Versatile method for AFM-tip functionalization with biomolecules: fishing a ligand by means of an *in situ* click reaction

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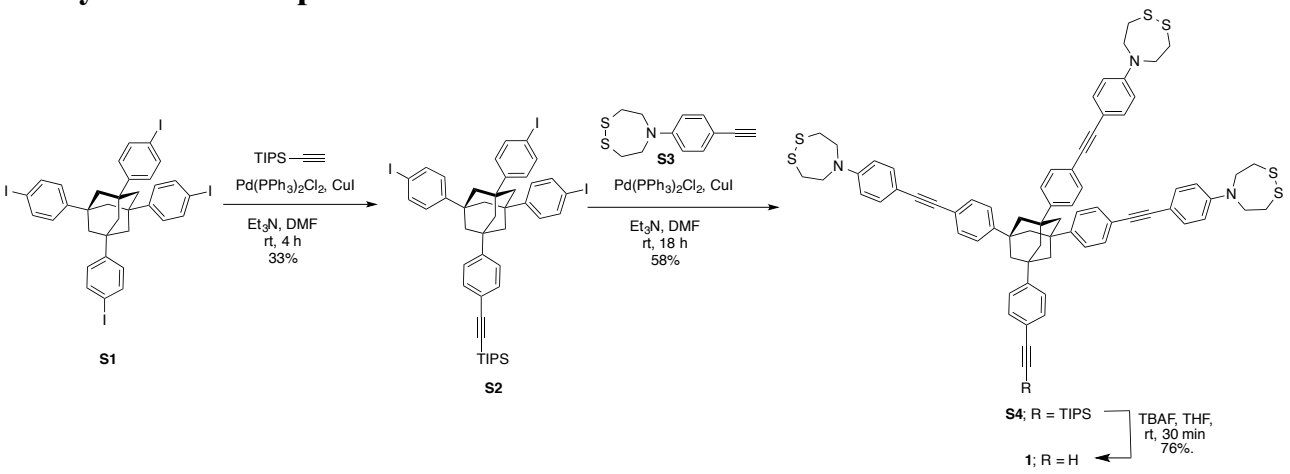
Supporting Information

1. Synthesis of tripods

General. NMR spectra were recorded on Varian 300 spectrometer (Varian Inc., CA, USA) and Bruker 400 spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany). HRMS analyses were performed using Bruker solariX ESI/MALDI-FTICR MS (Bruker Daltonics, GmbH, Bremen, Germany). FTIR spectra were recorded on a PerkinElmer Spectrum One FT-IR Spectrometer with Universal ATR Sampling Accessory (PerkinElmer Inc., Waltham, MA, USA). Melting points were measured with MEL-TEMP (Laboratory Devices, Holliston, MA, USA). All the reagents purchased from corresponding suppliers were used without further purification. NH_2–PEG_{2000}–N_3 was purchased from Laysan Bio, Inc. (Arab, Al, USA). $Pd(PPh_3)_2Cl_2$ and $[Cu(CH_3CN)_4]PF_6$ were purchased from ABCR GmbH & Co. KG, Germany. Wherever needed, solvents were dried by solvent system (Innovative Technology Inc., FL, USA) and triethylamine was distilled over CaH_2. Column chromatography and analytical TLC were performed on SILICYCLE SilicaFlash® F60 (230–400 mesh) and Silica gel 60 F254 TLC (Merck KGaA, Darmstadt, Germany), respectively.

1.1 Synthesis of tripod 1



Scheme S1. Synthesis of tripod **1**

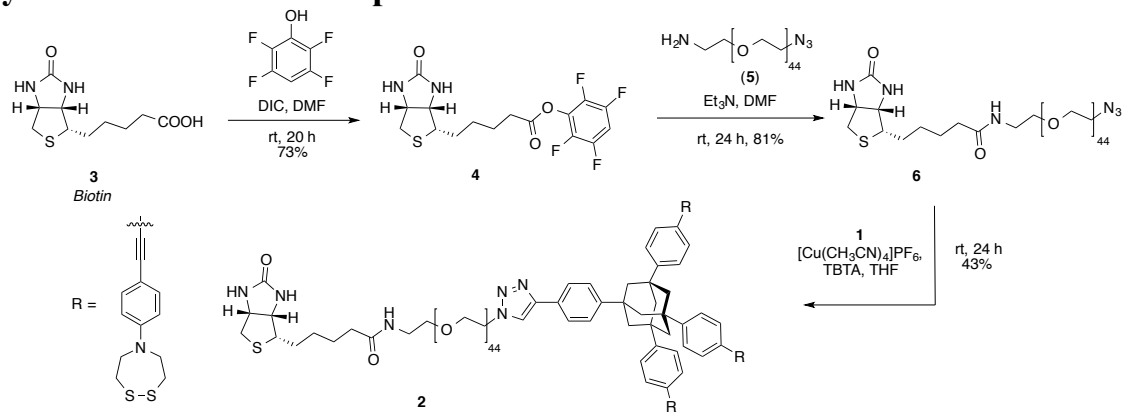
(a) Synthesis of triisopropyl(4-{3,5,7-tris-[4-iodophenyl]adamantan-1-yl}phenylethynyl)-silane (S2): Et_3N (3 mL), $Pd(PPh_3)_2Cl_2$ (37.2 mg, 0.053 mmol, 5 mol%) and CuI (20.2 mg, 0.106 mmol, 10 mol%) were added to a solution of tetrakis(4-iodophenyl)adamantane **S1** (1.0 g, 1.06 mmol, 1.0 molar equiv) in anhydrous DMF (10 mL), under a N_2 atmosphere. TIPS-acetylene (0.240 mL, 1.06 mmol, 1.0 molar equiv) in anhydrous DMF (2 mL) was added to the reaction mixture dropwise over 3 hours using a syringe. After the complete addition, the reaction mixture was stirred for one hour and then diluted with 30 mL CH_2Cl_2. 20 mL aqueous solution containing NH_4OH/NH_4Cl (1:9, v/v) was added and the layers were separated. The aqueous layer was extracted three times with CH_2Cl_2 (10 mL each time). The combined organic layer was washed with excess water and then brine solution. After drying over anhydrous Na_2SO_4, the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography using silica gel (mesh 230–400) and eluent: 10% CH_2Cl_2–hexanes to 100% CH_2Cl_2, to obtain 344 mg (33%) of **S2** as a white solid.

(b) Synthesis of triisopropyl(4-(3,5,7-tris-(4-{4-[1,2,5-dithiazepan-5-yl]phenylethynyl}phenyl)-adamantan-1-yl)phenylethynyl)silane (S4): Compound **S2** (0.438 g 0.439 mmol, 1.0 molar equiv) and compound **S3** (0.415 g, 1.75 mmol, 4.0 molar equiv) were dissolved in 10 mL anhydrous DMF.

To the mixed solution above, Et_3N (3 mL), $Pd(PPh_3)_2Cl_2$ (30.8 mg, 0.044 mmol, 10 mol%) and CuI (16.8 mg, 0.088 mmol, 20 mol%) were added under a N_2 atmosphere. The yellowish suspension was stirred at room temperature for 20 hour and then diluted with 50 mL CH_2Cl_2. Subsequently, a mixture of NH_4OH/NH_4Cl (1:9, v/v, 25 mL) was added and the layers were separated. The aqueous layer was extracted three times with CH_2Cl_2 (50 mL each time). The combined organic layer was washed with excess water and then brine solution. After drying over anhydrous Na_2SO_4, the solvent was removed under reduced pressure. The crude product was then purified by flash column chromatography using silica gel (mesh 230–400) and eluent: 60% toluene–hexanes to 100% toluene to get 336 mg (58%) of **S4** as a yellowish solid.

(c) Synthesis of tripod 1:[1] Tripod **S4** (240 mg, 0.181 mmol, 1.0 molar equiv) was dissolved in 8.0 mL anhydrous THF and tetrabutylammonium fluoride (1.0 M solution in THF, 0.180 mL, 1.0 molar equiv) was added. The reaction mixture was stirred at room temperature for 30 minutes and then diluted with 50 mL CH_2Cl_2. This solution was washed with 20 mL aqueous NH_4Cl solution followed by washing with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure. The yellowish solid obtained as a crude mixture was sonicated in 20 mL diethyl ether for 5 minutes. The solvent was removed and the solid dried again under reduced pressure to obtain 161.0 mg (76%) of tripod **1** as a yellowish solid.

1.2 Synthesis of molecular tip 2[1]



Scheme S2. Synthesis of N_3–PEG_{2000}–biotin **6** and molecular tip **2**

(a) Synthesis of compound 4:[1-3] To a mixture of biotin **3** (200 mg, 0.82 mmol, 1.0 molar equiv) and tetrafluorophenol (163 mg, 0.983 mmol, 1.2 molar equiv), anhydrous DMF (3.3 mL) was added. To the suspension obtained, *N,N*–diisopropylcarbodiimide, (0.140 mL, 0.901 mmol, 1.1 molar equiv) was added. The suspension was stirred at room temperature for 20 h to turn to be clear solution. The reaction mixture was diluted with $CHCl_3$ (20 mL) and water (10 mL). The organic layer was separated and the aqueous layer extracted twice with $CHCl_3$ (20 mL each time). The combined organic layer was washed with brine solution, dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure. The crude product was then purified by flash column chromatography using silica gel (mesh 230-400) and eluent: 5% MeOH–CH_2Cl_2, to obtain 287 mg (73%) of **4** as a sticky white solid. R*f* = 0.39 (5% MeOH–CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.05-6.94 (m, 1H, –OC_6F_4***H***), 5.46 (br s, 1H, –N***H***), 4.97 (br s, 1H, –N***H***), 4.55-4.51 (m, 1H, biotin –SCH_2C***H***NH–), 4.36-4.32 (m, 1H, biotin –SCH(alkyl)C***H***NH–), 3.22-3.16 (m, 1H, biotin, –SC***H***(alkyl)CH–), 2.94 (dd, *J* = 12.9 Hz, 5.0 Hz, 1H, biotin –SC(***H)***HCHNH–), 2.74 (overlapping d, *J* = 12.9 Hz, 1H, biotin –SC(***H)***HCHNH–), 2.71 (overlapping t, *J* = 7.4 Hz, 2H, –OC(O)C***H₂***CH_2–), 1.89–1.68 and 1.61–1.48 (m, 6H, alkyl chain); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.52, 162.71, 145.52 (2C, app dtd, *J* = 245.9, 12.4, 3.8 Hz), 140.0 (2C, dddd, *J* = 248.1, 15.1, 4.6, 2.4 Hz),

128.85-128.50 (m), 104.37 (t, J = 23.7 Hz), 61.03, 59.20, 55.26, 39.89, 32.37, 27.92, 27.69, 24.33; ^{19}F NMR (300 MHz, $CDCl_3$, $CFCl_3$): 139.37-139.53 (m), 153.48-153.64 (m).

