β-Cyclodextrin-bearing Gold Glyconanoparticles for the Development of Site Specific Drug Delivery Systems

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Supporting Information (SI)

(22 Pages)

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***S*-(12,15,18,21-Tetraoxa-23-{4-[4'-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyloxymethyl]-1*H*-1,2,3-triazol-1-yl}tricosanyl) thioacetate (7).** $(EtO)_3P{\cdot}CuI$ (20 mg, 0.056 mmol) was added to a solution of **4** (127 mg, 0.284 mmol) and **5** (108 mg, 0.284 mmol) in dry DMF (5 ml) under N_2 atmosphere, and the mixture was stirred for 30 min at room temperature and then 8 hours at 60 ºC. Solvent was then evaporated under reduced pressure and the crude was purified by column chromatography (EtOAc-Methanol 5:1) to yield compound **9** (198 mg, 0.235 mmol, 83 %) as a white solid. Mp: 219-226 ºC (dec.); $[\alpha]_D$ +8 (*c* 0.1, H_2O); IR (KBr) ν/cm^{-1} 3230, 2900, 2851, 1630, 1560, 1558, 1408, 1380, 1301, 1044, 1022, 892, 770, 719, 650; ^{1}H NMR (300 MHz, CD_3OD) δ 8.12 (s, 1H, H-5-C_2HN_3), 5.00 (d, 1H, ^2J = 12.4 Hz, C_2HN_3-C*H*O), 4.81 (d, 1H, ^2J = 12.4 Hz, C*H*O-C_2HN_3 overlapped with CD_3OD), 4.62 (t, 2H, *J* = 5.0 Hz, CH_2N), 4.47 (d, 1H, *J* = 7.8 Hz, H-1), 4.41 (d, 1H, *J* = 7.3 Hz, H-1'), 4.00-3.71 (m, 8H, H-3,4,4',5',6,6,6',6'), 3.66-3.50 (m, 18H, CH_2OEG, H-2,2',3',5), 3.49 (t, 2H, *J* = 6.6 Hz, OC*H*$_2(CH_2)_{10}$), 2.89 (t, 2H, *J* = 7.3 Hz, CH_2S), 2.34 (s, 3H, CH_3CO), 1.63-1.54 (m, 4H, $(CH_2)_2$), 1.47-1.33 (m, 14H, $(CH_2)_7$); ^{13}C NMR (75 MHz, CD_3OD) δ 197.6 (CO), 145.4 (C-4-C_2HN_3), 126.1 (C-5-C_2HN_3), 105.1 (C-1'), 103.4 (C-1), 80.6 (C-4), 77.0 (C-5'), 76.5 (C-5), 76.3 (C-3), 74.8 (C-3'), 74.6 (C-2), 72.5 (C-2'), 72.3, 71.5, 71.5, 71.5, 71.4, 71.1, 70.3 (*C*H_2OEG, OC$H_2(CH_2)_{10}$), 70.2 (C-4'), 63.0 (*C*H_2O- C_2HN_3), 62.5 (C-6'), 61.9 (C-6), 51.4 (CH_2N), 30.7, 30.7, 30.6, 30.6, 30.5, 30.2, 29.8, 29.7, 27.2 (*C*H_3CO, $(CH_2)_9$, CH_2S); MALDI-TOF-MS *m*/*z* calcd for $C_{36}H_{65}N_3O_{16}SNa$ 850.4, found 850.5 $(M + Na)^+$.

***S*-{12,15,18,21-Tetraoxa-23-[4-(cyclomaltoheptaos-2^I-*O*-ylmethyl)-1*H*-1,2,3-triazol-1-yl]tricosanyl} thioacetate (8).** $(EtO)_3P{\cdot}CuI$ (10 mg, 0.028 mmol) was added to a solution of **4** (62 mg, 0.138 mmol) and **6** (162 mg, 0.138 mmol) in dry DMF (3 ml) under N_2 atmosphere, and the mixture was stirred for 30 min at room temperature and

then 8 hours at 60 ºC. Solvent was then evaporated under reduced pressure and the crude was purified by column chromatography (10:5:1 CH_3CN-H_2O-30 % v/v aq NH_3) to yield compound **8** (172 mg, 0.106 mmol, 77 %) as a white solid. Mp 213°-215 ºC (dec.); $[\alpha]_D$ +47 (*c* 0.1, H_2O); IR (KBr) ν/cm^{-1} 3315, 2922, 2853, 1752, 1670, 1569, 1408, 1301, 1247, 1151, 1079, 1029, 946, 946, 853, 758, 704, 652, 623; ^{1}H NMR (300 MHz; DMSO-d_6) δ 8.09 (s, 1H, H-5-C_2HN_3), 5.94-5.90 (m, 2H, OH), 5.78-5.69 (m, 10H, OH), 4.90-4.78 (m, 9H, H-1^{I-VII}, CH_2O-C_2HN_3), 4.56-4.41 (m, 10H, OH), 3.82-3.79 (m, 3H, H-3^{I}, CH_2N), 3.62-3.46 (m, 43H, H-3^{II-VII},5^{I-VII},6^{I-VII},$6'^{I-VII}$,CH_2OEG,OC$H_2(CH_2)_{10}$), 3.36-3.30 (m, 18H, H-2^{I-VII},4^{I-VII} overlapped with HDO), 2.81 (t, 2H, ^3J= 7.2 Hz, CH_2S), 2.31 (s, 3H, CH_3CO), 1.50-1.44 (m, 4H, $(CH_2)_2$), 1.29-1.23 (m, 14H, $(CH_2)_7$); ^{13}C-NMR (75 MHz; DMSO-d_6): δ 195.4 (CO), 143.6 (C-4-C_2HN_3), 124.5 (C-5-C_2HN_3), 102.0 (C-1^{I-VII}), 81.7-81.4 (C-2^{I},4^{I-VII}), 73.2-71.7 (C-2^{II-VII},3^{I-VII},5^{I-VII}), 70.3, 69.8, 69.7, 69.6, 69.5, 68.8 (*C*H_2OEG, O*C*$H_2(CH_2)_{10}$), 64.4 (*C*H_2O-C_2HN_3), 60.0-59.7 (C-6^{I-VII}), 49.5 (CH_2N), 30.6 (*C*H_3CO), 29.2, 29.1, 29.0, 28.9, 28.8, 28.5, 28.4, 28.1, 25.6 ($(CH_2)_9$, CH_2S); MALDI-TOF-MS *m*/*z* calcd for $C_{66}H_{113}N_3O_{40}SNa$ 1642.7, found 1642.9 $(M + Na)^+$; calcd for $C_{64}H_{110}N_3O_{39}SNa$ 1600.6, found 1600.9 $(M - C_2H_3O + Na)^+$.

Bis(12,15,18,21-tetraoxa-23-{4-[4'-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyloxymethyl]-1*H*-1,2,3-triazol-1-yl}tricosanyl) disulfide (9). A solution of **7** (110 mg, 0.108 mmol) in aqueous 0.5 M KOH (5 ml) was stirred at room temperature for 16 h. Solvent was then evaporated under reduced pressure and the crude was purified by column chromatography (CH_3CN-H_2O 3:1) to yield **9** (93 mg, 0.057 mmol, 54 %) as a white solid. Mp 216-229 ºC (dec.); $[\alpha]_D$ +47 (*c* 0.1, H_2O); IR (KBr) ν/cm^{-1} 3272, 2922, 2851, 1669, 1568, 1558, 1408, 1347, 1301, 1241, 1044, 1022, 892, 782, 709, 648, 620; ^{1}H NMR (300 MHz, D_2O) δ 8.10 (s, 2H, H-5-C_2HN_3), 4.96 (d, 2H,

J = 12.6 Hz, C*H*O-C_2HN_3), 4.80 (bs, C*H*O-C_2HN_3 overlapped with HDO), 4.58-4.53 (m, 6H, H-1, CH_2N), 4.44 (d, 2H, *J* = 7.5 Hz, H-1'), 3.97-3.68 (m, 18H, H-2',3,4,4',5',6,6,6',6'), 3.63-3.52 (m, 28H, H-3',5, C*H*$_2$OEG), 3.44 (t, 4H, *J* = 6.2 Hz, OC*H*$_2$$(CH_2)_{10}$), 3.34 (t, 2H, *J* = 8.2 Hz, H-2), 2.69 (t, 4H, *J* = 6.7 Hz, CH_2S), 1.69 (bs, 4H, CH_2), 1.56 (bs, 4H, CH_2), 1.40-1.31 (m, 28H, $(CH_2)_7$); ^{13}C NMR (75 MHz; D_2O) δ 143.5 (C-4-C_2HN_3), 125.4 (C-5-C_2HN_3), 102.9 (C-1'), 101.4 (C-1),78.4 (C-4), 75.3 (C-5'), 74.8 (C-5), 74.3 (C-3), 72.7 (C-3'), 72.5 (C-2), 71.0 (C-2'), 70.9, 69.9, 69.8, 69.7, 68.8 (*C*H_2OEG, OC$H_2$$(CH_2)_{10}$), 68.5 (C-4'), 61.8 (*C*H_2O-C_2HN_3), 61.0 (C-6'), 60.1 (C-6), 49.9 (CH_2N), 38.9 (CH_2S), 29.8, 29.7, 29.5, 29.3, 29.1, 28.6, 26.1 ($(CH_2)_9$); ESI-TOF-MS m/z calcd for $C_{68}H_{124}N_6O_{30}S_2Na$ 1592.9, found 1592.8 $(M + Na)^+$.

