Supporting Information

A Versatile Platform for Adding Functional Properties to Amyloid Fibrils

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1. Biological Assays

1.1 Fibril Preparation

•Insulin
Recombinant human insulin expressed in yeast
(Sigma Aldrich #I2643-50, lot #SLBR9404V)

•Amyloid-β_{42} (Aβ42)
Amyloid beta42 (beta amyloid 1–42), TFA salt
(21st Century Biochemicals Inc.
#AB42-0001, lot # 6623)

•Lysozyme
Lysozyme from chicken egg white, lyophilized
(Sigma Aldrich #L-6876, lot #21C-8180)

•Thioflavin T Chloride (ThT)
(Sigma Aldrich #T3516-25)

•Sonicator
Ultrasonic Liquid Processor
(Sonics #VCX-750)

Insulin fibrils were made using a variation of our previously reported procedure.[1] Insulin (5.0 mg, 0.86 μmol) was dissolved in phosphate buffer (10 mM sodium phosphate, pH = 2.0, 5 mL, producing a final concentration of 1.0 mg/mL = 0.17 mM peptide monomer). The mixture was incubated (60 °C, 3 days). Fibril formation was confirmed by ThT fluorescence and by transmission electron microscopy (TEM) as described below. The fibril stock mixture was stored at ambient temperature.

Aβ42 fibrils were formed by the method of Fradinger *et al*.[2] Lyophilized Aβ42 peptide (1.0 mg, 0.22 μmol) was mixed with water (250 μL), and sodium hydroxide (1 M aqueous, 1.5 μL) was added to increase solubility. The mixture was diluted with phosphate buffer (20 mM sodium phosphate, pH = 7.4, 250 μL, producing a final concentration of 2.0 mg/mL = 0.44 mM peptide monomer) and then mixed with a sonicator (10 min, 22% power). Trace metal residue from the sonicator probe was pelleted by centrifugation (16,000 × *g*, 10 min). The supernatant was mixed gently (to suspend fibrils, but without disturbing the metal pellet), transferred into a fresh Eppendorf tube, and incubated (37 °C, 300 rpm agitation, 20 h). Fibril formation was confirmed by ThT fluorescence and by transmission electron microscopy as described below. The fibril stock mixture was stored at ambient temperature.

Lysozyme fibrils were made by a variation of our previously reported procedure.[1] Lysozyme (28.6 mg, 2.0 μmol) was mixed with phosphate buffer (10 mM sodium phosphate, pH = 7.4, 2.0 mL). The mixture was allowed to stand (24 h) and then centrifuged (16,000 × *g*, 10 min). The supernatant was isolated, and the final monomer concentration was determined to be 670 μM by absorbance at 280 nm using an extinction coefficient of 37750 $M^{-1}cm^{-1}$.[3] The mixture was incubated (60 °C, 3 days). Fibril formation was confirmed by ThT fluorescence and by transmission electron microscopy as described below. The fibril stock mixture was stored at ambient temperature.

ThT Fluorescence Procedure: ThT (approximately 0.5 mg) was dissolved in phosphate buffer (10 mM sodium phosphate, pH 7.4, 1 mL). The final concentration was more precisely determined to be 803 μM by its absorbance at 412 nm using an extinction coefficient of 36,000 $M^{-1}cm^{-1}$.[4] ThT and fibril stock solutions were diluted into phosphate buffer (10 mM, pH 7.4) to give mixtures with final volumes of 200 μL and final concentrations of 20 μM fibrils (or higher for lysozyme) and 20 μM ThT. Fluorescence spectra were recorded with an excitation wavelength of 440 nm and an emission range of 450–600 nm. Slit widths for excitation and emission were set to 5 nm each for insulin and 10 each for AB42 and lysozyme. As expected, the fluorescence outputs are highly enhanced for the fibril + ThT conditions versus fibril-only or ThT-only control conditions. These results are shown in Figures S1–S4.

TEM Procedure: To confirm the morphology of the fibrils, fibril stock mixtures (7 μL) were spotted on carbon-coated copper grids (Electron Microscopy Sciences, CF 150-Cu), allowed to sit (2 min, under a cover), and dried

[1] V. A. Ivancic, O. Ekanayake, N. D. Lazo, *ChemPhysChem*, 2016, **17**, 2461.
[2] E. A. Fradinger, S. K. Maji, N. D. Lazo, D. B. Teplow, Studying Amyloid β-Protein Assembly. In *Amyloid Precursor Protein: A Practical Approach*; W. Xia, H. Xu, Eds.; CRC Press: Boca Raton, 2005; pp 83-110.
[3] K. C. Aune, C. Tanford, *Biochem.*, 1969, **8**, 4579.
[4] G. V. de Ferrari, W. D. Mallender, N. C. Insetrosa, T. L. Rosenberry, *J. Biol. Chem.*, 2001, **276**, 23282.

with a Kimwipe. Uranyl acetate (1% solution, 7 µL) was then used as a stain, incubated on the grid (1 min, under a cover), and removed with a Kimwipe. The grids were air dried (3 min, under a cover) and stored in a grid box until imaging. Images were taken with a Phillips CM10 Transmission Electron Microscope at the Core Electron Microscope Facility at the University of Massachusetts Medical School. Figures S5–S7 show these images. Average fibril diameters were measured to be 7.8 nm, 9.6 nm, and 14.2 nm for insulin, Aβ42, and lysozyme, respectively.

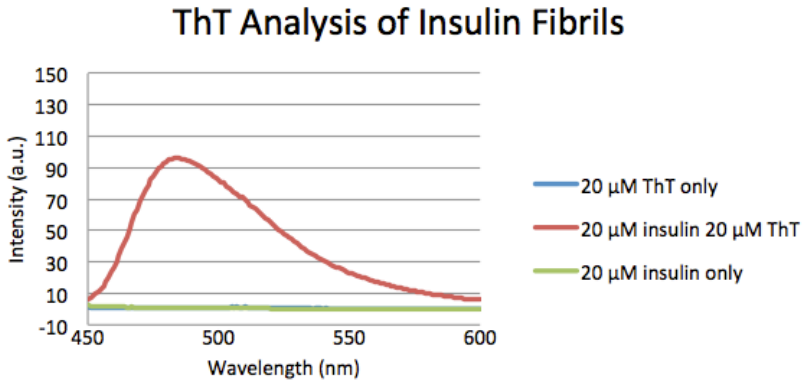


Figure S1. ThT analysis of insulin fibrils.

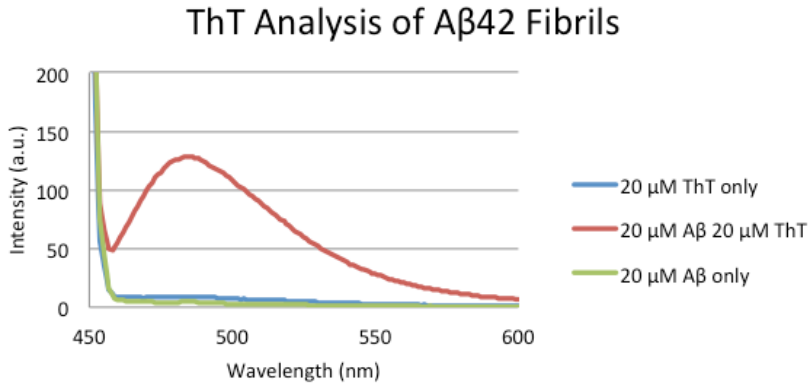


Figure S2. ThT analysis of Aβ42 fibrils.

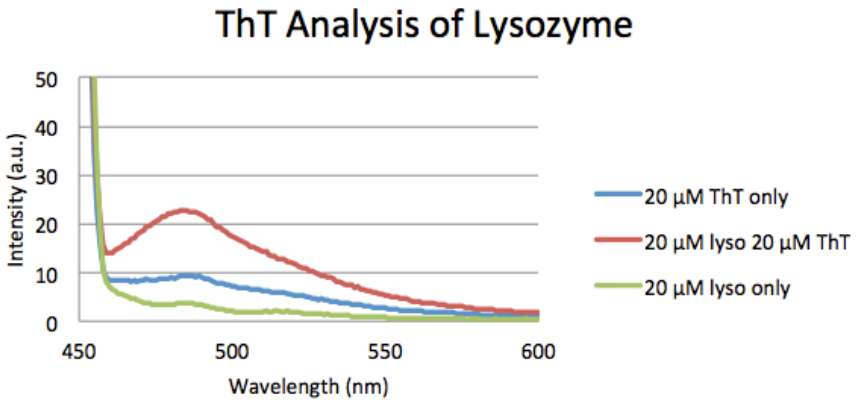


Figure S3. ThT analysis of lysozyme fibrils at standard concentration.

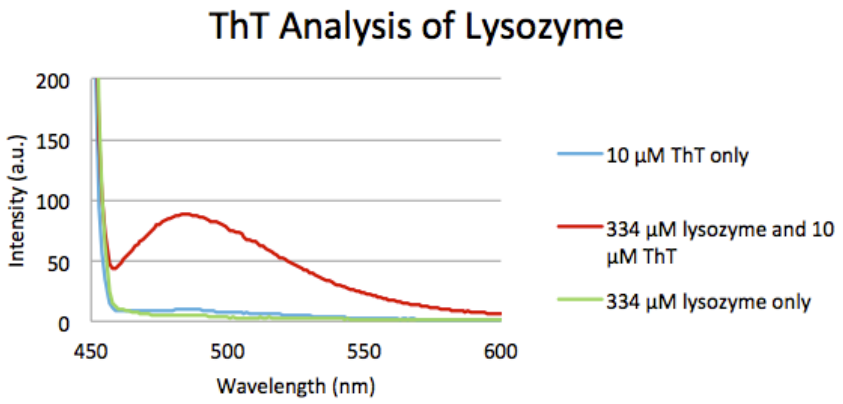


Figure S4. ThT analysis of lysozyme fibrils at a higher concentration.

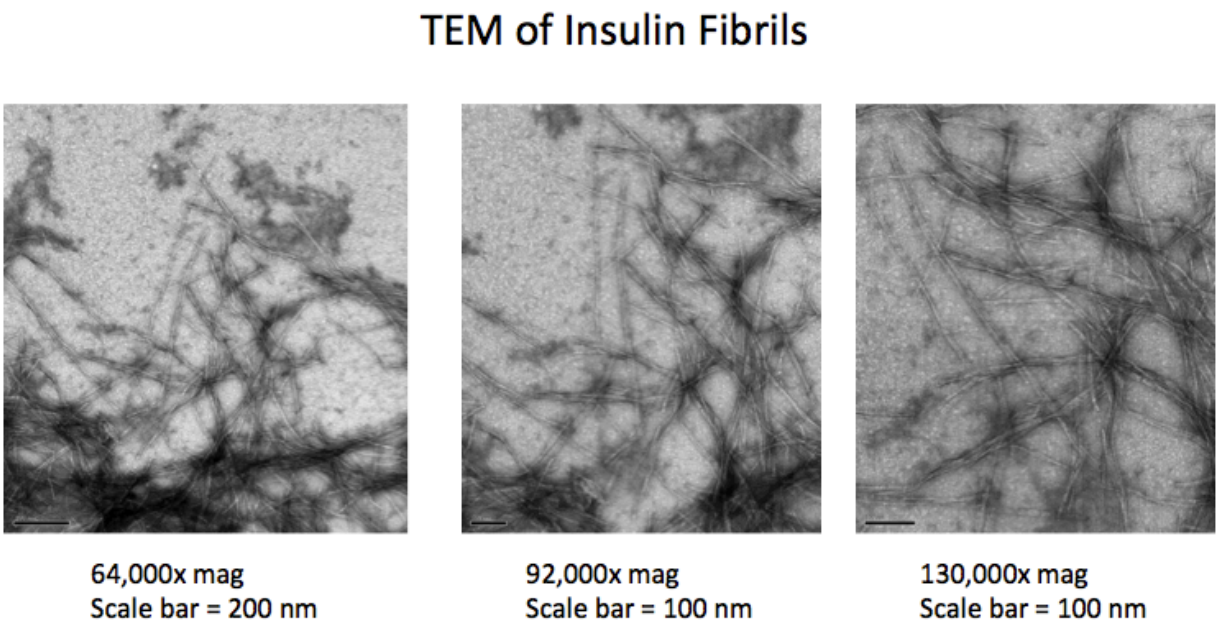


Figure S5. TEM analysis of insulin fibrils from a 170 µM stock solution (counted as monomer).

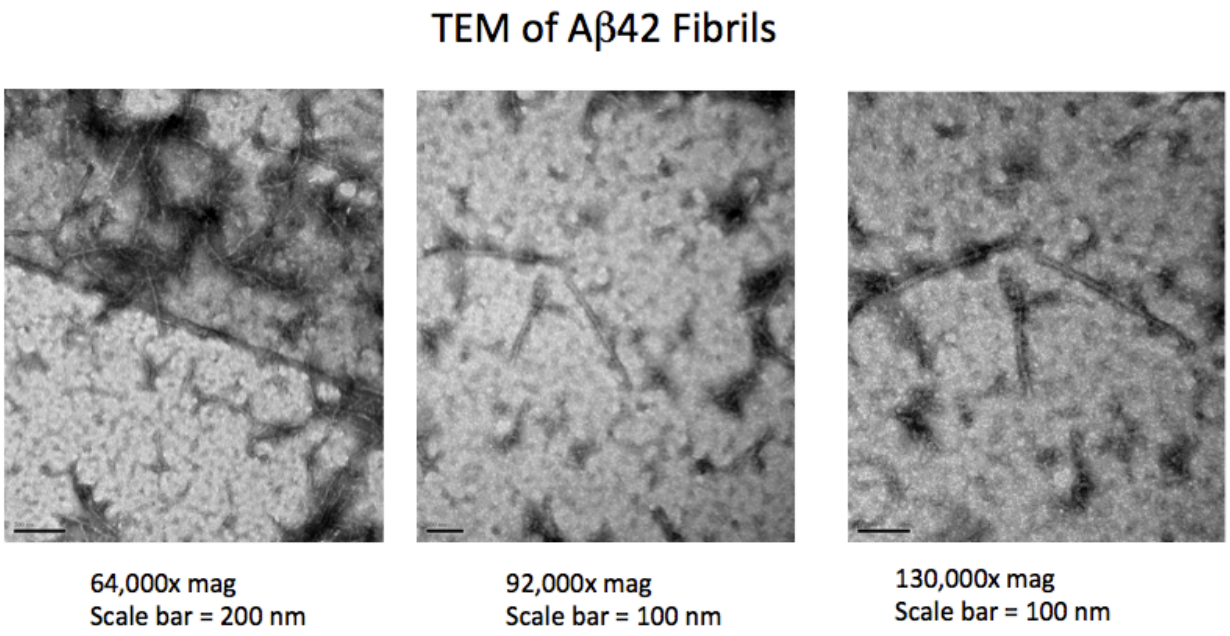


Figure S6. TEM analysis of Aβ42 fibrils from a 440 μM stock solution (counted as monomer).

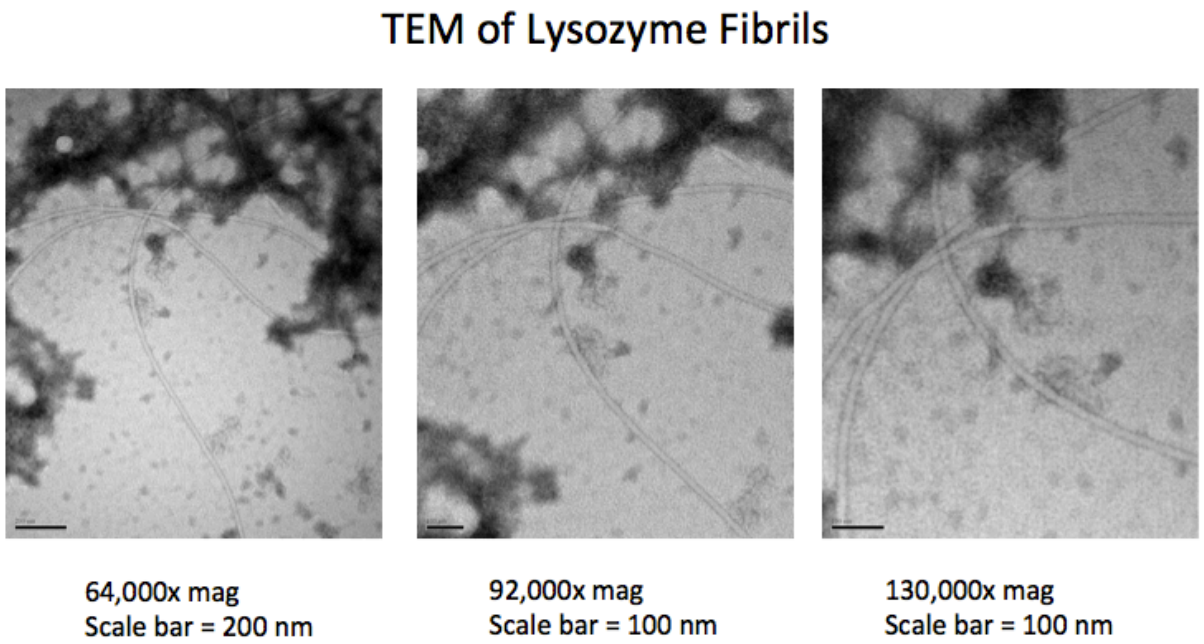


Figure S7. TEM analysis of lysozyme fibrils from a 670 μM stock solution (counted as monomer).

