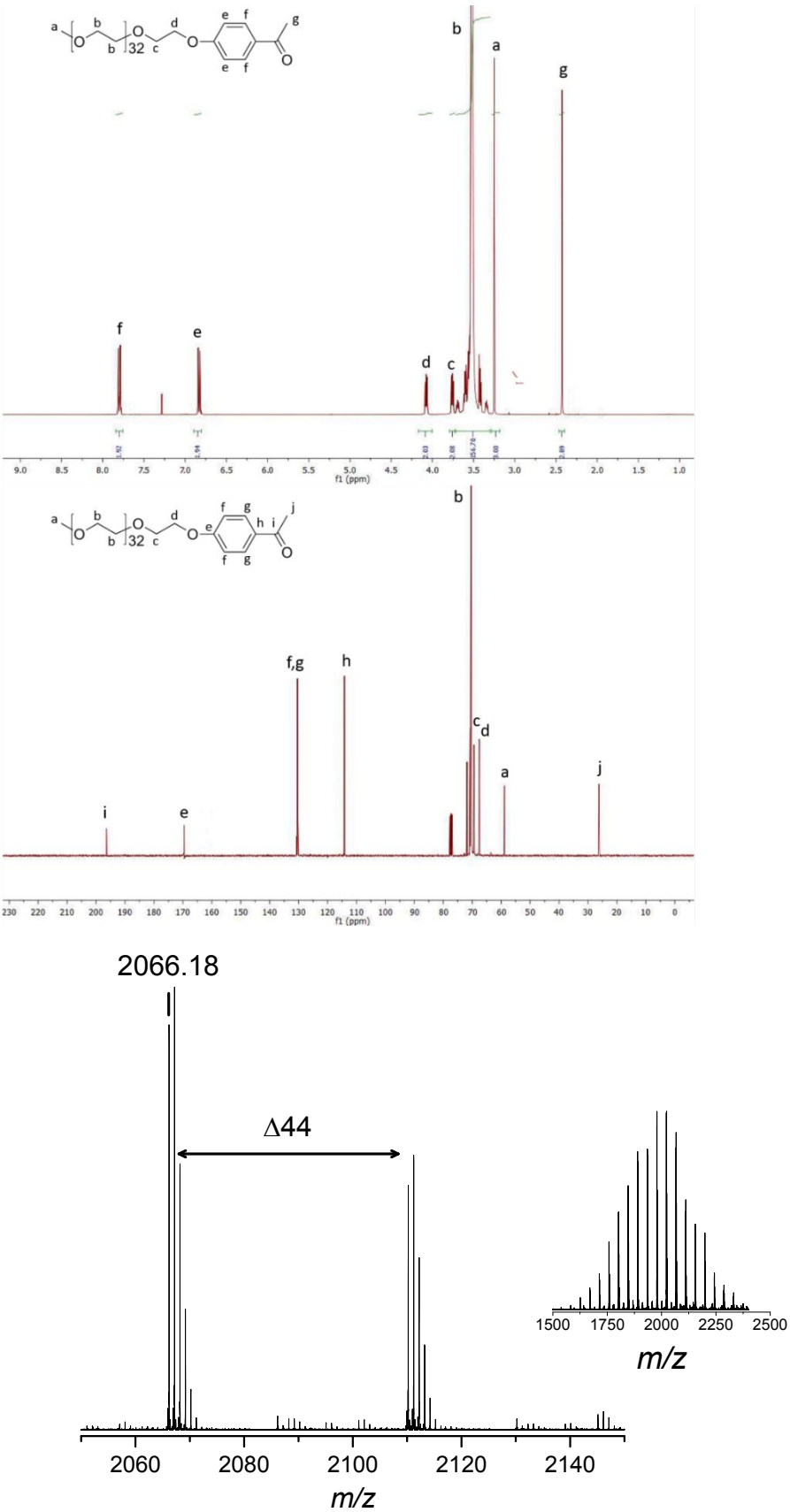


Figure S8. Assigned ^{1}H / ^{13}C NMR ($CDCl_3$) and mass spectra of compound **2** (2 kDa). HRMS (ESI-ion trap) m/z: Parent ions: $[M + Na]^+$ Calcd for $C_{95}H_{182}NaO_{45}$ 2066.18 (Found 2066.18).