Bis{12,15,18,21-Tetraoxa-23-[4-(cyclomaltoheptaos-2^I-*O*-ylmethyl)-1*H*-1,2,3-triazol-1-yl]tricosanyl} disulfide (10). A solution of **8** (132 mg, 0.082 mmol) in aqueous 0.5 M KOH (10 ml) was stirred at room temperature for 16 h. Solvent was then evaporated under reduced pressure and the crude was purified by column chromatography (CH_3CN-H_2O 2:1) to yield compound **10** (91 mg, 0.056 mmol, 69 %) as a white solid. Mp 294°-296 ºC (dec.); $[\alpha]_D$ +52 (*c* 0.1, H_2O); IR (KBr) ν/cm^{-1} 3349, 2919, 2859, 1664, 1456, 1406, 1356, 1299, 1137, 1080, 1028, 997, 941, 863, 756, 703, 639, 624; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.09 (s, 2H, H-5-C_2HN_3), 5.93-5.90 (m, 4H, OH), 5.76-5.70 (m, 20H, OH), 4.90-4.78 (m, 18H, H-1^{I-VII}, C*H*$_2$O-C_2HN_3), 4.58-4.40 (m, 20H, OH), 3.85-3.79 (m, 6H, H-3^I, CH_2N), 3.63-3.46 (m, 86H, H-3^{II-VII},5^{I-VII},6^{I-VII},6'$^{I-VII}$,CH_2OEG, OC$H_2$$(CH_2)_{10}$), 3.42-3.30 (m, 170H, H-2^{I-VII},4^{I-VII} overlapped with HDO), 2.72-2.66 (m, 4H, CH_2S), 1.65-1.55 (m, 4H, CH_2), 1.46 (t, 4H, ^3J = 6.3 Hz, CH_2), 1.35-1.17 (m, 28H, $(CH_2)_7$); ^{13}C NMR (75 MHz; DMSO-d_6) δ 143.5 (C-4-CH_2N_3), 124.4 (C-5-CH_2N_3), 102.0-101.7 (C-1^{I-VII}), 82.1-81.3 (C-4^{I-VII}), 79.3 (C-2^I), 73.4-71.7 (C-2^{II-VII},3^{I-VII},5^{I-VII}), 70.3, 69.9, 69.8, 69.7, 69.6, 69.5, 68.8 (*C*H_2OEG,

O*C*H_2(CH_2)$_{10}$), 64.4 (*C*H_2O-C_2HN_3), 60.2-59.8 (C-6^{I-VII}), 49.4 (CH_2N), 37.9 (CH_2S), 29.2, 29.0, 28.9, 28.8, 28.6, 28.5, 27.7, 25.7 ((CH_2)$_9$); MALDI-TOF-MS *m*/*z* calcd for $C_{128}H_{220}N_6O_{78}S_2Na$ 3177.3, found 3177.2 $(M + Na)^+$; calcd for $C_{64}H_{110}N_3O_{39}SNa$ 1600.6, found 1600.7 $(M/2 + Na)^+$.

AuNP 1. ^{1}H NMR (500 MHz, D_2O) δ 8.10 (s, 1H, H-5-C_2HN_3), 4.96 (d, 1H, *J* = 12.5 Hz, C*H*O-C_2HN_3), 4.80 (bs, C*H*O-C_2HN_3 overlapped with HDO), 4.58 (t, 2H, *J* = 4.6 Hz, CH_2N), 4.55 (d, 1H, *J* = 7.9 Hz, H-1), 4.44 (d, 1H, *J* = 7.7 Hz, H-1'), 3.97-3.66 (m, 9H, H-2',3,4,4',5',6,6,6',6'), 3.63-3.51 (m, 14H, H-3',5, C*H*$_2$OEG), 3.45 (t, 2H, *J* = 6.3 Hz, OC*H*$_2$(CH_2)$_{10}$), 3.34 (t, 1H, *J* = 8.4 Hz, H-2), 2.69 (t, 2H, *J* = 6.8 Hz, CH_2S), 1.69 (bs, 2H, CH_2), 1.56 (bs, 2H, CH_2), 1.41-1.31 (m, 14H, (CH_2)$_7$).

AuNP 2. ^{1}H NMR (500 MHz, D_2O) δ 8.12 (s, 1H, H-5-C_2HN_3), 5.28 (bs, 1H, H-1^I), 5.06-5.04 (H-1^{II-VII}), 4.80 (bs, C*H*$_2$O-C_2HN_3, CH_2N overlapped with HDO), 4.07 (t, 1H, *J* = 7.0 Hz, H-3^I), 3.96-3.70 (m, H-3^{II-VII},5^{I-VII},6^{I-VII},6'$^{I-VII}$), 3.64-3.32 (H-2^{I-VII},4^{I-VII}, OC*H*$_2$(CH2)10, CH_2OEG), 2.60 (t, 2H, *J* = 5.6 Hz, CH_2S), 1.61-1.60 (m, 4H, (CH_2)$_2$), 1.46-1.26 (m, 14H, (CH_2)$_7$).

AuNP 3. ^{1}H NMR (500 MHz, D_2O) δ 8.14 (s, 1H, H^a-5-C_2HN_3), 8.10 (s, 3H, H^b-5-C_2HN_3), 5.30 (d, 1H, *J* = 3.0 Hz, H-1^I), 5.10-5.05 (m, 6H, H-1^{II-VII}), 4.95 (d, 6H, *J* = 13.6 Hz, C*H*^{a}O-C_2HN_3), 4.80 (bs, C*H*^{a}O-C_2HN_3, C*H*$_2^b$O-C_2HN_3 overlapped with HDO), 4.64 (t, 2H, *J* = 4.5 Hz, CH_2^aN), 4.58 (bs, 6H, CH_2^bN), 4.55 (d, 3H, *J* = 7.9 Hz, H-1), 4.43 (d, 3H, *J* = 7.7 Hz, H-1'), 3.96-3.60 (m, 50H, H-2',3,3^{I-VII},4,4',5',5^{I-VII},6,6,6^{I-VII},6^{I-VII},6',6'), 3.65-3.58 (m, 98H, H-2^{I-VII},3',5,4^{I-VII}, C*H*$_2^{a,b}$OEG, OC*H*$_2^a$(CH_2)$_{10}$), 3.44 (bt, 6H, OC*H*$_2^b$(CH_2)$_{10}$), 3.34 (t, 3H, *J* = 8.2 Hz, H-2), 2.68 (bs, 6H, CH_2^bS), 2.62 (t, 2H, *J* = 6.6 Hz, CH_2^aS), 1.69 (bs, 6H, CH_2^b), 1.65-1.61 (m, 2H, CH_2^a), 1.56 (bs, 6H, CH_2^b), 1.41-1.30 (m, 52H, ($CH_2^{a,b}$)$_7$).