1.2 Biotin Display Assay

•Nitrocellulose Blotting Membrane
GE Healthcare Life Sciences
#10600093, lot #G5437139)

•Phosphate Buffer
10 mM sodium phosphate
pH 7.4

•TBS Buffer (tris-buffered saline)
25 mM Tris base
150 mM sodium chloride
pH 7.4

•TBST Buffer
TBS buffer + 0.5% Tween 20 (v/v)

•Blocking Solution
TBST buffer + 10 mg/mL bovine serum albumin

•Primary Antibody
Anti-biotin (rabbit) primary antibody solution:
1 mg/mL solution
(Rockland #600-401-098, Lot #23826)

•Primary Antibody Solution
1:2000 dilution in blocking solution

•Secondary Antibody
Goat anti-rabbit IgG (H&L) antibody with horseraddish peroxidase (HRP) conjugate:
1 mg/mL solution
(Invitrogen Life Technologies #A24531, Lot 50-28-112315)

•Secondary Antibody Solution
1:2000 in blocking solution

•ECL Western Blotting Substrate
(Pierce #32209, Lot RJ241236)

•Imaging instrument & software
Fujifilm LAS-4000 Imager
Image Reader LAS-4000 software v. 2.1
Multigauge software v.3.11

Into Eppendorf tubes were combined phosphate buffer (100 μL), biotin molecule **10** (2.5 μL of a 240 μM solution in DMSO, or pure DMSO as a negative control, or 10 mM biotin-NHS ester in DMSO as a positive control), and the insulin fibril stock mixture (100 μL of 2 μM stock suspension in phosphate buffer or a 1 μM suspension for the biotin-NHS ester control or pure phosphate buffer as a negative control). The final volumes were 200 μL with 1.25% DMSO (v/v), and the final concentrations were 3 μM biotin molecule **10** with 1 μM insulin or 125 μM biotin-NHS ester with 0.5 μM insulin. Each mixture was mixed by vortexing (3 sec) and allowed to incubate (ambient temperature, 1 h). To remove unbound small molecule, each mixture was concentrated using a spin column (Amicon Ultra: Ultracel 10 kDa centrifuge filter, 13 kRPM for 8 minutes), additional TBS buffer (500 μL) was added, and the samples were concentrated again (13 kRPM for 8 min) to yield approximately 20 μL of each fibril mixture.

Each mixture was dotted (4 replicates, 4 x 1 μL each replicate) onto a piece of nitrocellulose blotting membrane. The membrane was sequentially incubated in TBST blocking solution (15 mL, 4 °C, dark, 30 min), primary antibody solution (15 mL, 4 °C, dark, 60 min), TBST blocking solution (3 x 15 mL, 4 °C, dark, 5 min each), secondary antibody solution (15 mL, 4 °C, dark, 60 min), TBST buffer (2 x 15 mL, 4 °C, dark, 5 min each), and TBS buffer (15 mL, 4 °C, dark, 5 min). ECL solution (2 mL) was prepared and pipetted onto the membrane, and luminescence signals were collected with a 2 min exposure at 4 °C.

1.3 Amyloid Binding Assay

•Phosphate Buffer
10 mM sodium phosphate
pH 7.4

•Fluorometer
Cary Eclipse fluorescence spectrophotometer

•Fluorometer Micro Cell
3 mm, quartz
Starna Cells, 3-3.30-Q-3

•Cary Eclipse software 1.2

Various stock solutions of alkyne molecule **8** or biotin molecule **10** were prepared in DMSO (0.80, 2.0, 4.0, 8.0, 20, 40, 60, 80, 240, 400 μM). An aliquot of the appropriate fibril stock mixture was diluted in phosphate buffer to the desired concentration. Into Eppendorf tubes were added phosphate buffer (97.5 μL), a solution of alkyne molecule **8** or biotin molecule **10** (2.5 μL, various concentrations in DMSO), and the appropriate fibril stock mixture (100 μL, 2 μM insulin fibril or 6 μM Aβ42 fibril or 200 μM lysozyme fibril in phosphate buffer). The final volumes were 200 μL with 1.25% DMSO (v/v), and the final concentrations were 1 μM insulin or 3 μM Aβ42 or 100 μM lysozyme based on monomer and 0.010, 0.025, 0.050, 0.10, 0.25, 0.50, 0.75, 1.0, 3.0, or 5.0 μM alkyne molecule **8** or biotin molecule **10**. Control experiments that replace fibrils, small molecule, or both with the phosphate buffer or DMSO were also prepared. Samples were mixed by vortexing (2 sec) and allowed to stand (1 h, covered with foil). Each sample was mixed by vortexing (3 sec), transferred into a cuvette, and analyzed by fluorescence (ambient temperature, 382 nm excitation, 400–600 nm emission range, 10 nm slit widths for excitation and emission).

Replicate conditions (typically triplicate or duplicate) were averaged and analyzed for standard deviation. Averaged emission spectra for all conditions were corrected for solvent by subtracting the signal produced by a DMSO-only control. Solvent-corrected spectra from the test conditions (mixtures of fibril and small molecule) were then correction for background by subtracting the solvent-corrected spectra from both the corresponding small-molecule-only control and the corresponding fibril-only control. Figures S8–S11 show the background-corrected emission spectra for each fibril / small-molecule combination.

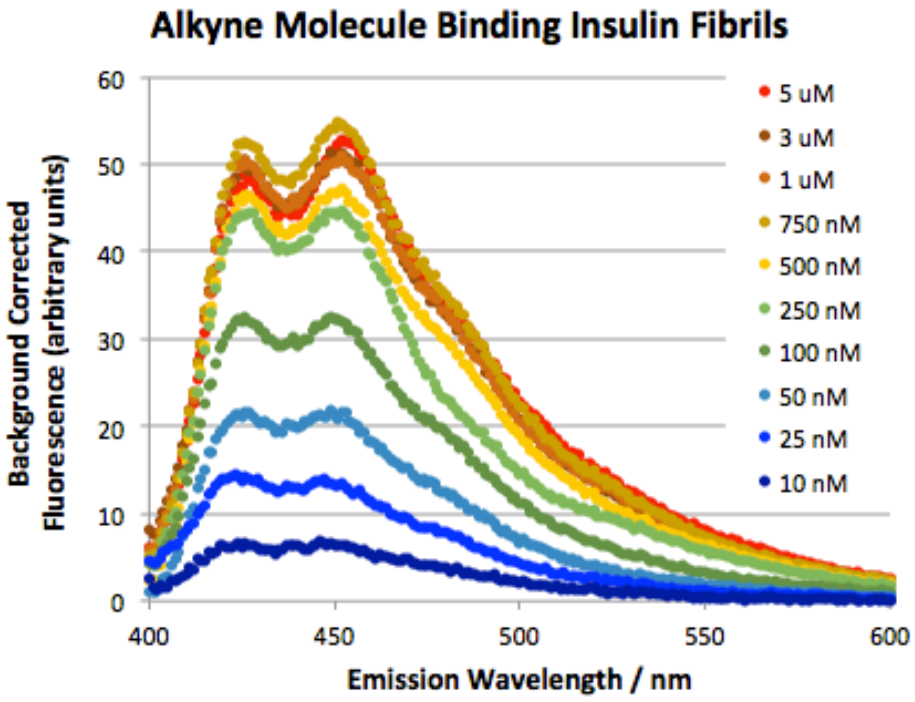


Figure S8. Fluorescence enhancement from alkyne **8** interacting with 1 μM insulin fibrils (counted as monomer). Averages of triplicate data.

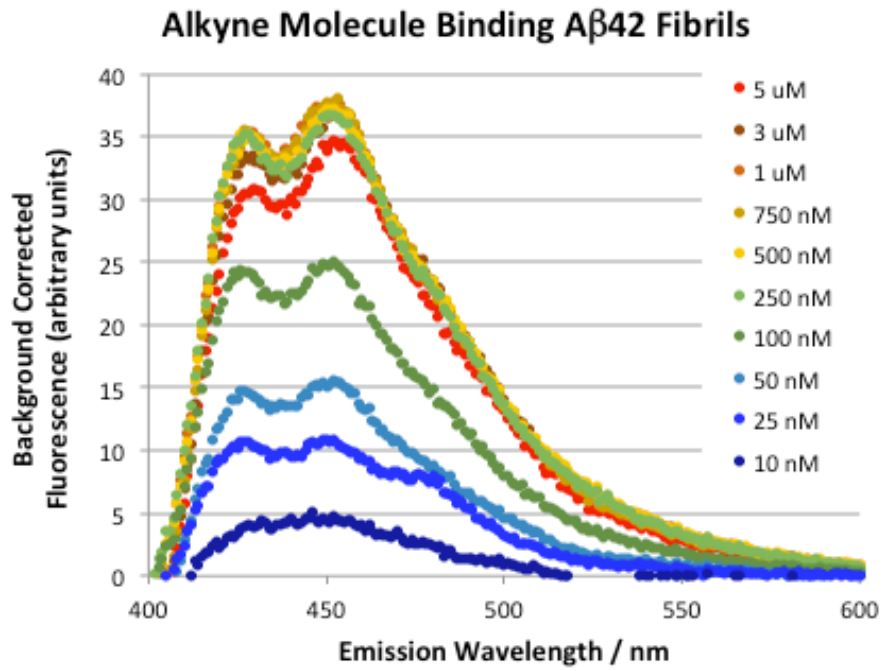


Figure S9. Fluorescence enhancement from alkyne **8** interacting with 3 μM Aβ42 fibrils (counted as monomer). Averages of triplicate data.

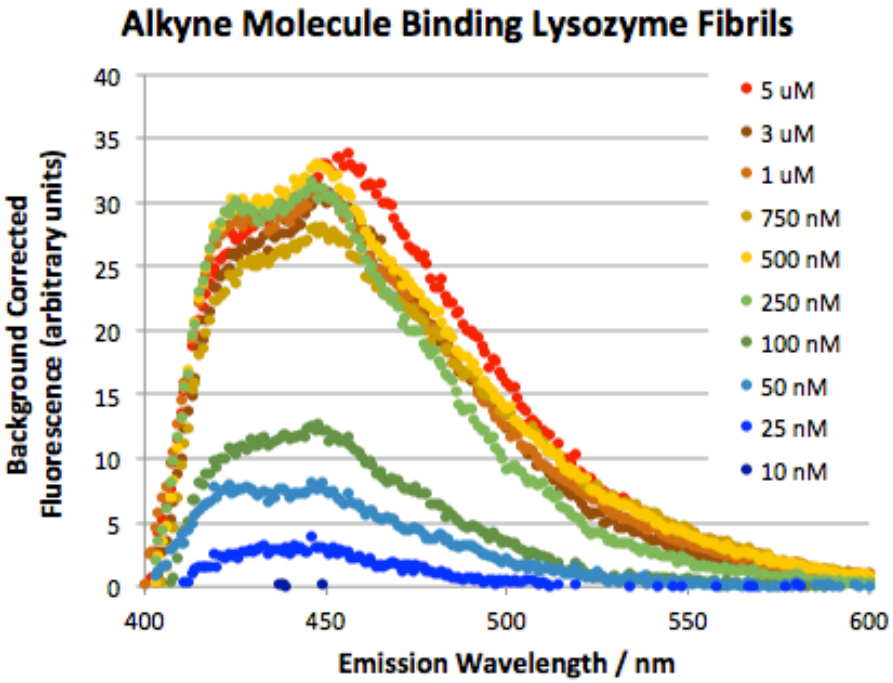


Figure S10. Fluorescence enhancement from alkyne **8** interacting with 100 μM lysozyme fibrils (counted as monomer). Averages of duplicate data.

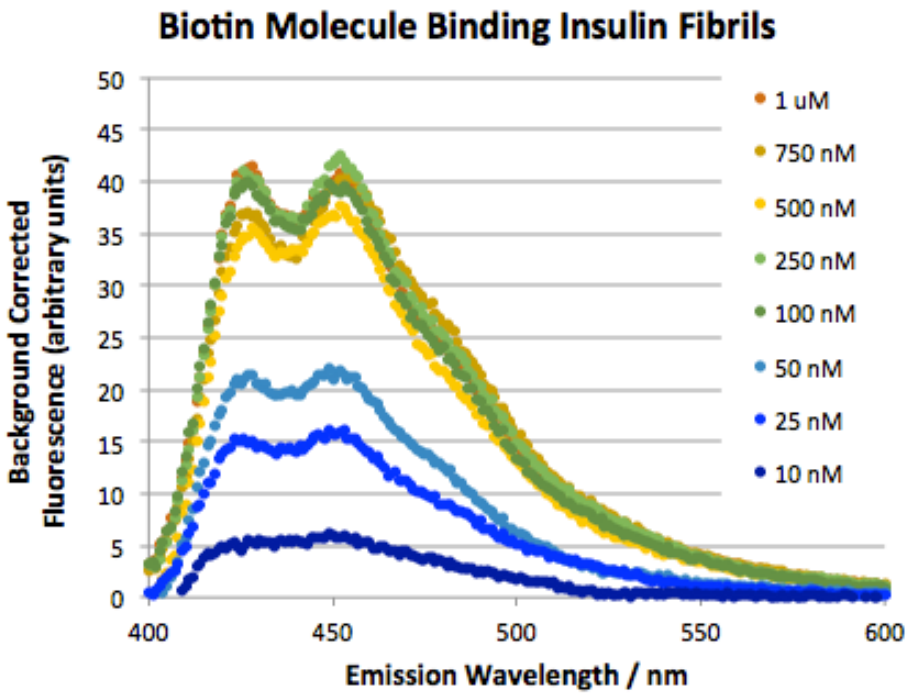


Figure S11. Fluorescence enhancement from biotin **10** interacting with 1 μM insulin fibrils (counted as monomer). Averages of triplicate data.

For each molecule–fibril pair, an emission wavelength near the 450 nm maximum was selected for further analysis (452 nm for insulin, 450 nm for Aβ42, and 449 nm for lysozyme) (Figures 2B, 3A–B, 4A). To extract approximate values for the binding constants and number of available binding sites, the quadratic solution to the binding equilibrium (eq S8) was used. Theoretical plots were compared to the experimental data until suitable fits were identified. The key parameters of each fit are listed in Table S1.

L_T = total ligand
L = free ligand
Y = background subtracted fluorescence readout
Y_{MAX} = readout plateau

P_T = total protein binding sites
P = free protein binding site
P•L = occupied binding sites

$$P + L \rightleftharpoons P{\bullet}L \qquad \text{(eq S1)}$$

$$K_D = \frac{P \times L}{P{\bullet}L} \qquad \text{(eq S2)}$$

$$P_T = P + P{\bullet}L \qquad \text{(eq S3)}$$

$$L_T = L + P{\bullet}L \qquad \text{(eq S4)}$$

$$Y = \frac{P{\bullet}L}{P_T} \times Y_{MAX} \qquad \text{(eq S5)}$$

combine eqs S2, S3, and S4:

$$K_D = \frac{(P_T - P{\bullet}L) \times (L_T - P{\bullet}L)}{P{\bullet}L} \qquad \text{(eq S6)}$$

rearrange:

$$0 = (P{\bullet}L)^2 - (K_D + P_T + L_T) \times (P{\bullet}L) + (P_T \times L_T) \qquad \text{(eq S7)}$$

solve quadratic equation and substitute into eq S5:

$$Y = \frac{(K_D + P_T + L_T) \pm \sqrt{(K_D + P_T + L_T)^2 - (4 \times P_T \times L_T)}}{2 \times P_T} \times Y_{MAX} \qquad \text{(eq S8)}$$

Table S1. Summary of binding properties.