(b) Synthesis of biotin linker 6: In a dry vial compound **4** (20 mg, 0.051 mmol, 1.0 molar equiv) and NH_2-PEG_{2000}-N_3 (**5)** (102 mg, 0.051 mmol, 1.0 molar equiv) were dissolved in 400 µL anhydrous DMF and then Et_3N (10 µL) was added using a micro syringe. The vial was sealed with a rubber septum bearing a nitrogen balloon and the reaction mixture stirred at room temperature for 24 h. The solvent was removed under reduced pressure. The crude mixture was dissolved in a small volume of CH_2Cl_2 and the product precipitated by slow addition into Et_2O. The precipitate was collected by centrifuge and the supernatant was concentrated. The precipitation was repeated with the mixture obtained from mother liquor with CH_2Cl_2–Et_2O and the precipitate was combined to obtain 91 mg (81%, white solid) of compound **6**. Melting point: 37-39 °C; IR (neat) ν_{max} (cm^{-1}): 3260 (w), 2882 (m), 2100 (w), 1793 (w), 1702 (m), 1524 (w), 1466 (m), 1359 (w), 1341 (s), 1279 (m), 1240 (m), 1146 (w), 1099 (s), 1060 (w), 956 (s) 841 (s); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.75 (t, J = 5.1 Hz, 1H, amide N***H***), 6.03 (br s, 1H, biotin ureido N***H***), 5.22 (br s, 1H, biotin ureido N***H***), 4.48 (dd, 1H, J = 7.6, 5.1 Hz, biotin –SCH_2C***H***NHCONH–), 4.30 (dd, 1H, J = 6.8, 4.8 Hz, biotin –$SCH(CH_2$–)C***H-***NHCONH), 3.81-3.43 (m, 176 H, PEG –OC***H$_2$***C***H$_2$***O–), 3.42-3.40 (m, 2H, PEG –OCH_2C***H$_2$***NHCO–), 3.37 (t, J = 5.1 Hz, 2H, PEG –OCH_2C***H$_2$***N_3), 3.15-3.10 (m, 1H, biotin –SC***H***(CH_2–)CHNH–), 2.88 (dd, J = 12.8 Hz, 5.0 Hz, 1H, biotin –SC***H***(H)CHNH–), 2.72 (d, J = 12.8 Hz, 1H, biotin –SCH(***H***)CHNH–), 2.21 (td, J = 7.3 Hz, 1.9 Hz, 2H, biotin –C***H$_2$***CONH–), 1.78–1.59 and 1.46–1.37 (m, 6H, biotin –C***H$_2$***C***H$_2$***C***H$_2$***CH_2CONH–); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.31, 163.67, 77.43, 71.1, 70.87–70.57 multiple PEG peaks, 70.28, 70.20, 70.09, 61.90, 60.29, 55.59, 50.85, 40.69, 39.32, 36.03, 28.30, 28.27, 25.69. HRMS (MALDI, matrix: DCTB): $[M+Na]^+$ calculated for $C_{100}H_{196}N_6O_{46}SNa$ 2272.2795 (44 PEG units), found 2272.2846.

(c) Synthesis of compound 2: In a dry vial, 26 mg of **6** (11.68 µmol, 1.0 molar equiv) and 20 mg of **1** (17.52 µmol, 1.5 molar equiv) were dissolved in anhydrous 3 mL THF. $[Cu(CH_3CN)_4]PF_6$ (2.2

mg, 5.84 μmol, 0.5 molar equiv) and TBTA (2.9 mg, 5.84 μmol, 0.5 molar equiv) were added to this solution. Vial was filled with nitrogen and sealed with a Teflon cap. Reaction mixture was stirred at room temperature for 24 h and then diluted with 20 mL CH_2Cl_2. Organic solution was washed twice with NH_4OH/NH_4Cl (1:9, v/v) aqueous solution (5 mL each time), followed by washing with excess water and brine. The organic layer was dried over anhydrous Na_2SO_4 and solvent was removed under reduced pressure. The crude product was purified by silica gel preparative TLC, using three plates and eluting with 10% MeOH–CH_2Cl_2. 17.2 mg (43%) of molecular tip **2** was obtained as a yellowish semi-solid compound. R*f* = 0.32 (10% MeOH–CH_2Cl_2); IR (neat) ν_{max} (cm^{-1}): 3356 (br), 2921 (m), 2863 (m) 1703 (w), 1661 (w), 1601 (w), 1522 (m), 1456 (w), 1400 (w), 1350 (m), 1292 (w), 1217 (w), 1086 (s), 1037 (w), 946 (s), 900 (w) 838 (m); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 (s, 1H, Ar***H***), 7.84 (d, 2H, *J* = 8.6 Hz, Ar***H***), 7.54 (d, 2H, *J* = 8.6 Hz, Ar***H***), 7.50-7.44 (m, 12H, Ar***H***), 7.40 (d, 6H, *J* = 9.0 Hz, Ar***H***), 6.59 (d, 6H, *J* = 9.1 Hz, Ar***H***), 6.48 (t, *J* = 5.0 Hz, 1H, amide N***H***), 5.48 (br s, 1H, biotin ureido N***H***), 4.82 (br s, 1H, biotin ureido N***H***), 4.59 (t, 2H, *J* = 5.0 Hz, PEG–OCH_2C***H_2***–triazole), 4.50 (dd, 1H, *J* = 7.5, 5.3 Hz, biotin–SCH_2C***H***NHCONH–), 4.32 (dd, 1H, *J* = 7.5, 4.8 Hz, biotin –SCH(CH_2–)C***H***NHCONH–), 3.98 (t, 12H, *J* = 5.5 Hz, –NCH_2C***H_2***SS), 3.92 (t, 2H, *J* = 5.0 Hz, PEG–OC***H_2***CH_2–triazole), 3.82-3.42 (m, 176 H, PEG –OC***$H_2C H_2$***O–), 3.18-3.13 (m, 1H, biotin –SC***H***(CH_2–)CHNH–), 3.09 (t, 12H, *J* = 5.5 Hz, –NC***H_2***CH_2SS–), 2.92 (dd, *J* = 12.8 Hz, 5.0 Hz, 1H, biotin –SC***H***(H)CHNH–), 2.73 (d, *J* = 12.7 Hz, 1H, biotin –SCH(***H***)CHNH–), 2.24-2.16 (m, 14H, biotin –C***H_2***CONH–, adamantane 6C***H_2***), 1.77–1.60 and 1.48–1.41 (m, 6H, biotin –C***$H_2C H_2C H_2$***CH_2CONH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.16, 163.21, 149.20, 148.84, 147.61, 146.49, 133.38, 131.57, 129.07, 125.96, 126.69, 125.23, 122.13, 121.09, 111.34, 110.85, 90.24, 87.61, 77.43, 70.85–70.68 multiple PEG peaks, 70.36, 70.09, 69.78, 61.96, 60.27, 55.43, 52.67, 50.59, 47.27, 47.15, 40.74, 39.53, 39.46, 39.37, 37.00, 35.98, 28.32, 28.28, 25.64. HRMS (MALDI, matrix: DCTB) $[M]^+$ calculated for $C_{172}H_{261}N_9O_{46}S_7$ 3412.6400 (44 PEG units), found 3412.6395.

2. Modification and characterization using Au surfaces

General. All gold surface substrates were cleaned by UV/ozone treatment using UV/Ozone ProCleaner™ Plus (BioForce Nanosciences) before functionalization and all solutions were filtered through 0.45 μm PTFE filters (CHROMAFIL® Xtra PTFE-45/25, Machery-Nagel GmbH & Co. KG, Düren, Germany) before using for functionalization of surface substrates.

2.1 Functionalization of Au surfaces and AFM imaging

Three ultra-flat gold surface substrates were cleaned by UV/ozone treatment for 1 hour to remove any adsorbed organic contamination. Immediately after cleaning, two substrates were soaked overnight in a filtered solution of tripod **1** (100 μM, 2.9 mg in 25 mL) in DMSO (UV spectroscopy grade, Fluka, analytical). Subsequently, these were washed with DMSO, water, toluene and ethanol and dried by a N_2 blowgun. One substrate was directly subjected to AFM imaging (Bruker Dimension Icon) using tapping-mode tips (AC-160, Olympus, Japan) and the second one was used for the surface click reaction. The tripod-functionalized gold surface substrate was soaked in the click solution at room temperature for 10 h. Subsequently; the substrate was thoroughly washed with THF, DMSO, water, toluene and ethanol. After drying under a N_2 stream it was subjected to AFM imaging. Another clean gold surface was washed with DMSO, water, toluene and ethanol. After drying by a N_2 blowgun, it was subjected to AFM imaging.

