Supporting Information

Improved Templated Fluorogenic Probes Enhance the Analysis of Closely Related Pathogenic Bacteria by Microscopy and Flow Cytometry

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Table of contents

General Information

Chemicals and reagents

Anhydrous solvents were purchased from Fisher Scientific and used without further purification. Chemicals were purchased from either Sigma-Aldrich or Acros and used without further purification. Chemicals used for the solid-phase synthesis of oligonucleotides such as

phosphoramidites, solid-supports, amino-modifiers, and synthesizer reagent-solutions were acquired from Glen Research. Bioreagents were purchased from VWR, and bacteria cultures from ATCC.

Instrumentation

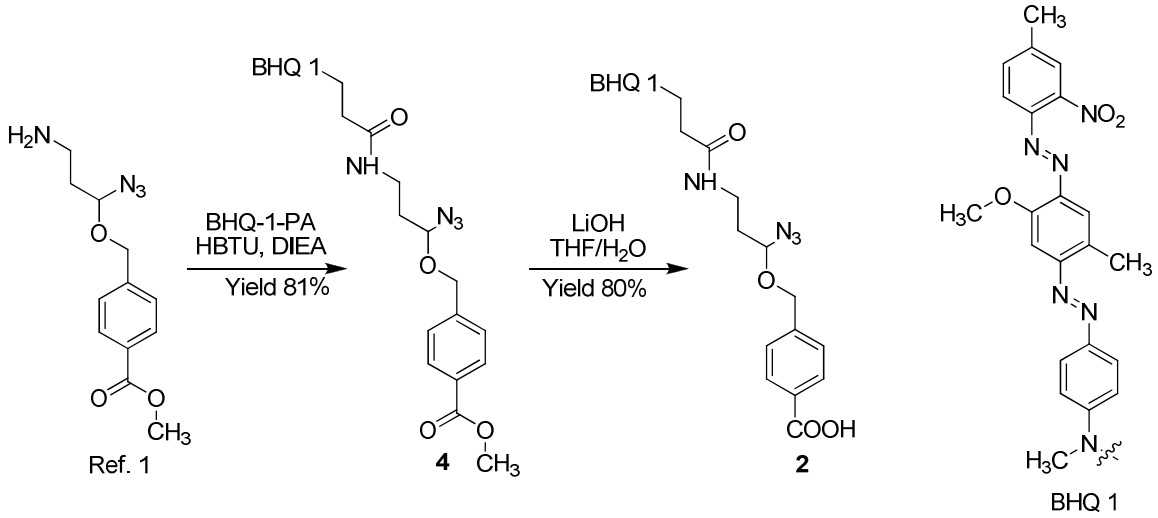
All ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Innova 500 MHz NMR spectrometer. ^{1}H- and ^{13}C-NMR Spectra were internally referenced to the residual solvent signal. High-resolution mass spectrometry analysis was performed by the UC Riverside Mass Spectrometry Facility. Analytical and semi-preparative high performance liquid chromatography was performed on a LC-CAD Shimadzu liquid chromatograph, equipped with a SPD-M10A VD diode array detector and a SCL 10A VP system controller and using reverse phase C18 columns. Fluorescence measurements were performed on a Fluorolog 3 Jobin Yvon fluorophotospectrometer equipped with an external temperature controller. Oligonucleotide masses were determined by the Stanford University Protein and Nucleic Acid Facility using a Perspective Voyager-DE RP Biospectrometry MALDI-TOF mass-spectrometry instrument using a 3-Hydroxypicolinic acid/di-ammonium hydrogen citrate matrix. Bacterial imaging was performed on a Nikon Eclipse E800 epifluorescence microscope equipped with a Nikon Plan AP 100x/1.40 oil immersion objective and a SPOT RT digital camera.

Bacteria culture

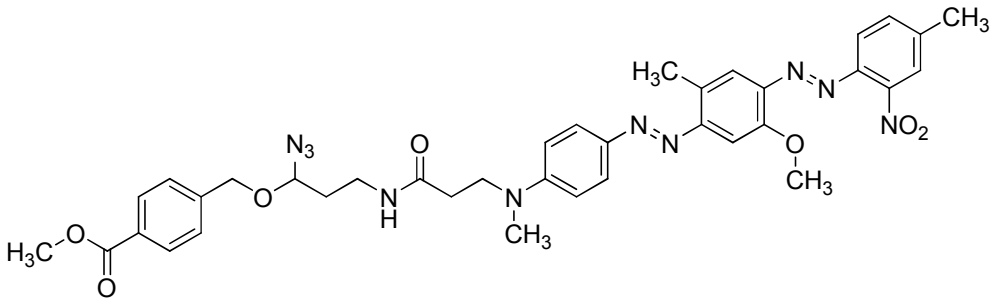
Growth medium (Lauria-Bertain), and glass containers were sterilized by autoclaving at 120˚C for 20 min. *E. coli K12* and *Salmonella enterica* were acquired from ATCC (catalog numbers 10798 and 700720 for *E.coli* and *Salmonella enteric,* respectively). Bacteria cultures were grown to mid-log phase in Luria-Bertani medium at 37˚C.

Synthetic procedures

Scheme S1. Synthesis of the α-azidoether BHQ-1 release linker **2**.