Fibril	Binder	Y_{MAX}	EC_{50}	K_D	Total Available Binding Sites (P_T)	Amyloid Monomer Concentration	Amyloid Monomers per Binding Site
Insulin	Alkyne **8**	51	60 nM	25 nM	80 nM	1 μM	12
Insulin	Biotin **10**	42	45 nM	5 nM	80 nM	1 μM	12
Aβ42	Alkyne **8**	39	65 nM	15 nM	100 nM	3 μM	30
Lysozyme	Alkyne **8**	31	110 nM	10 nM	200 nM	100 μM	500

2. Synthetic Procedures and Compound Data

2.1 Abbreviations

AIBN = azobisisobutyronitrile
DCM = dichloromethane
DMAP = 4-(dimethylamino)pyridine
DMF = *N*,*N*-dimethylformamide
DMSO = dimethylsulfoxide
EtOAc = ethyl acetate
Ether = diethyl ether
MeOH = methanol
TEA = triethylamine
TFA = trifluoroacetic acid
THF = tetrahydrofurane
y = yield

2.2 General Procedures

Column chromatography was performed with 60 Å 40–63 μm silia–P flash silica gel.

Solvents for reactions (DMF, DCM, THF, and toluene) were dried using a LC Technology Solutions purification system. Other solvents were used as received unless noted otherwise.

Chemicals were purchased from Fisher, VWR, or Sigma–Aldrich and used as received unless noted otherwise.

NMR Spectra were measured in $CDCl_3$ at ambient temperature unless otherwise noted.

^{1}H NMR spectra were recorded on either a 600 or 200 MHz Varian spectrometer. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane using the solvent as a reference ($CDCl_3$ = 7.26 ppm, DMSO-d_6 = 2.49 ppm, CD_3CN = 1.94 ppm, D_2O = 4.80 ppm, CD_3OD = 3.30). The following is an example data point: chemical shift (multiplicity [s = singlet, d = doublet, t = triplet, q = quartet, pent = pentet, sext = sextet, sept = septet, oct = octet, m = multiplet, br = broad, and combinations thereof], coupling constants [Hz], integration, assignment [if any]).

^{13}C NMR spectra were recorded on a 600 or 200 MHz (150 or 50 MHz) Varian spectrometer with complete proton decoupling. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane using the solvent or MeOH as a reference ($CDCl_3$ = 77.0 ppm, DMSO-d_6 = 39.5 ppm, CD_3CN = 1.39 ppm (and 118.69 ppm), CD_3OD = 49.0 ppm, MeOH = 49.5).

IR spectra were recorded on a Perkin Elmer Spectrum 100 FT–IR spectrometer with Perkin Elmer Spectrum software. Spectra are partially reported (υ_{max}, cm^{-1}).

MS were obtained either on an Agilent Technologies 6120 quadrupole LC/MS system with an Infinity 1260 liquid chromatography system at Clark University or at The University of Illinois Urbana–Champagne's Mass Spectrometry Center.

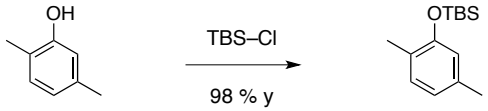
TLC was performed on 60 Å F_{254} pre-coated silica gel plates. Samples were visualized by either ultraviolet irradiation, potassium permanganate staining, or cerium ammonium molybdenate staining.

Optical Rotations were obtained with a Rudolph Research Autopol II automatic polarimeter.

Yield refers to isolated material.

Quantitative recovery means that mostly pure material was recovered in approximately the expected mass, and the material was used directly for the next step without purification.

2.3. Procedures, Data, and NMR Spectra



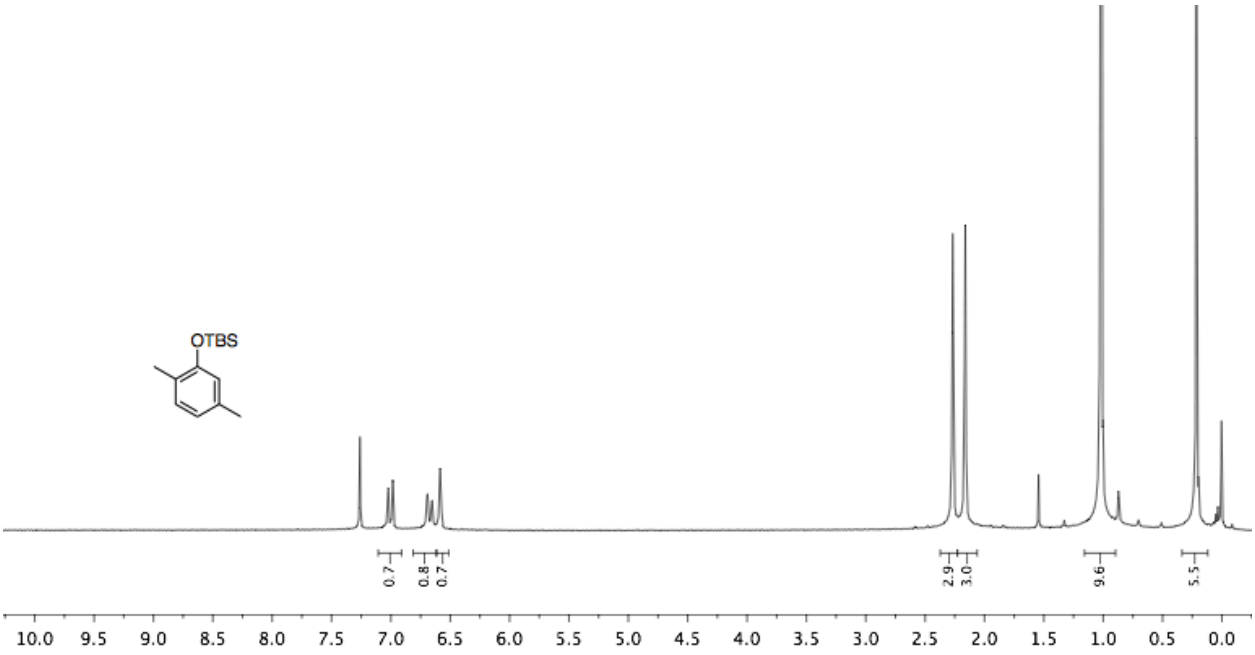
1A **1**

Synthesis of Silyl Ether 1: Into a flame-dried flask were added 2,5-dimethylphenol (**1A**, 2.00 g, 16.4 mmol, 1 equiv), dry DMF (16 mL, 1.0 M), imidazole (2.80 g, 41.2 mmol, 2.5 equiv), and *tert*-butyldimethylsilyl chloride (2.95 g, 19.7 mmol, 1.2 equiv.). The mixture was maintained under a nitrogen atmosphere, stirred (16 h), quenched with ammonium chloride (50% aqueous, 40 mL), extracted with hexane (3 x 40 mL), washed with sodium chloride (50% saturated, 50 mL), and dried with sodium sulfate. Volatiles were removed under pressure to yield pure silyl ether **1** (3.82 g, 16.2 mmol, 98% yield, colorless oil). Compound analysis is consistent with previously reported data.[5]

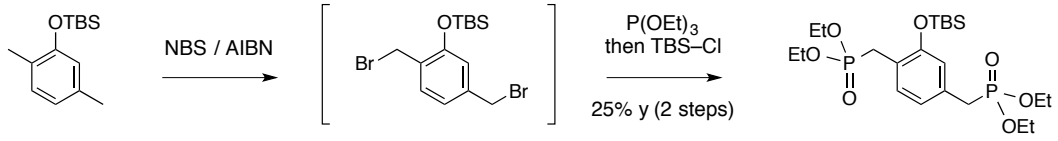
^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.01 (d, J = 7.4 Hz, 1H, Ar), 6.67 (d, J = 7.2 Hz, 1H, Ar), 6.58 (s, 1H, Ar), 2.27 (s, 3H, Me), 2.17 (s, 3H, Me), 1.01 (m, 9H, Si–*t*Bu), 0.21 (m, 6H, 2 x Si–Me).

TLC (14:1) Hexane/EtOAc, UV, R_f = 0.92.

^{1}H NMR Spectrum of 1



[5] R. E. Donaldson, P. L. Fuchs, *J. Org. Chem.*, 1977, **42**, 2032.



1 **2** **3**

Synthesis of Phosphonate Ester 3: Into a flask were added silyl ether **1** (2.10 g, 8.88 mmol, 1 equiv), carbon tetrachloride (30 mL, 0.3 M), *N*-bromosuccinimide (freshly recrystallized from boiling water, 3.41 g, 19.1 mmol, 2.2 equiv), and AIBN (151 mg, 0.920 mmol, 0.1 equiv). The flask was then degassed by a freeze-pump-thaw process (3x) under argon. A reflux condenser was attached, and the system was maintained under an argon atmosphere. The mixture was heated in the dark (75 °C, 6 h) and allowed to cool to ambient temperature. Insoluble material was removed by filtration and chased with hexane. The filtrate was dried with sodium sulfate, and volatiles were removed under reduced pressure to yield crude dibromide **2** (3.50 g, full mass recovery, yellow oil), which was used directly in following step without purification. NMR analysis of the crude mixture showed complete reaction of one of the benzylic positions (2.17 ppm), but only approximately 50% reaction of the other benzylic position (2.27 ppm) and the formation of small amounts of over-brominated products (7.9–7.7 ppm). Due to the high reactivity of benzylic bromides, purification was not attempted until after the subsequent reaction.

Into a flask were added crude dibromide **2** (8.88 mmol, 1 equiv) and triethylphosphite (5.87 mL, 34.3 mmol, 3.8 equiv). A reflux condenser was attached. The mixture was heated in the dark (160 °C, 16 h) and then allowed to cool. Volatiles were removed under reduced pressure (approximately 1 x 10^{-3} atm, 50 °C, 2 h) to yield crude phosphonate ester **3** (4.88 g, green oil with a red-orange sheen). Since this mixture contained substantial material in which the phenol protecting group had been removed, a reprotection step was performed.

Into a flame-dried flask were added crude phosphonate ester **3** (8.88 mmol, 1 equiv), dry DMF (12 mL, 0.7 M), imidazole (2.45 g, 36.01 mmol, 4.0 equiv), and *tert*-butyldimethylsilyl chloride (3.67 g, 24.4 mmol, 2.7 equiv). The mixture was maintained under nitrogen atmosphere, stirred (18 h), quenched with ammonium chloride (50% aqueous, 20 mL), extracted with hexane (3 x 40 mL), washed with sodium chloride (50% saturated, 40 mL), and dried with sodium sulfate. Volatiles were removed under reduced pressure, and the crude material was purified by column chromatography (125 mL silica gel, EtOAc to 20:1 EtOAc/methanol) to yield pure protected phosphonate ester **3** (1.12 g, 2.20 mmol, 25% yield from silyl ether **1**, fluorescent green oil) plus mixed fractions.

Crude dibromide intermediate **2**:

^{1}H NMR (major signals, $CDCl_3$, 600 MHz) δ 7.29 (d, J = 7.3 Hz, 1H, Ar), 6.94 (d, J = 7.3 Hz, 1H), 6.84 (s, 1H, Ar), 4.50 (s, 3H, CH_2Br), 4.41 (s, 3H, CH_2Br), 1.06 (s, 9H, Si–*t*Bu), 0.31 (s, 6H, 2 x Si–Me).

LRMS (methanol substitution product) calculated for $[C_{16}H_{28}O_3SiNa]^+$, requires m/z = 319.17, found m/z = 319.2 (ESI).

Phosphonate ester **3**:

^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.31 (dd, J_1 = 7.8 Hz, J_2 = 2.4 Hz, 1H, Ar), 6.83–6.79 (m, 2H, Ar), 4.05–3.94 (m, 8H, 4 x OEt), 3.17 (d, J = 21.7 Hz, 2H, CH_2–P), 3.07 (d, J = 21.7 Hz, 2H, CH_2–P), 1.22 (q, J = 6.7 Hz, 12H, 4 x OEt), 1.02 (s, 9H, 3 x Si–*t*Bu), 0.26 (s, 6H, 2 x Si–Me).

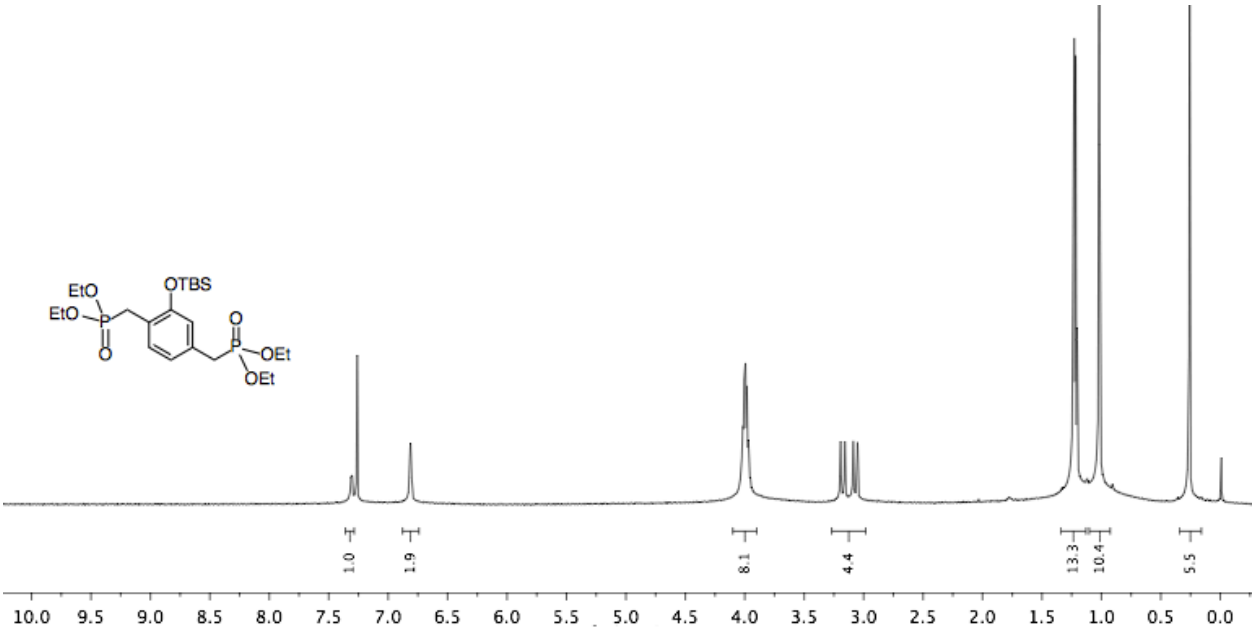
^{13}C NMR ($CDCl_3$, 50 MHz) δ 153.00 (dd, J_1 = 7.6 Hz, J_2 = 3.1 Hz, Ar), 130.88 (dd, J_1 = 8.9 Hz, J_2 = 3.8 Hz, Ar), 130.81 (overlapping multiple), 122.12 (dd, J_1 = 7.3 Hz, J_2 = 3.4 Hz, Ar), 120.45 (dd, J_1 = 8.7 Hz, J_2 = 3.7 Hz, Ar), 119.25 (dd, J_1 = 6.2 Hz, J_2 = 1.9 Hz, Ar), 61.52 (d, J =6.8 Hz, OEt), 61.32 (d, J =6.5 Hz, OEt), 32.86 (dd, J_1 = 140.1 Hz, J_2 = 1.7 Hz, C–P), 26.06 (dd, J_1 = 141.1 Hz, J_2 = 1.7 Hz, C–P), 35.35, 17.75, 15.96, 15.88, -4.75.

HRMS calculated for $[C_{22}H_{42}O_7P_2SiH^+]$), requires m/z = 509.2253, found m/z = 509.2250 (ESI).