A click solution was prepared as follows: 10 mg of N_3–PEG_{2000}–NH_2 **5** (5.0 μmole, 1.0 molar equiv) was dissolved in 4 mL THF (HPLC grade, Sigma-Aldrich). 9.3 mg $[Cu(CH_3CN)_4]PF_6$ (25.0 μmole, 5.0 molar equiv to **5**) and 13.3 mg tris-(benzyltriazolylmethyl)amine (TBTA) (25.0 μmole, 5.0 molar equiv to **5**) were dissolved in THF (1 mL each). Three solutions were mixed and filtered through a 0.45 μm PTFE filter.

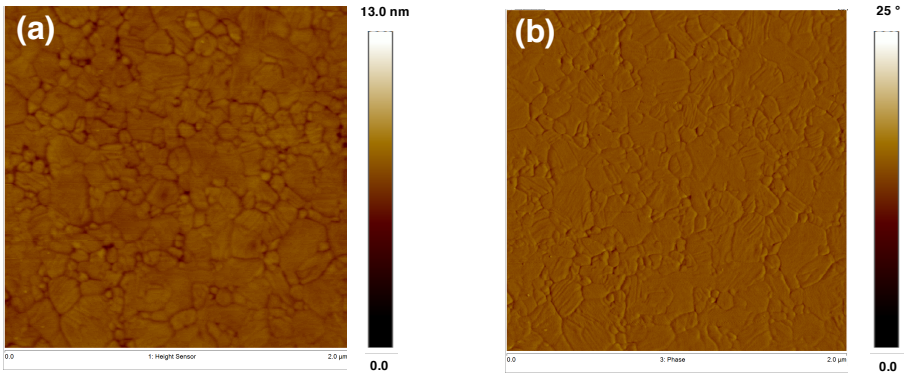


Figure S1. Tapping mode AFM images of ultraflat gold surface on mica (2×2 μm^2): (a) height (b) phase, (rms roughness of surface: 0.36 nm).

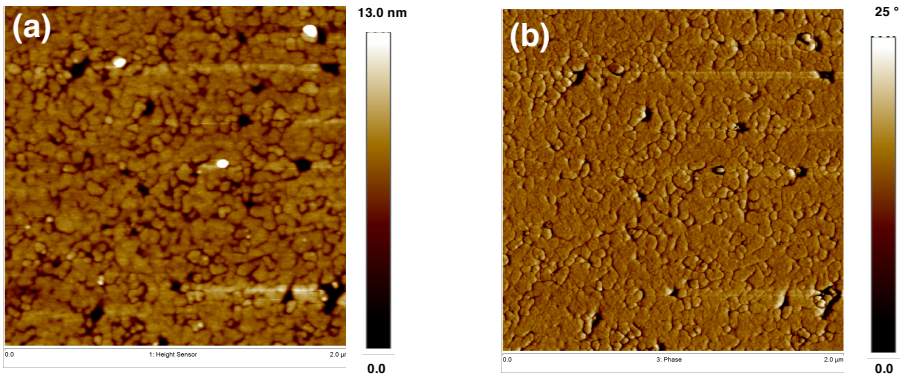


Figure S2. Tapping mode AFM images of gold surface (2×2 μm^2) after treatment with a DMSO solution of tripod **1**: (a) height (b) phase (rms roughness of surface: 1.60 nm).

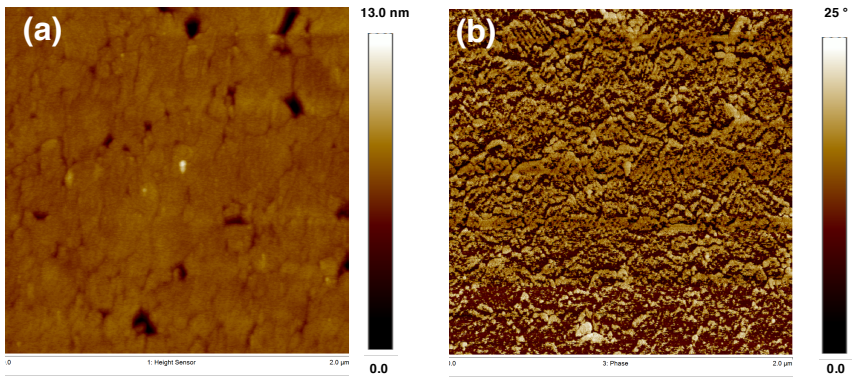


Figure S3. Tapping mode AFM images of gold surface (2×2 μm^2) after surface click reaction: (a) height (b) phase (rms roughness of surface: 0.60 nm).

2.2 PM-IRRAS measurement

Two ultraflat gold surface substrates were functionalized with tripod **1** and one of these substrates

was subjected to click reaction with compound **5** as described above except that times for tripod immobilization and click reaction were 2 h and 5 h respectively. Another ultraflat surface substrate was directly soaked in the click solution for 5 h. The PM-IRRAS spectra of three substrates are shown in Figure S4. Strong bands were observed in the sp^3 C–H stretching region (2800–3000 cm^{-1}) in all the substrates including the one that was directly soaked in the click solution without functionalization with tripod **1**. These bands possibly arise due to some contamination of the substrates and therefore were not considered for the analysis.

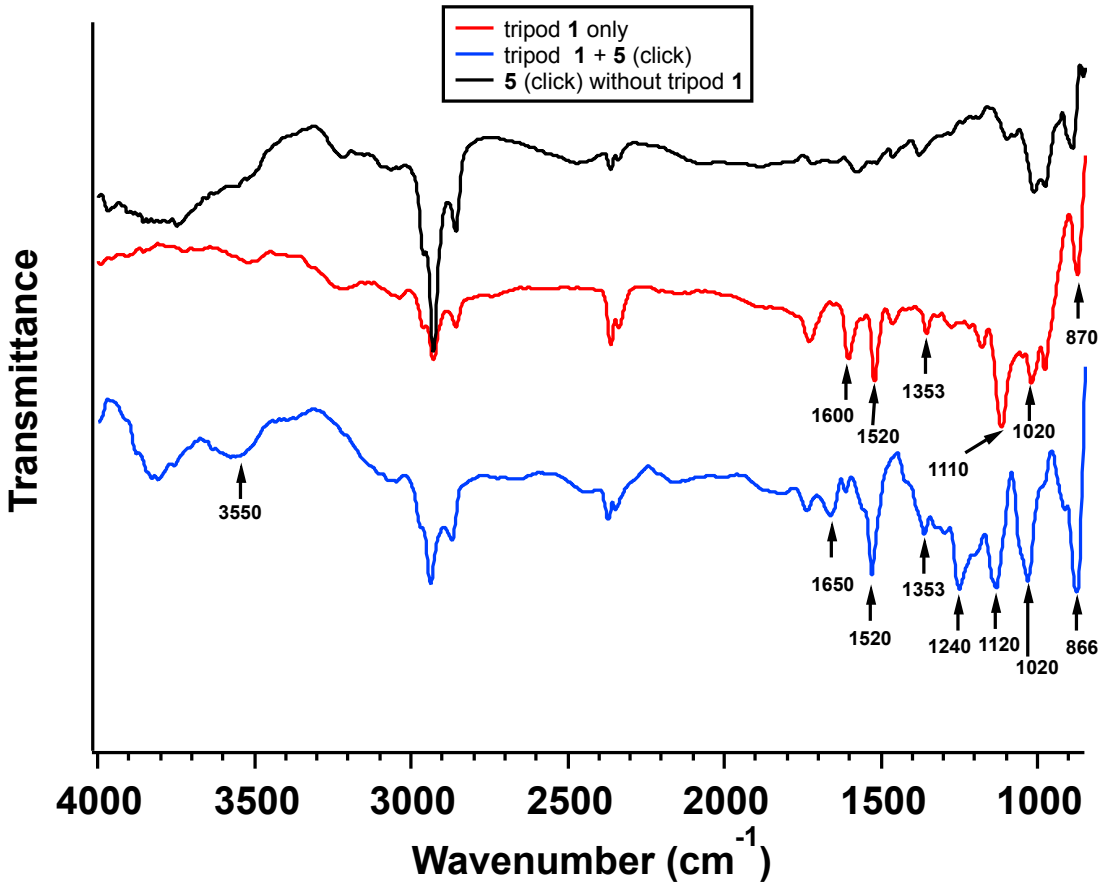


Figure S4. PM-IRRAS spectra (4000-850 cm^{-1}) of Au substrates (i) functionalized with tripod **1** (red line), (ii) subsequent click reaction with N_3–PEG_{2000}–NH_2 **5** (blue line), and (iii) soaked directly in click solution of **5** without treatment with tripod solution (black line). IR bands at 1240 cm^{-1} (CH_2 bending) and 3550 cm^{-1} (broad N–H stretch) were observed upon *in situ* click reaction tripod-functionalized substrate with **5**. These bands were not observed in the unfunctionalized substrate that was directly subjected to click reaction (control experiment).

2.3 Quartz Crystal Microbalance (QCM) measurement

QCM measurements were carried out using Q-sense AG, Gothenburg, Sweden. (Note: Manual injection of solutions in DMSO and THF, using plastic and glass syringes, respectively, was done as the tubing of pump were observed to be dissolving in these solvents). A gold-coated quartz crystal was cleaned by sonication in ethanol followed by UV/ozone treatment. DMSO (UV spectroscopy grade, Fluka, analytical) was injected using a plastic syringe to obtain a stable baseline. At t = 8 min, a filtered, 100 μM DMSO solution of tripod **1** was injected. A sharp drop in the oscillation frequency was observed that did not change further. At t = 55 min, pure DMSO was again injected for 20 min to wash out the unbound molecules. At t = 75 min, THF (HPLC grade, Sigma-Aldrich) was injected using a glass syringe. A sharp increase in the oscillation frequency was observed possibly due to the lower density of THF (0.889 g/cm^3) compared to DMSO (1.10 g/cm^3). Intermittent injection of THF was continued for 45 min until a stable baseline for THF was obtained. At t = 120 min, a filtered click solution containing N_3–PEG_{2000}–NH_2 (**5**) (10 mg, 5.0 μmole), 25 μmole of each $[Cu(CH_3CN)_4]PF_6$ (9.3 mg) and TBTA (13.3 mg) in 6 mL THF was injected using a glass syringe and a gradual decrease in the frequency was seen. After 85 min from the injection time of the click solution, no further decrease in frequency was noticed. At t = 205 min, pure THF was again injected for 20 min to wash out the unbound materials. At t = 225 min, pure DMSO was injected once and a sharp drop in oscillation frequency was observed. After 20 min (at t = 245 min) pure DMSO was re-injected and a further decrease in the frequency was observed. Upon further injection of DMSO the oscillation frequency did not change and remained constant.

