Supplementary Information for

Digital fluorescent pH sensors

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Experimental Section

Materials and Apparatus. *N*-{2-[(7-*N,N*-Dimethylaminosulfonyl)-2,1,3-benzoxadiazol-4-yl]-(methyl)amino}ethyl-*N*-methylacrylamide (DBD-AA)[S1] and *N*,2-dimethyl-*N*-(2-{methyl[7-(dimethyl-sulfamoyl)-2,1,3-benzoxadiazol-4-yl]amino}ethyl)propanamide (DBD-IA)[S2] were obtained as previously reported. *N,N*-Diethyl-1,3-propanediamine, *N*-[3-(dimethylamino)propyl]acrylamide (DMAPAM), *N*-isopropylacrylamide (NIPAM) and 3-morpholinopropylamine were obtained from Wako Pure Chemicals. Trisodium 8-hydroxypyrene-1,3,6-trisulfonate (HPTS) was purchased from Invitrogen. Water was purified using a Milli-Q reagent system, Direct-Q 3 UV (Millipore). All other reagents were of guaranteed reagent grade and used without further purification.

Infrared (IR) spectra were measured using a JASCO FT/IR-410 spectrometer. Proton nuclear magnetic resonance spectra (^{1}H-NMR, 400 MHz) and carbon nuclear magnetic resonance spectra (^{13}C-NMR, 100 MHz) were obtained using a Bruker AVANCE 400 spectrometer. The *J* values are given in hertz. Mass spectra with an electrospray ionization (ESI) system were obtained on a Bruker micrOTOF-05 spectrometer. The gel permeation chromatography apparatus (GPC) consisted of a JUSCO PU-2080 pump, a JASCO RI-2031 refractive index detector, a JASCO FP-2020 fluorescence detector, a JASCO CO-2060 column thermostat and a Shodex GPC KD-806M column. UV-visible absorption spectra were measured using a JASCO V-550 UV/VIS spectrophotometer. Fluorescence spectra were obtained using a JASCO FP-6500 spectrofluorometer with a Hamamatsu R-7029 optional photomultiplier tube (operative range, 200−850 nm) and were corrected using a JASCO ESC-333 substandard light source. The sample temperature was controlled by a JASCO ETC-273T temperature controller.

Synthesis of *N*-(3-morpholin-4-ylpropyl)acrylamide (MPAM).[S3] 3-Morpholinopropylamine (500 mg, 3.47 mmol) and triethylamine (485 μL, 3.47 mmol) were dissolved in toluene (5 mL). After the addition of acryloyl chloride (281 μL, 3.47 mmol) at 0 °C, the mixture was stirred at the same temperature for 1 h. The reaction mixture was then poured into an aqueous NaOH solution (0.5 mol/L, 100 mL) and the product was extracted with dichloromethane (100 mL × 2). The organic layer was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. Chromatography was performed on the residue on silica gel with dichloromethane-methanol (10 : 1) as an eluent to obtain MPAM (342 mg, 50 %) as a colorless oil: ν_{max}(neat)/cm^{-1} 3287, 3076, 2954, 2815, 1660, 1625 and 1556; ^{1}H-NMR ($CDCl_3$, δ) 7.22 (1H, br), 6.26 (1H, d, *J* 17.2), 6.06 (1H, dd, *J* 17.2, 10.4), 5.63 (1H, d, *J* 10.4), 3.72 (4H, t, *J* 4.4), 3.44 (2H, m), 2.48−2.51 (6H, m) and 1.72 (2H, tt, *J* 6.0); ^{13}C-NMR ($CDCl_3$, δ) 165.5, 131.2, 125.9, 67.1, 58.0, 53.7, 39.6 and 24.6; HR-ESI-MS *m/z*: calcd for $C_{10}H_{19}N_2O_2^+$ ([M+H]$^+$)

199.1447, Found 199.1447.

Synthesis of *N*-[3-(diethylamino)propyl]acrylamide (DEAPAM). *N,N*-Diethyl-1,3-propanediamine (500 mg, 3.84 mmol) and triethylamine (1.61 mL, 11.5 mmol) were dissolved in toluene (5 mL). After the addition of acryloyl chloride (312 μL, 3.84 mmol) at 0 °C, the mixture was stirred at the same temperature for 1 h. The reaction mixture was then poured into an aqueous NaOH solution (0.5 mol/L, 100 mL) and the product was extracted with dichloromethane (100 mL × 2). The organic layer was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. Chromatography was performed on the residue on silica gel with dichloromethane-methanol (100 : 15) as an eluent to obtain DEAPAM (199 mg, 28 %) as a colorless oil: ν_{max}(neat)/cm^{-1} 3279, 3077, 2969, 2803, 1658, 1626 and 1551; ^{1}H-NMR ($CDCl_3$, δ) 7.94 (1H, br), 6.22 (1H, d, *J* 17.2), 6.04 (1H, dd, *J* 17.2, 10.4), 5.59 (1H, d, *J* 10.4), 3.43 (2H, m), 2.49−2.56 (6H, m), 1.68 (2H, tt, *J* 6.0) and 1.04 (6H, t, *J* 7.2); ^{13}C-NMR ($CDCl_3$, δ) 165.4, 131.6, 125.3, 53.2, 46.9, 40.4, 25.0 and 11.9; HR-ESI-MS *m/z*: Calcd for $C_{10}H_{21}N_2O^+$ ($[M+H]^+$) 185.1654, Found 185.1650.