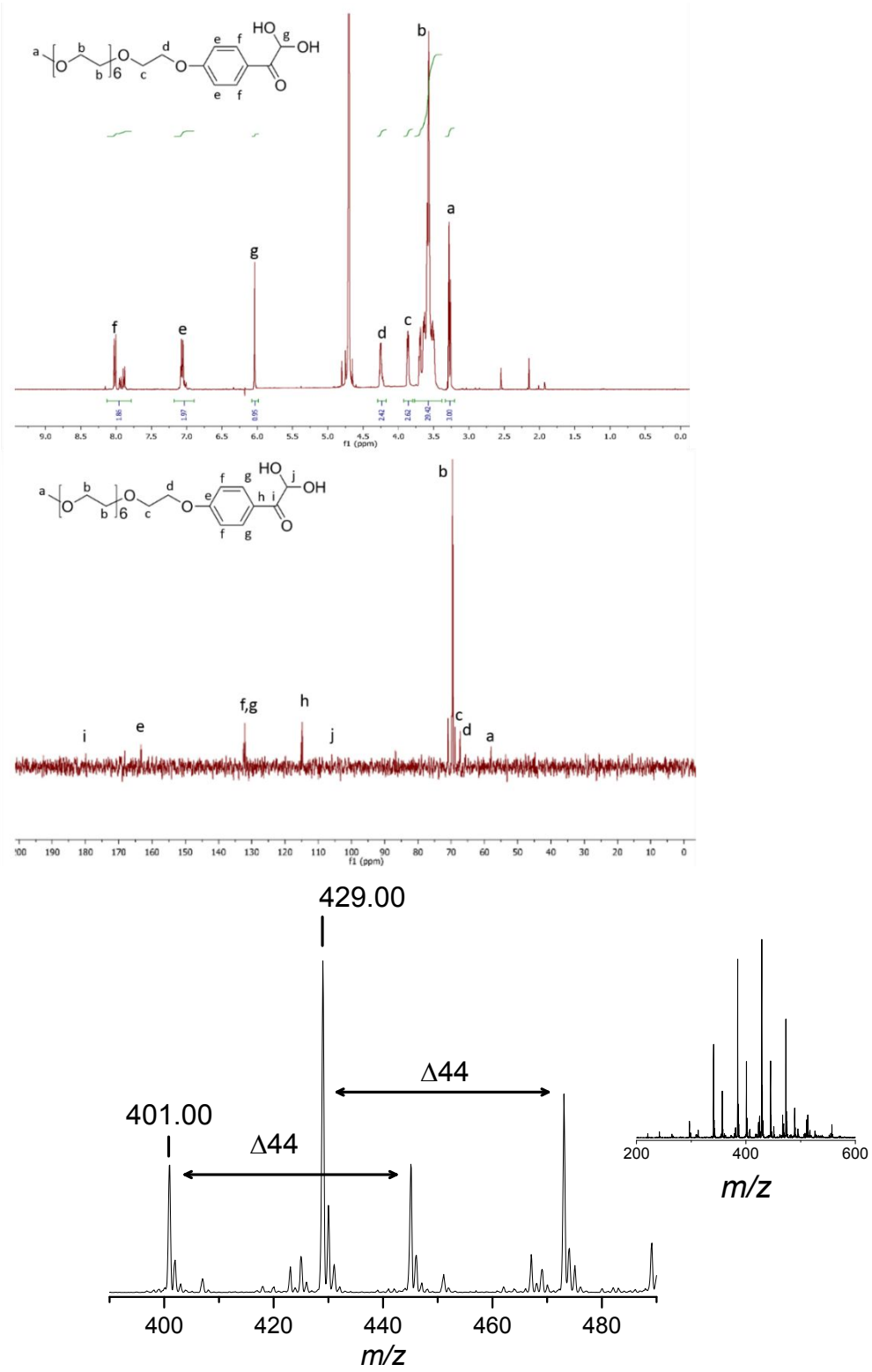


Figure S9. Assigned ^{1}H / ^{13}C NMR (D_2O) and mass spectra of compound **4** (0.35 kDa). HRMS (ESI-ion trap) m/z: Parent ions: $[M + H]^+$ Calcd for $C_{21}H_{33}O_9$ 429.21 (Found 429.00). Fragment ions: $[M + H]+$ (α-cleavage at aldehyde) Calcd for $C_{20}H_{33}O_8$ 401.22 (Found 401.00).