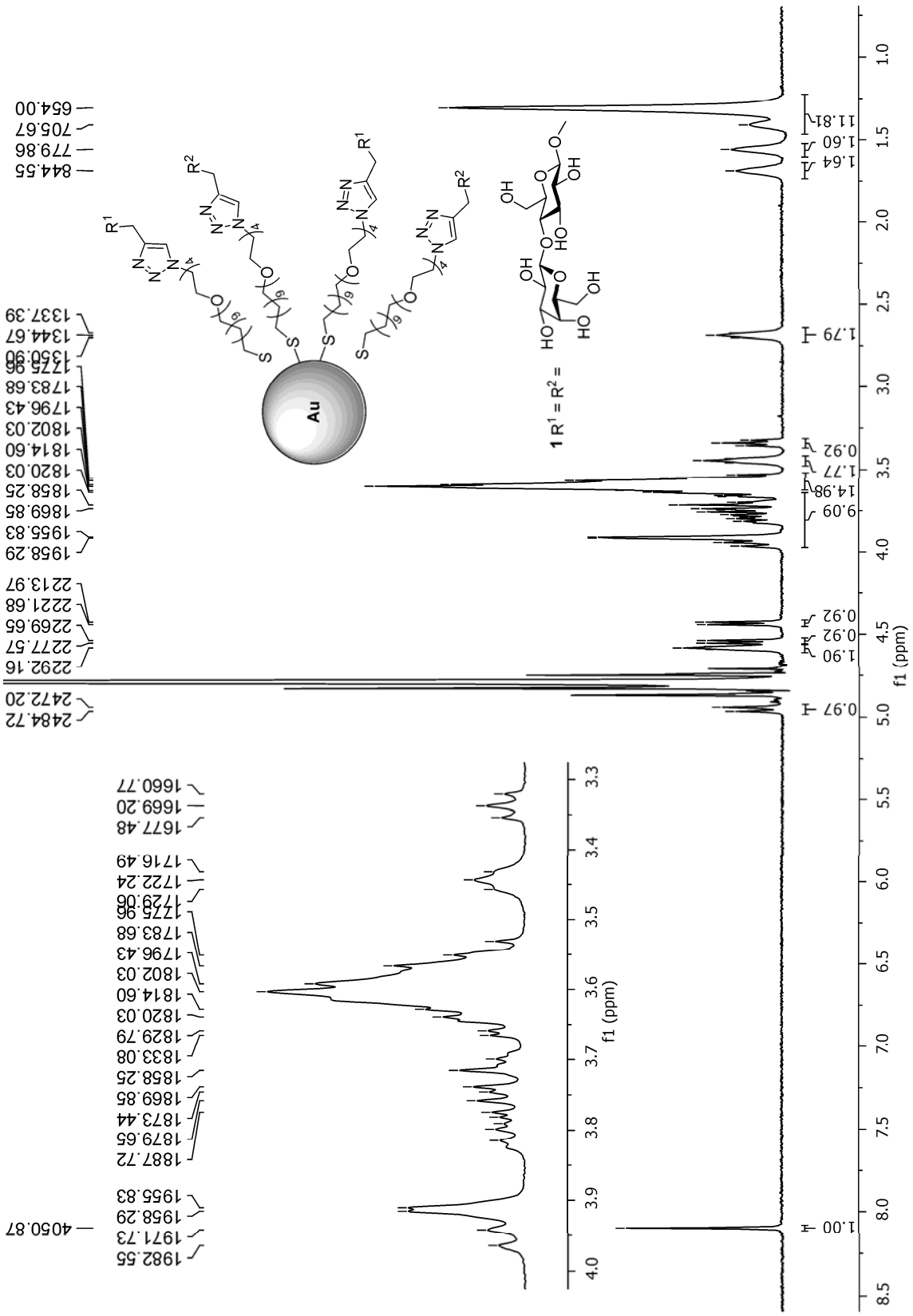


Fig S1 ^{1}H NMR spectrum (500 MHz, D_2O, 25 ºC) for AuNP **1**

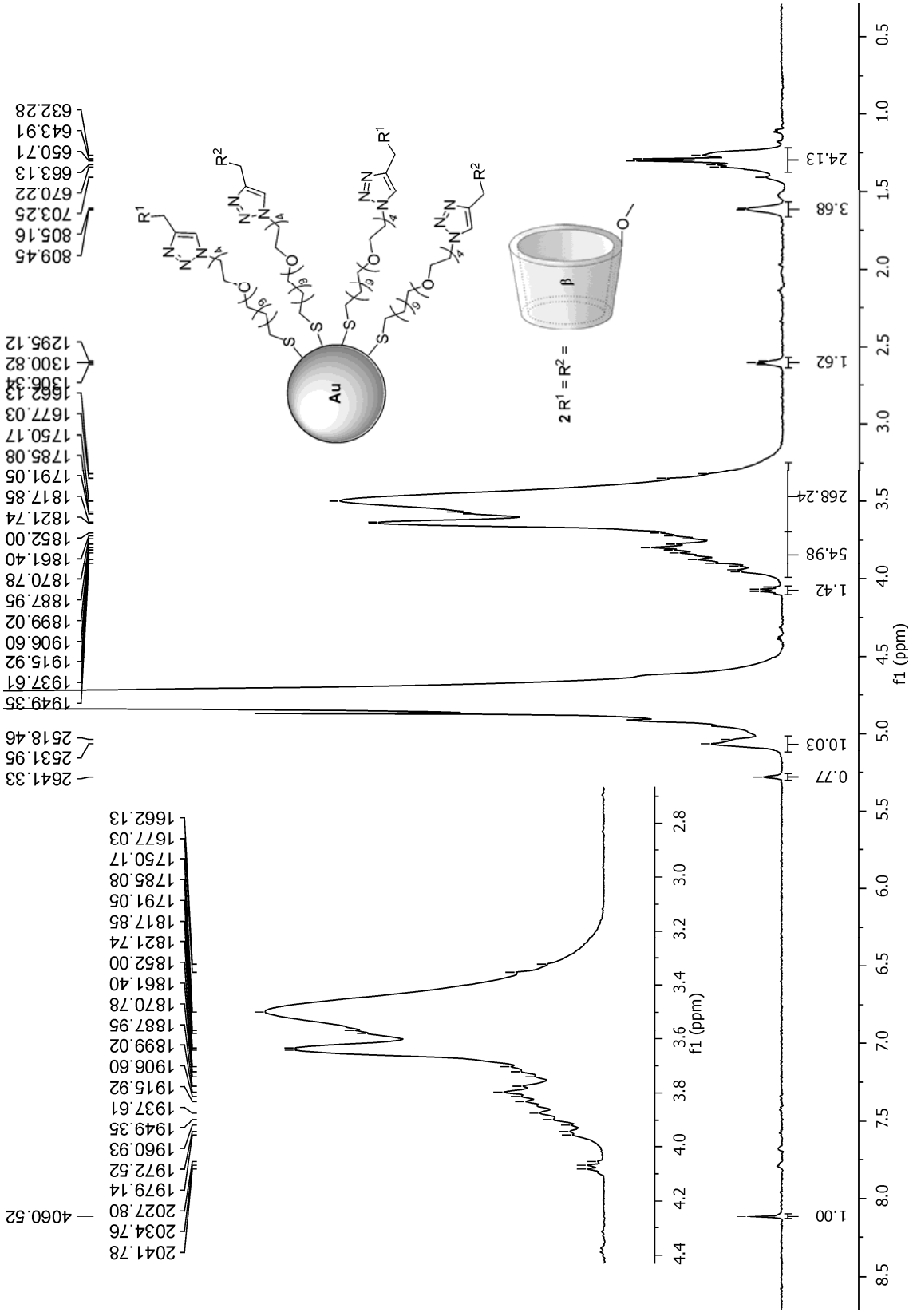


Fig S2 ^{1}H NMR spectrum (500 MHz, D_2O, 25 ºC) for AuNP **2**

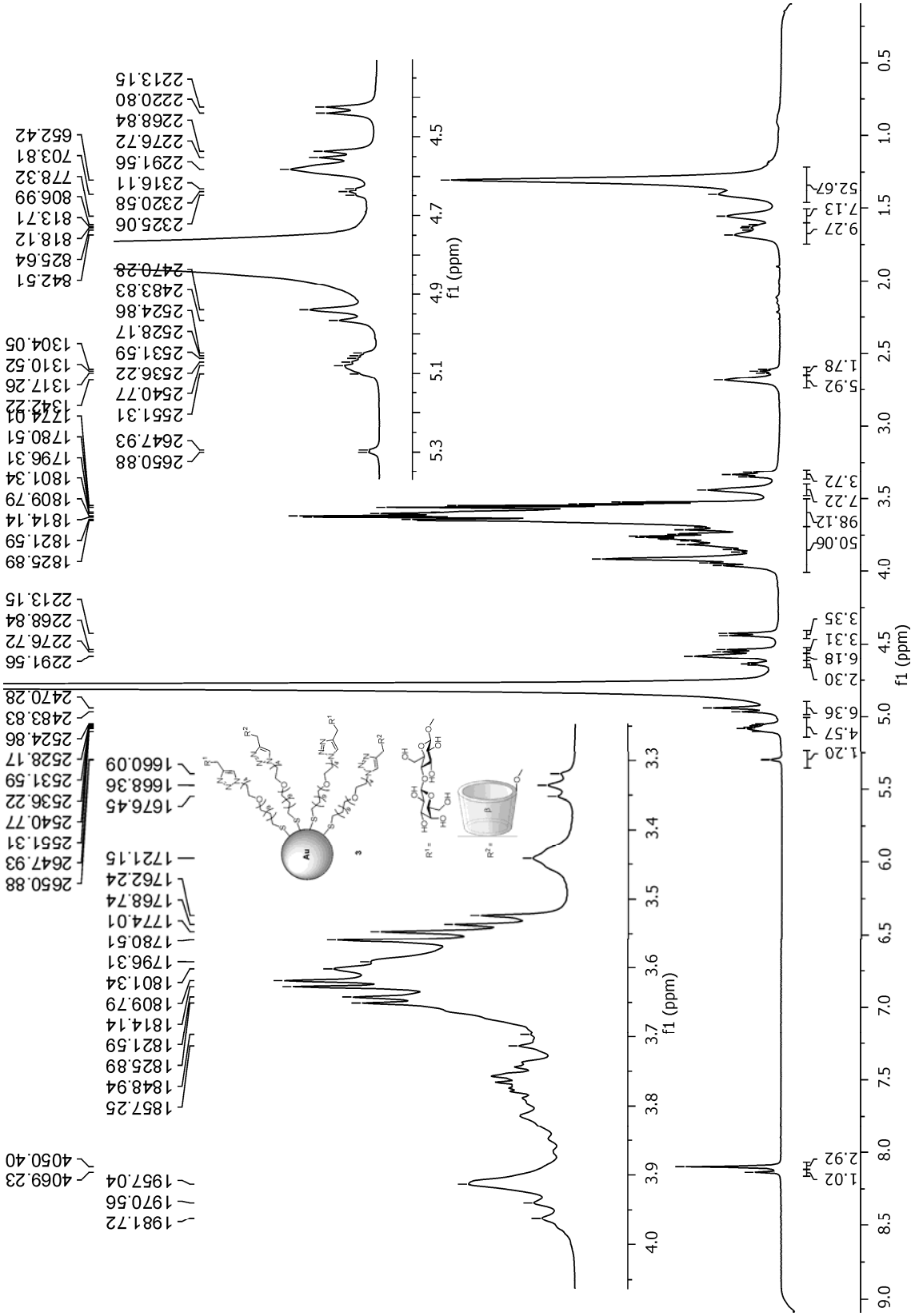