Polymerizations. Copolymers 1 and 3−5. NIPAM (4.5 mmol), an ionizable monomer (DMAPAM, MPAM, DEAPAM, or AA, 0.5 mmol), DBD-AA (5 μmol), and α,α'-azobisisobutyronitrile (AIBN, 50 μmol) were dissolved in 1,4-dioxane (10 mL), and the solution was bubbled with dry nitrogen or argon for 30 min to remove dissolved oxygen. Polymerization was performed at 60 °C for 6 h (for **3** and **4**) or 8 h (for **1** and **5**). The reaction mixture was cooled to room temperature and poured into diethyl ether (200 mL). The obtained copolymer was purified by reprecipitation using 1,4-dioxane (10 mL) and diethyl ether (200 mL). Yields: 83 % (**1**), 82 % (**3**), 71 % (**4**) and 83 % (**5**).

Copolymer 2. DMAPAM (5 mmol), DBD-AA (5 μmol), and AIBN (25 μmol) were dissolved in 1,4-dioxane (5 mL), and the solution was bubbled with dry argon for 30 min to remove dissolved oxygen. Polymerization was performed at 60 °C for 6 h. The reaction mixture was cooled to room temperature and poured into a mixture of *n*-hexane and acetone (9 : 1, v/v, 200 mL). The obtained copolymer was purified by reprecipitation using 1,4-dioxane (3 mL)-diethyl ether (200 mL) and dialysis. The yield was 21 %.

Characterization of 1-5. The contents of the NIPAM, DMAPAM, MPAM, DEAPAM, and AA units in the copolymers were determined from ^{1}H NMR spectra. The proportions of DBD-AA units in the copolymers were determined from the absorbances of the copolymers in methanol, in comparison with DBD-IA (ε = 10,800 M^{-1}cm^{-1} at 444 nm)[S2] as a model compound. The molecular weights of the copolymers were determined by means of GPC measurements. A calibration curve was obtained using a polystyrene standard, and 1-methyl-2-pyrrolidinone containing LiBr (5 mM) was adopted as the eluent (0.5 mL min^{-1}). A small peak at 22.5 min in the chromatograms of **2** and **4** was due to a trace amount of unpolymerized DBD-AA (less than 0.15 % of total area).

Fluorescence measurements. The fluorescence spectra of the copolymers (0.01 w/v%) were obtained in a Britton-Robinson buffer with an excitation of 450 nm. After pH adjustments at room temperature, the sample was equilibrated at a specific temperature for at least 500 s. The fluorescence

spectra of HPTS (1 μM) and DBD-IA (10 μM) were also measured in a Britton-Robinson buffer with excitations at 454 and 450 nm, respectively. These fluorometric measurements were performed at least in triplicate. The fluorescence quantum yields (Φ_f) were determined by the following equation:

$$\Phi_{f,S} = \Phi_{f,R} F_S A_R n_S^2 / F_R A_S n_R^2 \quad \text{(S1)}$$

where F is the area under the fluorescence spectrum with an excitation of 458 nm, A is the absorbance at 458 nm, n is the refractive index of the solvent, and the subscripts R and S represent reference and sample, respectively. 4-Methylamino-7-nitro-2,1,3-benzoxadiazole in acetonitrile ($\Phi_f = 0.38$ with an excitation at 458 nm[S4]) was used as a reference.

Acknowledgement. We thank Dr. N. Kumagai for taking IR spectra.