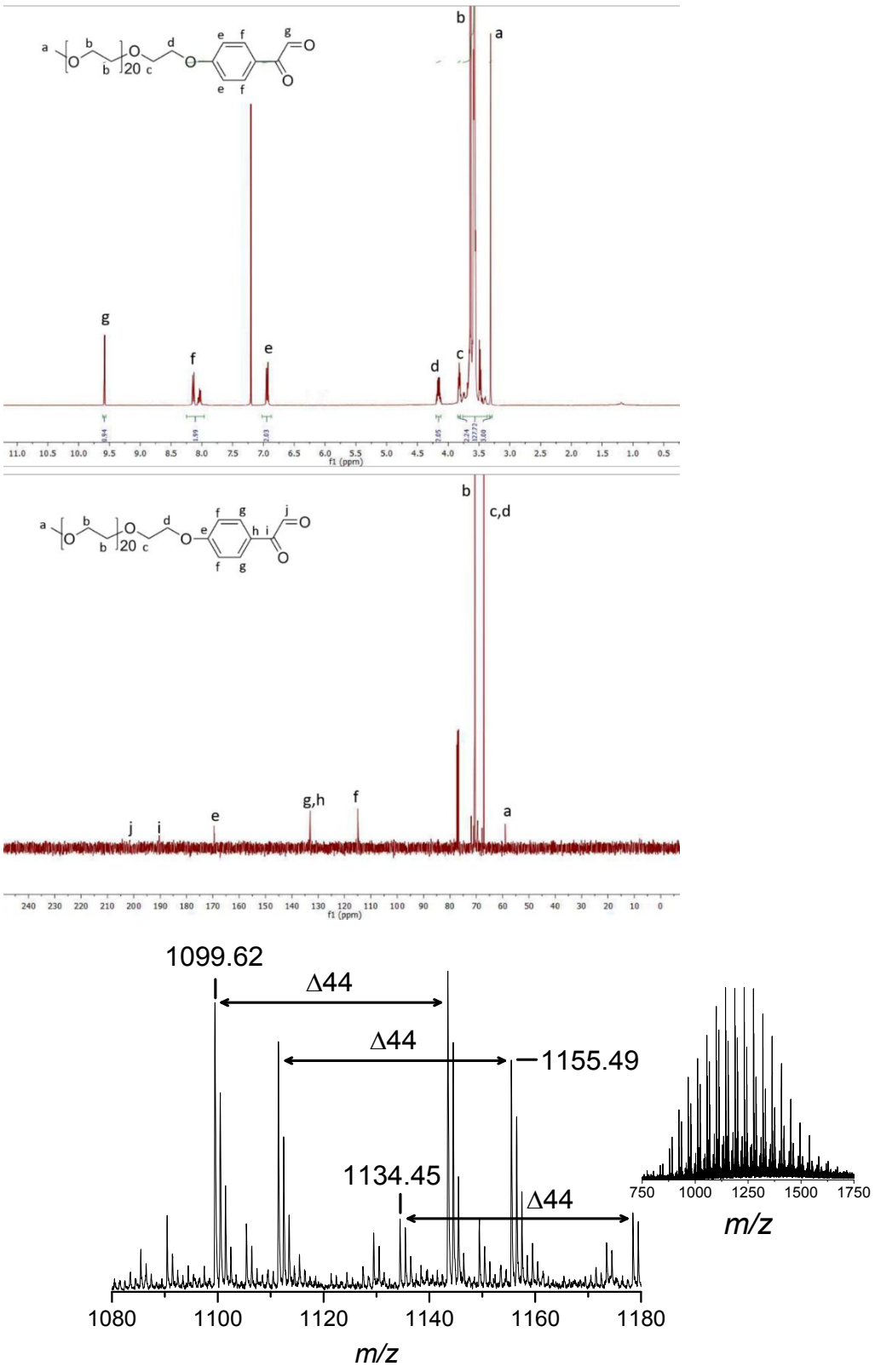


Figure S10. Assigned ^{1}H / ^{13}C NMR ($CDCl_3$) and mass spectra of compound **4** (1 kDa). HRMS (MALDI-TOF) m/z: Parent ions: $[M + H]^+$ Calcd for $C_{53}H_{97}O_{25}$ 1133.63 (Found 1134.45); $[M + Na]^+$ Calcd for $C_{53}H_{96}NaO_{25}$ 1155.61 (Found 1155.49). Fragment ions: $[M + Na]^+$ (α-cleavage at ketone) Calcd for $C_{51}H_{96}NaO_{23}$ 1099.62 (1099.62).