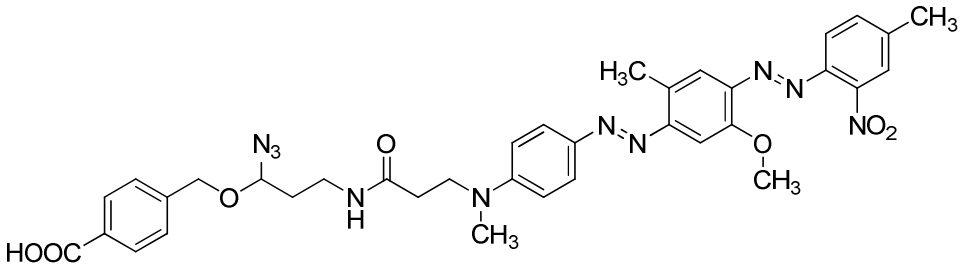


Methyl 4-[(1-azido-3-(3-(BHQ-1)-propionamido)propoxy)methyl]benzoate (**4**)



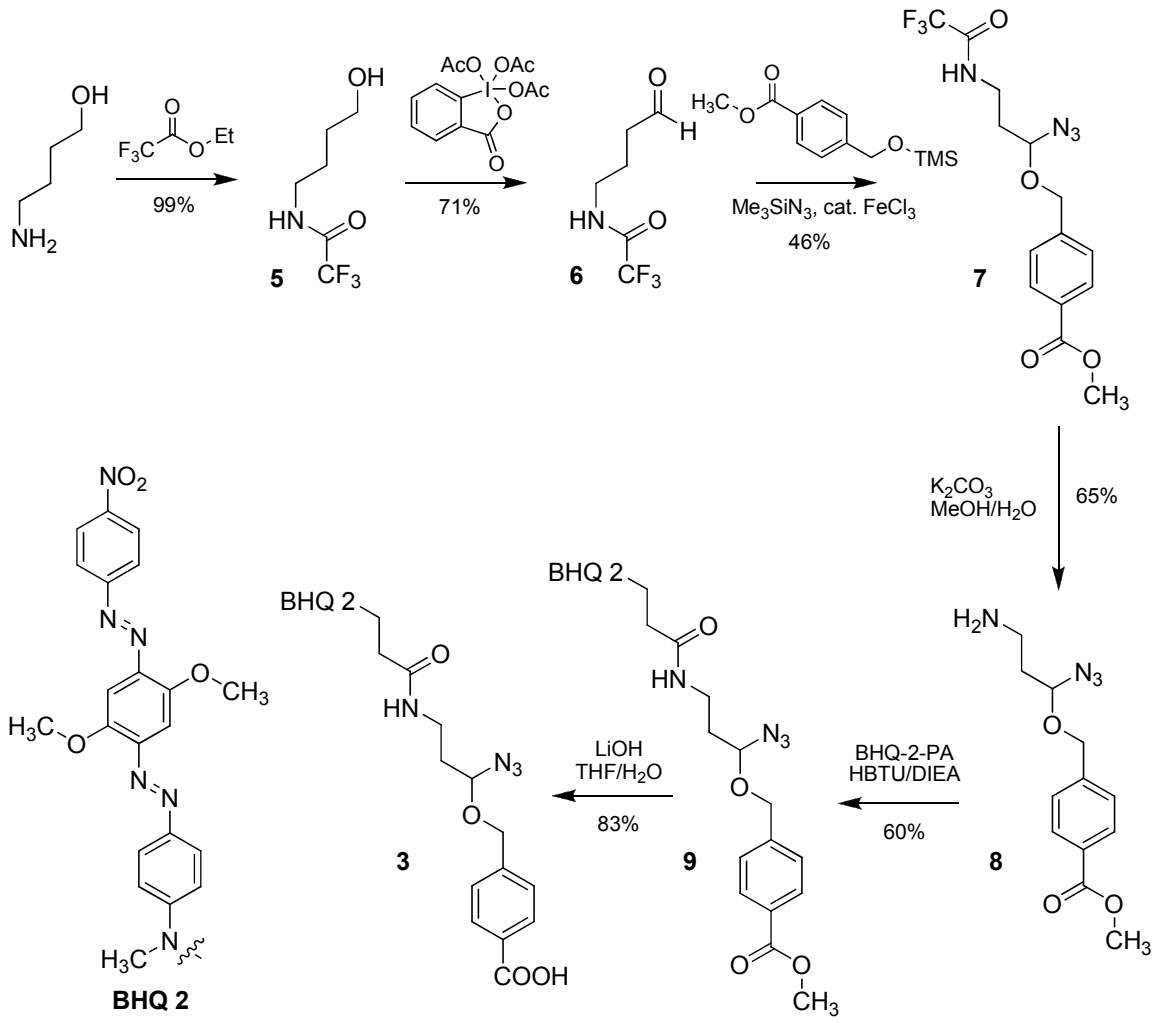
A solution of BHQ-1-propionic acid (220 mg; 0.46 mmol), HBTU (175 mg; 0.46 mmol) and DIEA (60 mg; 0.46 mmol) in DMF (5 mL) was kept at room temperature for 5 min and then added to a solution of methyl 4-(3-amino-1-azidopropoxymethyl)benzoate[1] (98 mg; 0.37 mmol) in DMF (1 mL). The solution was kept at room temperature for 30 min and volatiles evaporated. The residue was dissolved in DCM, washed with brine, dried over $MgSO_4$, filtrated and evaporated. The residue was purified by column chromatography (DCM: MeCN 9:1 + 2% MeOH) to give the product as a black solid in a yield of 220 mg (81%).
^{1}H-NMR (CDCl3, 500MHZ): 1.90-2.00 ppm (m, 2H), 2.38 ppm (t, J^3(H,H) = 6.75 Hz, 2H), 2.48 ppm (s, 3H), 2.68 ppm (s, 3H), 3.02 ppm (s, 3H), 3.26-3.36 ppm (m, 1H), 3.36-3.44 ppm (m, 1H), 3.75 ppm (t, J^3(H,H) = 6.75 Hz, 2H), 3.88 ppm (s, 3H), 3.99 ppm (s, 3H), 4.51 ppm (dd, J^3(H,H) = 5.75 Hz, 1H), 4.55 ppm (d, J^3(H,H) = 12.0 Hz, 1H), 4.82 ppm (d, J^3(H,H) = 12.0 Hz, 1H), 6.01 ppm (dd, J^3(H,H) = 5.75 Hz, 1H), 6.71 ppm (d, J^3(H,H) = 9.5 Hz, 2H), 7.37 ppm (d, J^3(H,H) = 8.0 Hz, 2H), 7.37 ppm (s, 1H), 7.44 ppm (d, J^3(H,H) = 8.5 Hz, 1H), 7.55 ppm (s, 1H), 7.63 ppm (d, J^3(H,H) = 8.5 Hz, 1H), 7.67 ppm (s, 1H), 7.88 ppm (d, J^3(H,H) = 9.0 Hz, 2H), 7.99 ppm (d, J^3(H,H) = 8.0 Hz, 2H). ^{13}C-NMR ($CDCl_3$, 500 MHz): 16.75, 21.30, 34.10, 34.15, 35.55, 38.94, 48.86, 52.22, 56.28, 70.33, 90.45, 99.34, 111.52, 118.95, 119.07, 124.32, 125.84, 127.61, 129.90, 132.99, 133.55, 141.66, 141.83, 143.40, 144.71, 145.15, 147.44, 151.08, 151.17, 154.81, 166.72, 170.93 ppm.