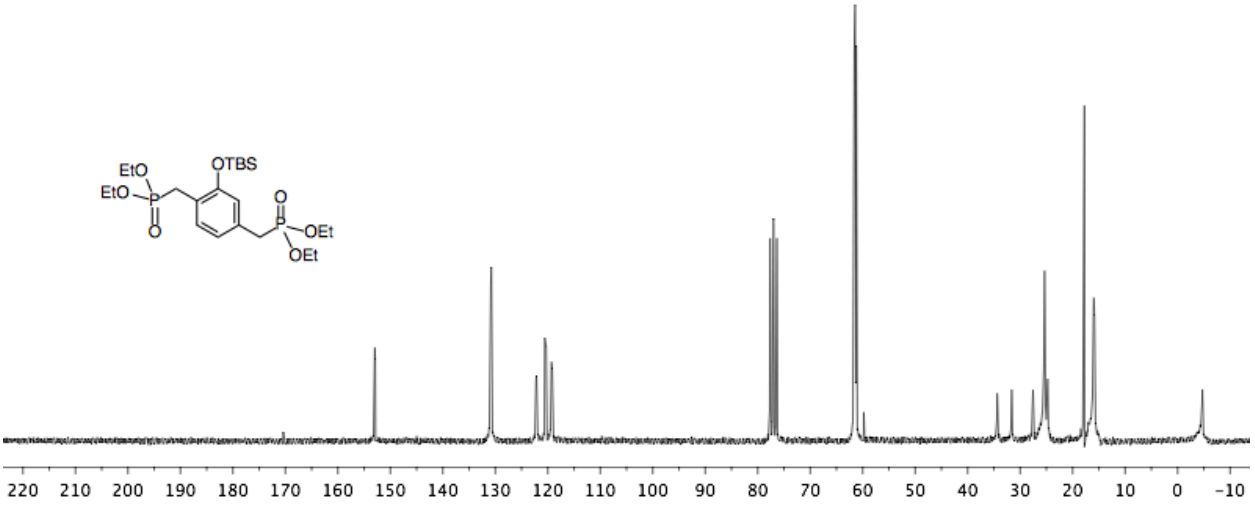
IR (ν, cm^{-1}) 2980, 2957, 2931, 2905, 2859, 1504, 1425, 1250, 1022, 955, 839, 780.

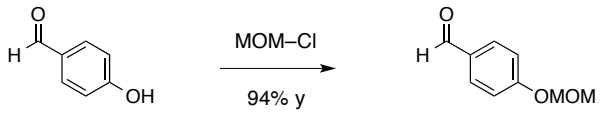
TLC ethyl acetate, permanganate and UV, R_f = 0.25.

^{1}H NMR Spectrum of 3



^{13}C NMR Spectrum of 3





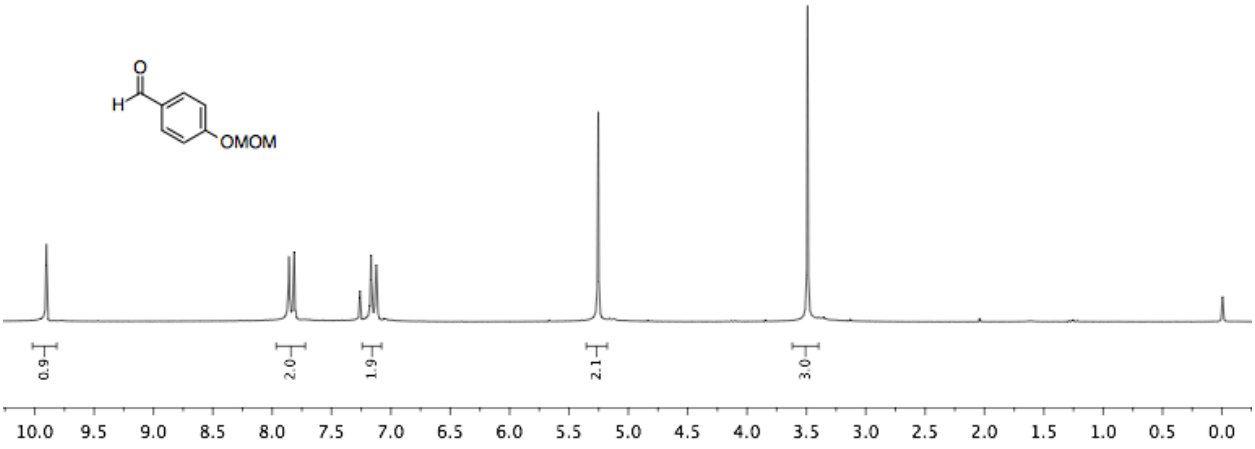
4A **4**

Synthesis of Aldehyde 4: Into a flame-dried flask were added 4-hydroxybenzaldehyde (**4A**, 1.00 g, 8.19 mmol, 1 equiv), THF (16 mL, 0.5 M), triethylamine (5.50 mL, 39.5 mmol, 4.8 equiv), and chloromethyl methyl ether (1.86 mL, 24.6 mmol, 3 equiv). The flask was vented through a Drierite-packed syringe, stirred (17 h), quenched with ammonium chloride (50% saturated, 20 mL), extracted with ethyl acetate (3 x 30 mL), washed with 30 mL of a 1:1 mixture of brine and potassium phosphate 5 (1 M, pH 7), and dried with sodium sulfate. Volatiles were removed under reduced pressure to yield pure aldehyde **4** (1.30 g, 7.69 mmol, 94% yield, colorless oil). Compound analysis is consistent with previously reported data.[6]

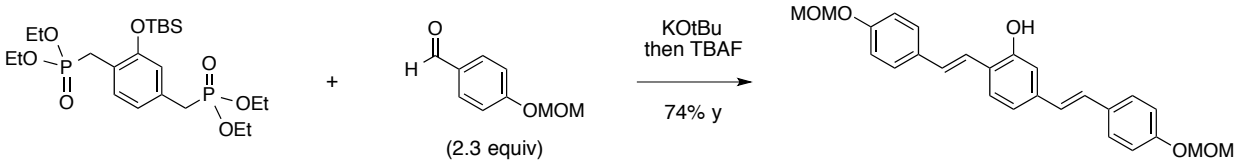
^{1}H NMR ($CDCl_3$, 200 MHz) δ 9.90 (s, 1H, O=CH), 7.84 (d, J = 8.6 Hz, 2H, Ar), 7.14 (d, J = 9.0 Hz, 2H, Ar), 5.25 (s, 2H, O–CH_2–O), 3.49 (s, 3H, OMe).

TLC (2:1) Hexane:EtOAc, permanganate and UV, R_f = 0.56.

^{1}H NMR Spectrum of 4



[6] N. Otto, D. Ferenc, T. Opatz, *J. Org. Chem.*, 2017, **82**, 1205.



3 **4** **5**

Synthesis of Phenol 5: Into a flame-dried flask were added phosphonate ester **3** (374 mg, 0.736 mmol, 1 equiv) and aldehyde **4** (288 mg, 1.67 mmol, 2.3 equiv). Potassium *tert*-butoxide (1.6 M in THF, 1.20 mL, 1.92 mmol, 2.6 equiv) was then added dropwise (producing a final phosphonate ester concentration of 0.6 M). The mixture was maintained under a nitrogen atmosphere, stirred (20 h), quenched with potassium phosphate (1 M, pH 5, 10 mL), stirred (5 min), extracted with EtOAc (3 x 15 mL), washed with sodium chloride (50% saturated, 25 mL), and dried with sodium sulfate. Volatiles were removed under reduced pressure to yield a red oil (508 mg). The oil was dissolved in dry THF (8.5 mL, 0.09 M) and cooled in an ice bath. Tetrabutylammonium fluoride (1 M in THF, 1.00 mL, 1.00 mmol, 1.4 equiv) was added. The mixture was stirred (0 °C, 1 h), allowed to warm to ambient temperature (additional 3 h), quenched with potassium phosphate buffer (1 M, pH 5, 5 mL), stirred (5 min), diluted with brine (2 mL), extracted with EtOAc (3 x 30 mL), washed with sodium chloride (50% saturated, 20 mL), and dried with sodium sulfate. Volatiles were removed under reduced pressure. The crude material was triturated with hexane (3 x 5 mL) and then purified by column chromatography (40 mL silica gel, 7:1:1 toluene/hexane/EtOAc) to yield pure phenol **5** (226 mg, 0.542 mmol, 74% yield from phosphonate ester **3**, yellow-orange solid). ^{1}H NMR signals were partially assigned by ^{1}H–^{1}H COSY.

^{1}H NMR (DMSO-d_6, 600 MHz) δ 9.75 (brs, 1H, OH), 7.54 (d, J = 8.7 Hz, 3H, 2 x Ar–OMOM + 1 Ar–OH), 7.48 (d, J = 8.4 Hz, 2H, Ar–OMOM), 7.27 (d, J = 16.4, 1H, vinyl), 7.17 (d, J = 16.4 Hz, 1H, vinyl), 7.08 (d, J = 16.4 Hz, 1H, vinyl), 7.06 (dd, J_1 = 7.6 Hz, J_2 = 1.1 Hz, 1H, Ar–OH), 7.01 (d, J = 8.7 Hz, 2H, Ar–OMOM), 7.01 (d, J = 17.5 Hz, 1H, vinyl), 7.00 (d, J = 8.6 Hz, 2H, Ar–OMOM), 7.00 (s, 1H, Ar–OH), 5.20 (s, 2H, O–CH_2–O), 5.19 (s, 2H, O–CH_2–O), 3.38 (s, 6H, OMe).

^{13}C NMR (DMSO-d_6, 150 MHz) δ 156.39, 156.14, 154.85, 137.29, 131.53, 130.74, 127.71, 127.49, 127.34, 127.05, 126.50, 126.40, 123.40, 121.58, 117.61, 116.37, 116.32, 113.36, 93.81, 55.56.

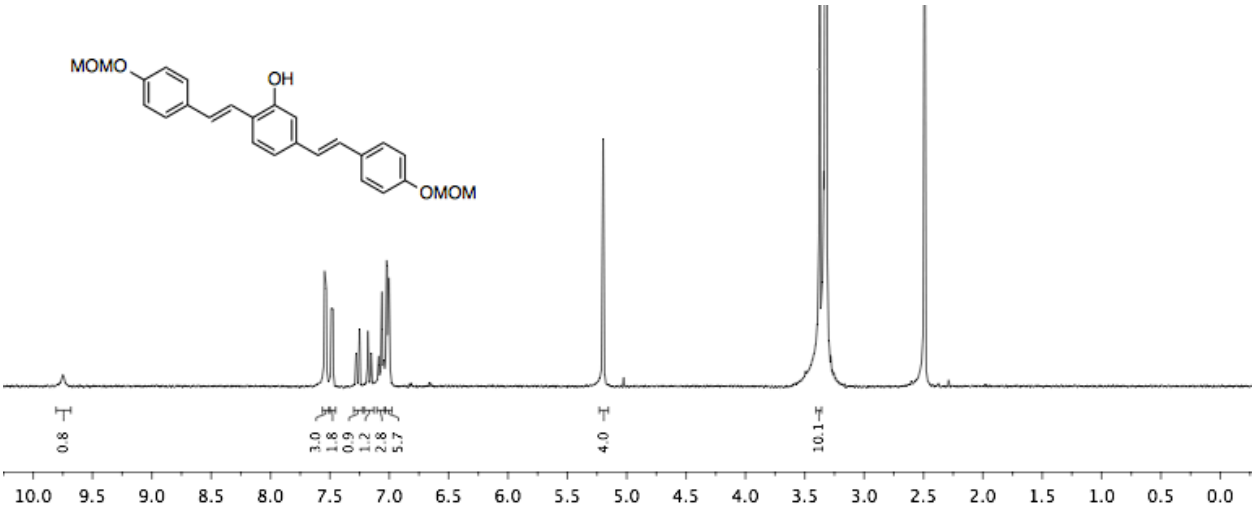
HRMS calculated for $[C_{26}H_{25}O_5]^-$, requires m/z = 417.1702, found m/z = 417.1699 (ESI negative mode).

IR (ν, cm^{-1}) 3524, 3501, 1603, 1515, 1505, 1238, 1150, 1099, 1076, 986, 963, 830

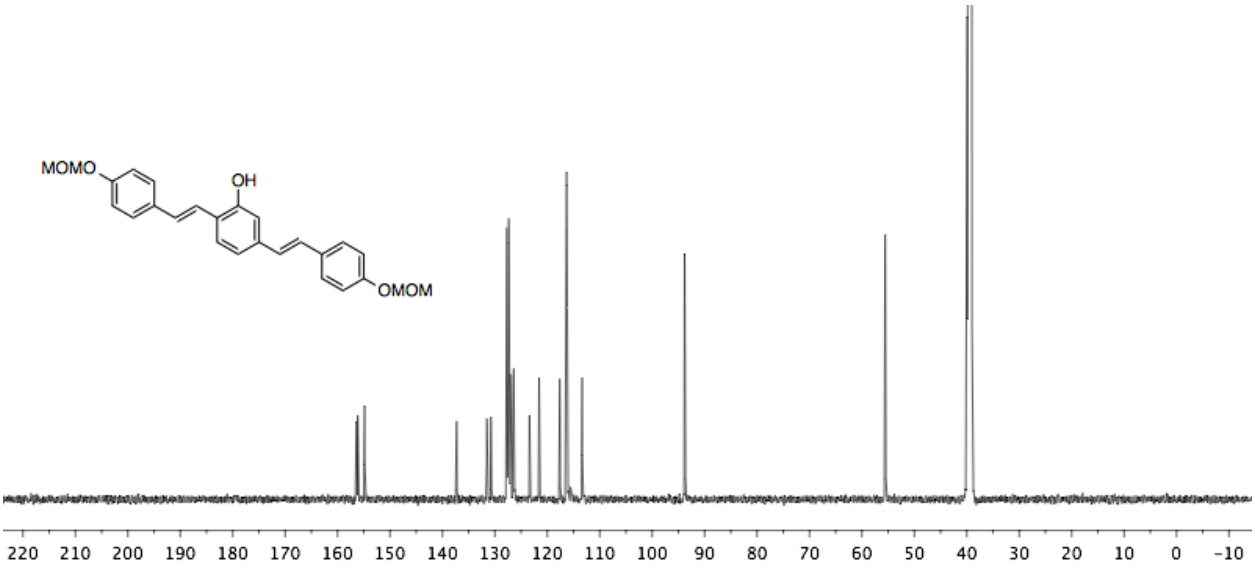
TLC (7:1:1) Toluene/hexane/EtOAc, permanganate and UV (blue), R_f = 0.32.

MP 164 °C (partial decomposition), 194–195 °C (further decomposition).

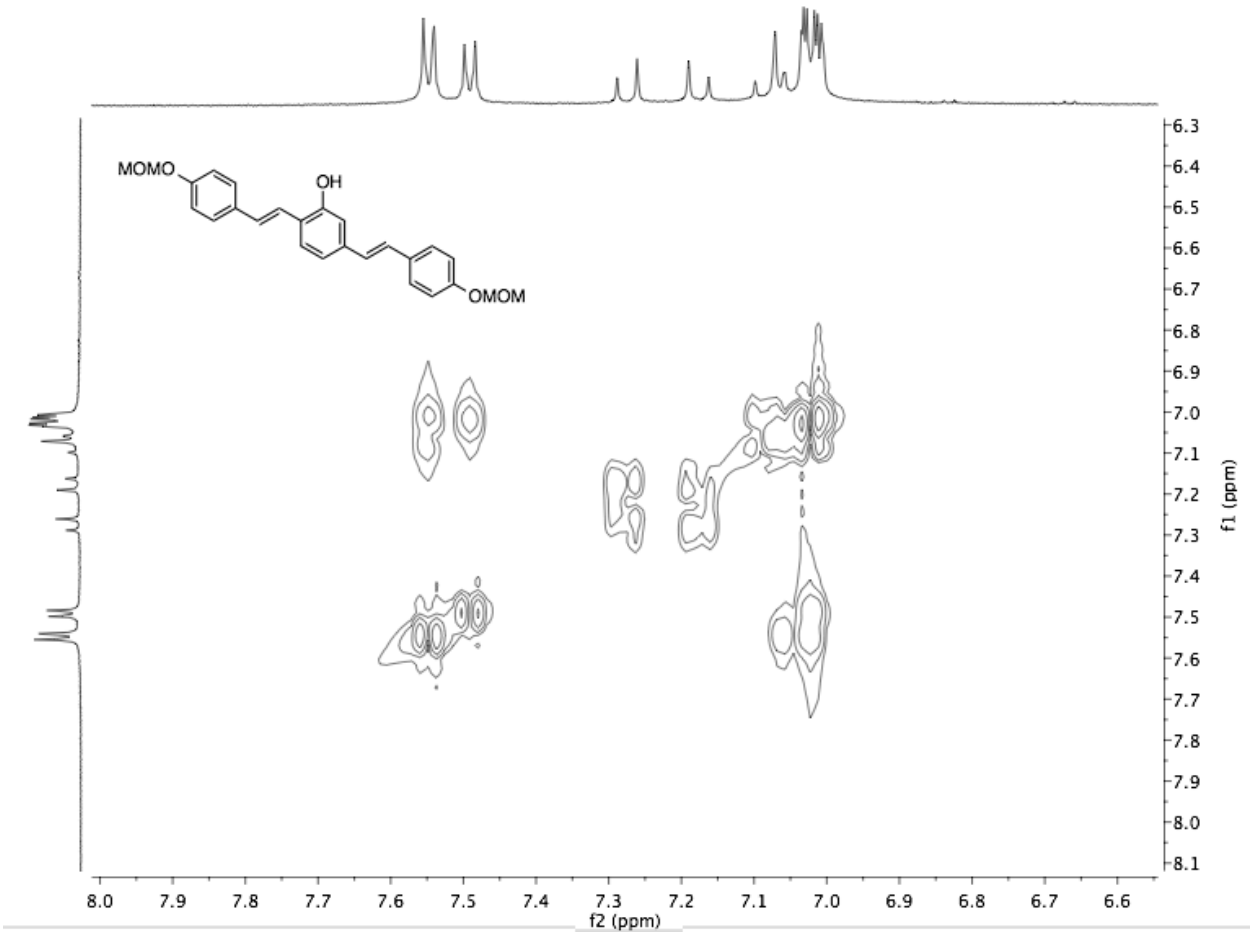
^{1}H NMR Spectrum of 5 (DMSO-d_6)