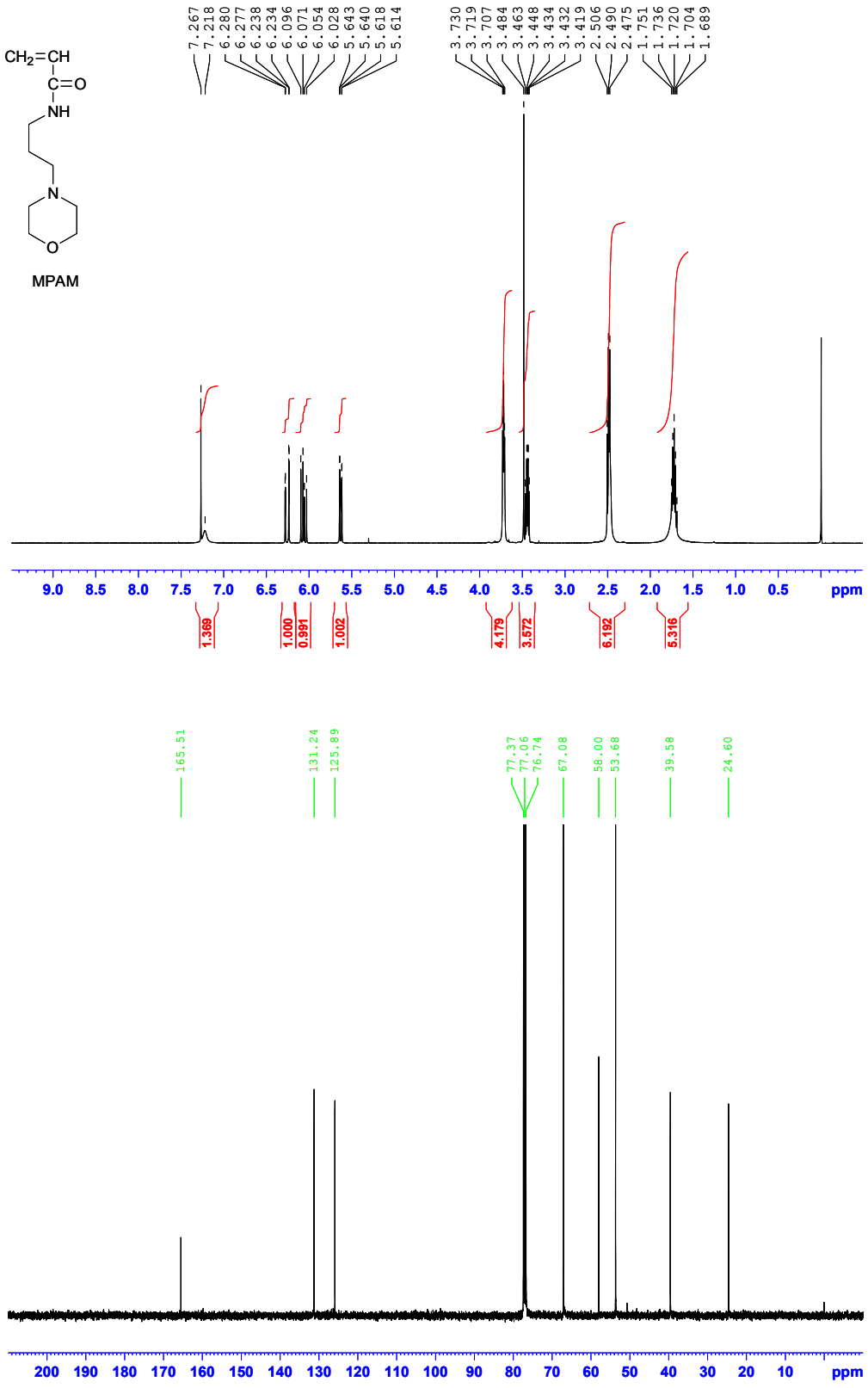
References

S1 C. Gota, S. Uchiyama and T. Ohwada, *Analyst*, 2007, **132**, 121–126.

S2 C. Gota, S. Uchiyama, T. Yoshihara, S. Tobita and T. Ohwada, *J. Phys. Chem. B*, 2008, **112**, 2829–2836.

S3 S. Ito, *Kobunshi Ronbunshu*, 1990, **47**, 467–474.