4-[(1-azido-3-(3-(BHQ-1)-propionamido)propoxy)methyl]benzoic acid (**2**)

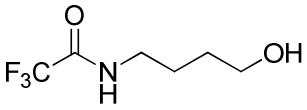


To a solution of **4** (180 mg; 0.24 mmol) in THF (3 mL) was added a solution of lithium hydroxide monohydrate (180 mg; 4.3 mmol) in water (1 mL) was added and the mixture stirred vigorously at 40˚C for 6 h. The mixture was diluted with water, acidified with little acetic acid and extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtrated and volatiles evaporated. The resiude was dissolved in little DCM and precipitation with hexanes provided the product as a fine black powder in a yield of 142 mg (80%).
^{1}H-NMR (DMSO, 500MHz): 1.76-1.90 ppm (m, 2H), 2.37 (t, J^3(H,H) = 6.75 Hz, 1H), 2.47 ppm (s, 3H), 2.60 ppm (s, 3H), 3.01 ppm (s, 3H), 3.12-3.20 ppm (m, 2H), 3.70 ppm (t, J^3(H,H) = 7.0 Hz, 2H), 3.90 ppm (s, 3H), 4.64 ppm (d, J^3(H,H) = 12.5 Hz, 1H), 4.74 ppm (dd, J^3(H,H) = 6.0 Hz, 1H), 4.79 ppm (d, J^3(H,H) = 12.5 Hz, 1H), 6.84 ppm (d, J^3(H,H) = 9.5 Hz, 2H), 7.26 ppm (s, 1H), 7.45 ppm (d, J^3(H,H) = 8.5 Hz, 2H), 7.472 ppm (s, 1H), 7.73 ppm (d, J^3(H,H) = 8.0 Hz, 1H), 9.0 Hz), 7.91 ppm (s, 1H), 7.93 ppm (d, J^3(H,H) = 8.0 Hz, 2H), 8.07 ppm (dd, J^3(H,H) = 5.5 Hz, 1H),
HRMS [+ Scan]; calculated m/z for C36G39N10O7 723.2998; observed mass 723.3002.

Scheme S2. Synthesis of the α-azidoether BHQ-2 release linker **3**.

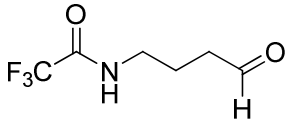


N-(4-hydroxybutyl)trifluoroacetamide (**5**)



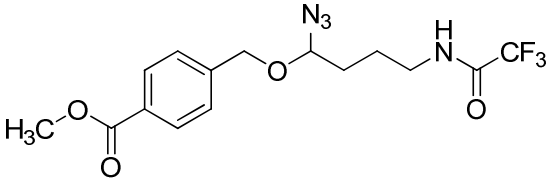
Ethyl trifluoroacetate (3.3 mL; 28 mmol) was added dropwise to cooled (0˚C) 4-amino-1-butanol (2.0 g; 22.4 mmol). The mixture was stirred for 2 h at 0˚C and evaporated. The clear liquid was dried on the high vacuum. The product was obtained in a yield of 4.1 g (99%).
^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.61-1.67 ppm (m, 2H), 1.68-1.74 ppm (m, 2H), 2.31 (bs, 1H), 3.38 ppm (dt, 2H), 3.72 ppm (t, 2H), 7.40 ppm (bs, 1H).
^{13}C-NMR (500 MHz, $CDCl_3$): δ = 26.02, 29.70, 40.01, 62.48, 116.20 ppm (q, *J* = 1144.0 Hz), 157.66 ppm (q, *J* = 146.5 Hz).
HRMS [+ Scan]; calculated m/z for C6H11NO2F3 186.0747; observed mass: 186.0741.