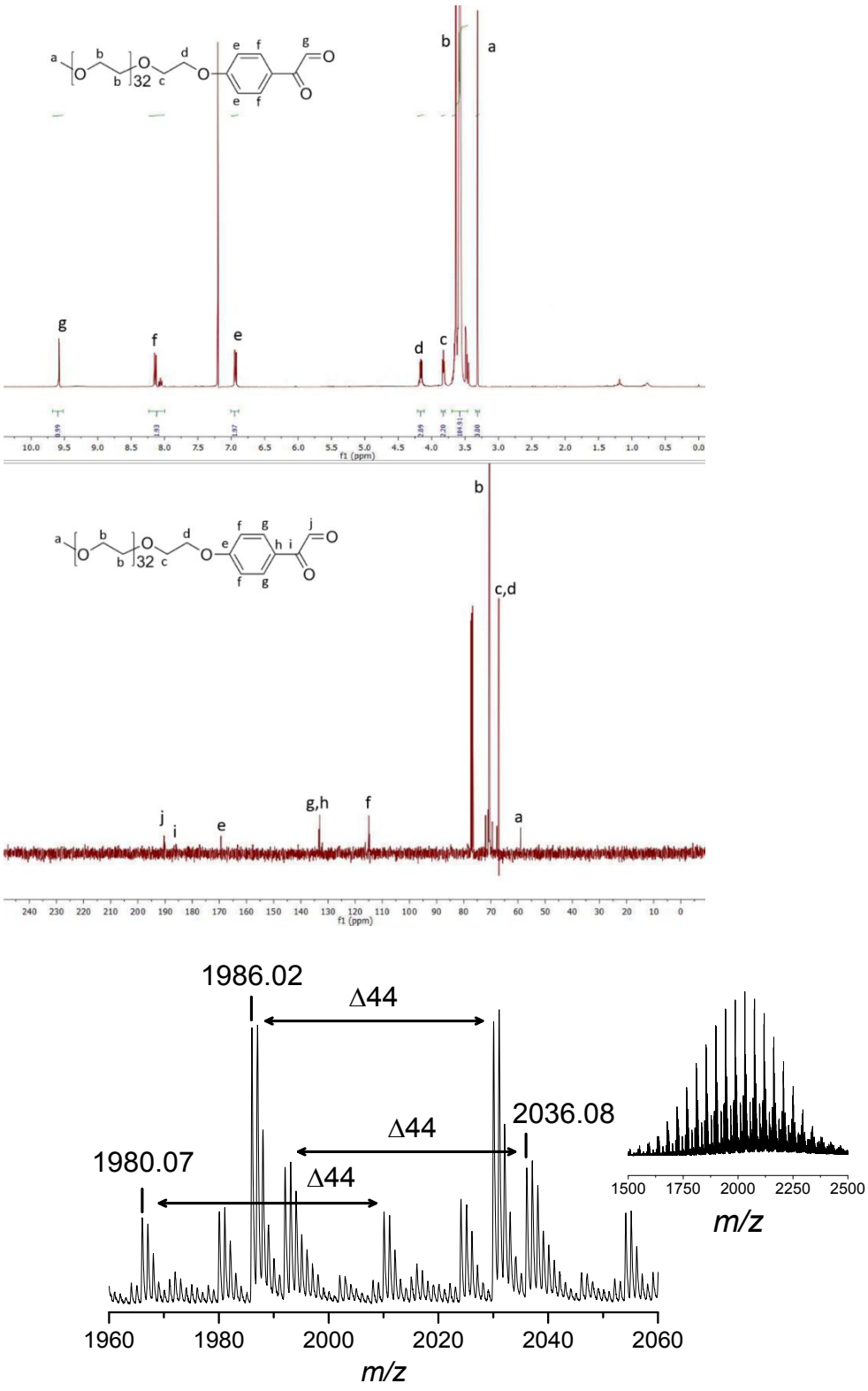


Figure S11. Assigned ^{1}H / ^{13}C NMR ($CDCl_3$) and mass spectra of compound **4** (2 kDa). HRMS (MALDI-TOF) m/z: Parent ions: $[M + Na]^+$ Calcd for $C_{93}H_{176}NaO_{45}$ 2036.14 (Found 2036.08). Fragemnt ions: $[M + H]^+$ (α-cleavage at aldehyde) Calcd for $C_{92}H_{177}O_{44}$ 1986.16 (Found 1986.02); $[M + Na]^+$ (α-cleavage at ketone) Calcd for $C_{91}H_{176}NaO_{43}$ 1980.15 (Found 1980.07).