S4 S. Uchiyama, T. Santa and K. Imai, *J. Chem. Soc., Perkin Trans. 2*, 1999, 2525–2532.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NH
N
O
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6.071
6.054
6.028
5.643
5.640
5.618
5.614
3.730
3.719
3.707
3.484
3.463
3.448
3.434
3.432
3.419
2.506
2.490
2.475
1.751
1.736
1.720
1.704
1.689
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.369
1.000
0.991
1.002
4.179
3.572
6.192
5.316
165.51
131.24
125.89
77.37
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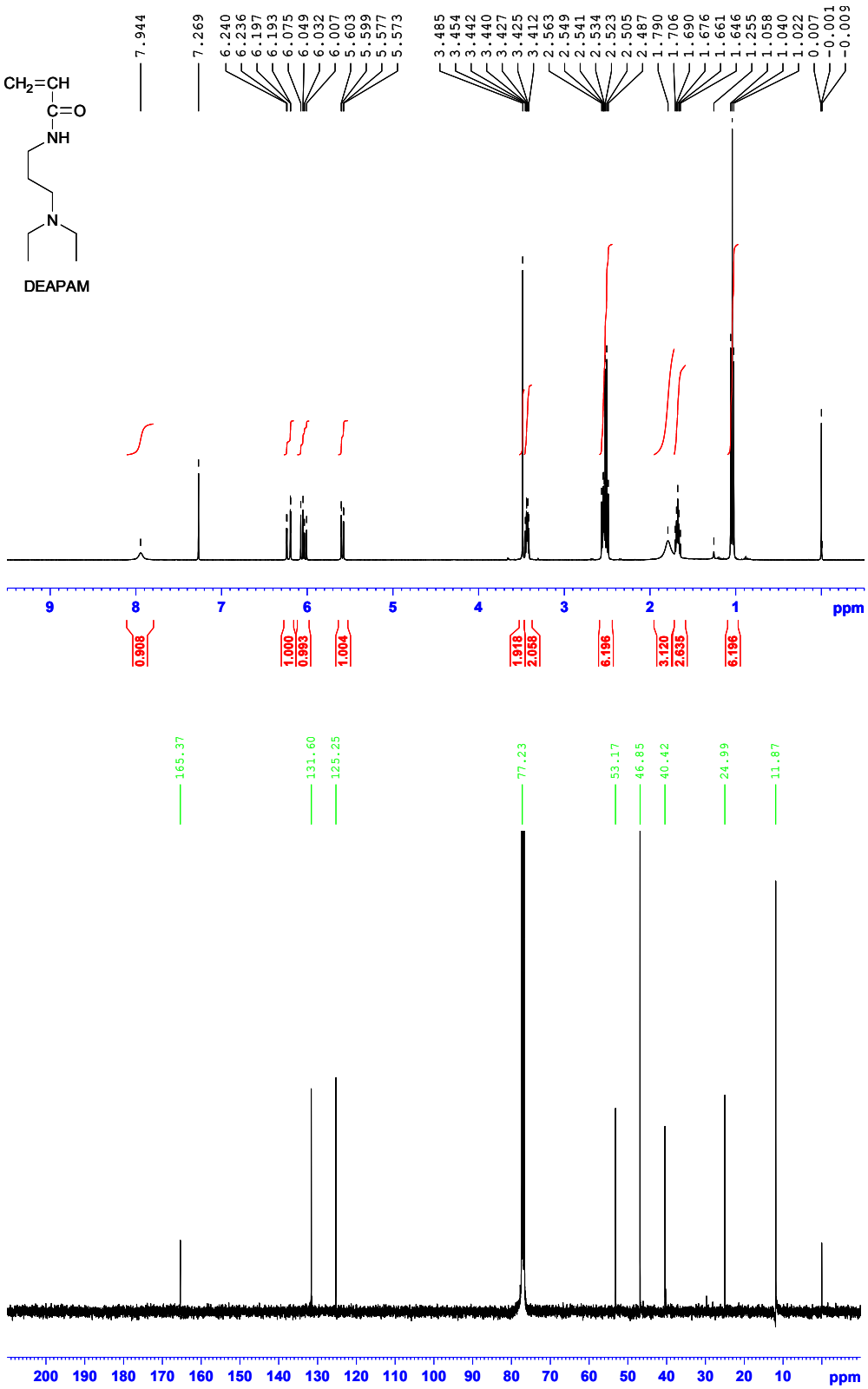
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5.603
5.599
5.577
5.573
3.485
3.454
3.442
3.440
3.427
3.425
3.412