N-(4-oxobutyl)trifluoroacetamide (**6**)



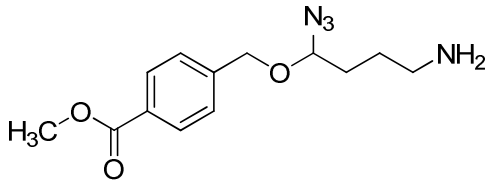
N-(4-hydroxybutyl)trifluoroacetamide (1.65 g; 8.9 mmol) was dissolved in anhydrous DCM (9 mL) and cooled to 0˚C. A solution of Dess-Martin periodinane (15% in DCM; 30 mL; 14.0 mmol) was added to the cooled solution and stirred first at 0˚C for 10 min and then at room temperature for 2.5 h. The reaction was quenched with 1:1 10% sodium thiosulfate and saturated $NaHCO_3$ and the biphasic mixture vigorously stirred for 30 min. The layers were separated and the aqueous layer extracted with DCM. The combined organic layers were dried over $MgSO_4$, filtrated and evaporated. The residue was purified by column chromatography (Hex:EtOAc 2:1 + 1% MeOH) providing *N*-(4-oxobutyl)trifluoroacetamide in a yield of 1.15 mg (71%).
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.85-1.95 ppm (m, 2H), 2.61 ppm (t, *J* = 5.2 Hz, 2H), 3.35-3.40 (m, 2H), 6.95 ppm (bs, 1H), 9.79 ppm (s, 1H). The ^{1}H-NMR spectrum confirms with literature reports.[2]

Methyl 4-[(1-azido-4-trifluoroacetamidobutoxy)methyl]benzoate (**7**)



A solution of *N*-(4-oxobutyl)trifluoroacetamide (1.0 g; 5.5 mmol) and a catalytic amount of anhydrous ferric chloride (44 mg; 0.27 mmol) in anhydrous MeCN (6 mL) was cooled to -40˚C. A solution of methyl 4-[(trimethylsilyloxy)methyl]benzoate[1] (1.56 g; 6.6 mmol) and azidotrimethylsilane (0.94 g; 8.2 mmol) in MeCN (5 mL) was added dropwise to the cooled solution. The reaction mixture was stirred at -40˚C for 60 min and quenched with buffered phosphate saline (pH 7.2). The aqueous solution was extracted with Et_2O. The organic layer was washed with brine, dried over $MgSO_4$, filtrated and evaporated. The residue was purified by column chromatography (Hex:DCM:EtOAc 7:2:1) to provide methyl 4-[(1-azido-4-trifluoroacetamidobutoxy)methyl]benzoate in a yield of 0.95 g (46%).
^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.72-1.78 ppm (m, 2H), 1.83-1.90 ppm (m, 2H), 3.36-3.44 (m, 2H), 3.92 ppm (s, 3H), 4.51 ppm (t, *J* = 6.0 Hz, 1H), 4.62 ppm (d, *J* = 12.5 Hz, 1H), 4.88 ppm (d, *J* = 12.0 Hz, 1H), 6.50 ppm (bs, 1H), 7.41 ppm (d, *J* = 8.0 Hz, 2H), 8.04 ppm (d, *J* = 8.0 Hz, 2H).
^{13}C-NMR (500 MHz, $CDCl_3$): δ = 21.14, 31.71, 39.42, 52.27, 70.35, 91.70, 115.85 ppm (q, *J* = 1146.5 Hz), 127.69, 129.96, 141.80, 157.40 ppm (q, *J* = 146.5 Hz), 166.86 ppm.
HRMS [+ Scan]; calculated m/z for C15H21N5O4F3 392.1540; observed mass: 392.1543.

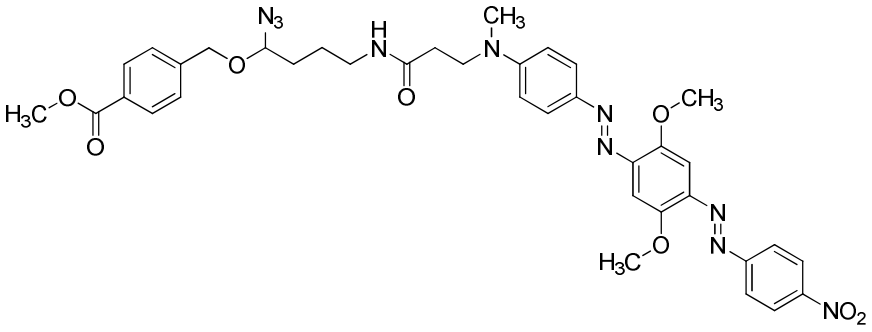
Methyl 4-[(4-amino-1-azidobutoxy)methyl]benzoate (**8**)



To a solution of methyl 4-[(1-azido-4-trifluoroacetamidobutoxy)methyl]benzoate (0.4g; 1.1 mmol) in MeOH (4.6 mL) was added a solution of potassium carbonate (0.4 g; 2.9 mmol) in water (2.4

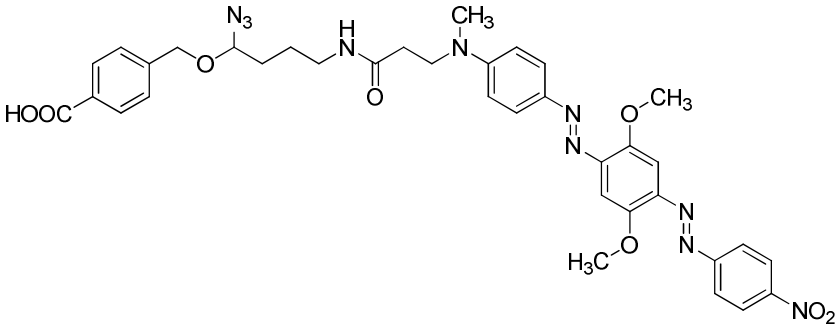
mL) and the mixture stirred at room temperature for 4 h. The mixture was diluted with water and extracted with EtOAc. The organic layers were combined, dried over $MgSO_4$, filtrated and evaporated. The residue was purified by column chromatography (MeCN + 5% MeOH) to provide methyl 4-[(4-amino-1-azidobutoxy)methyl]benzoate in 0.19 g yield (65%).
^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.51-1.69 ppm (m, 2H), 1.77-1.91 ppm (m, 2H), 2.70 ppm (bs, 2H), 2.74 ppm (t, *J* = 7.0 Hz, 2H), 3.90 ppm (s, 3H), 4.45 ppm (t, *J* = 6.0 ppm, 1H), 4.60 ppm (d, *J* = 12.5 Hz, 1H), 4.86 ppm (d, *J* = 12.5 Hz, 1H), 7.41 ppm (d, *J* = 8.5 Hz, 2H), 8.02 ppm (d, *J* = 8.0 Hz, 2H).
^{13}C-NMR (500 MHz, $CDCl_3$): δ = 27.95, 31.90, 41.34, 52.21, 70.12, 91.77, 127.55, 129.76, 129.87, 142.19, 166.87 ppm.