3 AFM tips modifications and analysis[1]

General. Similar to the surface functionalization, all AFM tips were cleaned by UV/ozone treatment using UV/Ozone ProCleaner™ Plus (BioForce Nanosciences) before functionalization and all solutions were filtered through 0.45 μm PTFE filters (CHROMAFIL® Xtra PTFE-45/25, Machery-Nagel GmbH & Co. KG, Düren, Germany) before using for functionalization of AFM tips.

3.1 Immobilization of molecular tripods on the AFM tips[1]

Commercially available gold-coated silicon nitride cantilevers (NPG, Veeco Instruments, Santa Barbara CA) were cleaned by UV/ozone treatment for 30 min to remove any adsorbed organic materials and immediately after cleaning, these were soaked overnight in DMSO (UV spectroscopy grade, Fluka analytical) solutions of tripod **1** (100 μM, 2.9 mg in 25 mL) and molecular tip **2 (**200 μM, 6.8 mg in 10 mL), using a homemade Teflon-coated cantilever holder. Subsequently, these were washed with DMSO, water, toluene and ethanol and dried using a N_2 blowgun. Molecular tip **2** was directly subjected to force measurements with NeutrAvidin.

3.2 Click chemistry on AFM tip functionalized with tripod 1

A solution of N_3–PEG_{2000}–biotin (**6**) (10 mg, 5.0 μmole), 25 μmole of each $[Cu(CH_3CN)_4]PF_6$ (9.3 mg) and TBTA (13.3 mg) in 6 mL THF (HPLC grade, Sigma-Aldrich) was filtered and an AFM tip modified with tripod **1** was soaked in this solution overnight at room temperature. Subsequently, the cantilever was thoroughly washed with THF, DMSO, water, toluene and ethanol. After drying under a N_2 stream it was subjected to force measurements with NeutrAvidin.

3.3 AFM force spectroscopy measurements and analysis [1]

All the force measurements were carried out with a Molecular Force Probe (MFP-3D AFM,

Asylum Research, Santa Barbara, CA) using. Spring constants were calibrated by the thermal noise method before and were generally between 100-150 pN/nm. NeutrAvidin coated agarose beads (immobilized NeutrAvidin Protein 29200, Pierce Biotechnology Inc., Rockford, IL) were placed on a glass slide in 20 mM HEPES buffer (pH 7.4). Force–distance measurements with the modified gold-coated AFM tips were carried out on top of the beads with a scan rate of 0.12 Hz, tip velocity of 500 nm/s and at a distance of 2 μm.

3.4 Control experiments

(1) After the force measurements with NeutrAvidin on cross-linked agarose beads, an excess of a filtered biotin solution containing 0.22 mg/mL in HEPES buffer was added and the force measurements were carried out as mentioned above.

(2) Force–distance measurements were also carried out (a) using an AFM tip functionalized with tripod **1** (without biotin) *vs* NeutrAvidin in HEPES; (b) using an AFM tip functionalized with molecule **2** *vs* glass substrate in HEPES.

Table S1. Data for force measurements: probability of biotin-receptor interactions and control experiments

Force measurement experiment (AFM tip modified by)	Conditions	Number of total measurements carried out	Number measurements with any force[a]	Probability of force observation[b]	Total number of forces observed	Average number of forces observed per measurement[c]
Molecular tip **2** *vs* NeutrAvidin	In buffer	315	283	90%	629	2.22
	In presence of biotin in buffer	197	58	29%	85	1.46
Tip modified by *in situ* click *vs* NeutrAvidin	In buffer	288	213	74%	368	1.73
	In presence of biotin in buffer	200	52	26%	61	1.17
Tripod **1** *vs* NeutrAvidin	In buffer	200	32	16%	39	1.22
Molecular tip **2** *vs* glass	In buffer	176	128	73%	160	1.25

[a]Measurements with one or more detectable forces.
[b]Number of measurements with any detectable force divided by number of total number of measurements.
[c]Total number of forces observed divided by the number of measurements with any detectable force. Total of four AFM tips with spring constants between 100-150 pN/nm were used.

4. References

1. M. E. Drew, A. Chworos, E. Oroudjev, H. Hansma and Y. Yamakoshi, Langmuir, 2010, **26**, 7117–7125.
2. W. J. Kim, S. H. Choi, Y. S. Rho and D. J. Yoo, Bull. Korean Chem. Soc., 2011, **32**, 4171–4175.
3. K. E. Nelson, L. Gamble, L. S. Jung, M. S. Boeckl, E. Naeemi, S. L. Golledge, T. Sasaki, D. G. Castner, C. T. Campbell and P. S. Stayton, *Langmuir*, 2001, **17**, 2807–2816.

5. Spectral data

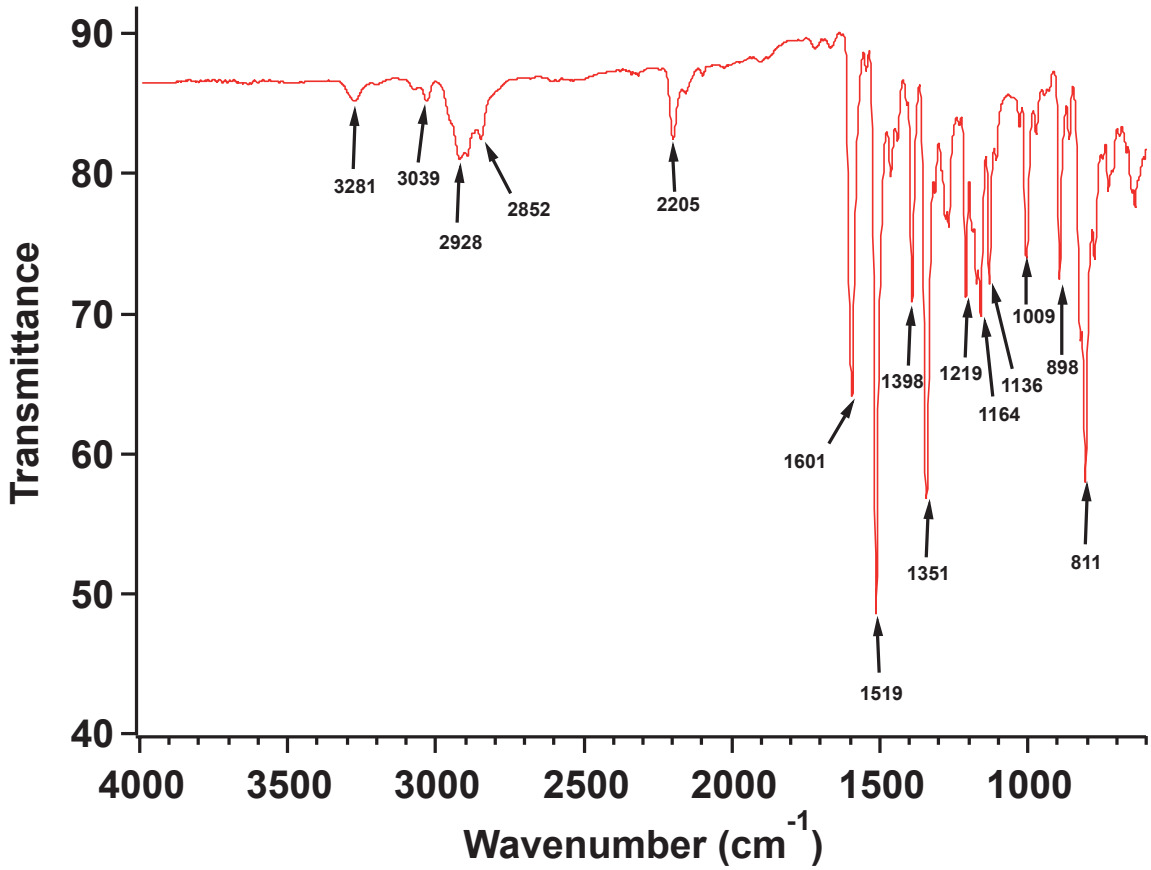


Figure S5. FT-IR spectrum of tripod **1**.

