**Supporting Information**

# Attach, Remove, or Replace: Reversible Surface Functionalization Using Thiol-Quinone Methide Photoclick Chemistry

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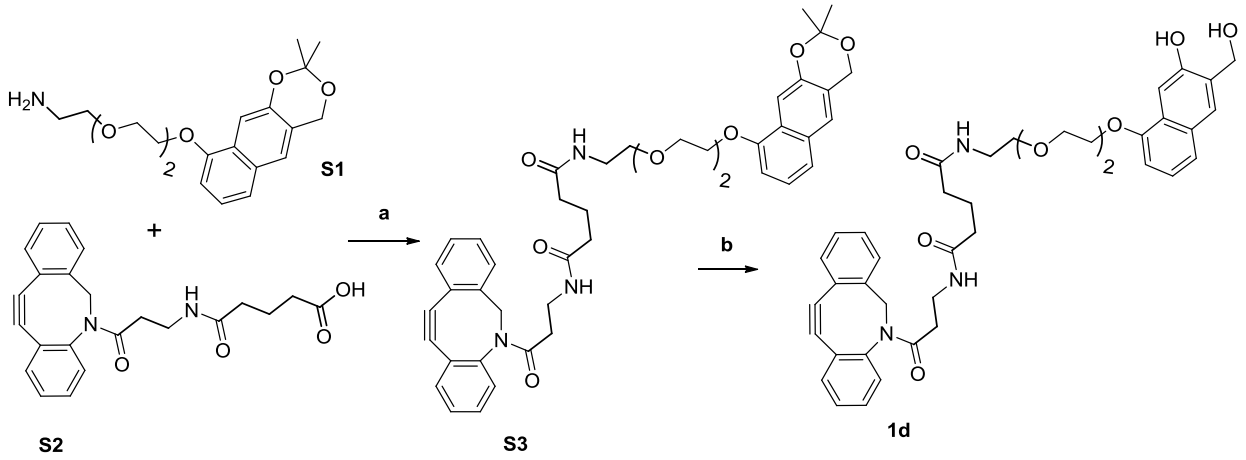
**General Information.** All organic solvents were dried and freshly distilled before use. Flash chromatography was performed using 40-63 μm silica gel. All NMR spectra were recorded on 400 MHz instruments in $CDCl_3$ and referenced to TMS unless otherwise noted. Solutions were prepared using HPLC grade water and acetonitrile.

**Fluorescent Imaging**. Images of patterned slides were obtained using Olympus IX71 inverted fluorescence microscope. The fluorescent intensity analysis of the images was conducted using Image J program (NIH).

**Materials**: A 12μM pitch TEM copper grid (G2000HS, Structure Probe Incorporated) was employed as a shadow mask for patterned irradiation of the slides. Thiol functionalized microscope glass slides were purchased from Xenopore Corporation (www.Xenopore.com). Methoxy PEG Maleimide (MW 2000) was purchased from JenKem Technology USA Inc.; FTIC-Avidin and Rhodamine B were purchased from Life Technologies. All other chemicals were purchased from Sigma-Aldrich and were used as received. 8-TEG-3-(hydroxymethyl) naphthalen-2-ol (NQMP-TEG, **1b**),[1] 9-(Amino-TEG)-2,2-dimethyl-4*H*-naphtho[2,3-d][1,3] dioxine (**S1**),[1] 8-(Biotin-TEG)-3-(hydroxymethyl)naphthalen-2-ol (NQMP-Biotin, **1c**),[1] ADIBO-carboxylic acid (**S2**),[2] 5-dansyloxy-3-hydroxynaphthalen-2-yl)methyl (DNS-NQMP, **1a**)[3] and azido Rhodamine B[4] were prepared following previously reported procedures.

**Scheme S1:** Synthesis of NQMP-ADIBO, **1d**




*Reagents and conditions:* (a) EDC, DMAP, DMF, 80%; (b) Amberlist-15, acetonitrile, 90%.