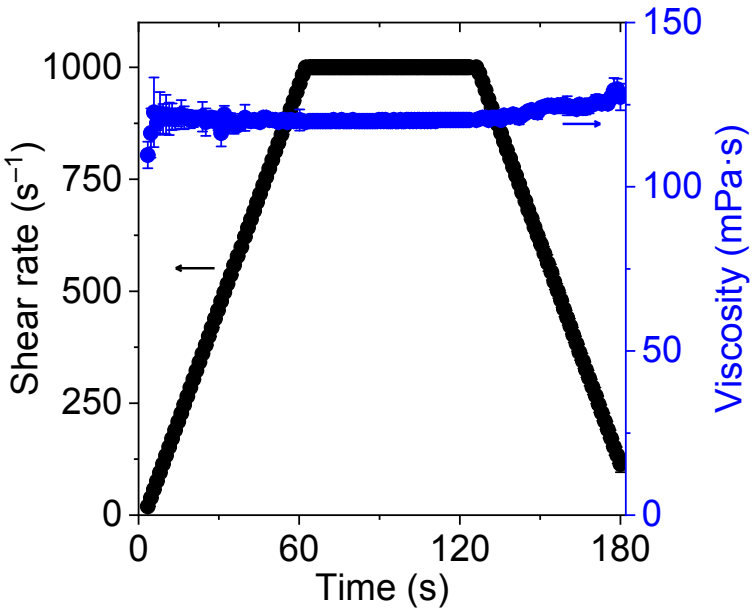


Figure S12. Viscosity for different shear rates over time for lot 1 of polyclonal IgG solution (460 mg·mL^{-1}) in ~16 mM sodium phosphate, ~100 mM NaCl, and ~100 mM sucrose, pH 7.4. The solution behaves like a Newtonian fluid over the entire range of shear rates examined. Data presented as Mean ± SD (n = 3).

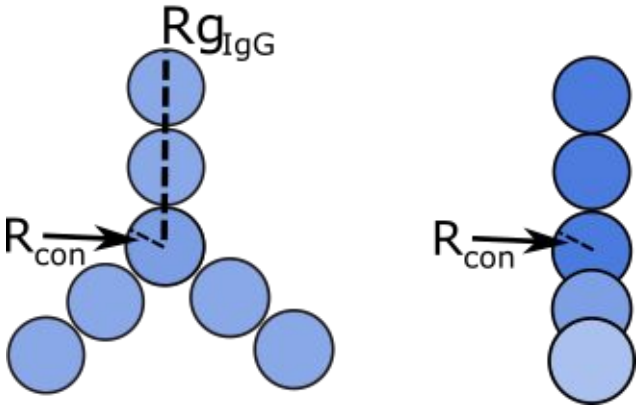


Figure S13. Schematic of IgG model for CG simulations.

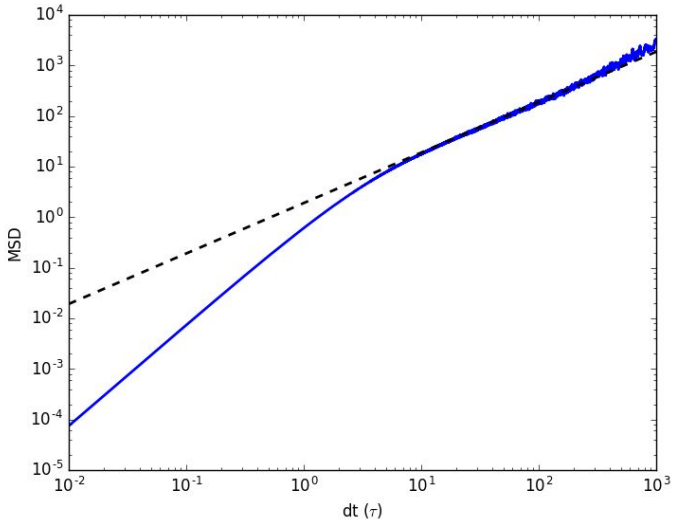


Figure S14. Mean squared displacement (MSD) of the additive per time increment within the simulation. The dashed line is a fit line to the diffusive regime and has a slope of 0.994, the intercept of this fit on the log–log plot gives the diffusion coefficient.