Fig S3 ^{1}H NMR spectrum (500 MHz, D_2O, 25 ºC) for AuNP **3**

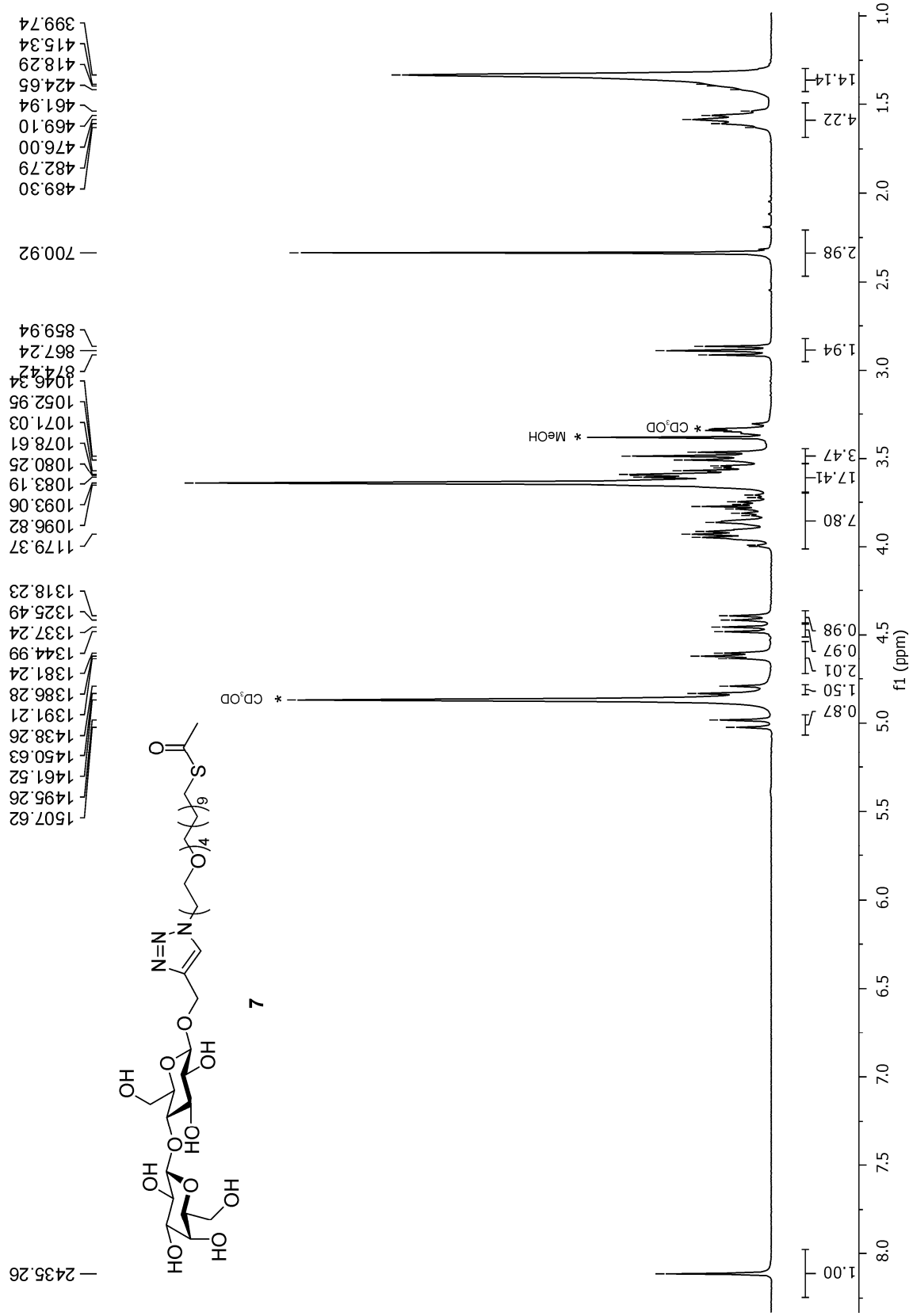


Fig S4 ^{1}H NMR spectrum (300 MHz, CD_3OD, 25 ºC) for compound **7**

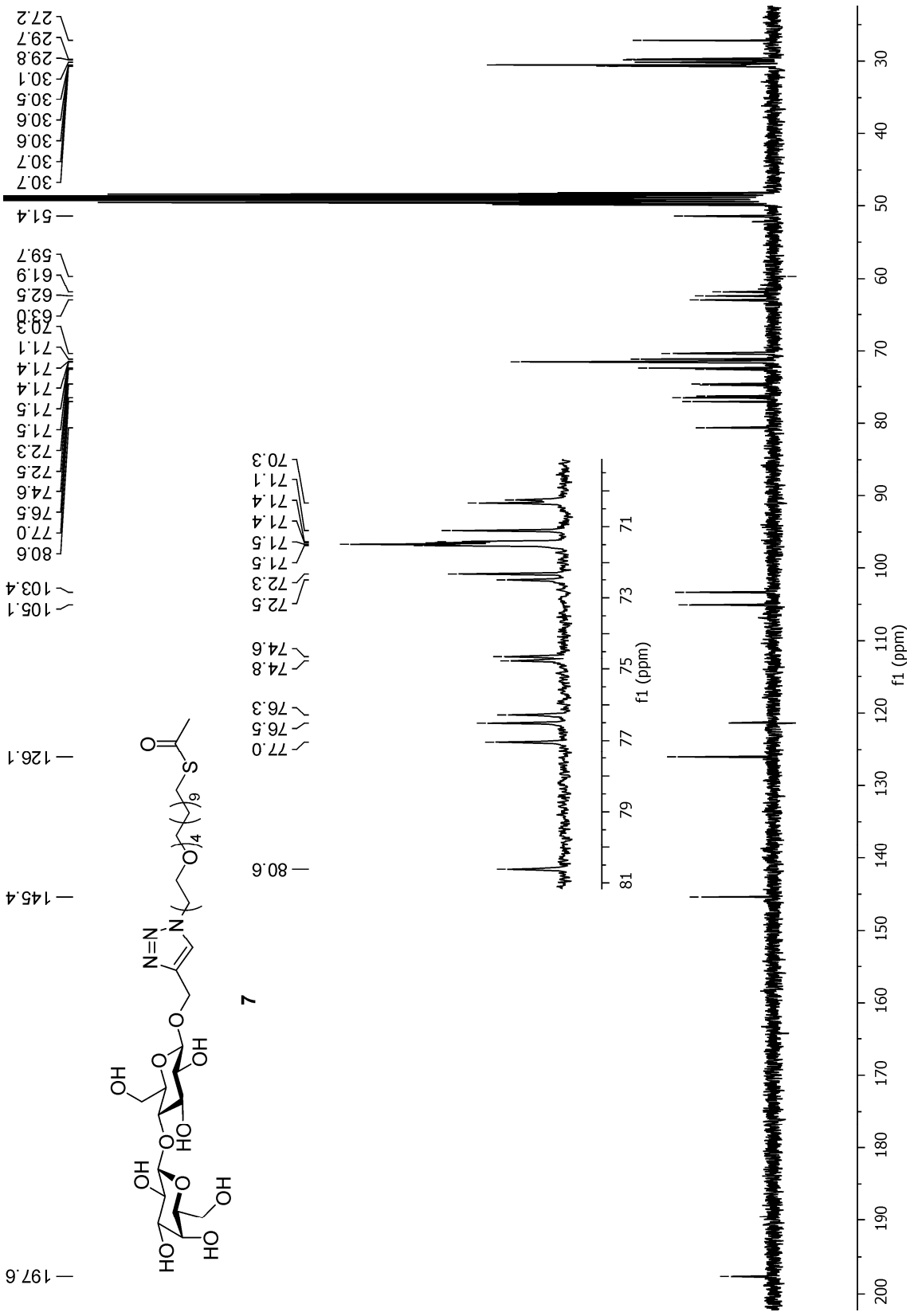