^{13}C NMR Spectrum of 5 (DMSO-d_6)



^{1}H–^{1}H COSY NMR Spectrum of 5 (DMSO-d_6)

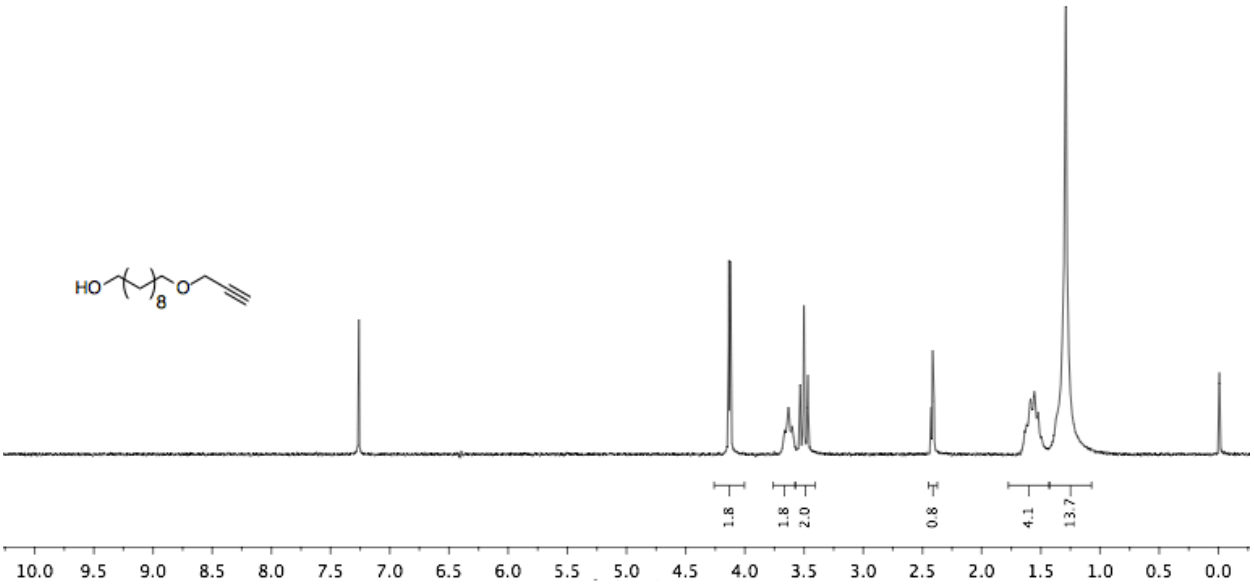


Synthesis of Ether 6C: Into a flame-dried flask was added sodium hydride (60% by weight in mineral oil, 226 mg, 5.66 mmol, 1.16 equiv) and dry DMF (10 mL). The mixture was stirred under a nitrogen atmosphere with a vent needle, and 1,10-decanediol (**6A**, 2.47 g, 14.2 mmol, 2.9 equiv) was added dropwise (evolving gas). The mixture was stirred (20 min) and then cooled in an ice bath. 3-bromo-1-propyne (**6B**, 80% = 9.2 M in toluene, 0.53 mL, 4.9 mmol, 1 equiv) was added dropwise. The mixture was allowed to warm to ambient temperature, stirred (18 h), quenched with potassium phosphate buffer (1 M, pH 5, 5 mL), stirred (5 min), extracted with EtOAc (4 x 15 mL), washed with water (15 mL) and then sodium chloride (50% saturated, 4 x 15 mL), and dried with sodium sulfate. Volatiles were removed under reduced pressure. The crude material was triturated with hexane (5 x 10 mL) and then purified by column chromatography (25 mL silica gel, 3:1 hexane/EtOAc) to yield pure ether **6C** (456 mg, 2.15 mmol, 44% yield, slightly yellow oil) plus mixed fractions. Compound analysis are consistent with previously reported data.[7]

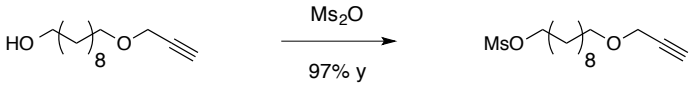
^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.12 (d, J = 2.2 Hz, 2H, CH_2–C≡C), 3.63 (t, J = 6.2 Hz, 2H, CH_2–O), 3.50 (t, J = 6.3 Hz, 2H, CH_2–O), 2.41 (t, J = 2.2 Hz, 1H, alkyne), 1.67–1.44 (m, 4H, 2 x CH_2), 1.40–1.18 (m, 12H, 6 x CH_2).

TLC (3:1) Hexane/EtOAc, permanganate, R_f = 0.25.

^{1}H NMR Spectrum of 6C



[7] D. Basak, S. Christensen, S. K. Surampudi, C. Versek, D. T. Toscano, M. T. Tuominen, R. C. Hayward, D. Venkataraman, *Chem. Commun.*, 2011, **47**, 5566.



6C **6**

Synthesis of Mesylate 6: Into a flame-dried flask were added propargyl ether **6C** (295 mg, 1.39 mmol, 1 equiv), dry DCM (3 mL, 0.5 M), 2,6-lutidine (0.50 mL, 4.3 mmol, 3.1 equiv), and DMAP (142 mg, 1.16 mmol, 0.83 equiv). The flask was maintained under a nitrogen atmosphere and cooled in an ice bath. Methanesulfonic anhydride (634 mg, 3.64 mmol, 2.6 equiv) was added. The mixture was stirred (4.5 h), quenched with water (5 mL), extracted with DCM (3 x 10 mL), washed with sodium bisulfate (1 M, 10 mL) and then a 1:1 mixture of 1 M sodium bisulfate and brine (10 mL), and dried with sodium sulfate. Volatiles were removed under reduced pressure to yield pure mesylate **6** (392 mg, 1.35 mmol, 97% yield, orange oil).

^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.22 (t, J = 6.6 Hz, 2H, CH_2–OMs), 4.12 (d, J = 2.3 Hz, 2H, O–CH_2–C≡C), 3.50 (t, J = 6.6 Hz, 2H, CH_2–O), 3.00 (s, 3H, Me) 2.42 (t, J = 22 Hz, 1H, C≡CH), 1.85–1.68 (m, 2H, CH_2–C–OMs), 1.68–1.50 (m, 2H, CH_2–C–O), 1.46–1.23 (m, 12H, 6 x CH_2).

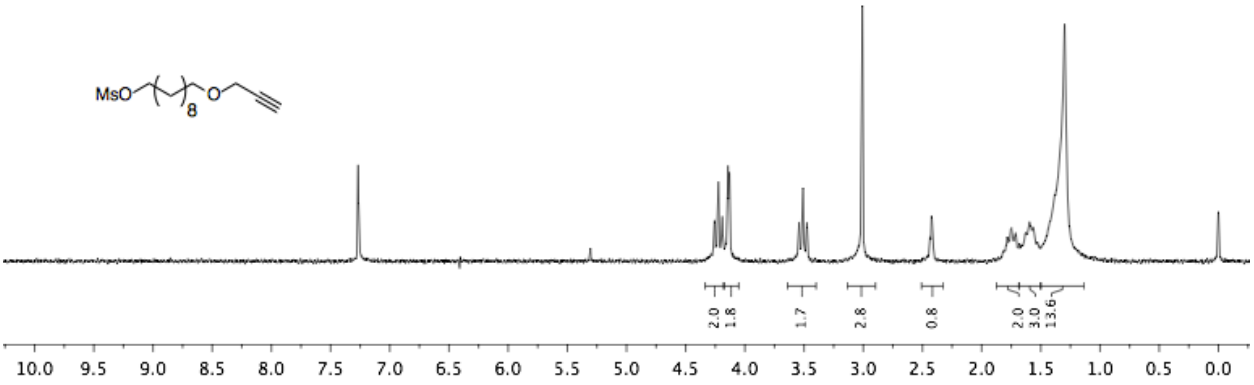
^{13}C NMR ($CDCl_3$, 200 MHz) δ 80.00, 74.01, 70.21, 70.15, 57.96, 37.30, 29.44, 29.34, 29.29, 29.06, 28.93, 26.00, 25.35.

HRMS calculated for $[C_{14}H_{26}O_8SNa]^+$, requires m/z = 313.1449, found m/z = 313.1442

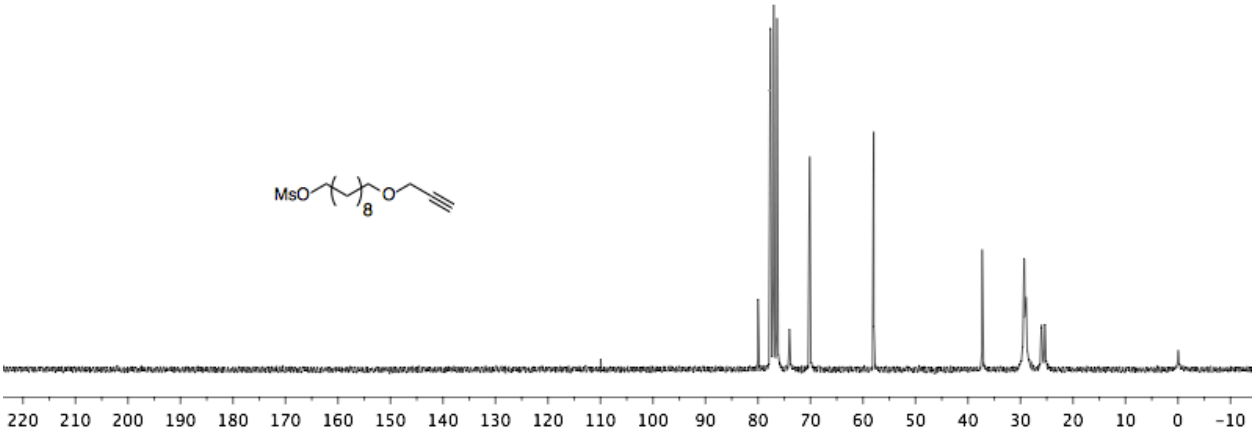
IR (ν, cm^{-1}) 3281, 2927, 2854, 1466, 1350, 1172, 1098, 930

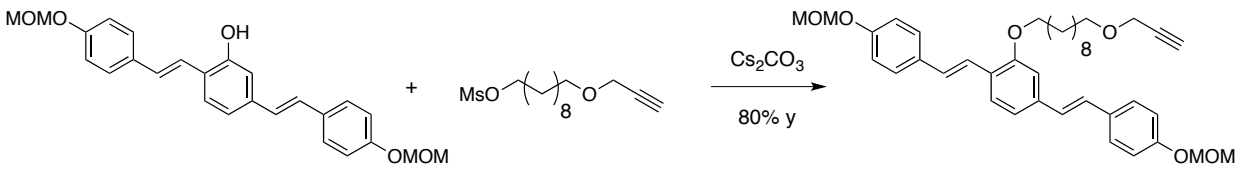
TLC (1:1) Hexane/EtOAc, permanganate, R_f = 0.60

^{1}H NMR Spectrum of 6



^{13}C NMR Spectrum of 6





5 **6** **7**

Synthesis of Ether 7: Into a flame-dried flask were added phenol **5** (54 mg, 0.13 mmol, 1 equiv), dry DMF (1.5 mL, 0.09 M), mesylate **6** (77 mg, 0.26 mmol, 2 equiv), and cesium carbonate (217 mg, 0.664 mmol, 5.2 equiv). The solution was maintained under a nitrogen atmosphere, heated in the dark (60 °C, 18 h), quenched with sodium bisulfate (1 M, 5 mL), extracted with EtOAc (15 + 2 x 10 mL), washed with water (4 x 10 mL) and then sodium chloride (50% saturated, 2 x 10 mL), and dried with sodium sulfate. Volatiles were removed under reduced pressure, and the crude material was purified by column chromatography (20 mL silica gel, DCM) to yield pure ether **7** (64 mg, 0.10 mmol, 80% yield, yellow solid). ^{1}H NMR signals were partially assigned by ^{1}H–^{1}H COSY.

^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.54 (d, J = 8.1 Hz, 1H, Ar–OC_{10}), 7.45 (d, J = 8.1 Hz, 4H, Ar–OMOM), 7.35 (d, J = 17.1 Hz, 1H, vinyl), 7.12 (d, J = 16.2 Hz, 1H, vinyl), 7.09 (d, J = 8.1 Hz, 1H, Ar–OC_{10}), 7.05 (d, J = 16.6 Hz, 1H, vinyl), 7.04 (d, J = 7.6 Hz, 2H, Ar–OMOM), 7.03 (d, J = 8.2 Hz, 2H, Ar–OMOM), 7.00 (s, 1H, Ar–OC_{10}), 6.96 (d, J = 16.0 Hz, 1H, vinyl), 5.20 (s, 4H, 2 x O–CH_2–O), 4.13 (d, J = 2.4 Hz, 2H, C≡C–CH_2O), 4.08 (t, J = 6.2 Hz, 2H, Ar–OCH_2), 3.50 (t, J = 7.1 Hz, 2H, CH_2–O), 3.49 (s, 6H, 2 x OMe), 2.40 (t, J = 2.4 Hz, 1H, C≡CH), 1.88 (pent, J = 7.1 Hz, 2H, Ar–O–C–CH_2), 1.58 (pent, J = 7.1 Hz, 2H, CH_2), 1.54 (pent, J = 7.1 Hz, 2H, CH_2), 1.41 (pent, J = 7.1 Hz, 2H, CH_2), 1.37–1.30 (m, 10H, 5 x CH_2).

^{13}C NMR ($CDCl_3$, 150 MHz) δ 156.90, 156.66, 156.58, 137.72, 132.18, 131.30, 128.10, 127.83, 127.66, 127.62, 127.05, 126.45, 126.06, 121.90, 119.05, 116.46, 116.41, 109.73, 94.43, 80.08, 73.98, 70.28, 68.51, 57.98, 56.01, 29.51, 29.39, 29.36, 26.24, 26.08.

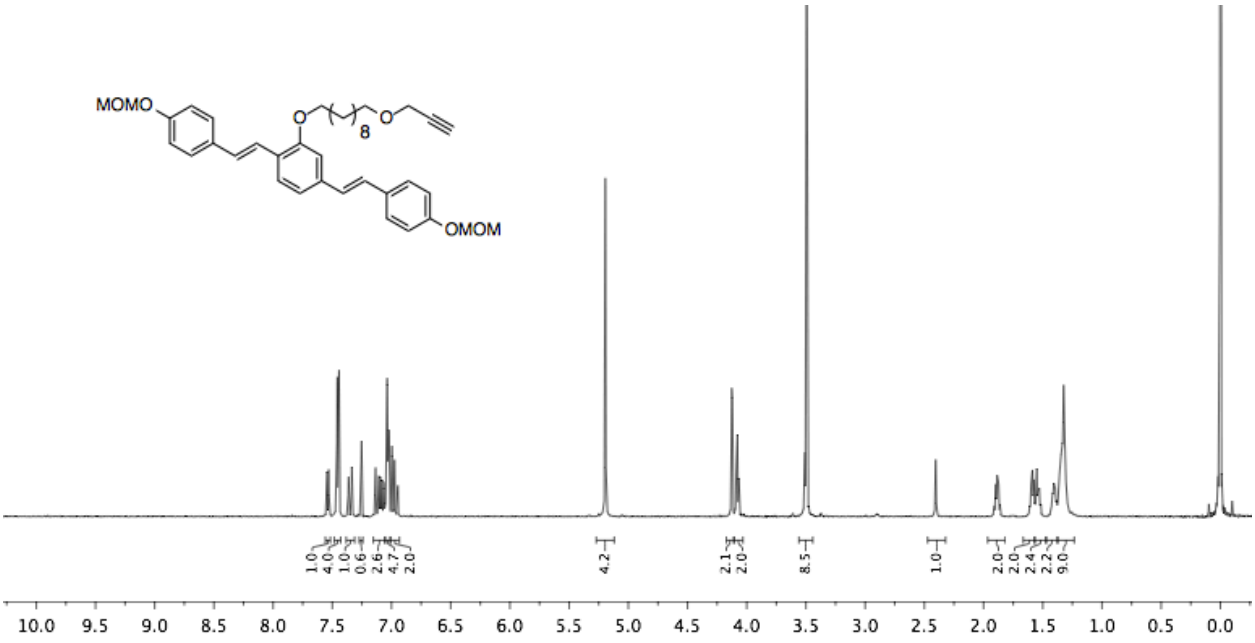
HRMS calculated for $[C_{39}H_{48}O_6Na]^+$, requires m/z = 635.3349, found m/z = 635.3363 (ESI).