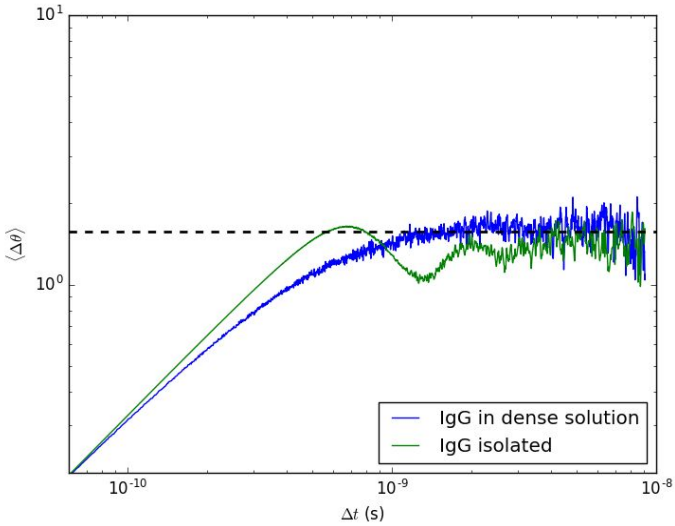


Figure S15. Mean angular displacement of IgG per time increment within the simulation. The mean angular displacement of the additive both isolated and the densely packed solution. The dotted line shows 90º.

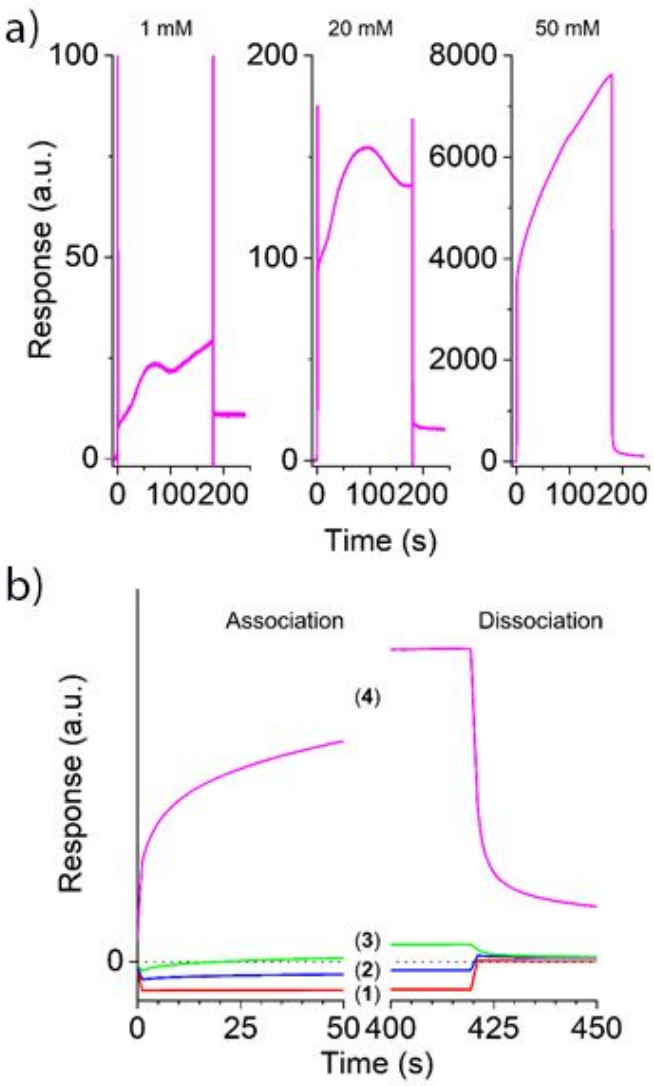


Figure S16. (**a**) SPR association/dissociation curves obtained for different concentrations of **4** (1–50 mM) towards immobilized infliximab. These curves are of poor quality, as can be established e.g., by the non-single-exponential profile of either the association or dissociation phases. (**b**) Zoom of association/dissociation curves for 100 mM **1–4** towards immobilized infliximab measured by surface plasmon resonance. The dotted line represents the baseline response, to best illustrate the presence of artefacts caused by high analyte concentration. For the dissociation phase of **4**, rapid release over 5 s was first observed, followed by a slower one (~120 s). Indeed, in addition to forming imines with lysine/arginine residues, **4** can also form di-hemiaminals with arginine over the course of minutes to hours. This process is enhanced because of the high concentration used in this particular experiment, yet is fully reversible at neutral pH (over a similar timeframe). Plots are representative of two identical repeats.