2.563
2.549
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2.523
2.505
2.487
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ppm
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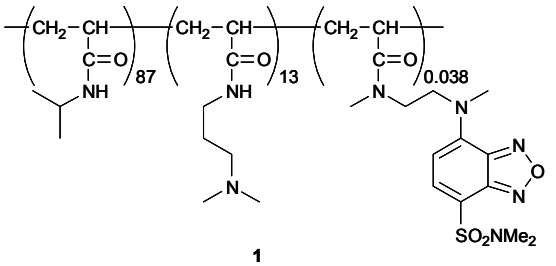
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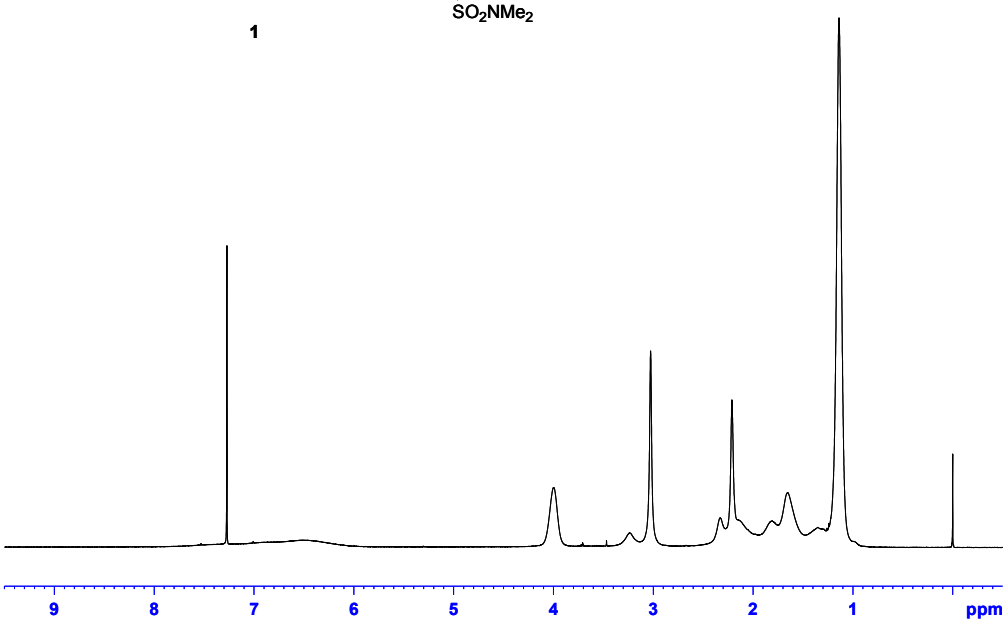
1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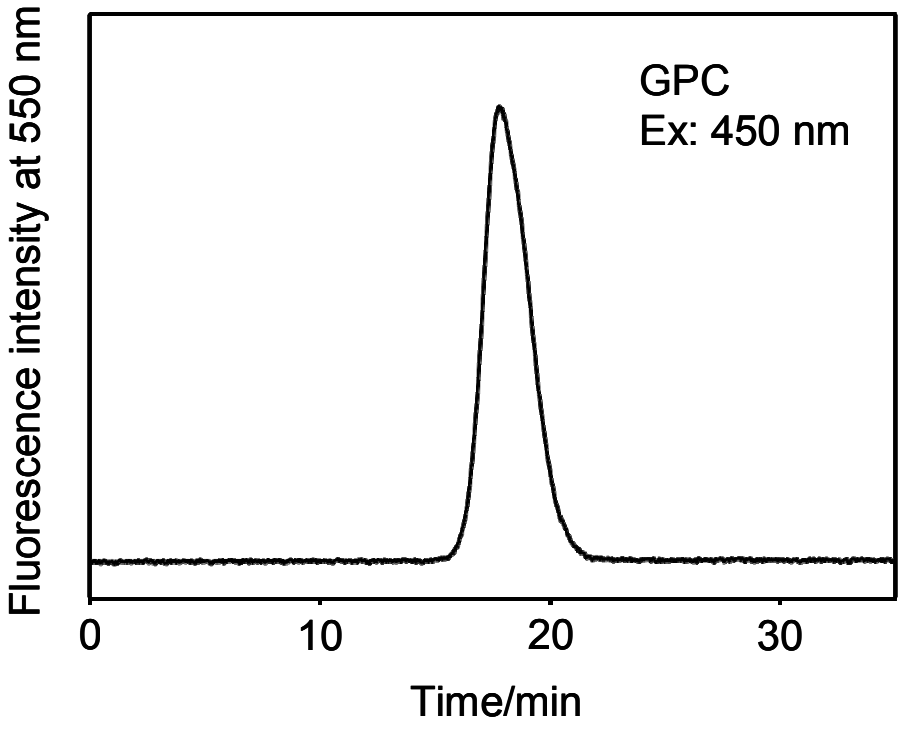
7
6
5
4
3
2
1
ppm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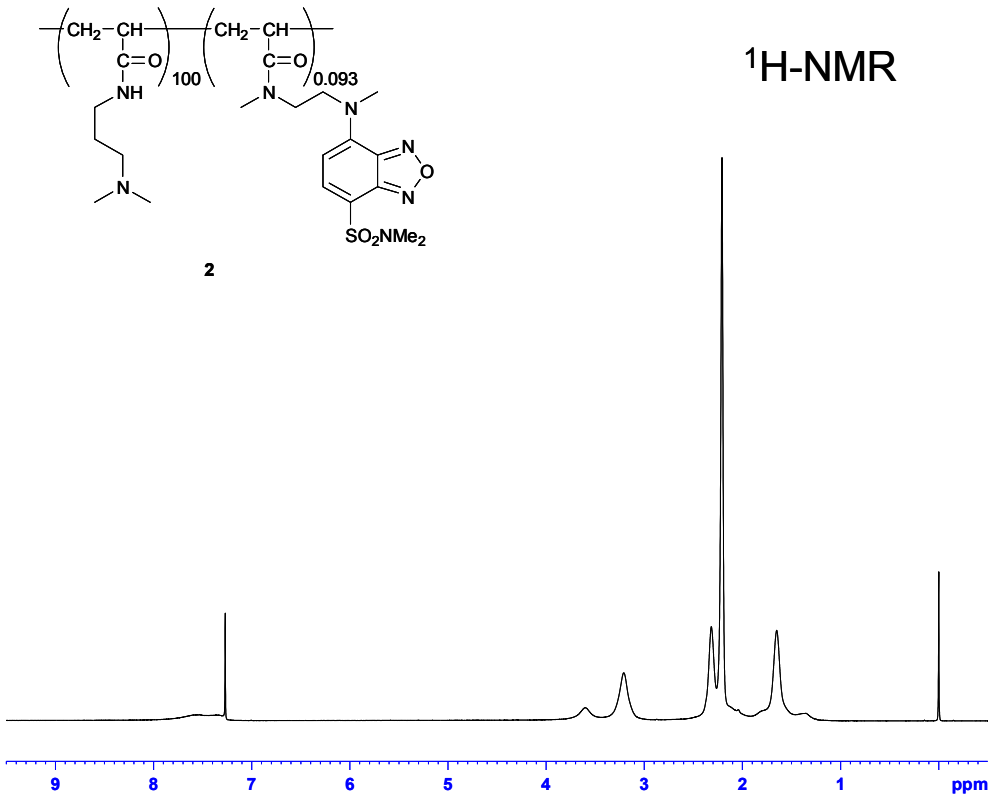
Fluorescence intensity at 550 nm
0
10
20
30
Time/min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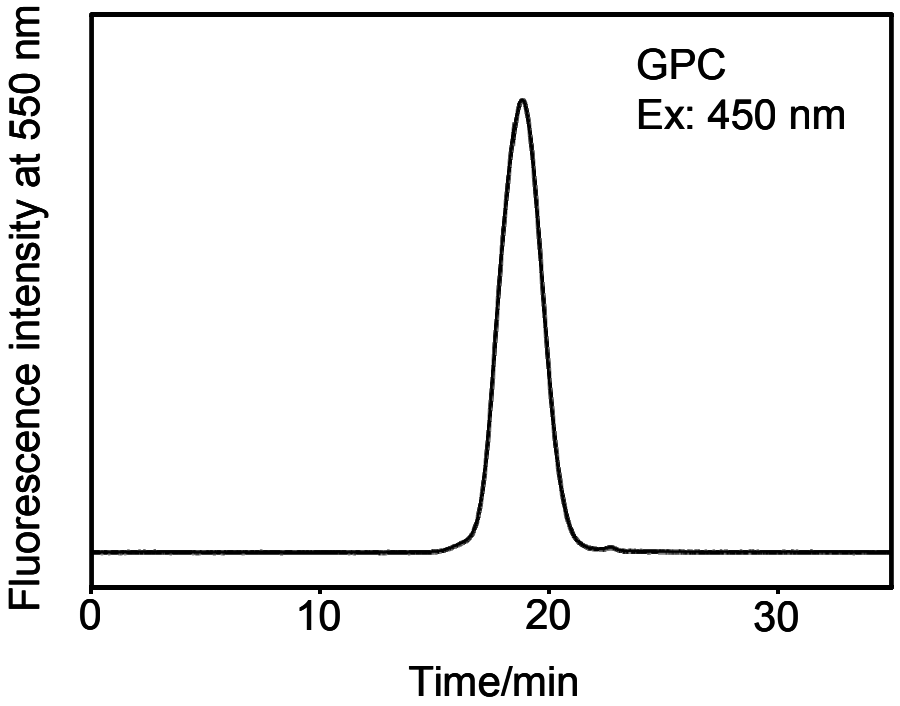
ppm