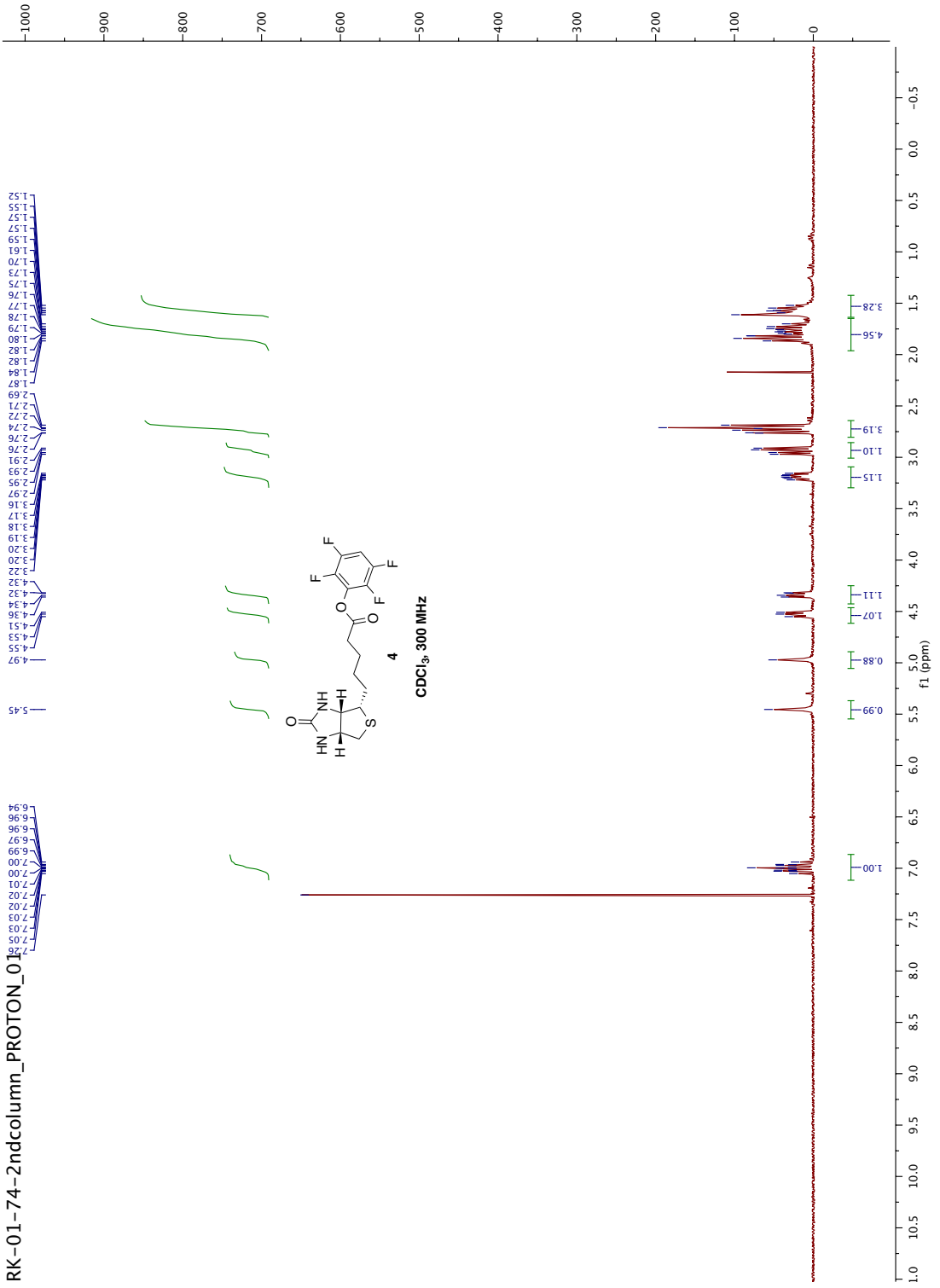


Figure S6. ^{1}H-NMR spectrum of compound **4**.

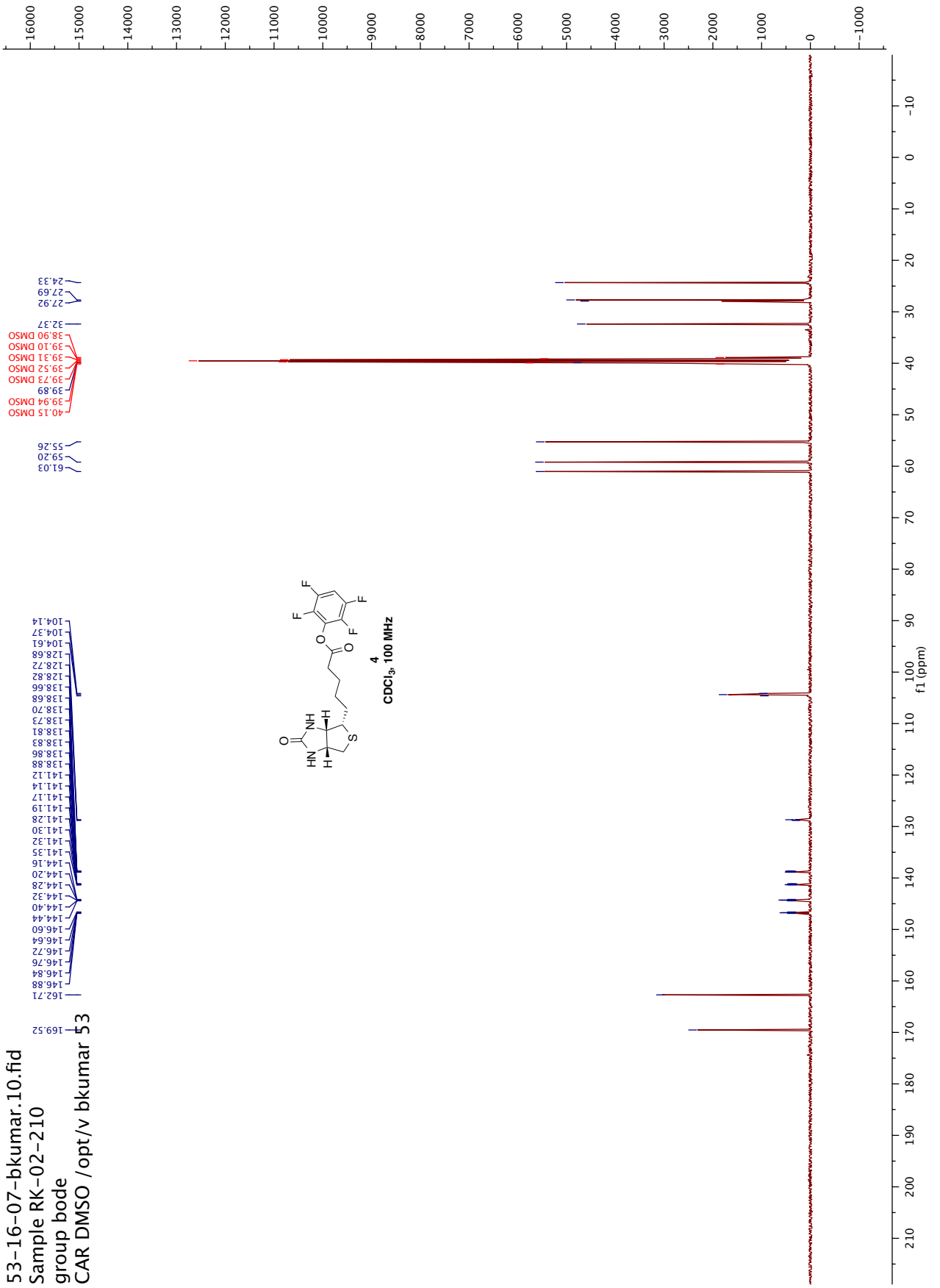


Figure S7.[13]C-NMR spectrum of compound **4**.

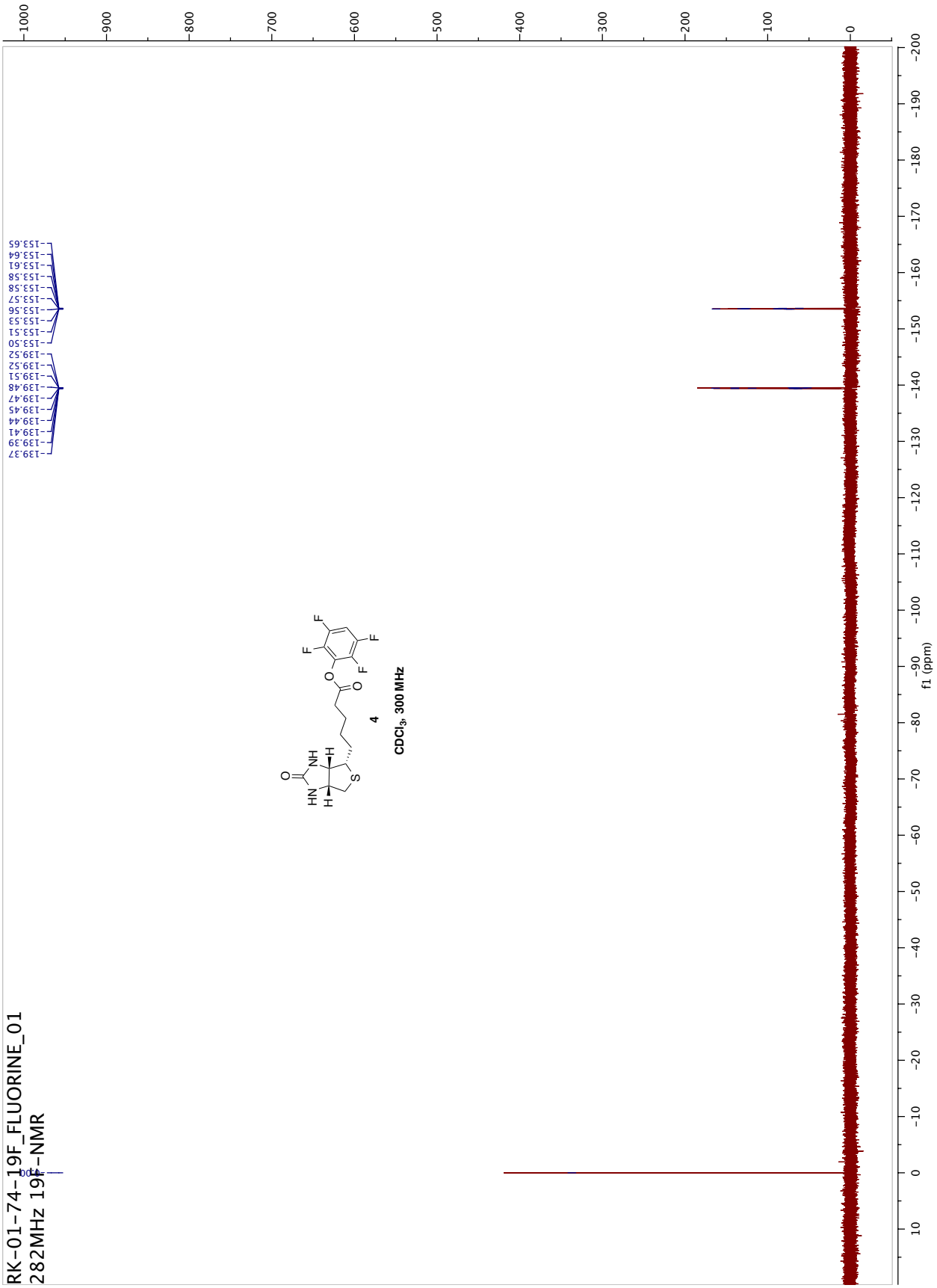


Figure S8.[19]F-NMR spectrum of compound **4**.