Methyl 4-[(1-azido-4-(3-(BHQ-2)-propionamido)butoxy)methyl]benzoate (**9**)



A solution of BHQ-2 propionic acid (255 mg; 0.52 mmol), *O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU, 196 mg; 0.52 mmol) and DIEA (134 mg; 1.03 mmol) in anhydrous DMF (7 mL) was added to methyl 4-[(4-amino-1-azidobutoxy)methyl]benzoate (0.12 g; 0.43 mmol). The mixture was stirred at room temperature for 30 min. The solvent was evaporated and the residue dissolved in DCM and washed twice with brine. The organic layer was dried over $MgSO_4$, filtrated and evaporated. The residue was purified by silica column chromatography (DCM:MeCN 9:1 + 1% MeOH) to provide (**9**) in a yield of 195 mg (60%).
^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.51-1.69 ppm (m, 2H), 1.77-1.91 ppm (m, 2H), 2.70 ppm (bs, 2H), 2.74 ppm (t, *J* = 7.0 Hz, 2H), 3.90 ppm (s, 3H), 4.45 ppm (t, *J* = 6.0 ppm, 1H), 4.60 ppm (d, *J* = 12.5 Hz, 1H), 4.86 ppm (d, *J* = 12.5 Hz, 1H), 7.41 ppm (d, *J* = 8.5 Hz, 2H), 8.02 ppm (d, *J* = 8.0 Hz, 2H).
^{13}C-NMR (500 MHz, $CDCl_3$): δ = 27.95, 31.90, 41.34, 52.21, 70.12, 91.77, 127.55, 129.76, 129.87, 142.19, 166.87 ppm.
HRMS [+ Scan]; calculated m/z for C37H41N10O8 753.3103; observed mass: 753.3111.

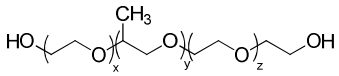
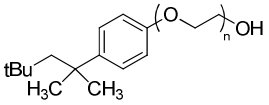
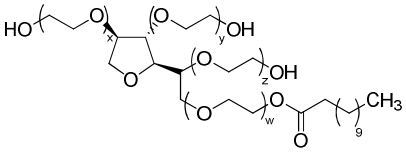
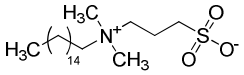
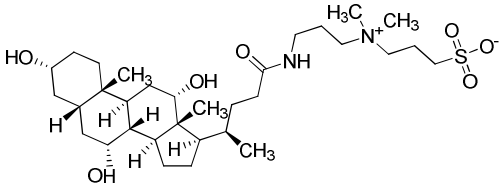
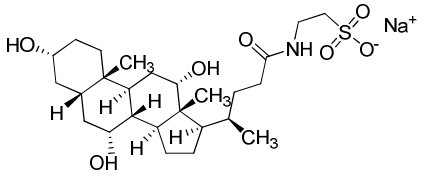
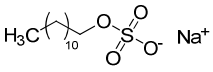
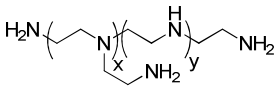
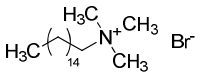
4-[(1-azido-4-(3-(BHQ-2)-propionamido)butoxy)methyl]benzoaic acid (**3**)



To a solution of **9** in THF (5 mL) was added a solution of lithium hydroxide monohydrate (320 mg; 7.6 mmol) in water (4 mL). The biphasic mixture was vigorously stirred at 40˚C for 5 h. The mixture was diluted with water, acidified with citric acid and extracted with DCM. The organic

layer was washed with brine, dried over $MgSO_4$, filtrated and evaporated. **13** was obtained in a yield of 129 mg (83%).
^{1}H-NMR (500 MHz, [D6]DMSO): δ = 1.43-1.50 ppm (m, 2H), 1.62-2.1.75 ppm (m, 2H), 2.38 ppm (t, *J* = 8.5 Hz, 2H), 3.03 ppm (s, 3H), 3.06 ppm (q, 8.0 Hz, 2H), 3.715 ppm (t, 8.5 Hz, 2H), 3.94 ppm (s, 3H), 3.99 ppm (s, 3H), 4.66 ppm (d, *J* = 16.0 Hz, 1H), 4.73 ppm (t, *J* = 7.5 Hz, 1H), 4.81 ppm (d, *J* = 15.5 Hz, 1H), 6.86 ppm (d, *J* = 11.5 Hz, 2H), 7.37 ppm (s, 1H), 7.44 ppm (s, 1H), 7.44 ppm (d, *J* = 10.0 Hz, 2H), 7.81 ppm (d, *J* = 12.0 Hz, 2H), 7.92 ppm (d, *J* = 10.0 Hz, 2H), 8.01 ppm (t, 1H), 8.06 ppm (d, *J* = 11.5 Hz, 2H), 8.46 ppm (d, *J* = 11.5 Hz, 2H).
HRMS [+ Scan]; calculated m/z for C36H39N10O8 739.2947; observed mass: 739.2953.

Table S1. Molecular structures of additives investigated in this study.