2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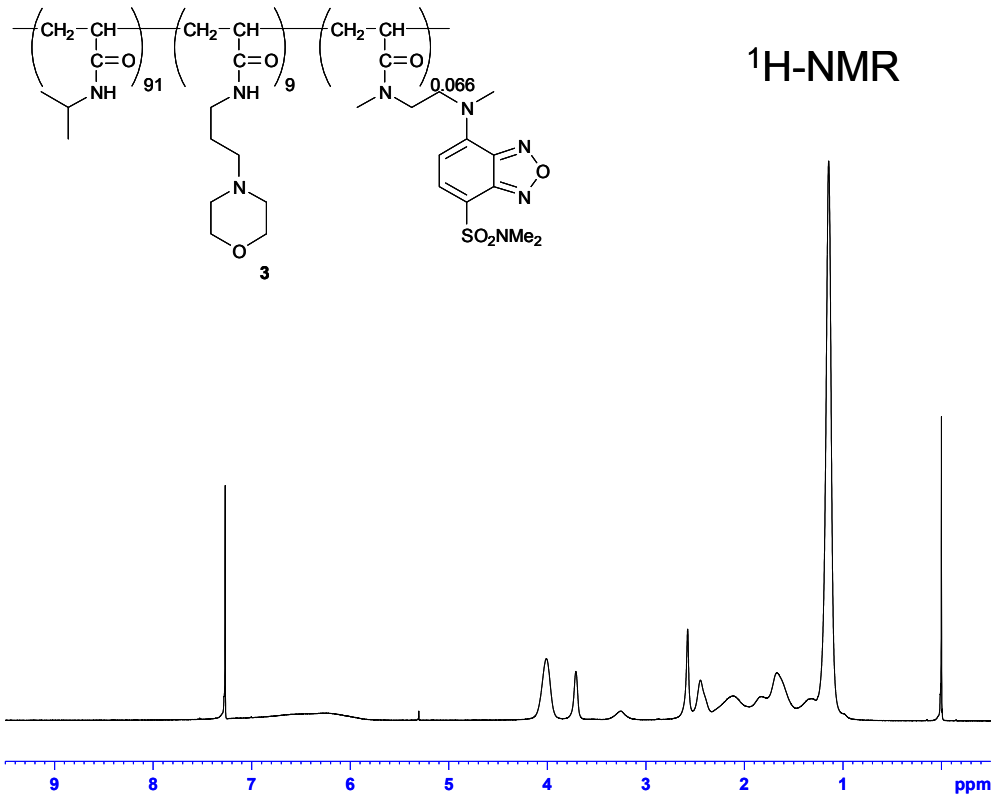
Fluorescence intensity at 550 nm
0
10
20
30
Time/min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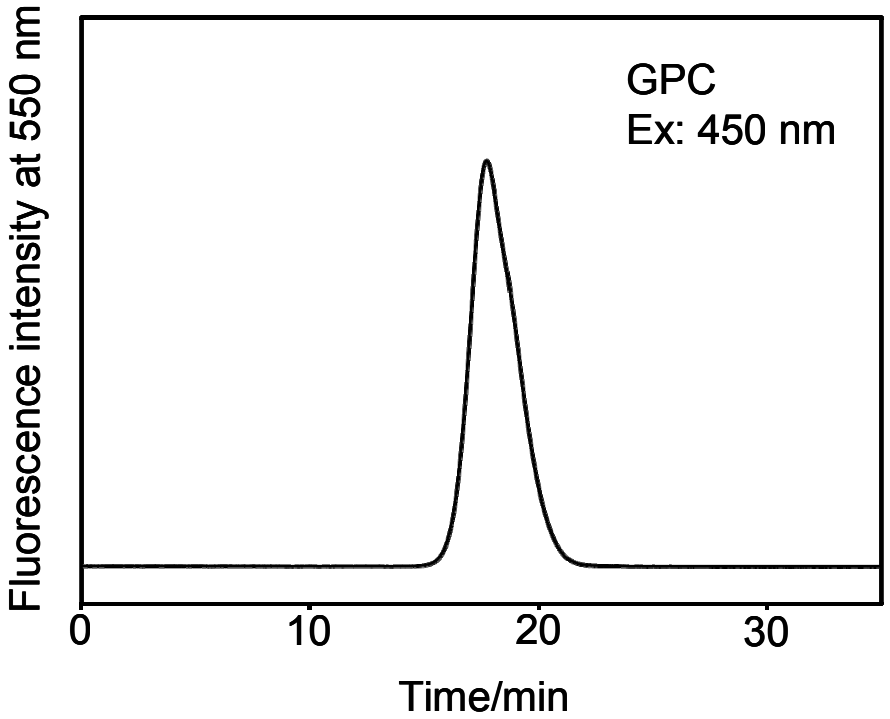
SO_2NMe_2
9
8
7
6
5
4
3
2
1
ppm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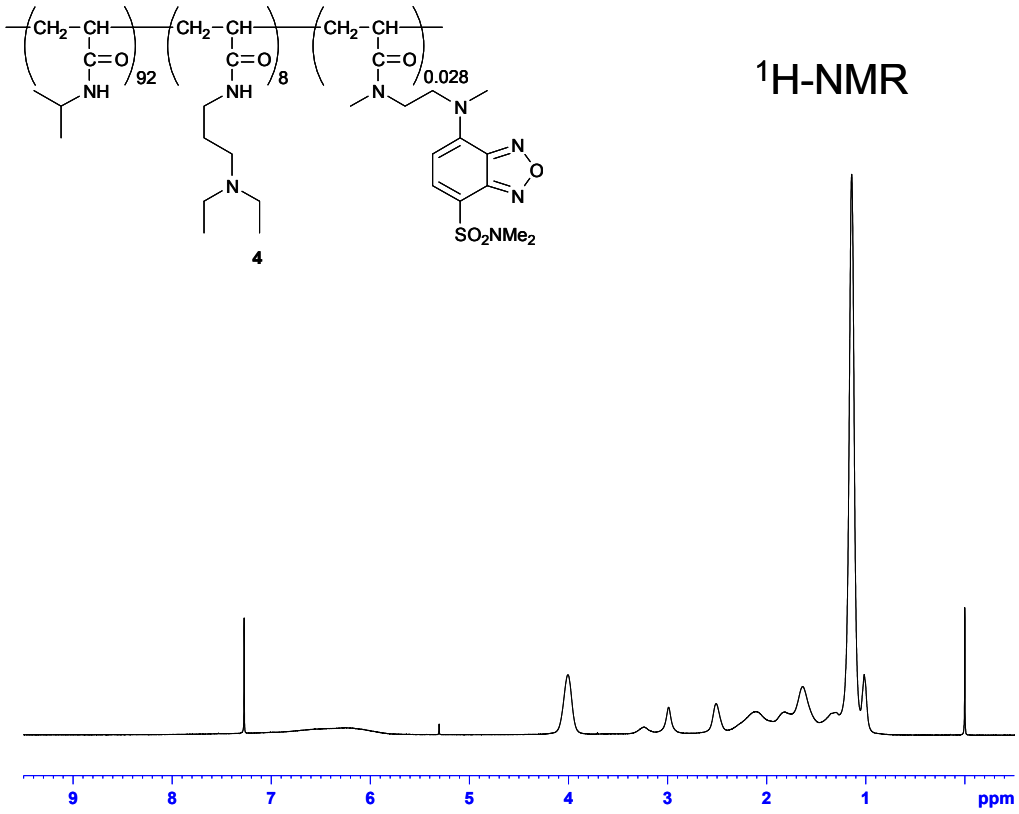
Fluorescence intensity at 550 nm