Fig S5 ^{13}C NMR spectrum (75 MHz, CD_3OD, 25 ºC) for compound **7**

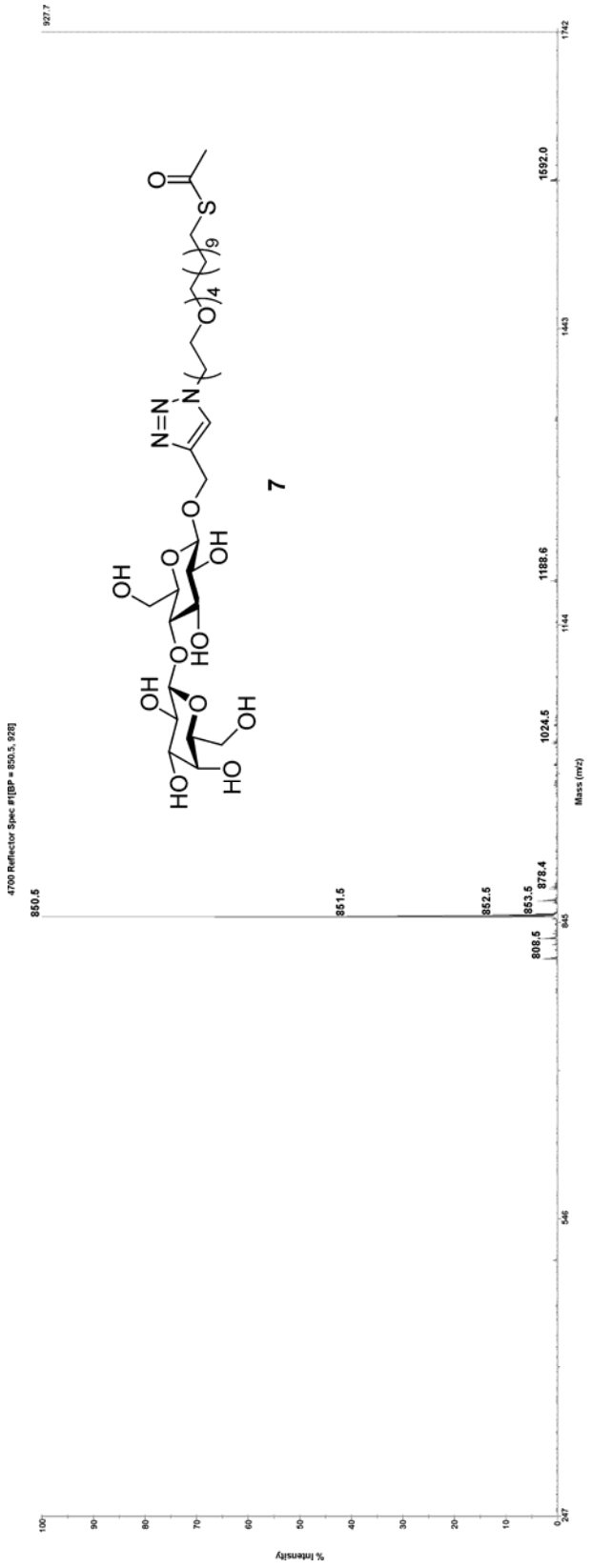


Fig S6 MALDI-TOF spectrum for compound **7**

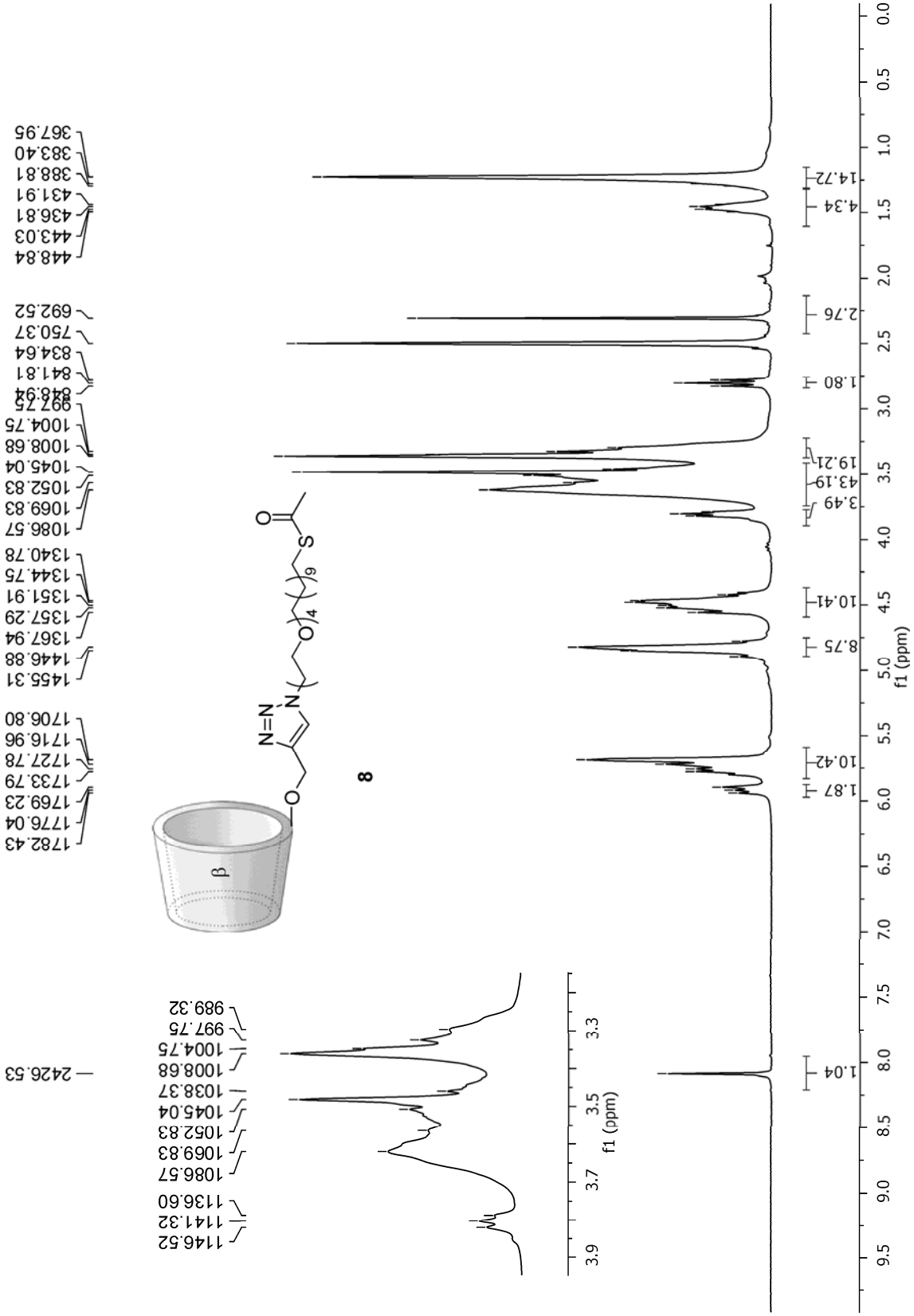