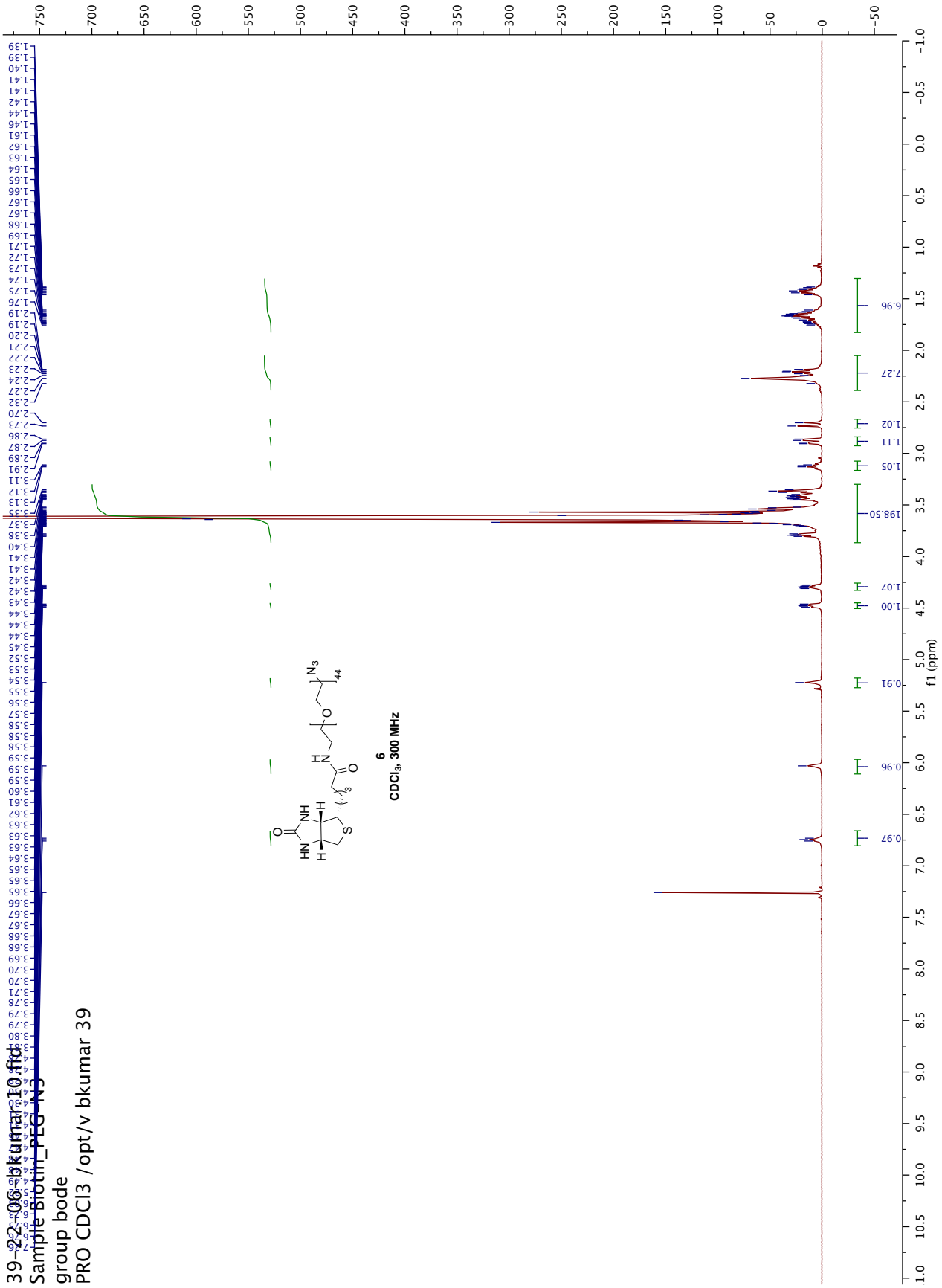


Figure S9. ^{1}H-NMR spectrum of compound **6**.

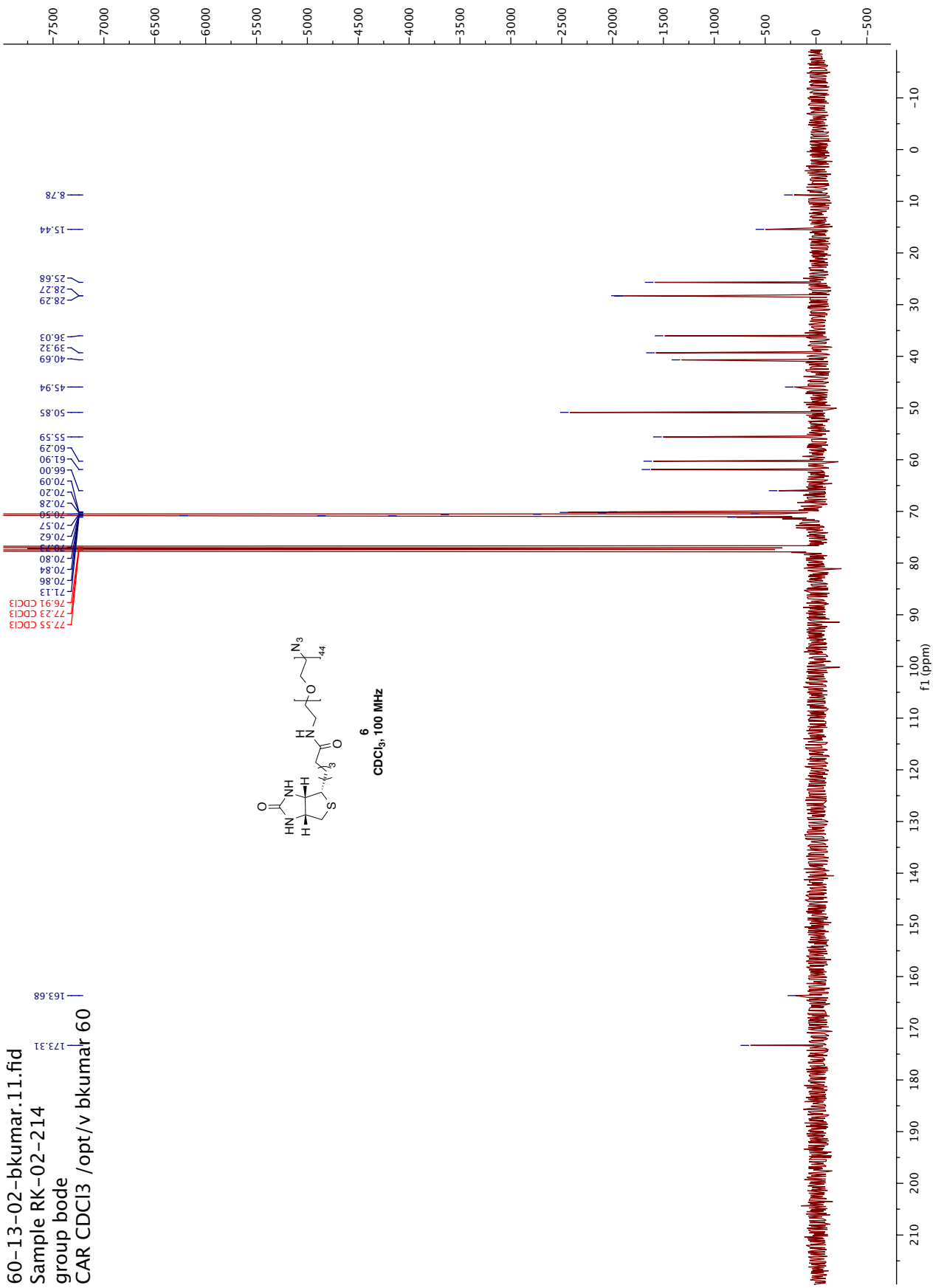


Figure S10. ^{13}C-NMR spectrum of compound **6**.