**Protected NQMP-TEG-ADIBO** (**S3**): 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (97 mg, 0.3 mmol) and catalytic amount of DMAP were added to a solution of ADIBO-carboxylic acid **S2** (150 mg, 0.38 mmol) in 8 mL of dry DMF, followed by a dropwise addition of a solution of amine **S1** (151 mg, 0.42 mmol) in 2mL of DMF. The mixture was stirred for 12h at rt, solvent was removed in vacuum, the residue was dissolved in DCM, washed with $NaHCO_3$ solution, brine, dried over anhydrous magnesium sulfate, and the concentrated under reduced pressure. The residue was purified by column chromatography using (10% MeOH in dichloromethane) to yield 225 mg (80%) of the ketal **S3**. $^{1}$H NMR: 7.55 (d, J = 7.9 Hz, 2H), 7.36 – 7.03 (m, 10H), 6.61 (d, J = 7.5 Hz, 1H), 6.48 (t, J = 5.6 Hz, 1H), 6.36 (t, J = 6.0 Hz, 1H), 5.02 (d, J=14 Hz, 1H), 4.95 (m, 2H), 4.14 (t, J = 4.8 Hz, 2H), 3.84 (dd, J = 5.6, 3.9 Hz, 2H), 3.70-3.64 (m, 2H), 3.60-3.53 (m, 3H), 3.52 – 3.43 (m, 2H), 3.40 – 3.05 (m, 4H), 2.44 – 2.29 (m, 2H), 2.04 – 1.62 (m, 6H), 1.50 (s, 6H). $^{13}$C NMR: 172.7, 172.6, 172.5, 153.7, 151.2, 149.7, 148.2 , 132.3, 129.7, 129.2, 128.8, 128.6, 128.4, 128.1, 127.4, 126.3, 125.8, 123.9, 123.4 , 123.2, 122.7, 121.8, 120.2, 115.0, 107.9, 107.2, 104.9, 100.0, 71.0, 70.4, 70.0, 69.9, 68.0, 61.3, 55.8, 53.7, 39.4, 35.6, 35.3, 35.2, 34.6, 25.2, 25.2, 22.1. FW calc [$(C_{43}H_{48}N_3O_8)H^+$):734.344; EI-HRMS: 734.3434.

**NQMP-ADIBO** (**1d**): About 75 mg of Amberlyst-15 resin was added to a solution of ketal **S3** (200 mg, 0.17 mmol) in 5 mL of acetonitrile and stirred for 2 h at rt. 10 mL of DCM was added to a reaction mixture, the resin was removed by filtration through a cotton plug, and the acetonitrile /DCM solution was passed through short silica gel column to yield 170 mg analytically pure ADIBO-NQMP (**1d**) in 90% yield. $^{1}$H NMR: 7.77 (d, J = 7.9 Hz, 2H), 7.56 – 7.23 (m, 10H), 6.82 (d, J = 7.5 Hz, 1H), 6.68 (t, J = 5.6 Hz, 1H), 6.55 (t, J = 6.0 Hz, 1H), 5.21 (d, J=14 Hz, 1H), 5.15 (m, 2H), 4.33 (t, J = 4.8 Hz, 2H), 4.05 (dd, J = 5.6, 3.9 Hz, 2H), 3.90-3.83 (m, 2H), 3.80-3.72 (m, 3H), 3.71 – 3.63 (m, 2H), 3.60 – 3.25 (m, 4H), 2.64 – 2.48 (m, 2H), 2.24 – 1.82 (m, 6H). $^{13}$C NMR: 170.8, 170.5, 170.5, 151.7, 149.2, 147.6, 146.2, 130.3, 127.7, 127.2, 126.8, 126.6, 130.4, 126.1, 125.4, 124.3, 123.8, 121.9, 121.4, 121.2, 120.7, 119.8, 119.2, 105.9, 105.1, 102.9, 98.1, 69.0, 68.4, 68.0, 67.9, 66.0, 59.3, 53.8, 51.7 , 37.4 , 33.6, 33.3, 33.2, 32.6, 20.1. FW calc [$(C_{40}H_{43}N_3O_8)H^+$):694.3128; EI-HRMS: 694.3120.

**Methods: Photochemical derivatization of Thiol-functionalized glass slides**

*Uniform derivatization*: Slides were immersed in 0.2 mM aqueous solution of NQMP precursor **1a-d** and irradiated for 2 min using mini-Rayonet photochemical reactor equipped with 8 fluorescent UV lamps (4W, 350 nm).

*Patterned derivatization*:

- *Method A:* A 1 x 1 inch thiol derivatized glass slide was placed on a elastic support and covered with a thin layer of an aqueous solution of **1a-d** (0.2 mM), TEM grid mask was gently placed over the solution and a cover glass plate (quartz) was placed over the mask to keep it fixed its position. When placing the cover plate, care should be taken not to squeeze out the reaction solution. Irradiation was carried out through the cover glass using hand held UV fluorescent lamp (4 W, 350 nm) for 4 min.
- *Method B:* The TEM grid was affixed to the backside (non-derivatized) of the glass slide using screw clamps. This set up was placed over 4 W UV lamp with the thiol functionalized slide facing upwards. A thin layer of an aqueous solution of NQMP precursor **1a-d** (0.2 mM) was placed over the thiol surface and the irradiation were carried out from the bottom of the glass slide.