Fig S7 ^{1}H NMR spectrum (300 MHz, DMSO-d_6, 25 ºC) for compound **8**

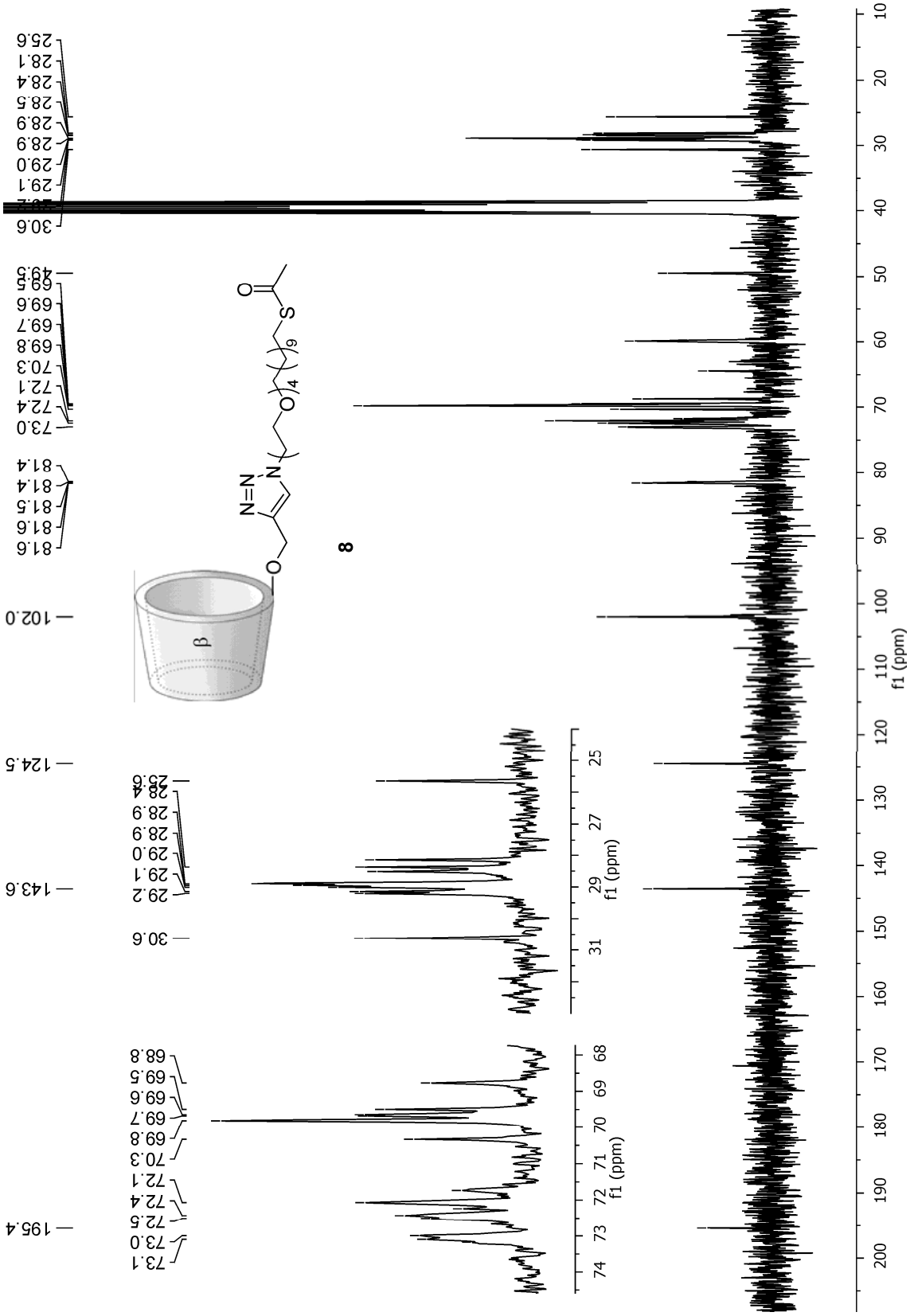


Fig S8 ^{13}C NMR spectrum (75 MHz, DMSO-d_6, 25 ºC) for compound **8**

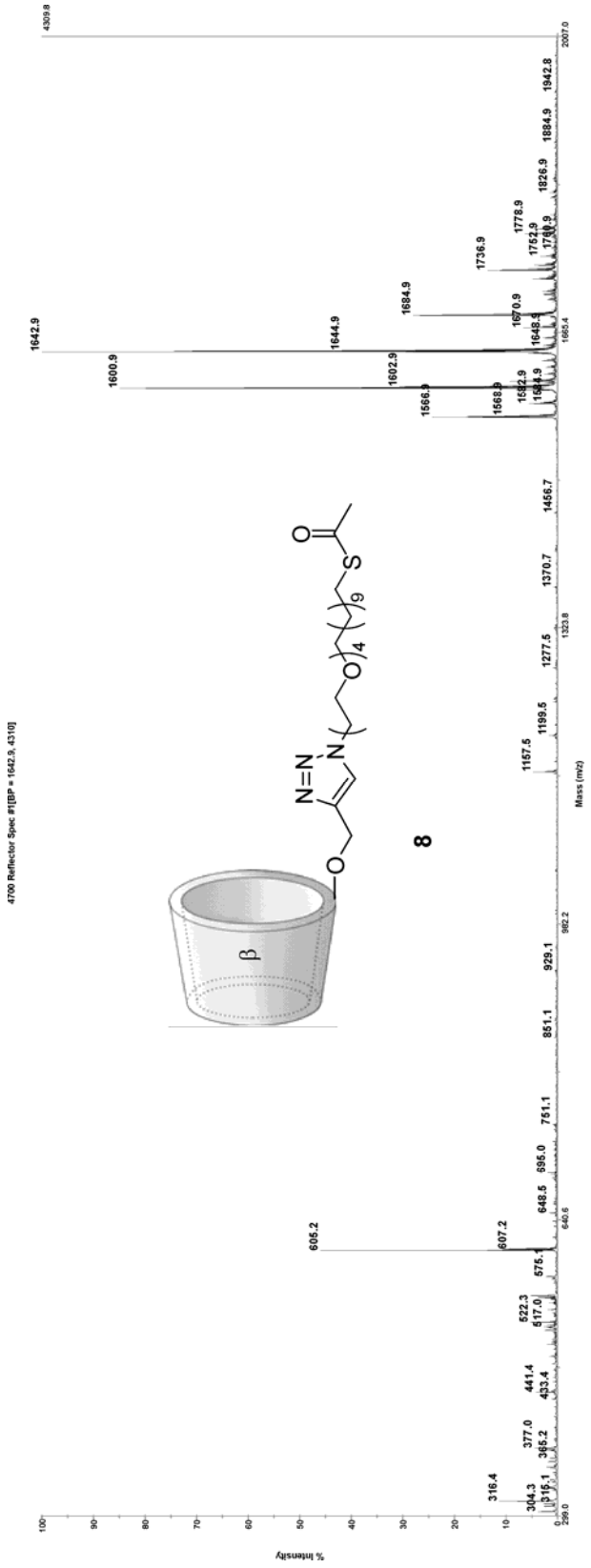


Fig S9 MALDI-TOF spectrum for compound **8**