TLC (2:1) Hexane:ethyl acetate, permanganate and UV (blue), R_f = 0.58.

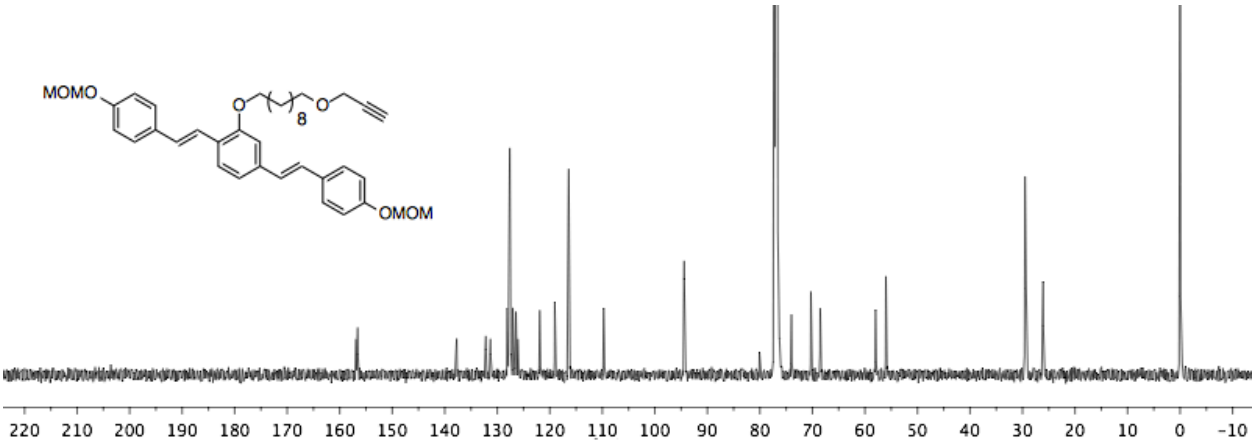
IR (ν, cm^{-1}) 2917, 2850, 1601, 1509, 1236, 1148, 1074, 999, 968, 825

MP 68–70 °C

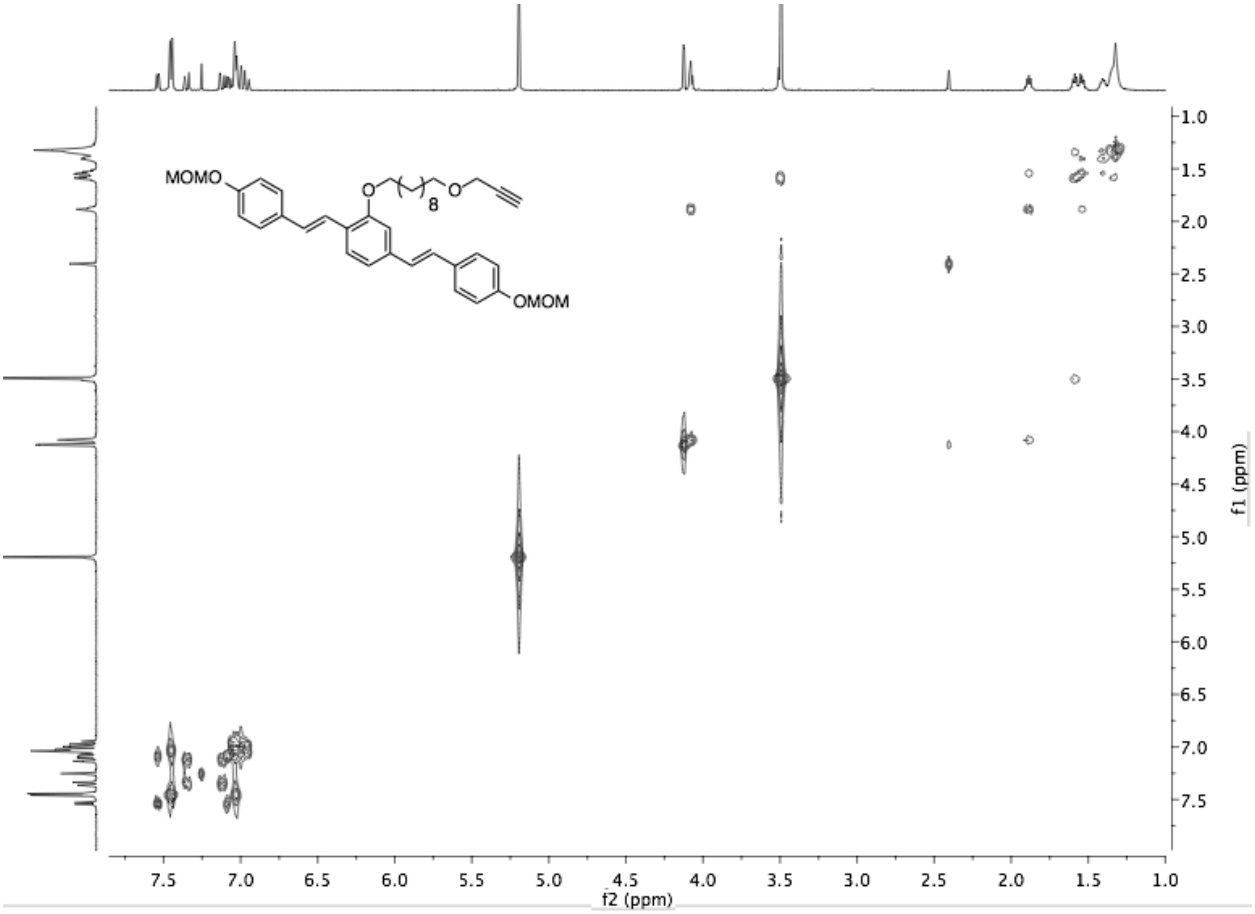
^{1}H NMR Spectrum of 7

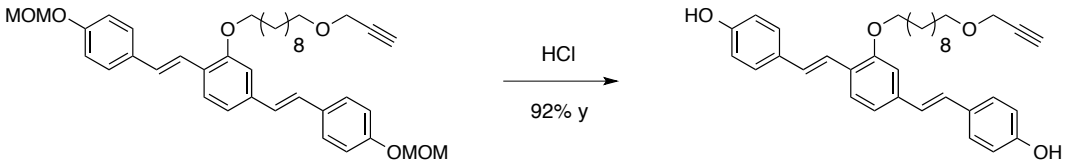


^{13}C NMR Spectrum of 7



^{1}H–^{1}H COSY NMR Spectrum of 7





7 **8**

Synthesis of Phenol 8: Into a flask were added ether **7** (23.5 mg, 0.0384 mmol, 1 equiv), methanol (1.5 mL, 0.03 M), and hydrochloric acid (4 M in dioxane, 1.3 mL, 5.2 mmol, 135 equiv). The solution was stirred (1 h), quenched with sodium hydroxide (1 M, 5 mL), diluted with potassium phosphate buffer (1 M, pH 7, 5 mL), extracted with EtOAc (3 x 10 mL), washed with a mixture (10 mL, 1:1) of potassium phosphate buffer (1 M, pH 5) and brine, and dried with sodium sulfate. Volatiles were removed under reduced pressure, and the crude mixture was purified by column chromatography (5 mL silica gel, 3:1 toluene/EtOAc) to yield pure phenol **8** (18.5 mg, 0.0353 mmol, 92% yield, yellow solid). ^{1}H NMR signals were partially assigned by ^{1}H–^{1}H COSY

^{1}H NMR (MeCN-d_3, 600 MHz) δ 7.54 (d, J = 8.1 Hz, 1H, Ar–OC$_{10}$), 7.42 (d, J = 7.9 Hz, 2H, Ar–OH), 7.40 (d, J = 7.4 Hz, 2H, Ar–OH), 7.29 (d, J = 15.8 Hz, 1H, vinyl), 7.16 (d, J = 16.8 Hz, 1H, vinyl), 7.15 (d, J = 16.4 Hz, 1H, vinyl), 7.13 (s, 1H, OH), 7.12 (d, J = 1.0 Hz, 1H, Ar–OC$_{10}$), 7.10 (dd, J_1 = 7.8 Hz, J_2 = 1.0 Hz, 1H, Ar–OC$_{10}$), 7.09 (s, 1H, OH), 6.99 (d, J = 16.4 Hz, 1H, vinyl), 6.82 (d, J = 8.7 Hz, 2H, Ar–OH), 6.80 (d, J = 9.0 Hz, 2H, Ar–OH), 4.10 (t, J = 6.8 Hz, 2H, Ar–OCH$_2$), 4.07 (d, J = 2.1 Hz, 2H, C≡C–CH$_2$O), 3.43 (t, J = 7.3 Hz, 2H, CH$_2$–O), 2.65 (t, J = 2.3 Hz, 1H, C≡CH), 1.85 (pent, J = 6.8 Hz, 2H, Ar–O–C–CH$_2$), 1.58–1.47 (m, 4H, 2 x CH$_2$), 1.41 (pent, J = 7.5 Hz, 2H, CH$_2$), 1.37–1.25 (m, 8H, 4 x CH$_2$).

^{13}C NMR (MeCN-d_3, 150 MHz) δ 157.92, 157.72, 157.62, 139.17, 131.16, 130.5, 129.45, 129.27, 128.94, 128.77, 127.46, 126.82, 126.77, 121.67, 120.07, 116.64, 116.60, 110.85, 81.39, 75.32, 70.83, 69.50, 58.47, 30.38, 30.30, 30.19, 30.12, 27.10, 26.92.

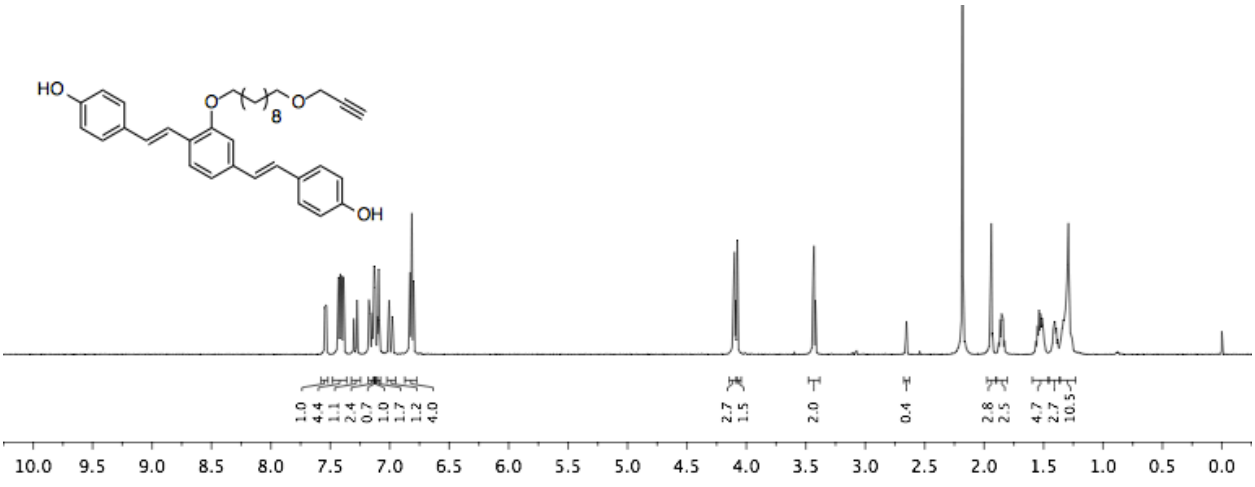
HRMS calculated for $[C_{35}H_{39}O_4]^-$, requires m/z = 523.2848, found m/z = 523.2848 (ESI negative mode).

TLC (3:1) Toluene/EtOAc, permanganate and UV (blue), R_f = 0.43.

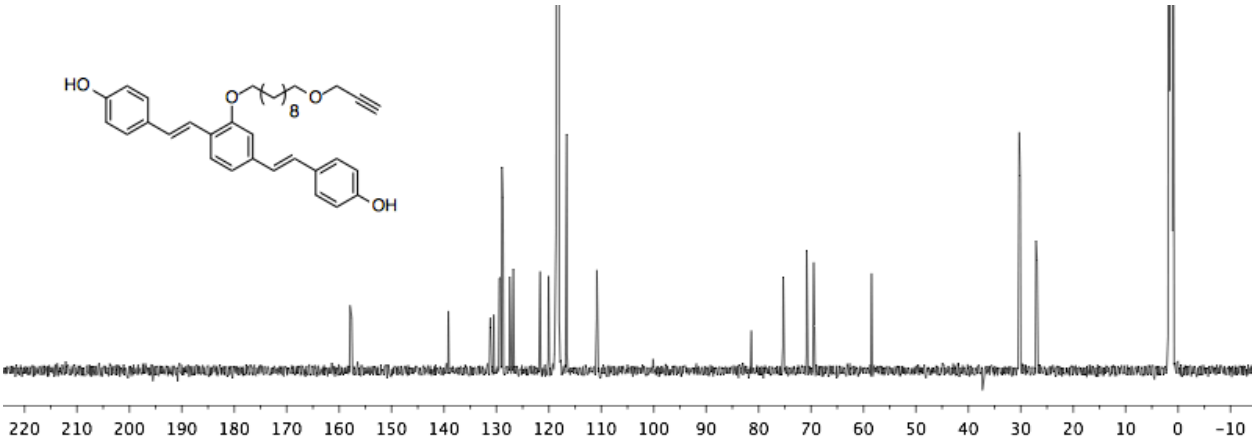
IR (ν, cm^{-1}) 3285, 2917, 2850, 1592, 1510, 1449, 1238, 1171, 1101, 962, 824