0
10
20
30
Time/min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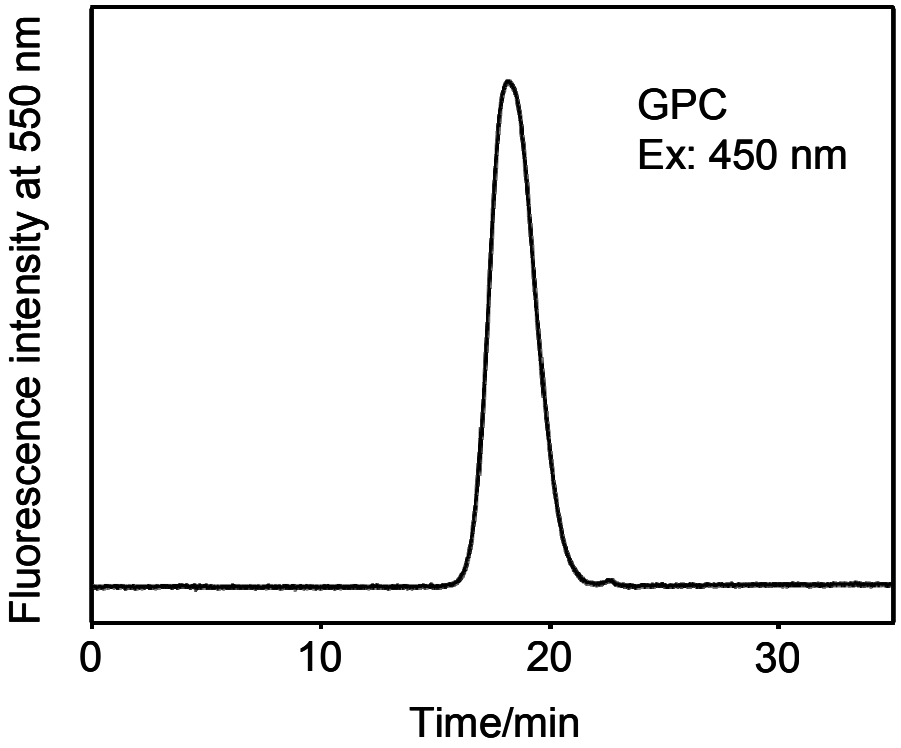
9
8
7
6
5
4
3
2
1
ppm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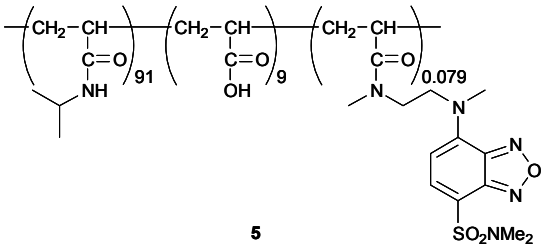
Fluorescence intensity at 550 nm
0
10
20
30
Time/min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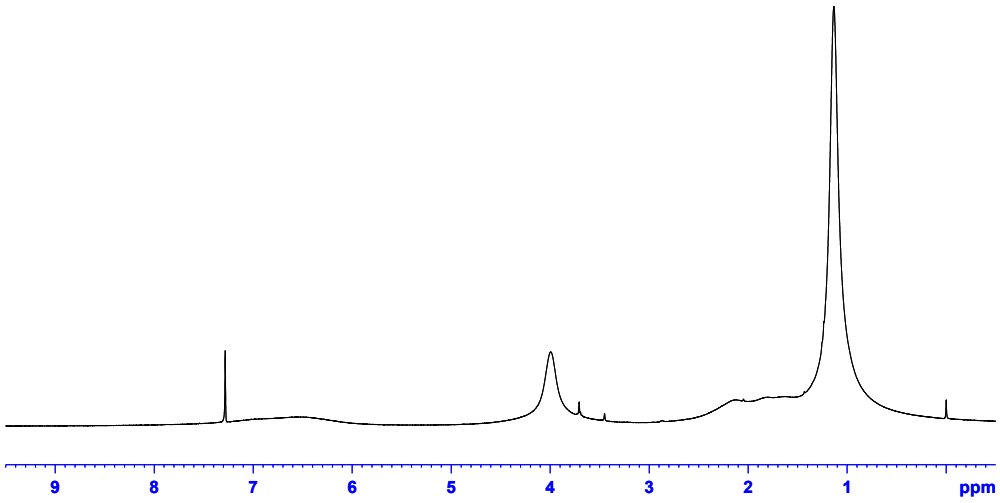
SO2NMe2