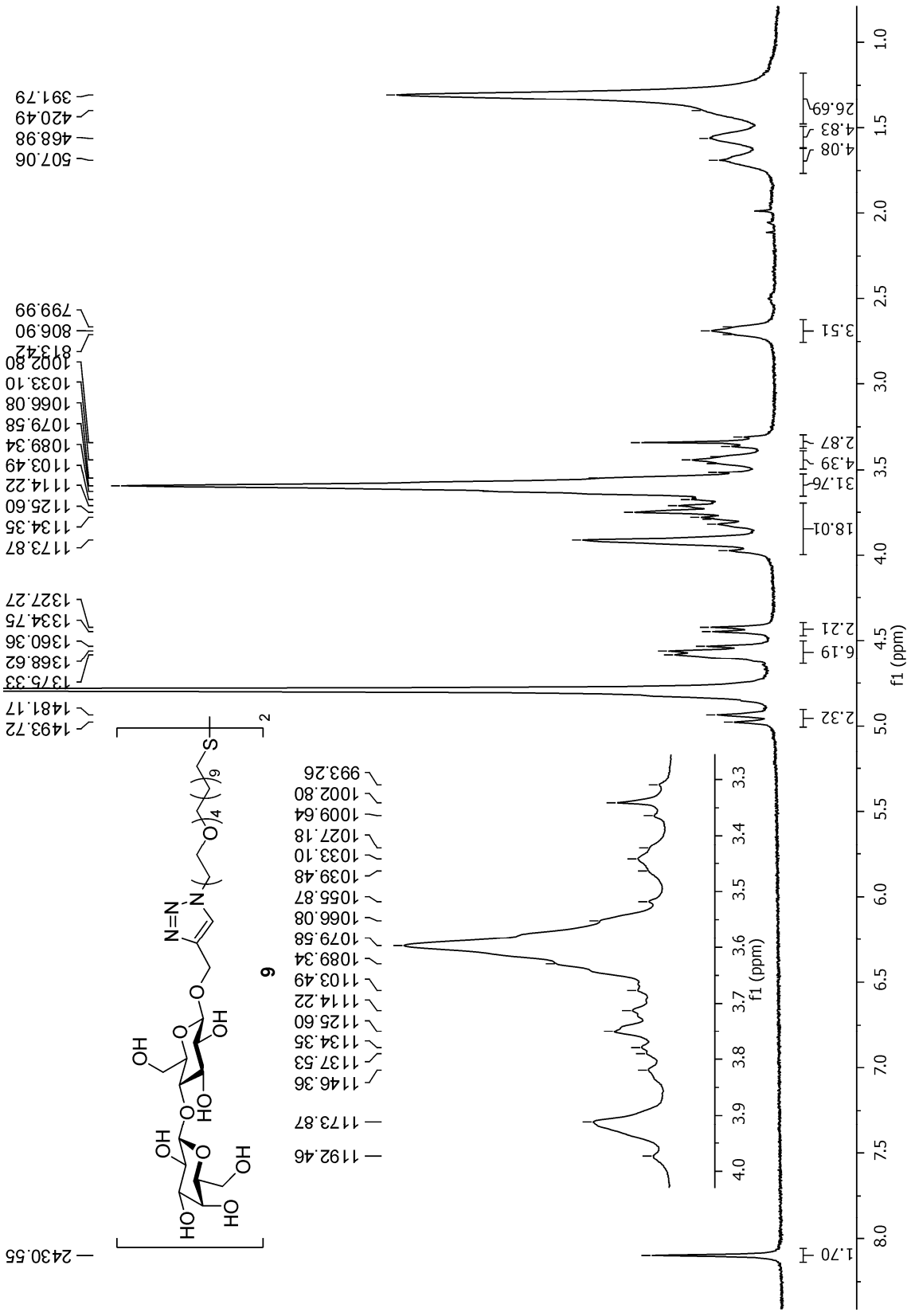


Fig S10 ^{1}H NMR spectrum (300 MHz, D_2O, 25 ºC) for compound **9**

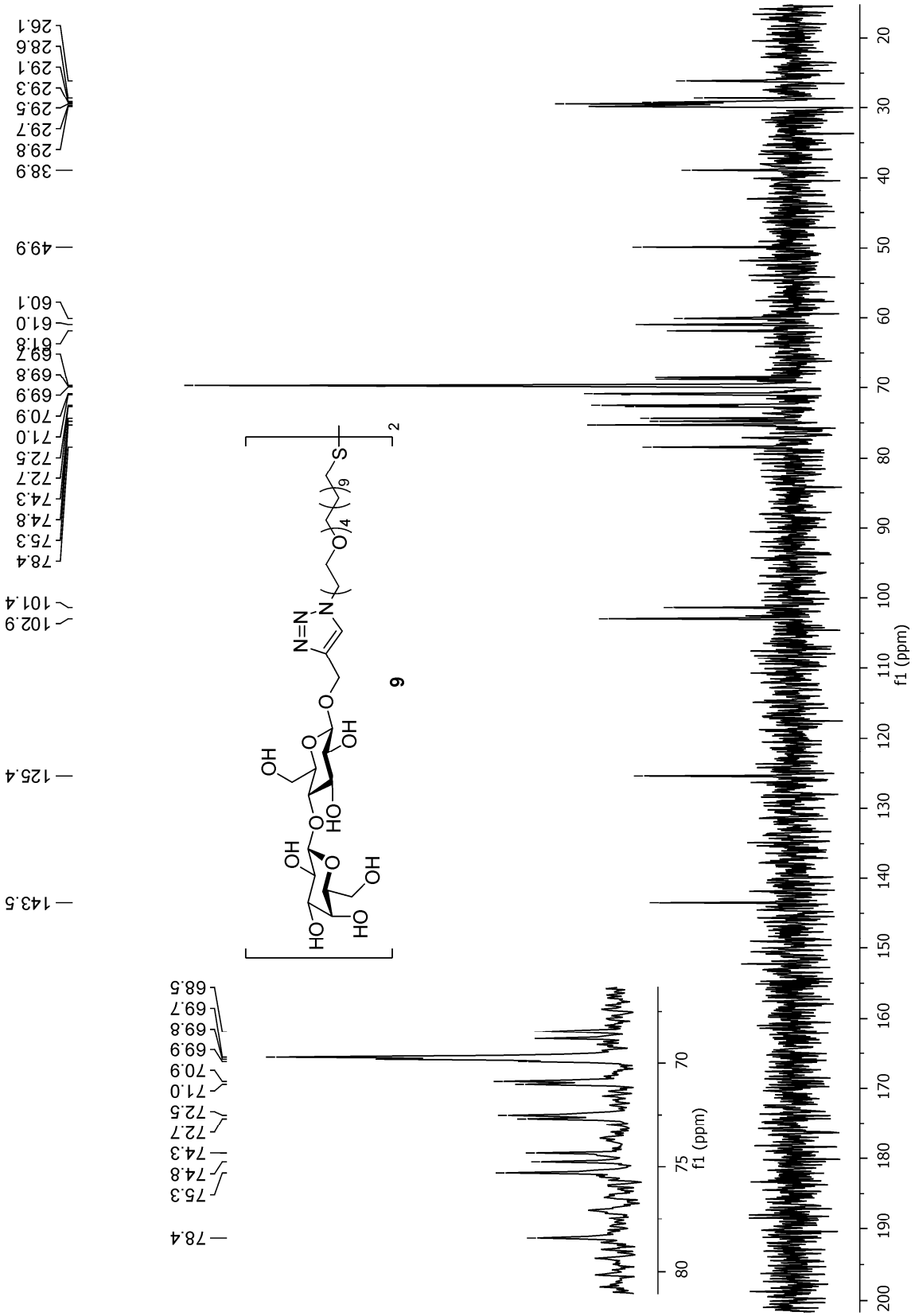
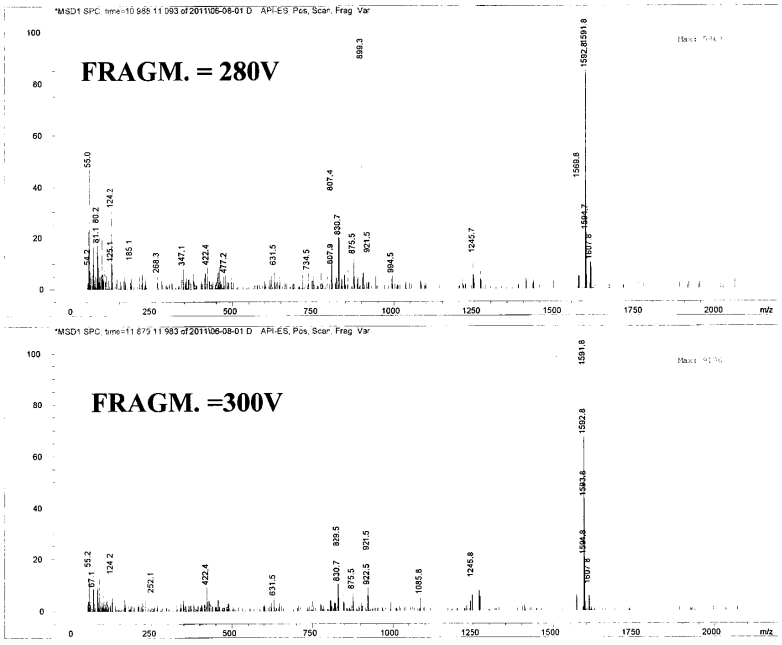
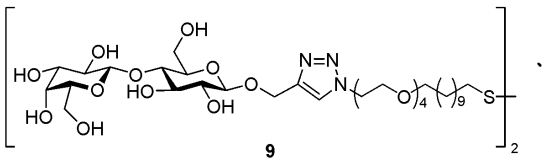