MP 157–160 °C

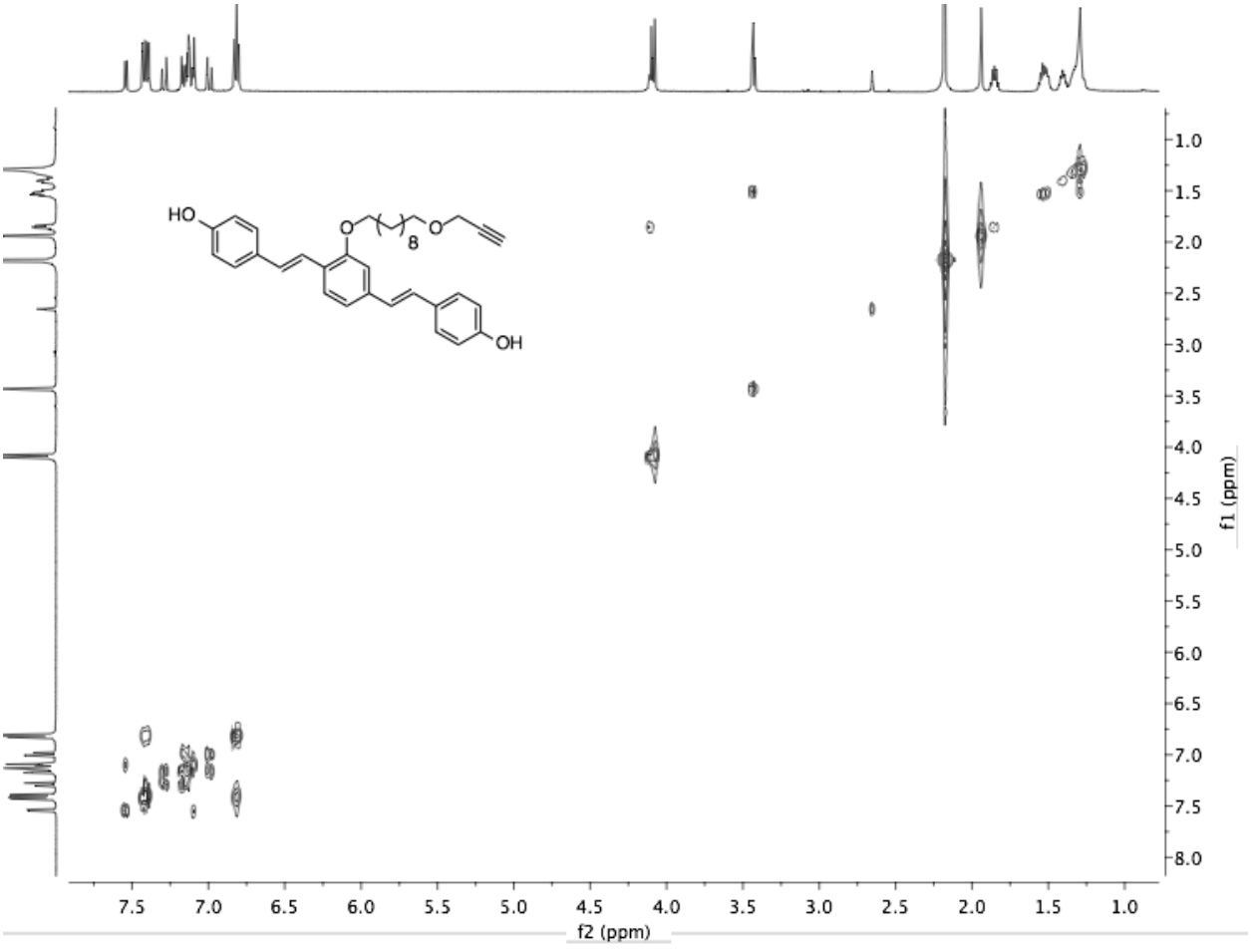
^{1}H NMR Spectrum of 8 (MeCN-d_3)

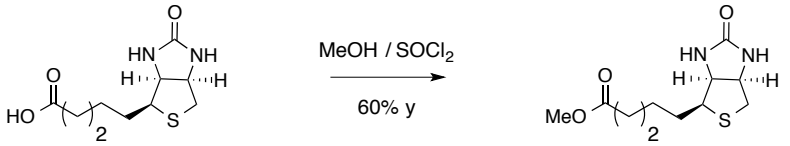


^{13}C NMR Spectrum of 8 (MeCN-d_3)



^{1}H–^{1}H COSY NMR Spectrum of 8 (MeCN-d_3)



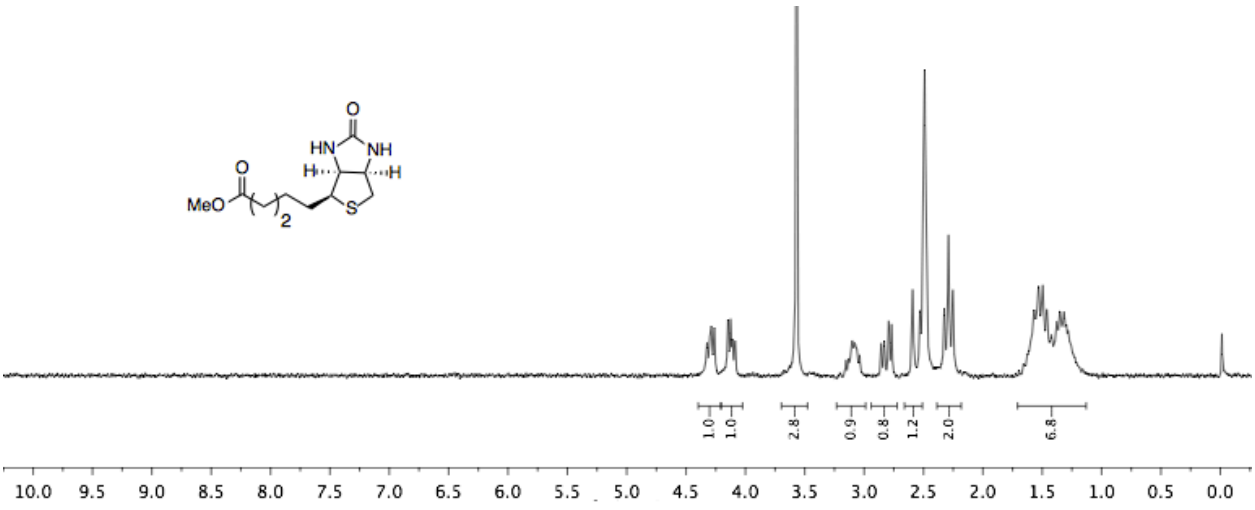


9A **9B**

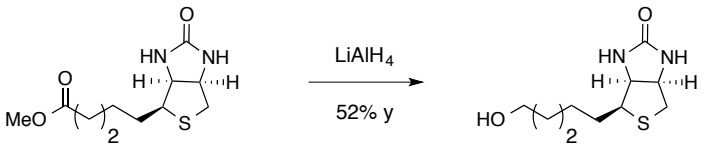
Synthesis of Ester 9B: Methanol was dried over 3 Å molecular sieves for 17 h. Into a flame-dried flask were added (*D*)-biotin (**9A**, 1.90 g, 7.77 mmol, 1 equiv) and dry methanol (15 mL, 0.5 M). The flask was fitted with a reflux condenser and rubber septum and vented through a Drierite-packed syringe. Thionyl chloride (1.70 mL, 23.5 mmol, 3 equiv) was added. The mixture was heated to reflux and stirred (2 h). The reflux condenser was exchanged for a distillation head, and volatiles were removed by distillation and chased with methanol (10 mL). Approximately 1/3 of the material was accidentally spilled. Remaining volatiles were removed under reduced pressure to yield methyl ester **9B** (1.21 g, 4.69 mmol, 60% yield, white solid). Compound analysis is consistent with previously reported data.[8]

^{1}H NMR (DMSO-d_6, 200 MHz) δ 4.29 (dd, J_1 = 7.8 Hz, J_2 = 5.8 Hz, 1H, CH–N), 4.12 (dd, J_1 = 7.8 Hz, J_2 = 4.6 Hz, 1H, CH–N), 3.57 (s, 3H, OMe), 3.17–3.01 (m, 1H, CH–S), 2.81 (dd, J_1 = 12.3 Hz, J_2 = 5.0 Hz, 1H, CH_2–S), 2.56 (d, J = 12.8 Hz, 1H, CH_2–S), 2.29 (t, J = 7.2 Hz, 2H, CH_2–C=O), 1.68–1.22 (m, 8H, 4 x CH_2).

^{1}H NMR Spectrum of 9B (DMSO-d_6)



[8] T. P. Soares da Costa, W. Tieu, M. Y. Yap, N. R. Pendini, S. W. Polyak, D. S. Pedersen, R. Morona, J. D. Turnidge, J. C. Wallace, M. C. J. Wilce, G. W. Booker, A. D. Abell, *J. Biol. Chem.*, 2012, **287**, 17823.



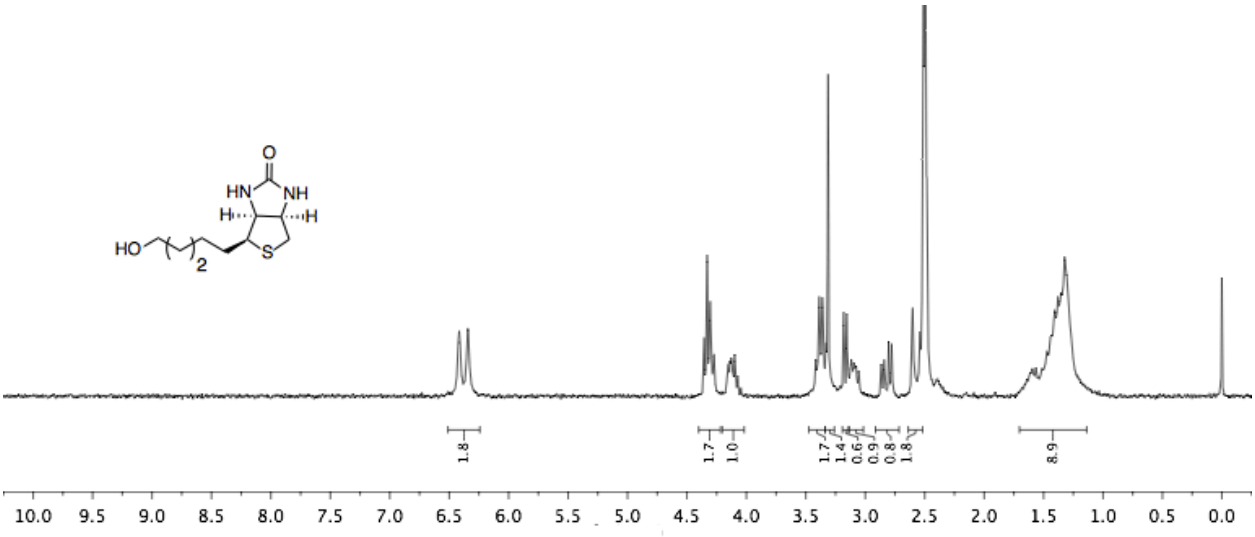
9B **9C**

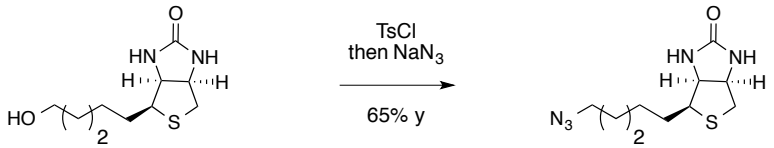
Synthesis of Alcohol 9C: Into a flame-dried flask was added lithium aluminum hydride (573 mg, 15.1 mmol, 3.8 equiv) and THF (30 mL). The flask was maintained under a nitrogen atmosphere and cooled in an ice bath. Ester **9B** (1.02 g, 3.95 mmol, 1 equiv) was added gradually (first as a solid and then chased with THF, although it is not especially soluble in that solvent). The mixture was allowed to return to ambient temperature, stirred (6 h), cooled in an ice bath, quenched by dropwise addition of a mixture (1:1, 8 mL) of water and methanol, stirred (15 min), diluted with potassium sodium tartrate (saturated, 25 mL) and EtOAc (50 mL), and stirred (15 h). The organic phase was isolated, the aqueous phase was further extracted with EtOAc (7 x 50 mL), and the combined organic phase was washed with sodium chloride (50% saturated, 50 mL) and dried with sodium sulfate. Volatiles were removed under reduced pressure, and the crude material was purified by column chromatography (50 mL silica gel, [3–15]% MeOH in DCM) to yield alcohol **9C** (468 mg, 2.03 mmol, 52% yield, white powder) plus trace MeOH. Compound analysis is consistent with previously reported data.[8]

^{1}H NMR (DMSO-d_6, 200 MHz) δ 6.41 (s, 1H, NH), 6.35 (s, 1H, NH), 4.38–4.24 (m, 1H, CH–N), 4.18–4.05 (m, 1H, CH–N), 3.37 (q, J = 5.4 Hz, 2H, CH_2–O), 3.13–3.04 (m, 1H, CH–S), 2.82 (dd, J_1 = 12.1 Hz, J_2 = 4.7 Hz, 1H, CH_2–S), 2.57 (d, J = 13.2 Hz, 1H, CH_2–S), 1.71–1.14 (m, 8H, 4 x CH_2).

TLC (15% Methanol in DCM), permanganate, R_f = 0.48.

^{1}H NMR Spectrum of 9C (DMSO-d_6)





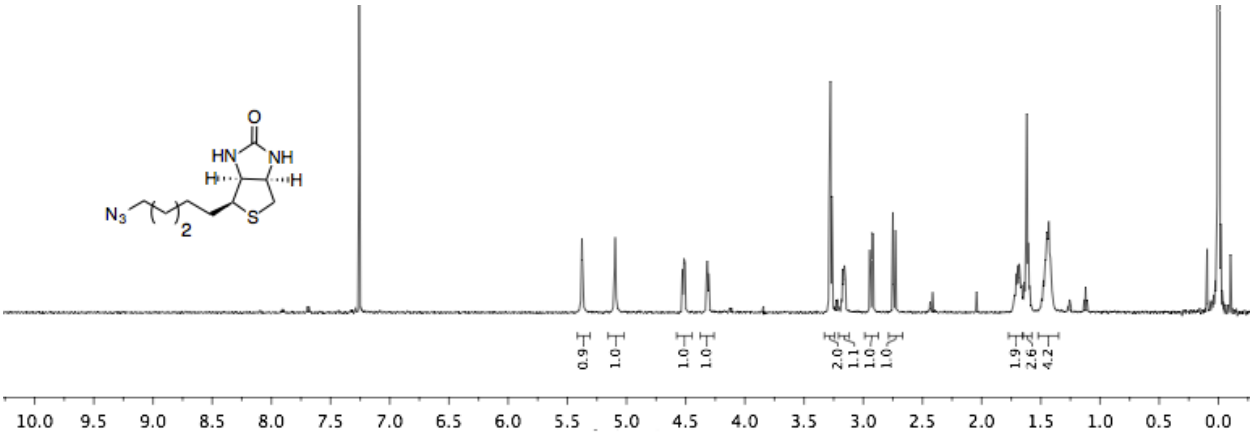
9C **9**

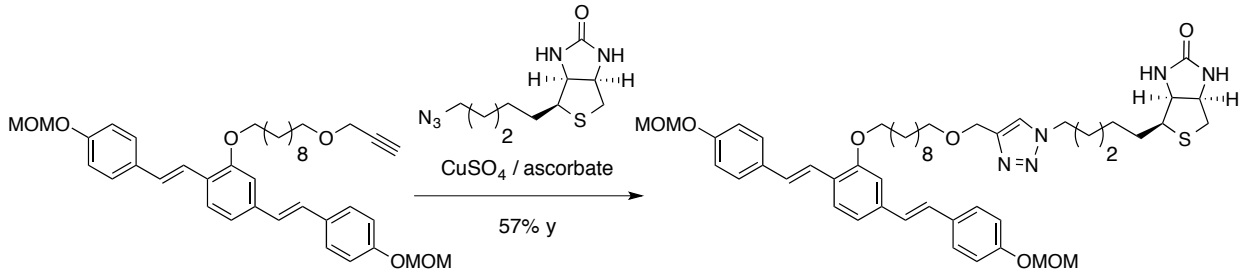
Synthesis of Azide 9: Into a flame-dried flask were added alcohol **9C** (424 mg, 1.84 mmol, 1.0 equiv), dry DCM (18 mL, 0.1 M), triethylamine (0.640 mL, 4.60 mmol, 2.5 equiv), and DMAP (46.5 mg, 0.381 mmol, 0.2 equiv). The solution was maintained under a nitrogen atmosphere and cooled in an ice bath. *p*-toluenesulfonyl chloride (534 mg, 0.381 mmol, 1.5 equiv) was added. The mixture was allowed to warm to ambient temperature, stirred (21 h), quenched with sodium bicarbonate (saturated, 15 mL), stirred (5 min), extracted with EtOAc (2 x 30 mL), and washed sequentially with a mixture (1:1, 2 x 30 mL) of saturated sodium bicarbonate and brine, then a mixture (1:1, 2 x 30 mL) of sodium bisulfate (10% aqueous) and brine, and finally a mixture (1:1, 30 mL) of water and brine. The mixture was dried with sodium sulfate, and volatiles were removed under reduced pressure to yield an orange powder.

Into a flame-dried flask were added most of the orange powder (94%, 1.73 mmol expected), sodium azide (388 mg, 5.97 mmol, 3.5 equiv), and DMSO (16 mL, 0.1 M). The mixture was maintained under a nitrogen atmosphere, heated (90 °C, behind a blast shield, 6 h), allowed to cool to ambient temperature, diluted with sodium bicarbonate (saturated, 10 mL), extracted with EtOAc (2 x 30 mL), washed with sodium chloride (50% saturated, 2 x 20 mL), and dried with sodium sulfate. Volatiles were removed under reduced pressure to yield mostly-pure azide **9** (303 mg, 1.19 mmol, 65% yield from alcohol **9C**, orange solid). Compound analysis is consistent with previously reported data.[8]

^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.38 (s, 1H, NH), 5.09 (s, 1H, NH), 4.52 (dd, J_1 = 6.9 Hz, J_2 = 5.8 Hz, 1H, CH–N), 4.32 (dd, J_1 = 7.5 Hz, J_2 = 5.3 Hz, 1H, CH–N), 3.28 (t, J = 6.9 Hz, 2H, CH_2–N_3), 3.19–3.14 (m, 1H, CH–S), 2.93 (dd, J_1 = 13.3 Hz, J_2 = 5.8 Hz, 1H, CH_2–S), 2.74 (d, J = 13.4 Hz, 1H, CH_2–S), 1.75–1.55 (m, 4H, 2 x CH_2), 1.50–1.39 (m, 4H, 2 x CH_2).