^{1}H-NMR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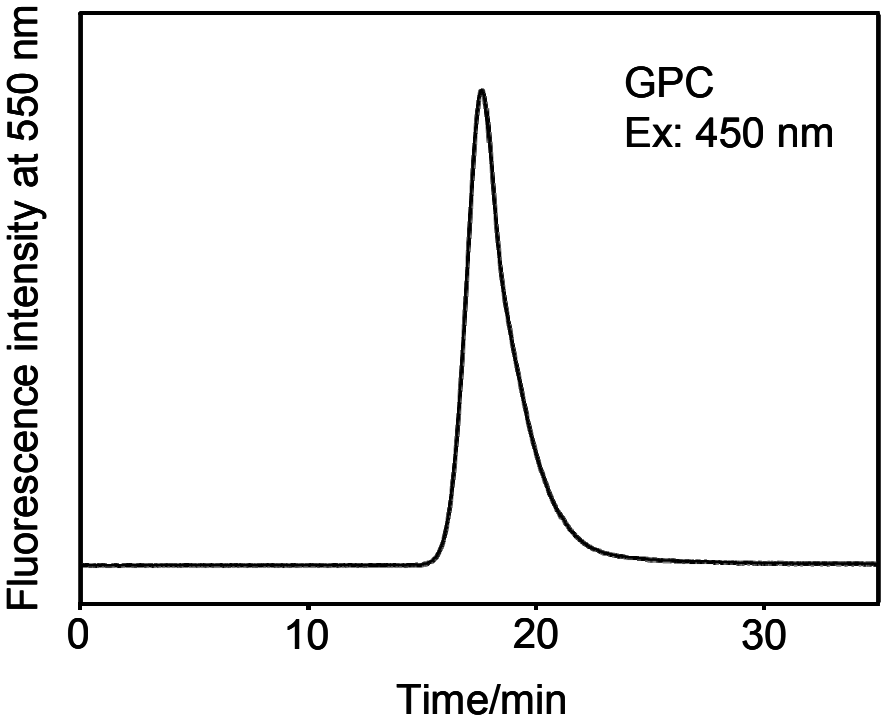
4
3
2
1
ppm


GPC
Ex: 450 nm
Fluorescence intensity at 550 nm
0
10
20
30
Time/min