*Patterned immobilization of FTIC-avidin***:** NQMP-Biotin conjugate **1c** was micro-patterned on thiol functionalized glass slide following the procedure "B". The resulting biotinylated slide was immersed into a solution of FITC-Avidin (50 µL of 2 mg/mL in 10 mL PBS) at 2 °C for 15 min. The non-specifically bound FITC-Avidin was removed by sonicating the glass slides in PBS solution for 30 min followed by overnight incubation in fresh phosphate buffer.[1]

*Double Click Derivatization*: NQMP-TEG-ADIBO (**1d**) was micro-patterned on thiol functionalized glass slide following the the procedure "B". Then the patterned surfaces were incubated in 1 mM solution of azido Rhodamine B (1mM) for 1 h, washed with DMF and methanol.

The Dansyl and FITC-Avidin derivatized slides were handled under the ambient light but stored in the dark at room temperature. No significant changes to the contrast and clarity of the fluorescent image were observed after 1.5 month.

**References**

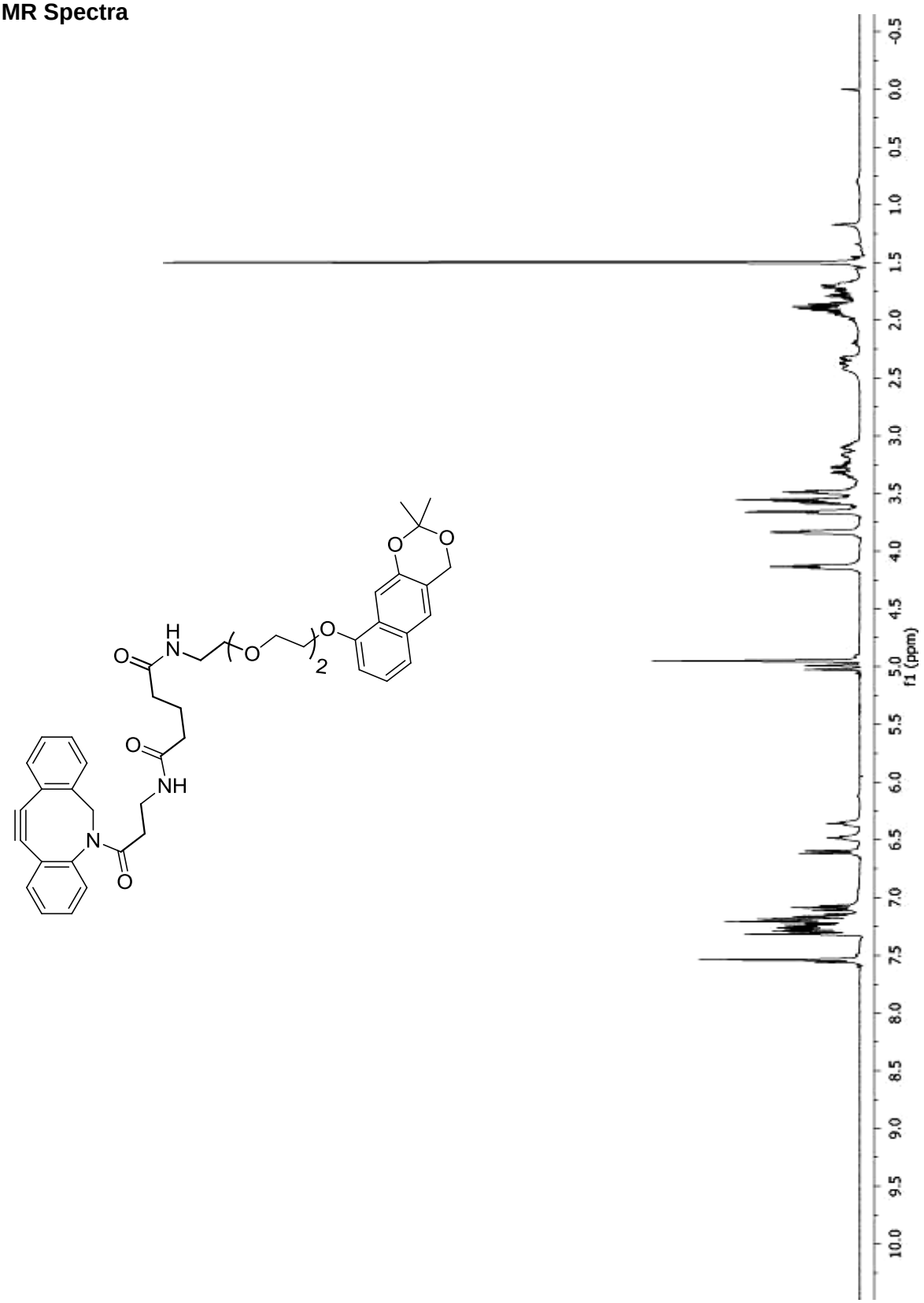

[1] Arumugam, S.; Popik, V.V. *J. Am. Chem. Soc.* **2011***, 133*,15730.

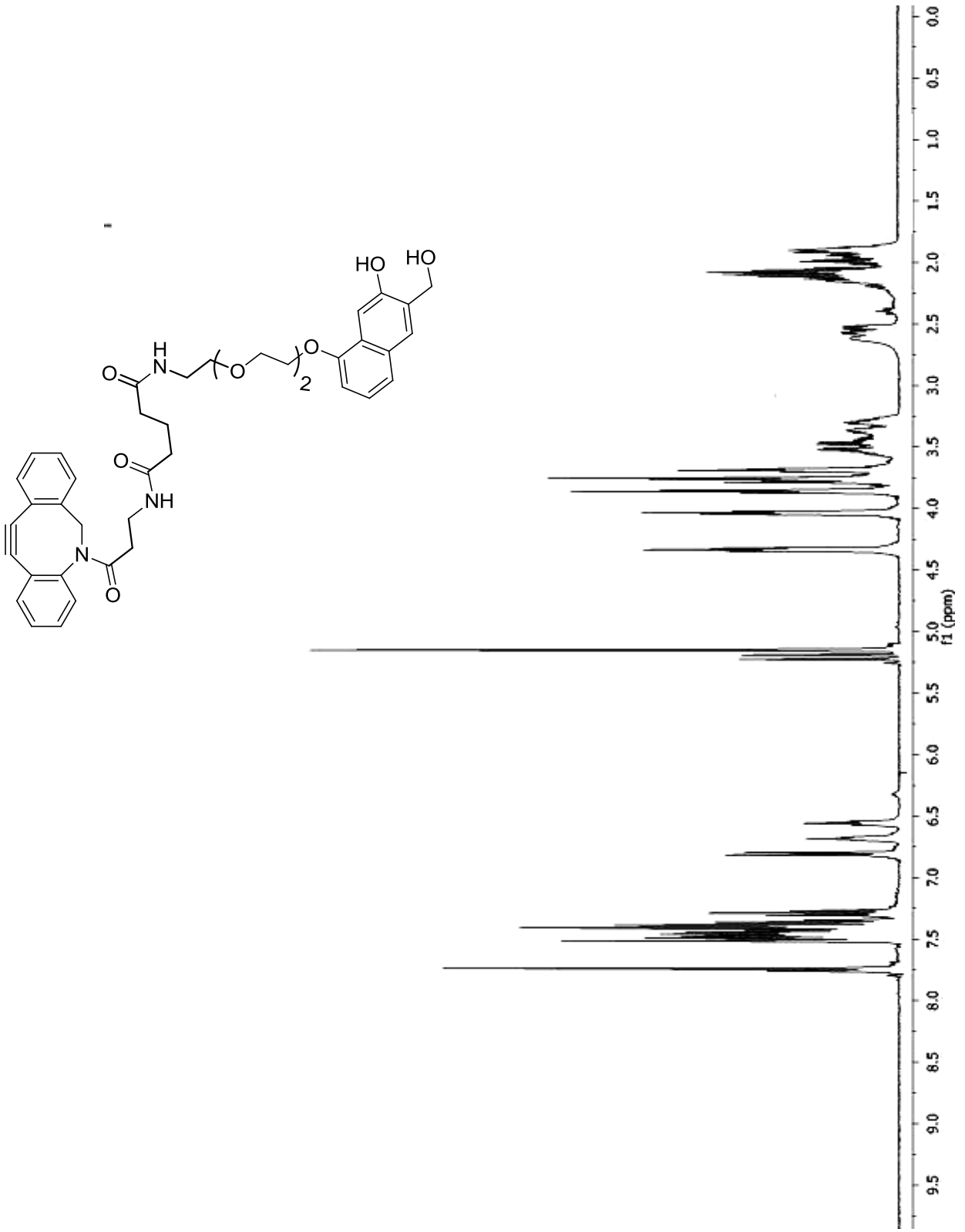
[2] Cheng, Z. Elias, D. R., Kamat, N. P., Johnston, E. D., Poloukhtine, A., Popik, V. V., Hammer, D. A., and Tsourkas, A. *Bioconjugate Chem.*, **2011**, *22*, 2021.