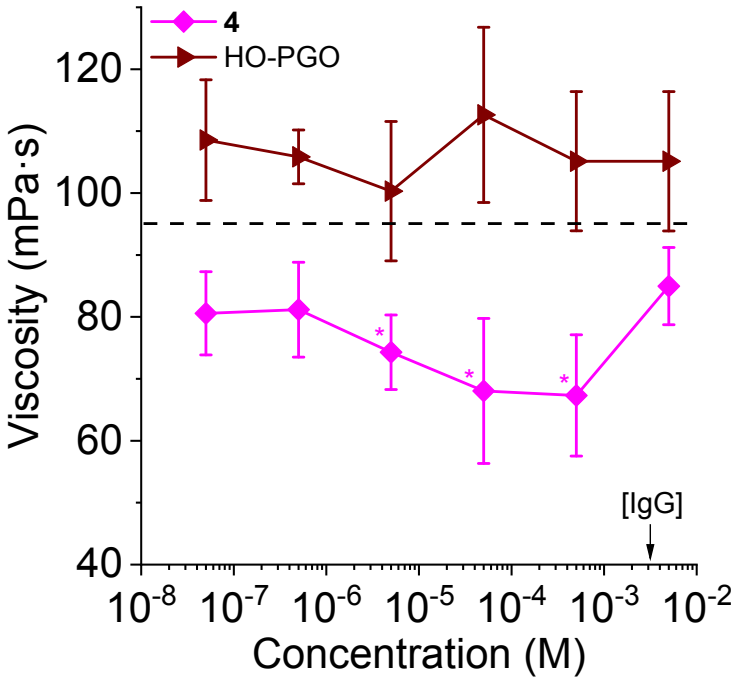


Figure S17. Effect of mPEG moiety on viscosity-reducing effect. Effect of the concentration of 4-hydroxyphenylglyoxal or **4** (0.35 kDa) on the viscosity of a 406 mg·mL^{-1} solutions of IgG (lot 2). The viscosity of the additive-free solution was 95 ± 10 mPa·s, and is represented as the dashed horizontal line. Data shown as Mean ± SD (n = 3). (*) denotes a significant difference as compared to the additive-free sample (ANOVA, Tukey, $p < 0.05$).

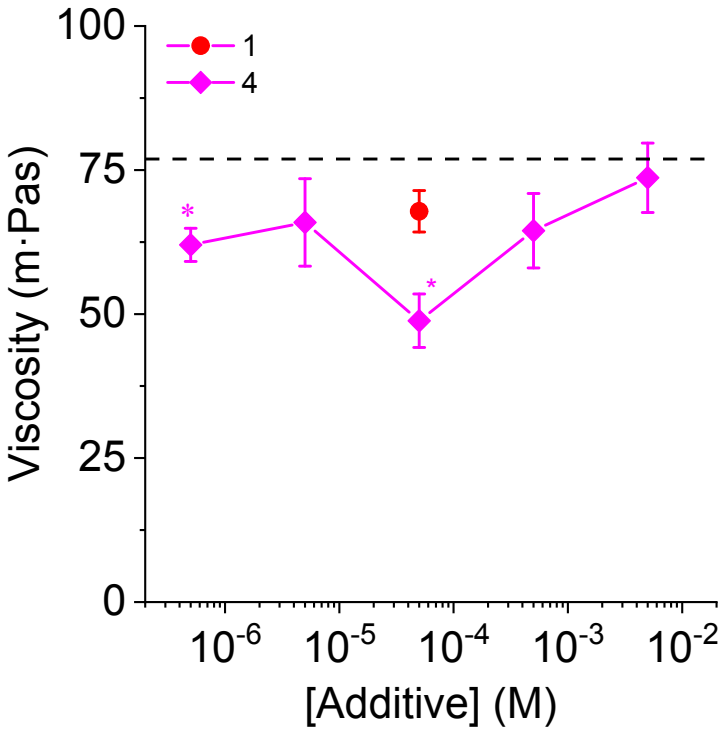


Figure S18. Dynamic PEGylation reduces the viscosity of concentrated infliximab. Effect of the concentration of **1** or **4** (0.35 kDa) on the viscosity of a 150 mg·mL^{-1} solutions of infliximab. The viscosity of the additive-free solution was 77 ± 2 mPa·s, and is represented as the dashed horizontal line. Data shown as Mean ± SD (n = 3). (*) denotes a significant difference as compared to the additive-free sample (ANOVA, Tukey, $p < 0.05$).