^{1}H NMR Spectrum of 9





7 **9** **10A**

Synthesis of Triazole 10A: Into a flask were added alkyne **7** (53.6 mg, 0.0875 mmol, 1 equiv), azide **9** (21.5 mg, 0.0843 mmol, 0.96 equiv), and THF (1.6 mL, 0.06 M). In a separate flask were combined copper sulfate pentahydrate (0.1 M aqueous, 0.8 mL, 0.08 mmol, 0.9 equiv, blue) and sodium ascorbate (0.1 M aqueous, 1.6 mL, 0.16 mmol, 1.8 equiv) to produce an orange mixture, which was transferred to the alkyne-containing flask. The mixture was stirred vigorously (18 h, gradually becoming a bluish-yellow color and producing a yellow–green precipitate), diluted with water (3 mL) and ammonium hydroxide (concentrated aqueous, 3 mL), extracted with EtOAc (2 x 5 mL), washed with sodium chloride (50% saturated, 2 x 10 mL), and dried with sodium sulfate. Volatiles were removed under reduced pressure, and the crude material was purified by column chromatography (7 mL silica gel, [2–4]% methanol in DCM) to yield pure triazole **10A** (43.5 mg, 0.0501 mmol, 57% yield, yellow–green crystalline solid).

^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.54 (d, J = 7.8 Hz, 1H, Ar–OC_{10}), 7.53 (s, 1H, triazole), 7.45 (d, J = 8.2 Hz, 4H, Ar–OMOM), 7.34 (d, J = 16.1 Hz, 1H, vinyl), 7.12 (d, J = 16.6 Hz, 1H, vinyl), 7.09 (d, J = 8.2 Hz, 1H, Ar–OC_{10}), 7.05 (d, J = 15.8 Hz, 1H, vinyl), 7.03 (d, J = 8.3 Hz, 2H, Ar–OMOM), 7.02 (d, J = 8.8 Hz, 2H, Ar–OMOM), 7.00 (s, 1H, Ar–OC_{10}), 6.96 (d, J = 16.6 Hz, 1H, vinyl), 5.53 (s, 1H, NH), 5.20 (s, 2H, O–CH_2–O), 5.19 (s, 2H, O–CH_2–O), 4.94 (s, 1H, NH), 4.61 (s, 2H, O–CH_2–triazole), 4.49–4.46 (m, 1H, CH–N), 4.32 (t, J = 7.5 Hz, 2H, CH_2–triazole), 4.29–4.25 (m, 1H, CH–N), 4.08 (t, J = 6.4 Hz, 2H, Ar–OCH_2), 3.51 (t, J = 6.5 Hz, 2H, CH_2–O), 3.50 (s, 3H, OMe), 3.49 (s, 3H, OMe), 3.13–3.08 (m, 1H, CH–S), 2.89 (dd, J_1 = 12.9 Hz, J_2 = 4.6 Hz, 1H, CH_2–S), 2.70 (d, J = 13.0 Hz, 1H, CH_2–S), 1.94–1.84 (m, 4H, 2 x CH_2), 1.73–1.27 (m, 20H, 10 x CH_2).

^{13}C NMR ($CDCl_3$, 150 MHz) δ 163.16, 156.90, 156.66, 156.58, 145.55, 137.73, 132.16, 131.28, 128.09, 127.85, 127.66, 127.61, 127.02, 126.45, 126.04, 122.22, 121.89, 119.05, 116.47, 116.41, 109.73, 94.42, 70.93, 68.52, 64.38, 61.96, 60.04, 56.05, 55.40, 50.11, 40.48, 29.91, 29.64, 29.56, 29.53, 29.46, 29.39, 29.35, 28.45, 28.35, 26.38, 26.24, 26.12.

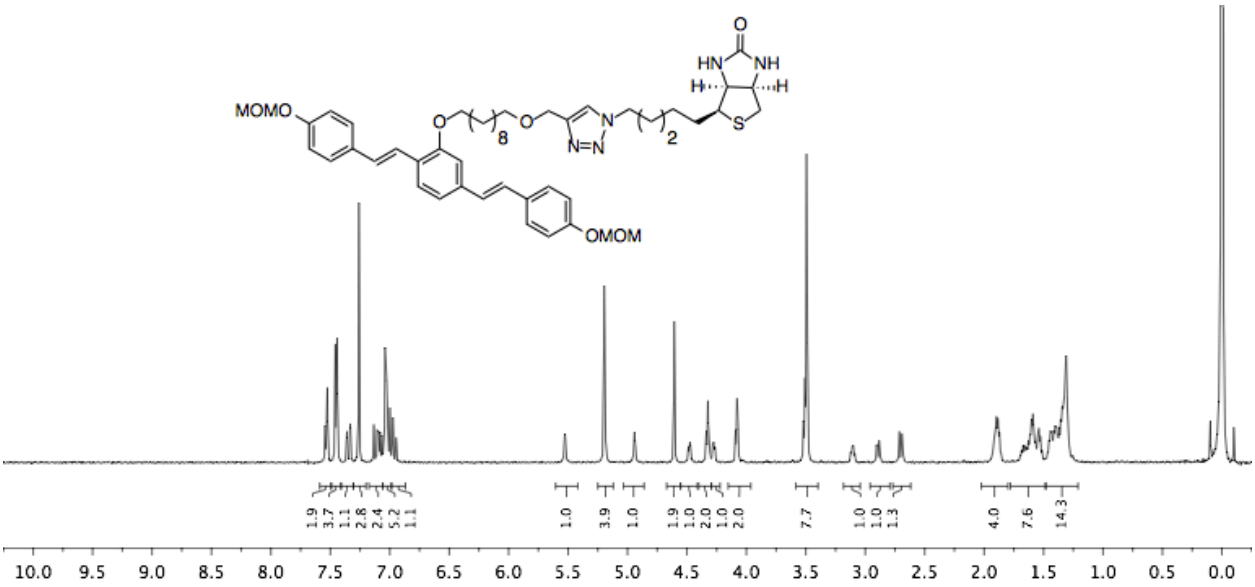
HRMS calculated for $[C_{49}H_{65}N_5O_7SH]^+$, requires m/z = 868.4683, found m/z = 868.4670 (ESI).

TLC (10% Methanol in DCM), permanganate and UV (blue), R_f = 0.44.

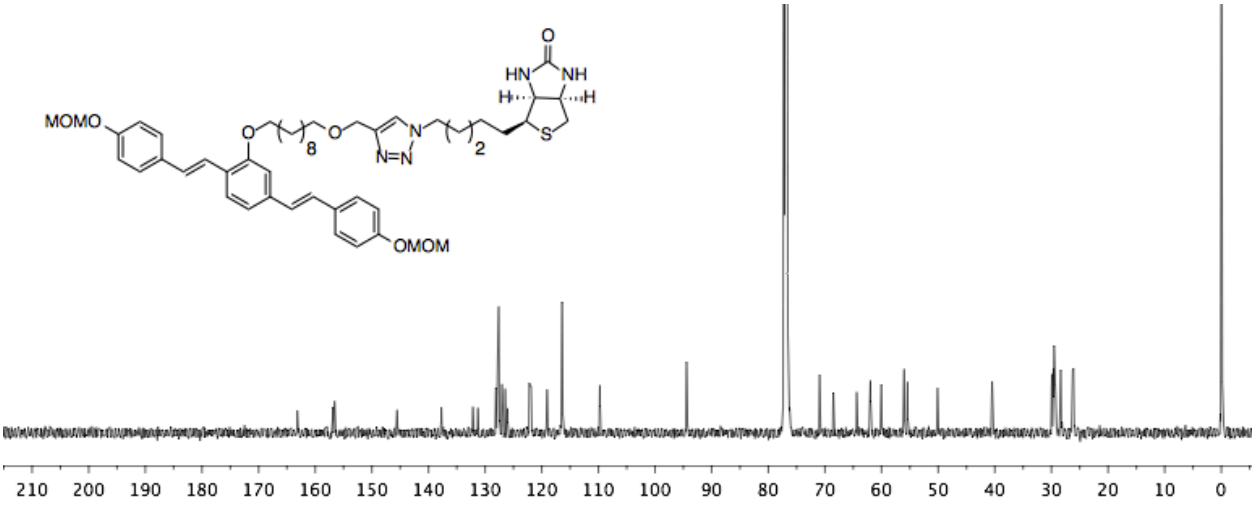
IR (ν, cm^{-1}) 3208, 2923, 2851, 1698, 1602, 1510, 1235, 1150, 1057, 993, 827

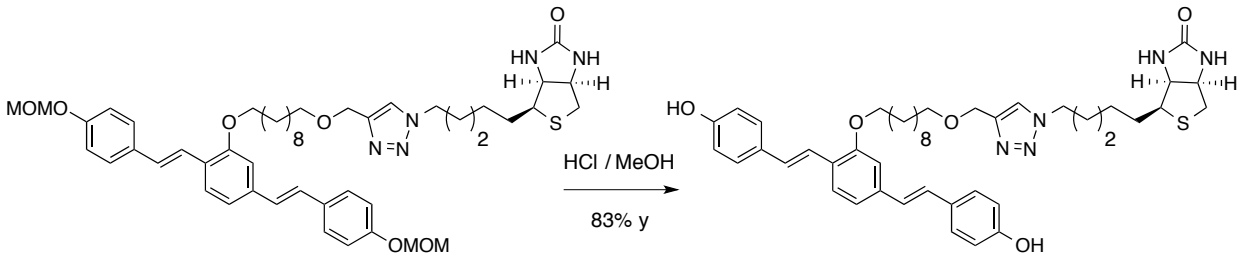
MP 103–105 °C

^{1}H NMR Spectrum of 10A



^{13}C NMR Spectrum of 10A





10A **10**

Synthesis of Phenol 10: Into a flask were added ether **10A** (34.9 mg, 0.0402 mmol, 1 equiv), methanol (2 mL, 0.02 M), and hydrochloric acid (4 M in dioxane, 1 mL, 4 mmol, 100 equiv). The solution was stirred (1.5 h, dark, becoming a yellow solution), quenched potassium phosphate bufer (1 M, pH 5, 5 mL), diluted with potassium phosphate buffer (1 M, pH 5, 10 mL), extracted with EtOAc (3 x 15 mL), washed with sodium chloride (50% saturated, 20 mL), and dried with sodium sulfate. Volatiles were removed under reduced pressure, and the crude mixture was purified by column chromatography (7 mL silica gel, [4–7]% MeOH in DCM) to yield pure phenol **10** (26.1 mg, 0.0335 mmol, 83% yield, yellow–green solid).

^{1}H NMR ([1:9] CD_3OD / $CDCl_3$, 600 MHz) δ 7.57 (s, 1H, triazole), 7.52 (d, J = 8.0 Hz, 1H, Ar–OC_{10}), 7.38 (d, J = 8.1 Hz, 4H, Ar–OH), 7.30 (d, J = 16.8 Hz, 1H, vinyl), 7.10 (d, J = 16.8 Hz, 1H, vinyl), 7.08 (d, J = 8.2 Hz, 1H, Ar–OC_{10}), 7.03 (d, J = 16.2 Hz, 1H, vinyl), 7.00 (s, 1H, Ar–OC_{10}), 6.91 (d, J = 16.3 Hz, 1H, vinyl), 6.83 (d, J = 9.1 Hz, 4H, Ar–OH), 4.59 (s, 2H, O–CH_2–triazole), 4.47 (dd, J_1 = 7.6 Hz, J_2 = 4.9 Hz, 1H, CH–N), 4.33 (t, J = 7.0 Hz, 2H, CH_2–triazole), 4.26 (dd, 1H, J_1 = 8.1 Hz, J_2 = 4.9 Hz, CH–N), 4.09 (t, J = 6.2 Hz, 2H, Ar–OCH_2), 3.51 (t, J = 7.0 Hz, 2H, CH_2–O), 3.14–3.09 (m, 1H, CH–S), 2.89 (dd, J_1 = 13.0 Hz, J_2 = 4.8 Hz, 1H, CH_2–S), 2.72 (d, J = 13.6 Hz, 1H, CH_2–S), 1.94–1.84 (m, 4H, 2 x CH_2), 1.70–1.24 (m, 20H, 10 x CH_2).

^{13}C NMR ([1:9] CD_3OD / $CDCl_3$, 150 MHz) δ 163.77, 156.54, 156.32, 145.09, 137.61, 129.89, 129.06, 128.27, 128.02, 127.67, 127.59, 126.15, 125.93, 125.71, 122.55, 120.53, 118.86, 115.48, 115.44, 109.56, 70.84, 68.45, 63.83, 61.90, 59.95, 55.35, 50.10, 40.18, 29.78, 29.52, 29.36, 29.32, 29.24, 29.16, 28.30, 28.21, 26.13, 25.85.

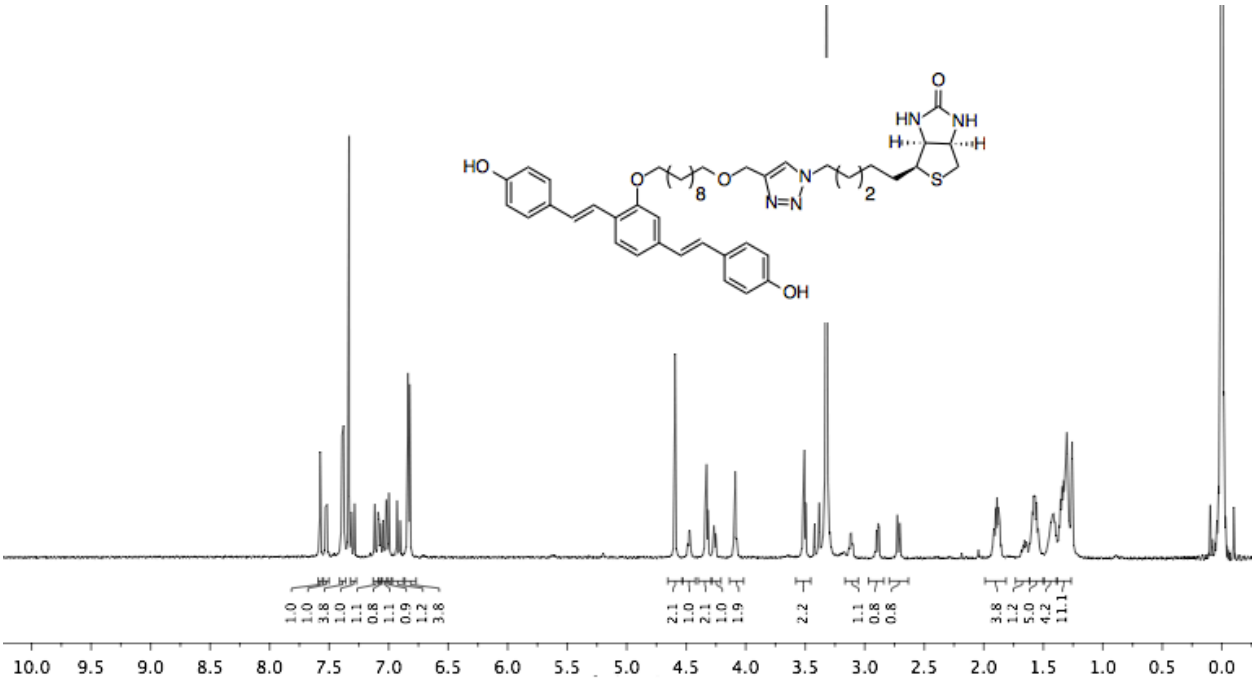
HRMS calculated for $[C_{45}H_{57}N_5O_5SH]^+$, requires m/z = 780.4159, found m/z = 780.4160 (ESI).

TLC (1:9) MeOH/DCM, permanganate and UV (blue), R_f = 0.41.

IR (ν, cm^{-1}) 3225, 2923, 2853, 1682, 1605, 1585, 1514, 1446, 1237, 1168, 826.

MP 65–70 °C (decomposition)

^{1}H NMR Spectrum of 10



^{13}C NMR Spectrum of 10