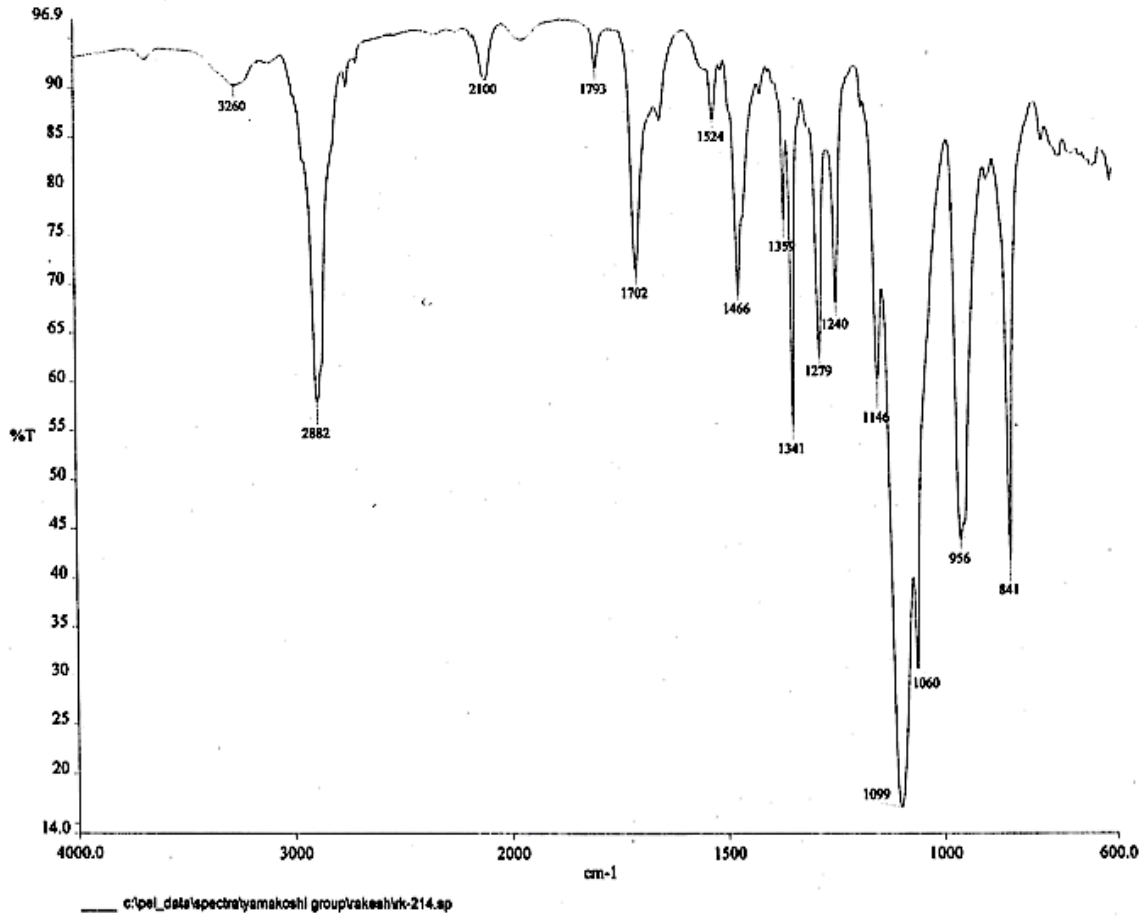


Figure S11. FT-IR spectrum of compound **6**.

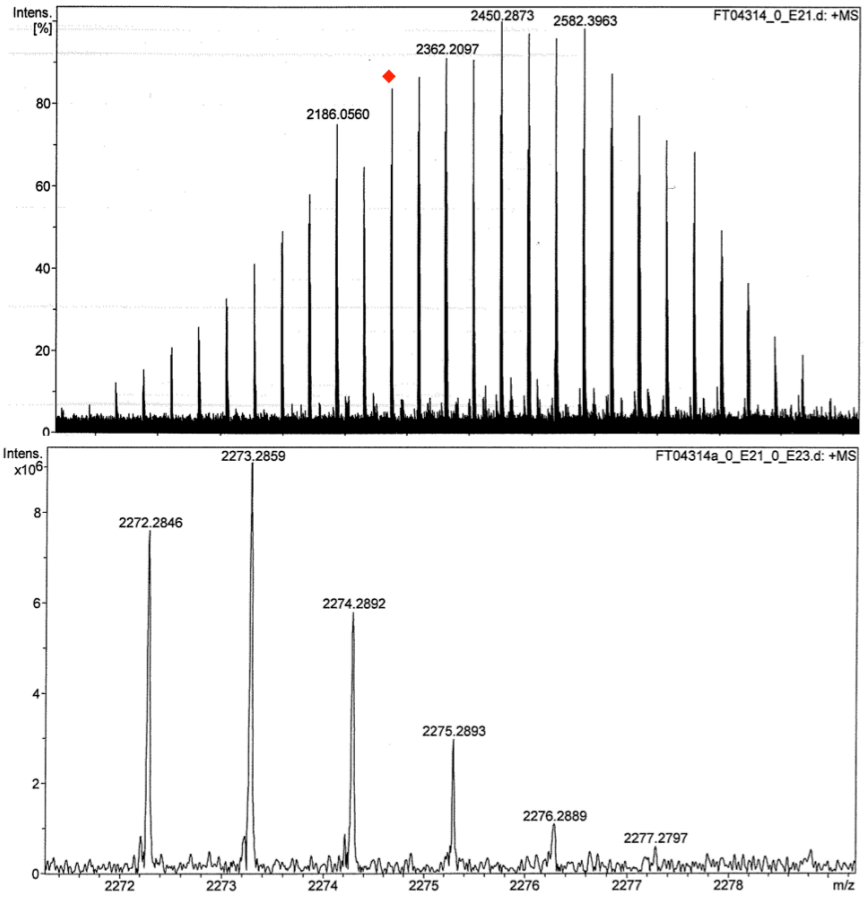


Figure S12. HRMS (MALDI) of compound **6** (matrix DCTB). Mass distribution of the PEG chains (top) and mass $[M+Na]^+$ for 44 PEG units (bottom).

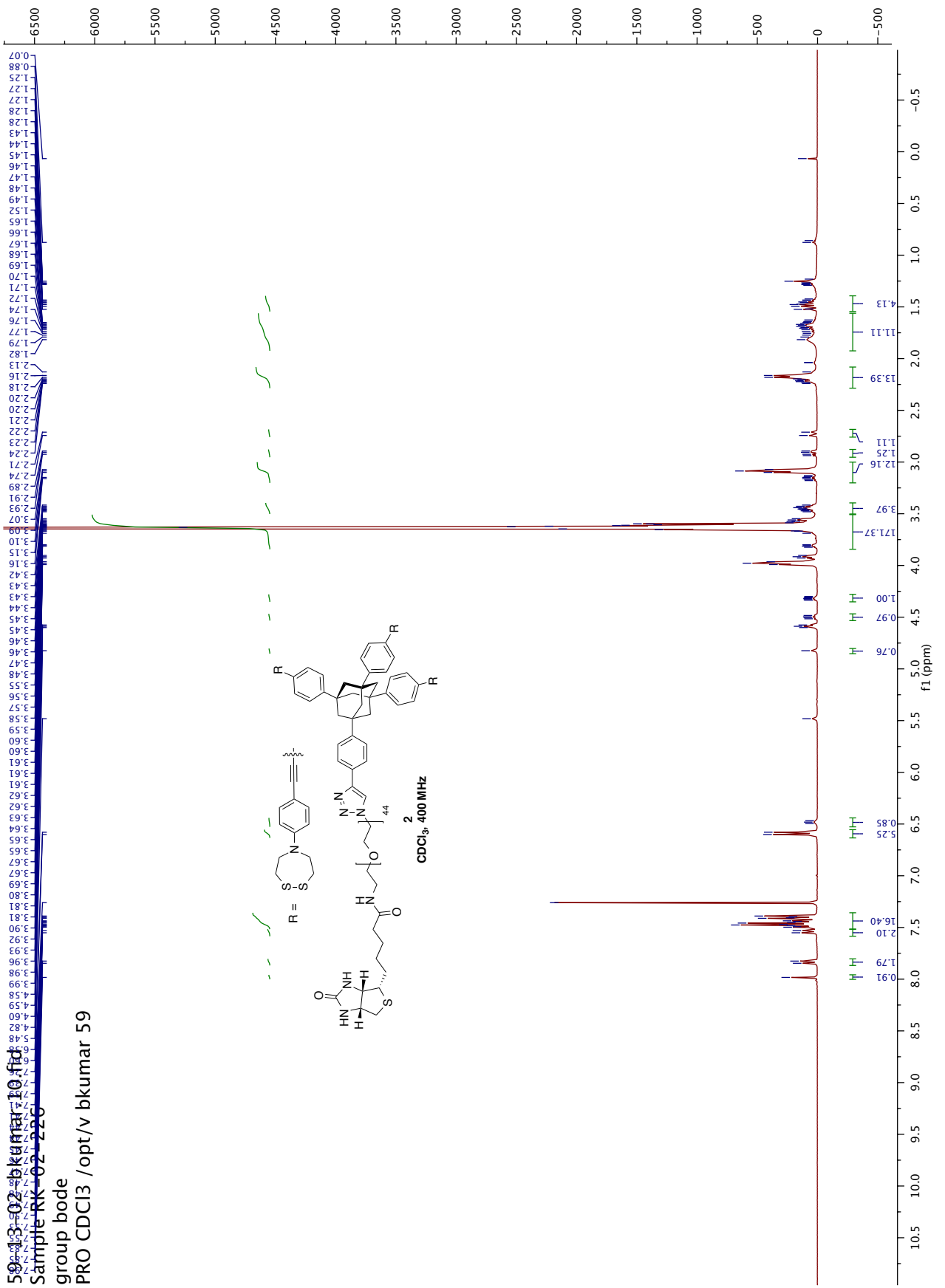


Figure S13. ^{1}H-NMR spectrum of compound **2**.