Pluronic F-68		Neutral polymer
Triton X-100		Neutral detergent
Tween 20		Neutral detergent
DPAPS[a]		Zwitterionic detergent
CHAPS[b]		Zwitterionic detergent
STDC[c]		Anionic detergent
SDS[d]		Anionic detergent
PEI[e]		Cationic polymer
HDTA[f]		Cationic detergent

[a]DPAPS = 3-(*N,N*-Dimethylpalmitylammonio)propane sulfonate; [b]CHAPS = 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate; [c]STDC = Sodium taurodeoxycholate; [d]SDS = Sodium dodecyl sulfate; [f]PEI = Polyethyleneimine; [f]HDTA = Hexadecyltrimethylammonium bromide.

Supplementary Experiments

Figure missing.

Figure S1. Effect of increasing concentrations of selected delivery additives on the growth of *E. coli* in a Petri dish assay. Conditions as described in experimental section.

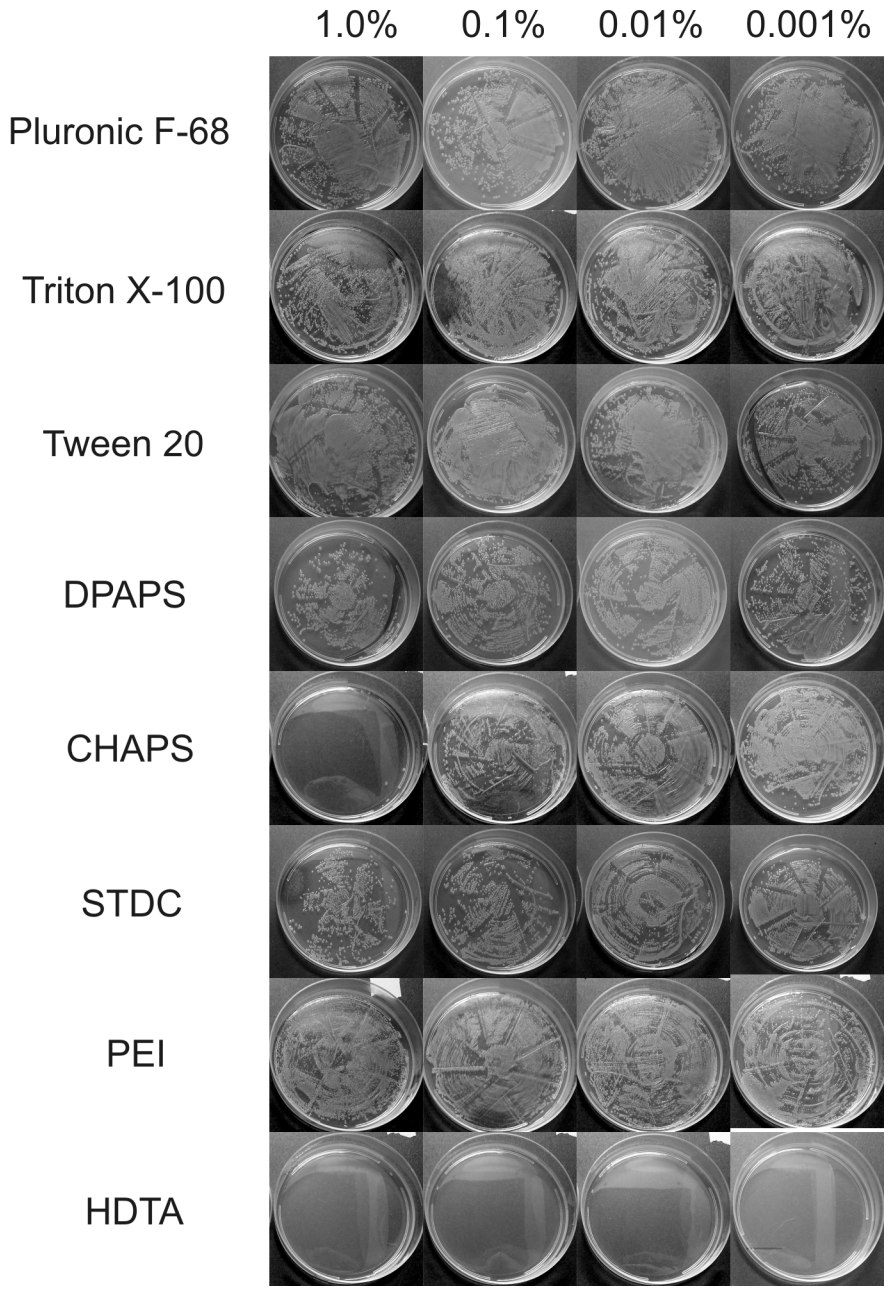


Figure S2. Reaction kinetics and mismatch discrimination of templated fluorescence activation of NIR STAR SE by TPP-DNA.