[3] Arumugam, S.; Popik V.V. *Photochem. Photobiol. Sci.* **2012***, 11*, 518.

[4] Orski, S. V., Poloukhtine, A. A., Arumugam, S., Mao, L., Popik, V. V., and Locklin, J. *J. Am. Chem. Soc.* **2010**, *132*, 11024.

**$^{1}$H NMR Spectra**

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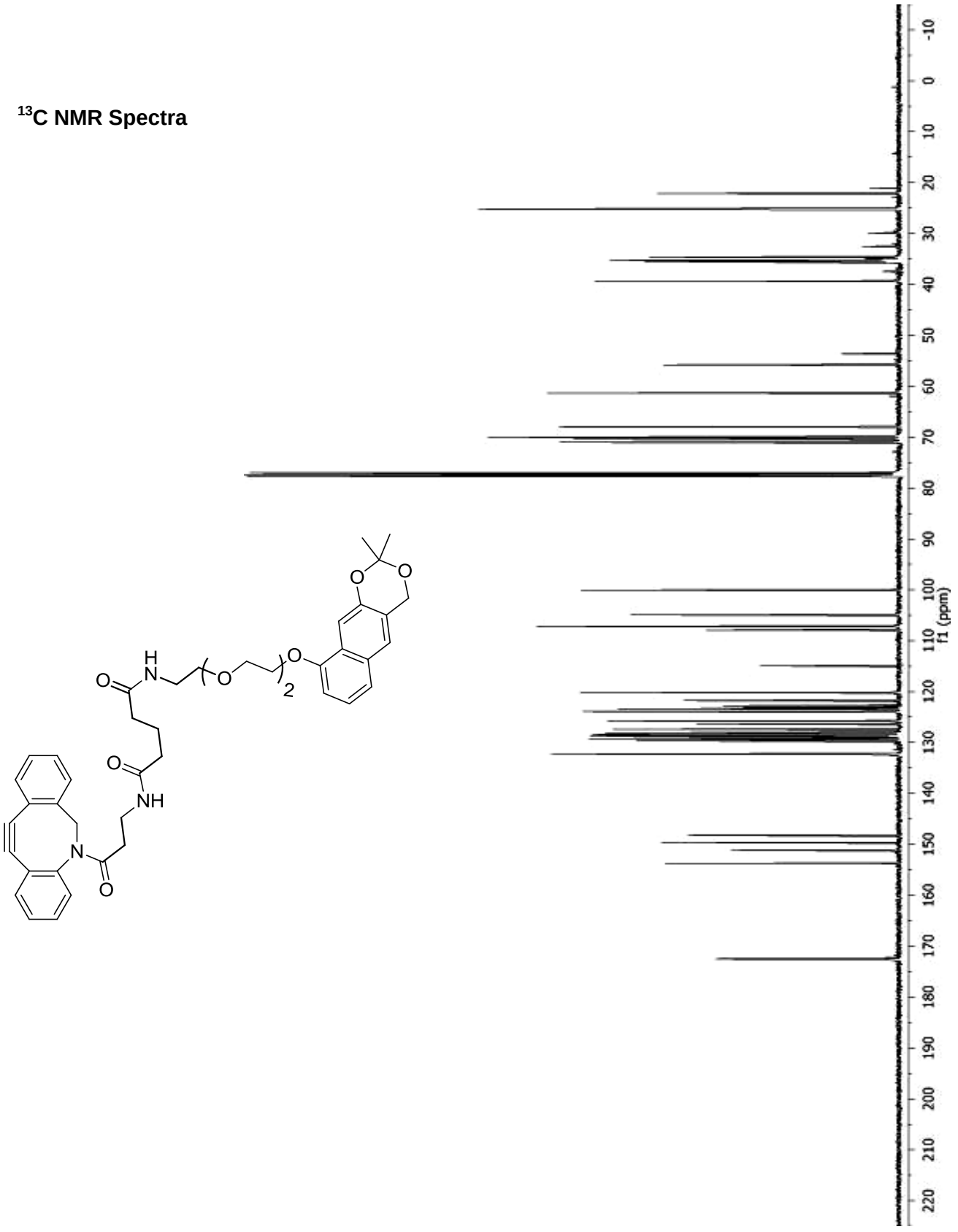
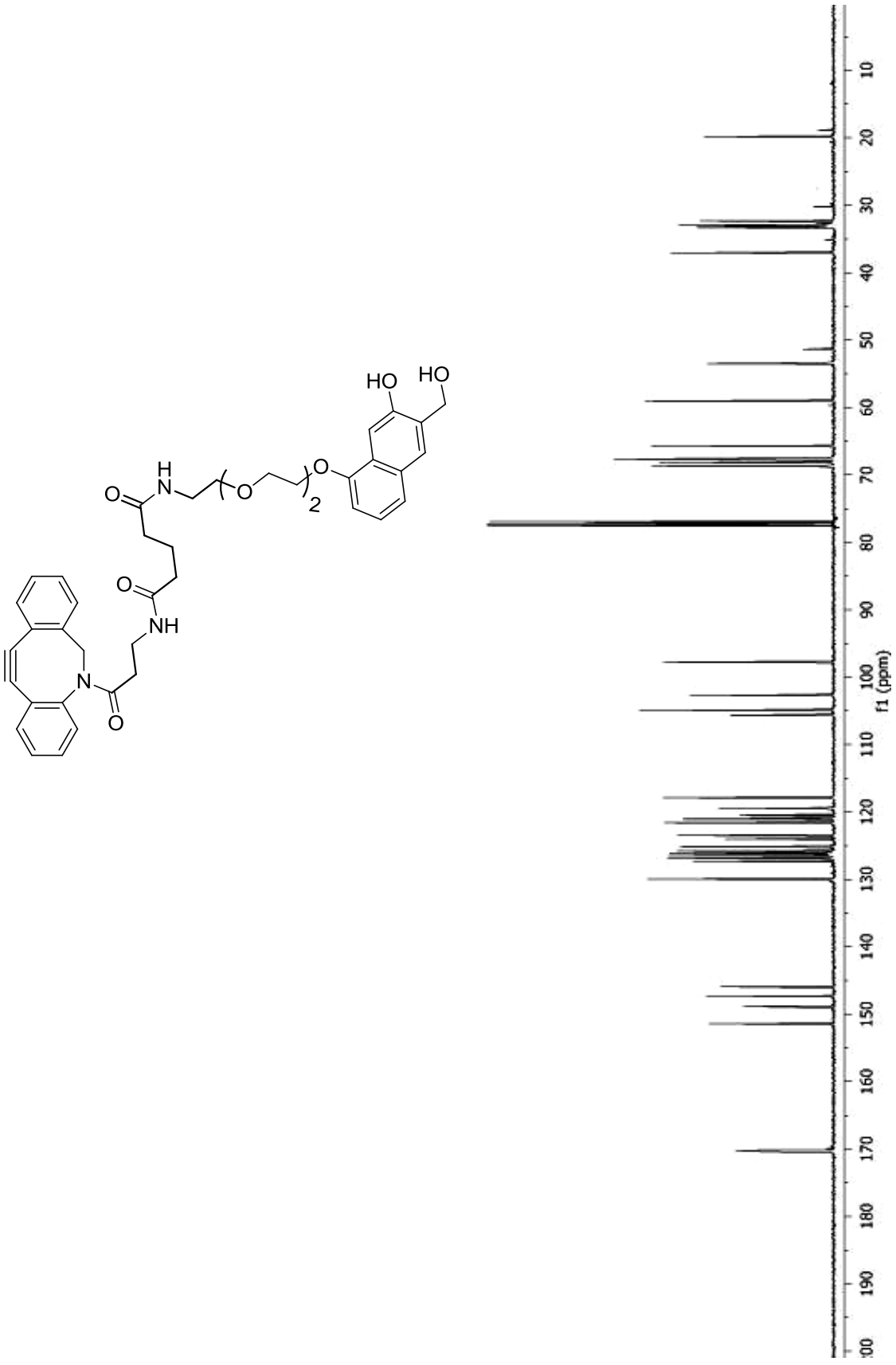
**$^{13}$C NMR Spectra**

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