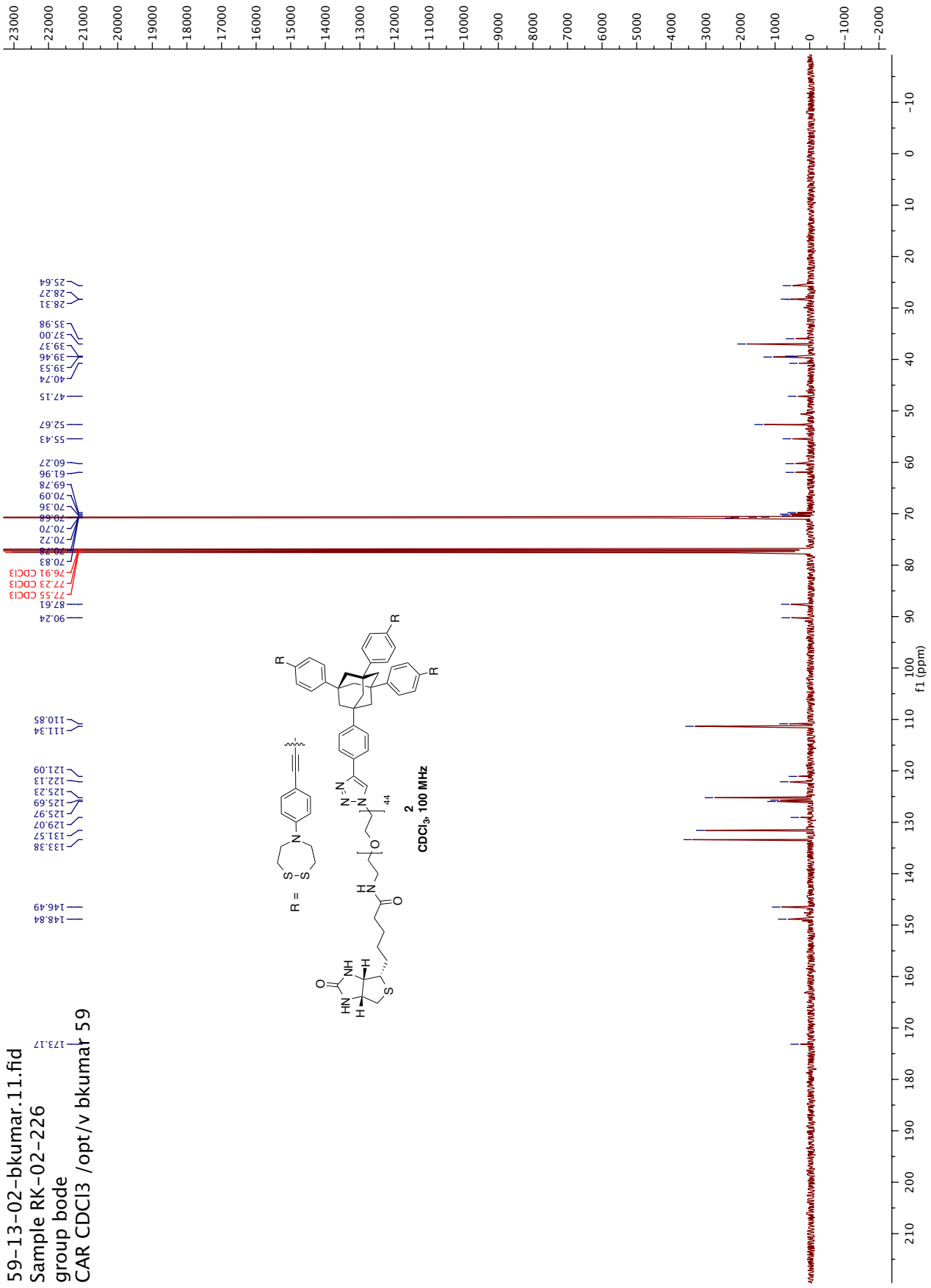


Figure S14. ^{13}C-NMR spectrum of compound **2**.

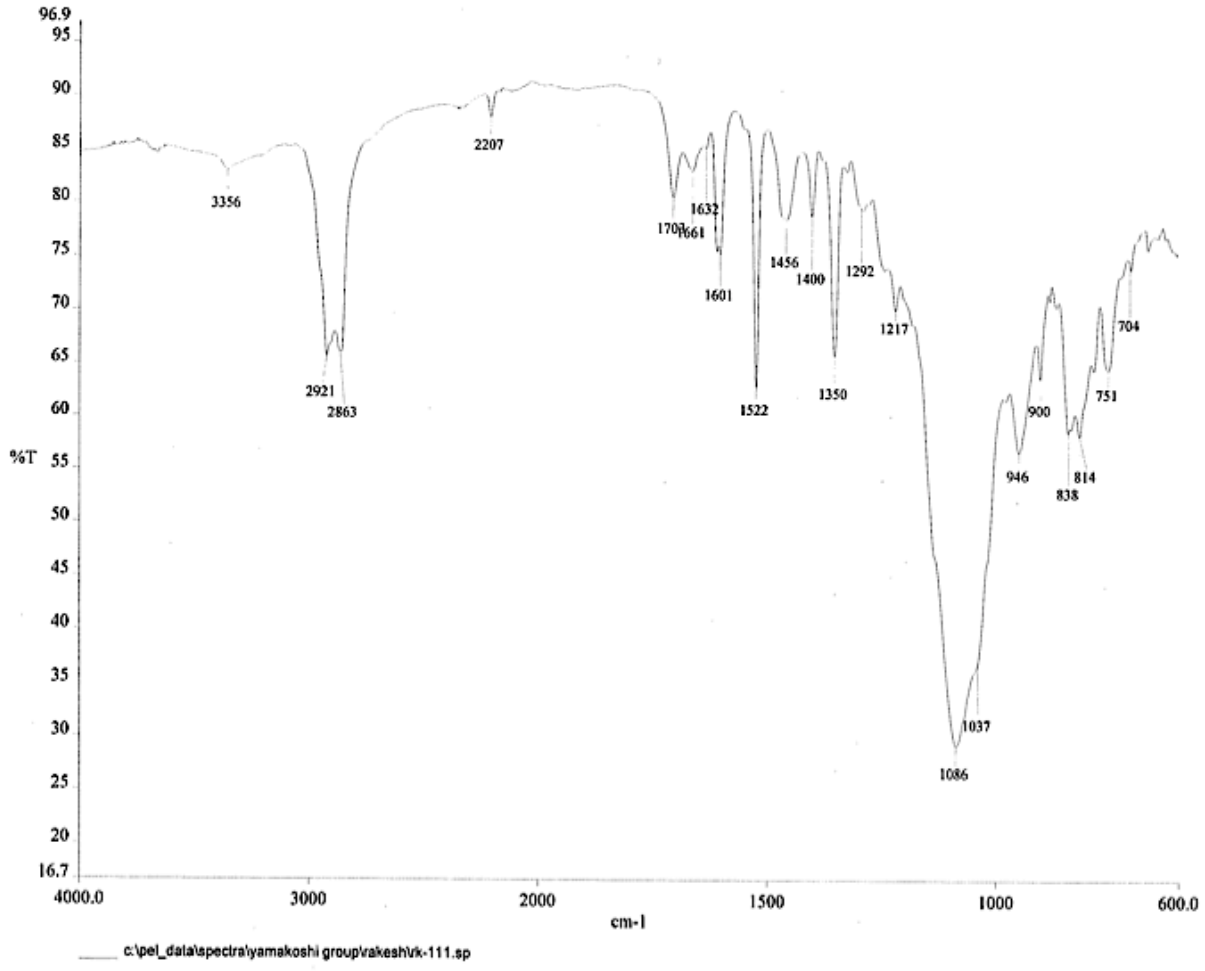


Figure S15. IR spectrum of compound **2**.

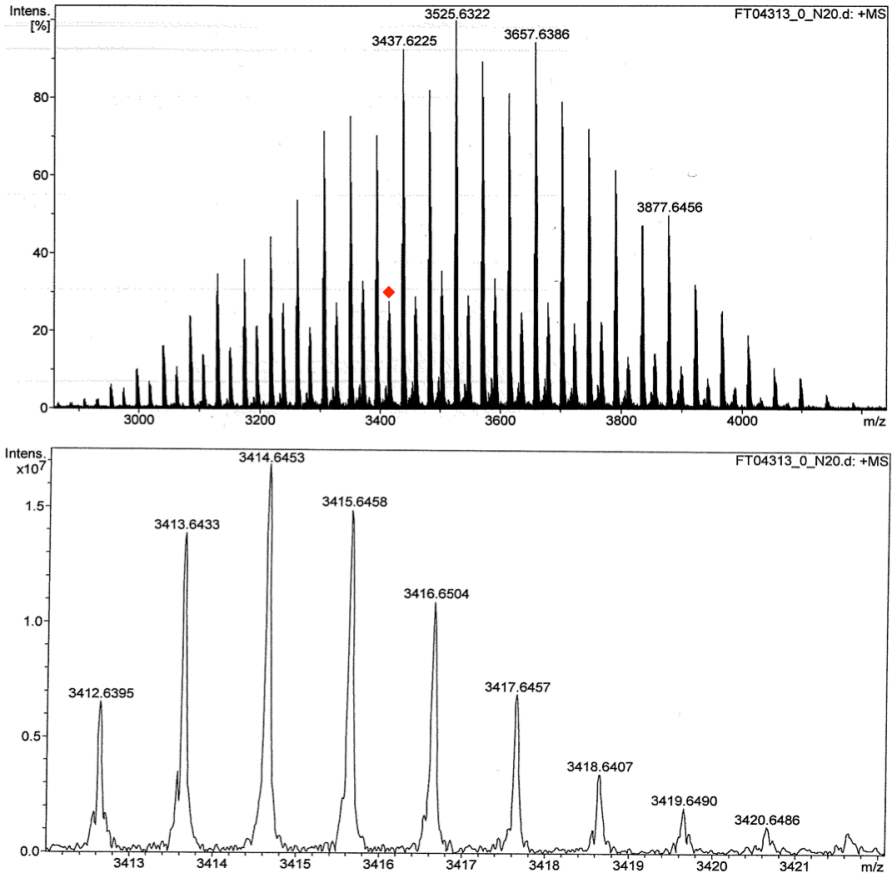


Figure S16. HRMS (MALDI) of compound **2** (matrix: DCTB). Mass distribution of the PEG chains (top) and mass for 44 PEG units (bottom). The main distribution in the top figure corresponds to $[M+Na]^+$ and the minor one corresponds to $[M]^+$.