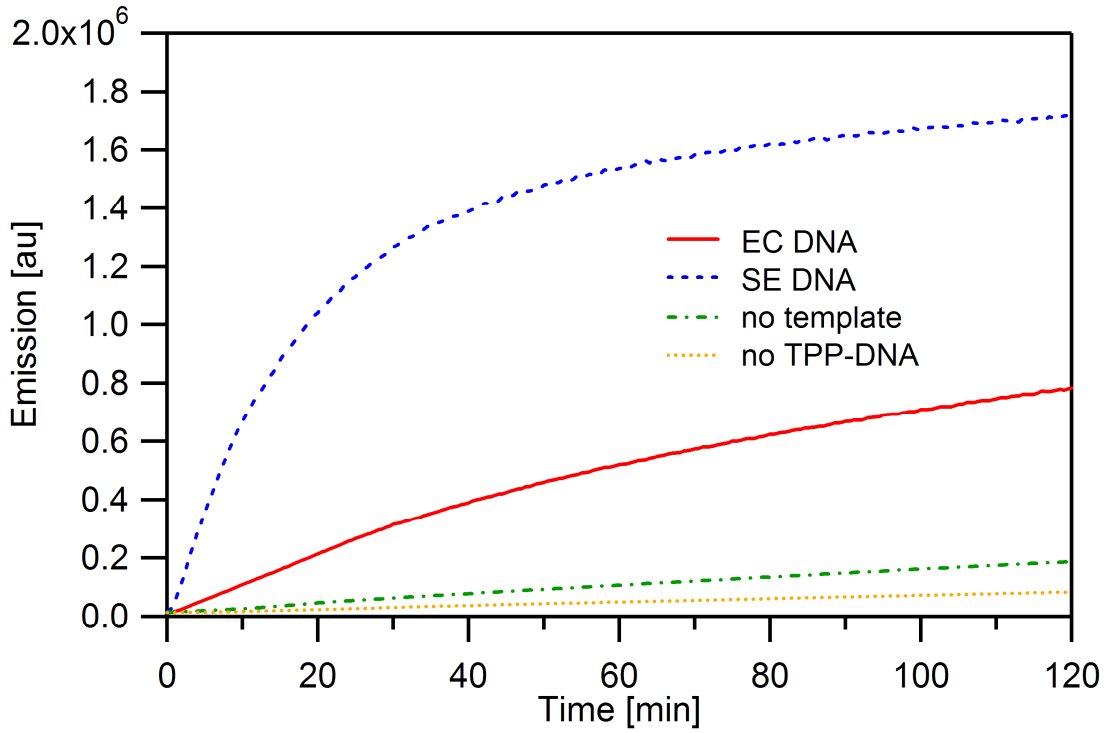
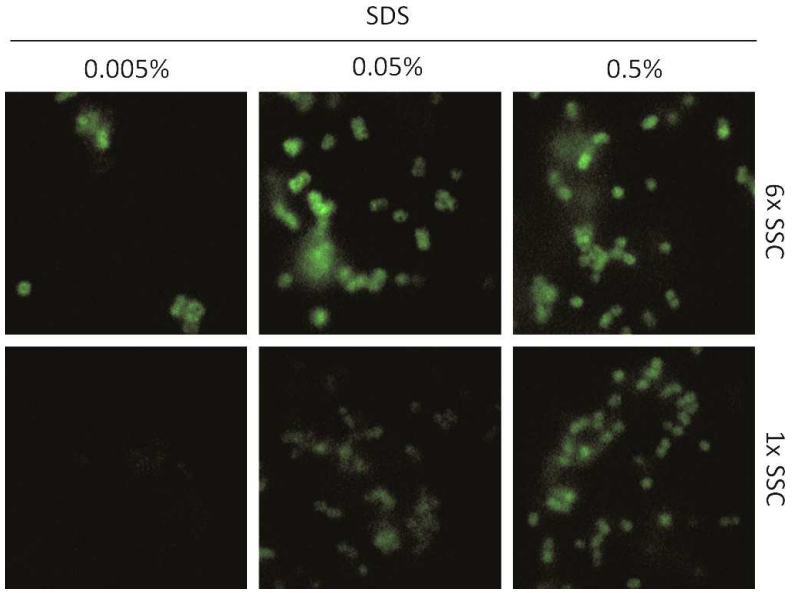


Figure S3. Delivery of template probes into *Escherichia coli* K12 under varied salinity and SDS detergent concentrations. Signal is generated by green STAR EC probes after 4 h incubation.



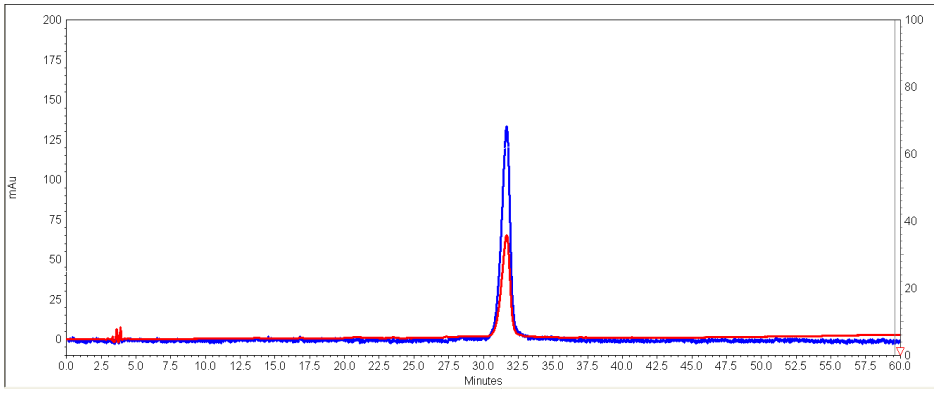
Characterization of Oligonucleotide Conjugates

Table S2. Summary of MALDI-TOF mass spectrometry data.

Strand	Calculated mass[a]	Observed mass
green STAR EC	3616 m/z	3616 m/z
green STAR SE	3640 m/z	3640 m/z
green STAR SE DAP	3655 m/z	3655 m/z
green STAR EC BHQ1	3802 m/z	3804 m/z
NIR STAR EC	3938 m/z	3938 m/z
NIR STAR SE	3962 m/z	3966 m/z
EC DNA	6544 m/z	6540 m/z
SE DNA	6519 m/z	6515 m/z
Helper 1	5519 m/z	5521 m/z
Helper 2	5443 m/z	5541 m/z
TPP-DNA	3082 m/z	3080 m/z