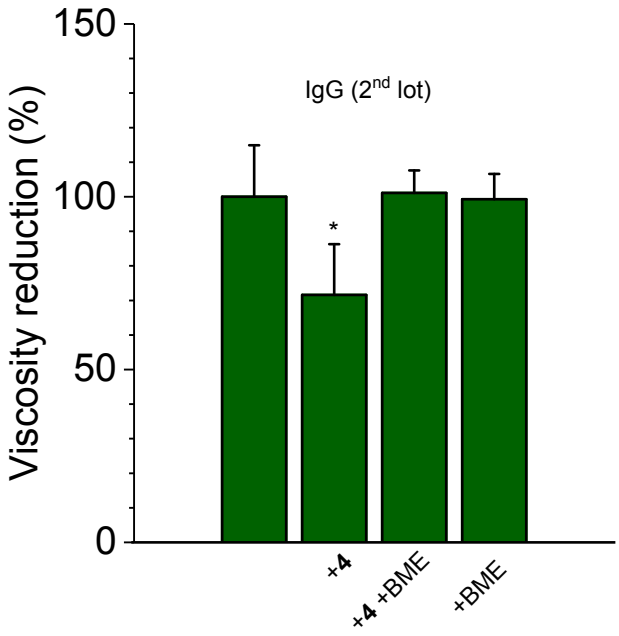


Figure S19. Deactivation of the phenylglyoxal with β-mercaptoethanol (BME) in 4, eliminates its viscosity-reducing effect. Comparison of the viscosity-reducing effect of **4** (5 μM) on the second lot of polyclonal IgG (460 mg·mL^{-1}) with and without pre-incubation with BME (numerical values in **Supplementary Table S3**). Data presented as Mean + SD (n = 3). (*) denotes statistically significant difference relative to corresponding additive-free solution (ANOVA, Tukey, $p < 0.05$).

Table S1. Simulation parameters. These parameters give the scaling for the molecular dynamics simulations. σ is the length scale used in simulation, normalized to the diameter of the simulated PEG particle. m' is the mass scale also normalized to the mass of PEG. kT is the product of Boltzmann constant and temperature, normalized to 1 at room temperature. τ is the simulation time scale, calculated using the other parameters.

Simulation	Experiment
1 σ =	1.2 nm
1 m' =	2.31×10^{-25} kg
1kT =	1.38×10^{-23} J·K^{-1} (at 293 K)
1 τ =	9.07×10^{-12} s

Table S2. Mean comparison table for Figure 2a-c and Figure 3d. '1' denotes a statistically-significant difference compared to the additive-free solution (ANOVA, Tukey, $p < 0.05$).

Concentration (M)	**1**	**2**	**3**	**4**	**1**	**4**	**1**	**3**	**4**	**1**	**3**	**4**
	0.35 kDa						1 kDa			2 kDa		
	20 min				24 h		20 min			20 min		
5×10^{-9}	1	1	1	1	–	–	1	0	0	1	0	1
5×10^{-8}	1	1	1	1	–	–	1	1	1	0	0	0
5×10^{-7}	1	0	1	1	1	1	1	0	1	1	0	1
5×10^{-6}	0	1	1	1	0	1	1	1	1	1	1	0
5×10^{-5}	1	1	1	1	0	1	1	1	1	1	1	1
5×10^{-4}	1	1	1	1	0	1	1	1	1	1	1	1
5×10^{-3}	1	1	1	1	1	1	1	1	0	1	1	1

Table S3. Solution composition for data shown in Figure 2d and Figure S19. HO-PGO is 4-hydroxypgenylglyoxal, BME is β-mercaptoethanol. Data presented as Mean ± SD (n = 3).

Protein	[Protein] ($mg \cdot mL^{-1}$)	Additive	[Additive] (M)	Viscosity (mPa·s)
IgG (lot 1)	460	–	–	120 ± 1
IgG (lot 1)	460	**4**	5×10^{-6}	75 ± 1
IgG (lot 2)	406	–	–	95 ± 10
IgG (lot 2)	406	**4**	5×10^{-5}	70 ± 10
IgG (lot 2)	406	HO-PGO	5×10^{-5}	113 ± 14
IgG (lot 2)	460	–	–	160 ± 10
IgG (lot 2)	460	**4**	5×10^{-6}	126 ± 7
IgG (lot 2)	460	**4** / BME	5×10^{-6} / 20×10^{-6}	165 ± 5
IgG (lot 2)	460	BME	20×10^{-6}	162 ± 7
Infliximab	150	–	–	73 ± 1
Infliximab	150	**4**	5×10^{-5}	48 ± 5