Fig S11 ^{13}C NMR spectrum (75 MHz, D_2O, 25 ºC) for compound **9**





9

Fig S12 ESI-TOF spectrum for compound **9**

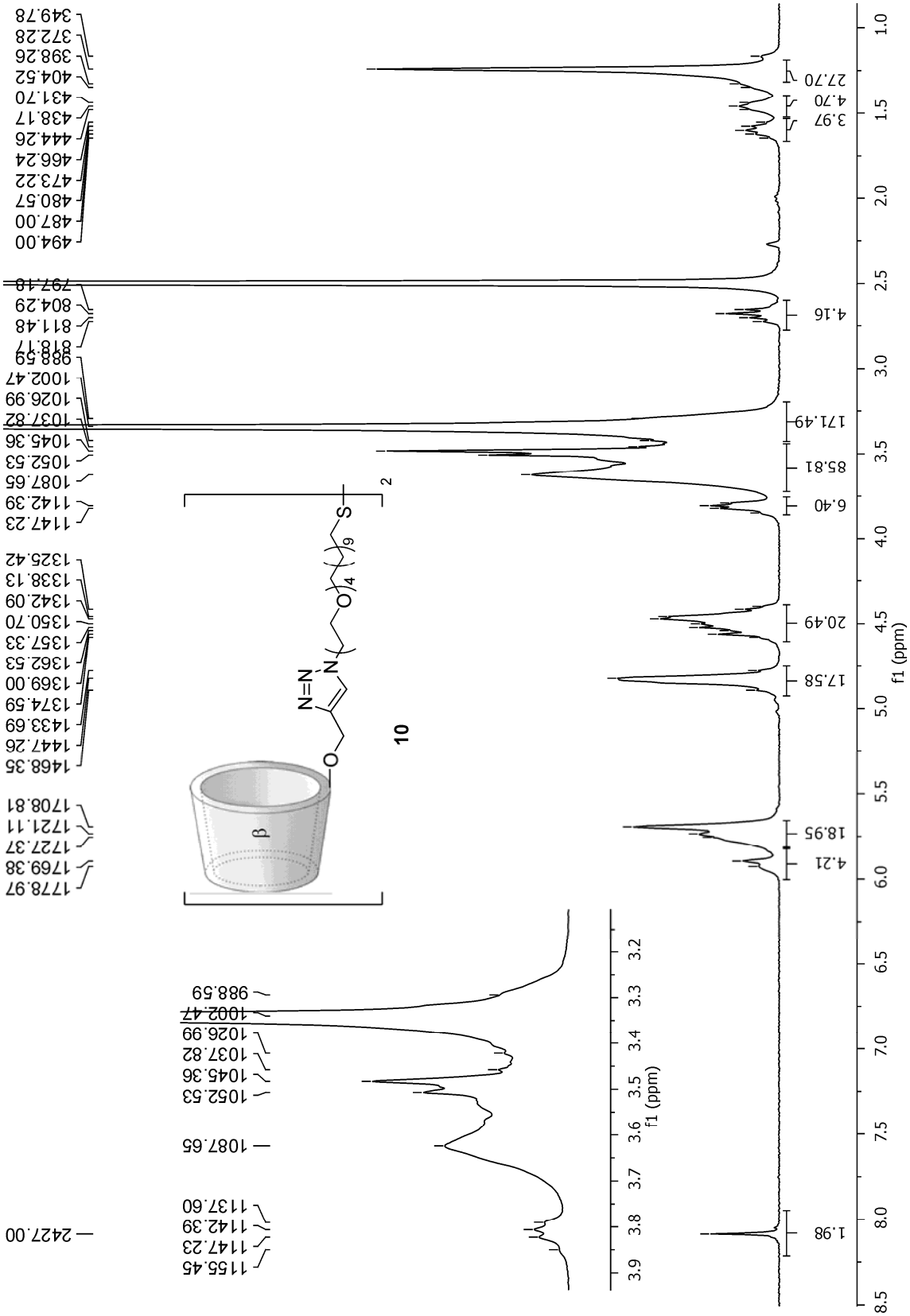


Fig S13 ^{1}H NMR spectrum (300 MHz, DMSO-d_6, 25 ºC) for compound **10**

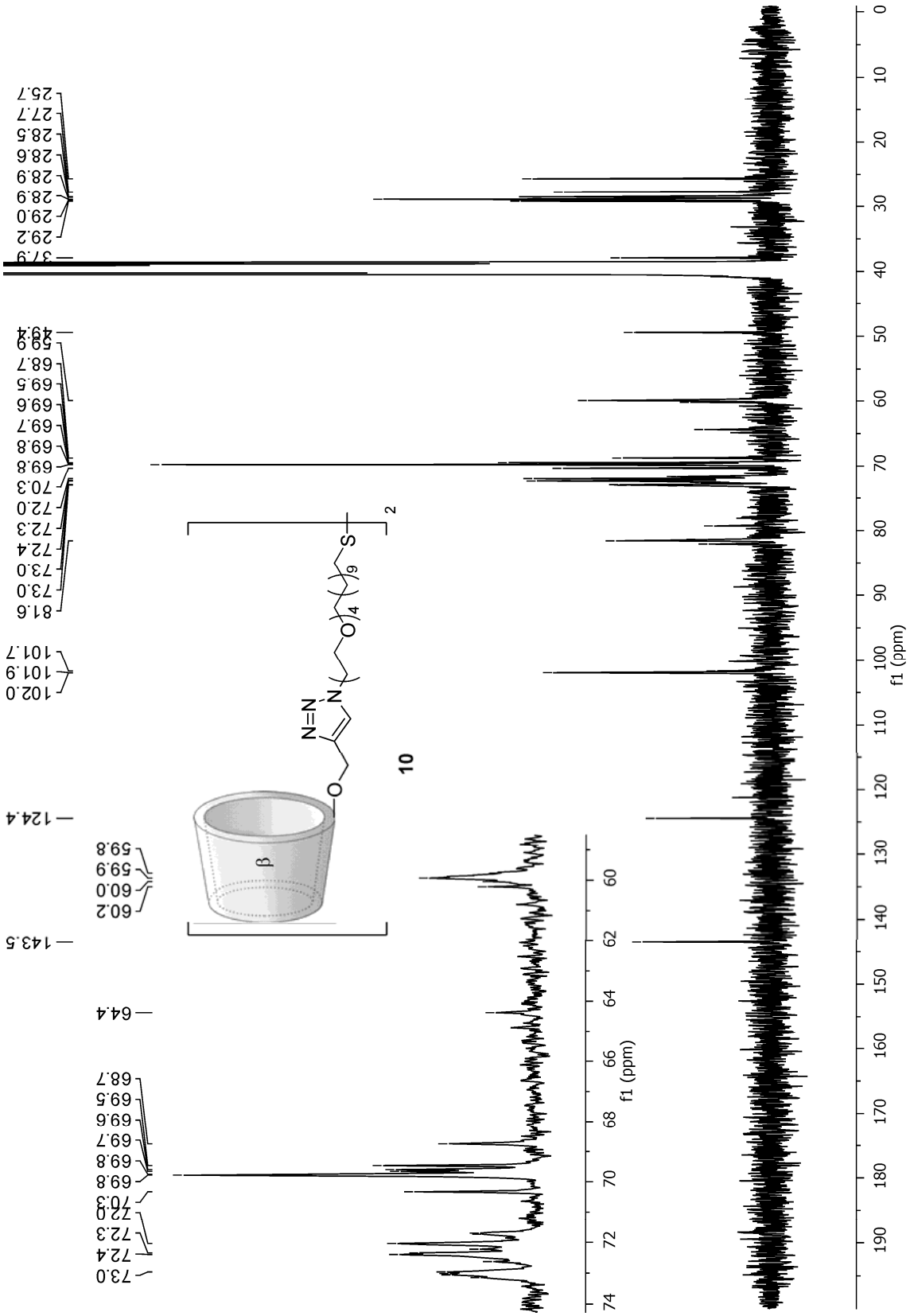


Fig S14 ^{13}C NMR spectrum (75 MHz, D_2O, 25 ºC) for compound **10**

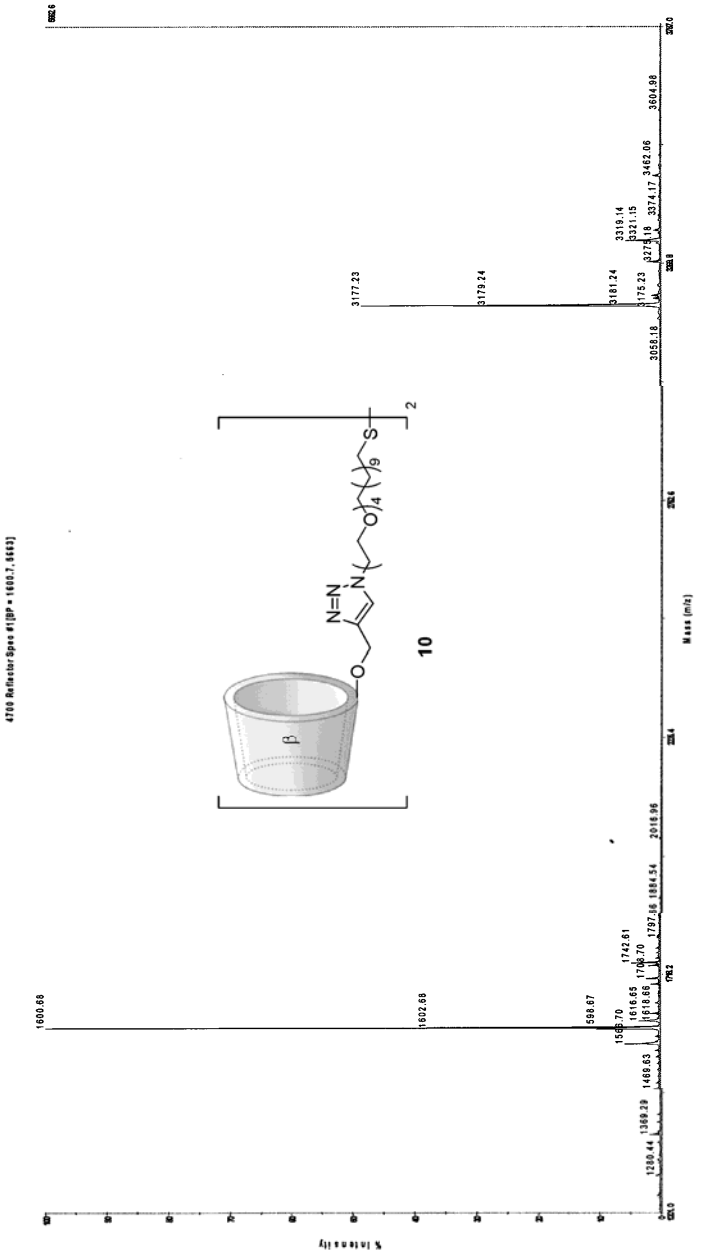


Fig S15 MALDI-TOF spectrum for compound **10**

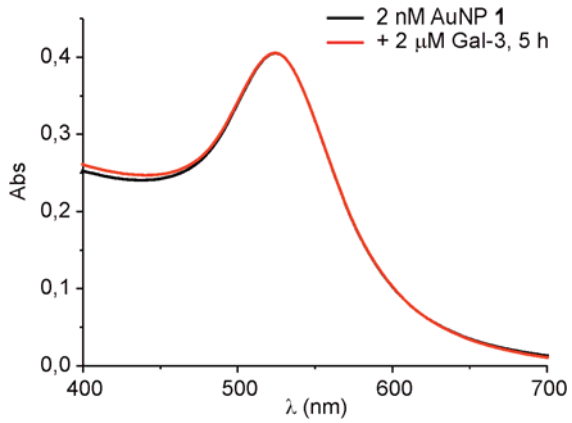


Figure S16 UV spectra for AuNP **1** (2 nM) in 10 mM phosphate buffer, pH 7.2, 20 mM NaCl in the absence (black line) and in the presence (orange line) of Gal-3 (2 μM) after 5 hours of incubation at room temperature in the dark.

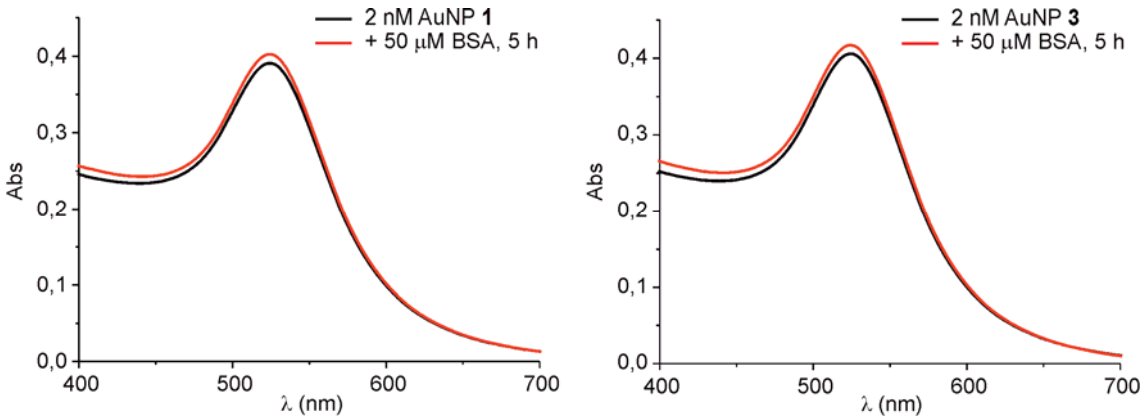


Figure S17 UV spectra for AuNP **1** (2 nM, left) and AuNP **2** (2 nM, right) in 10 mM phosphate buffer, pH 7.2, 20 mM NaCl in the absence (black line) and in the presence (orange line) of BSA (50 μM) after 5 hours of incubation at room temperature in the dark.

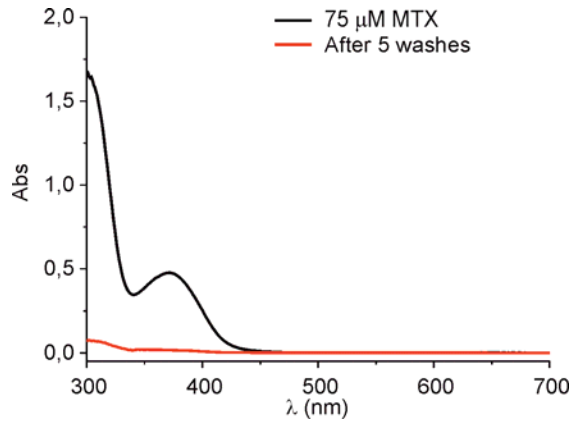


Figure S18 UV spectra for 75 μM MTX in 10 mM phosphate buffer, pH 7.2, 20 mM NaCl before (black line) and after (orange line) five centrifugal filtrations.