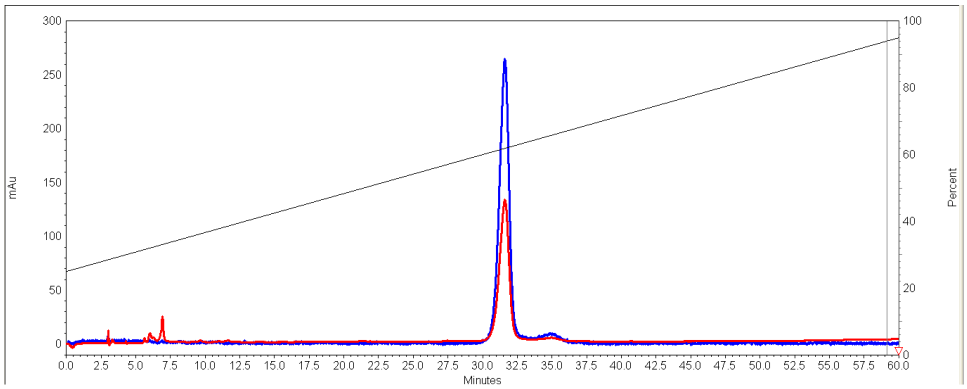
[a] The molecular peak of TPP-DNA is accompanied by a major peak + 16 m/z which was assigned to oxidation of the triphenylphosphine moiety during mass-spec sample preparation and measurement.

Figure S4. Analytical HPLC of Purified NIR STAR EC.



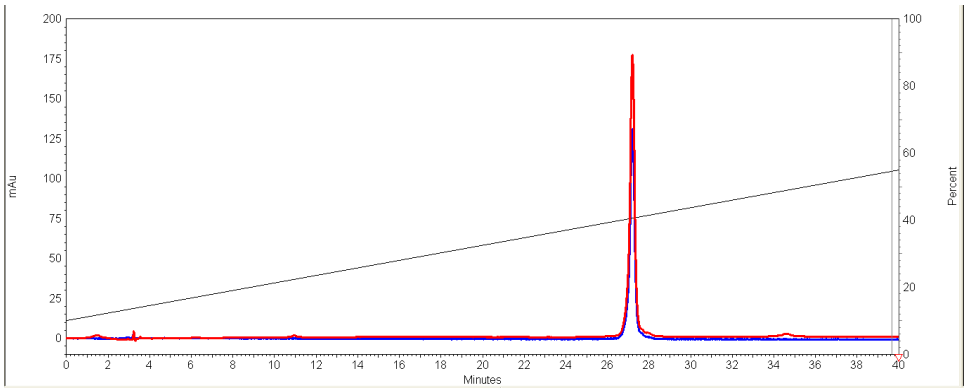
HPLC gradient: 50 mM TEAA buffer + 25 – 90 % MeCN in 60 minutes.
Blue trace: λ_{abs} = 644 nm; red trace: λ_{abs} = 260 nm.

Figure S5. Analytical HPLC of Purified NIR STAR SE.



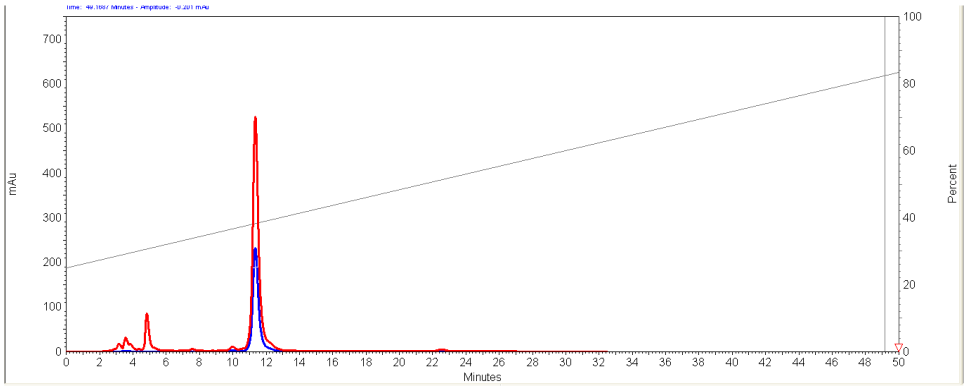
HPLC gradient: 50 mM TEAA (triethylammonium acetate, pH 8.5) buffer + 25 – 90 % MeCN in 60 minutes. Blue trace: λ_{abs} = 644 nm; red trace: λ_{abs} = 260 nm.

Figure S6. Analytical HPLC of Purified green STAR SE DAP.



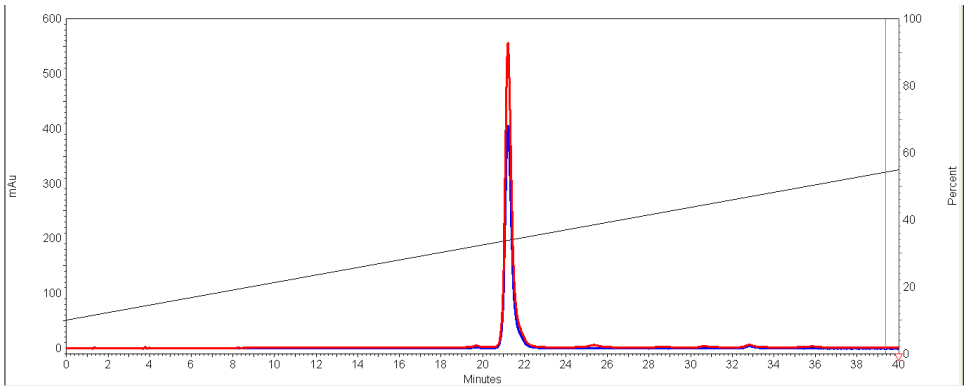
HPLC gradient: 50 mM TEAA buffer + 10 – 55 % MeCN in 40 minutes.
Blue trace: λ_{abs} = 494 nm; red trace: λ_{abs} = 260 nm.

Figure S7. Analytical HPLC of Purified green STAR EC



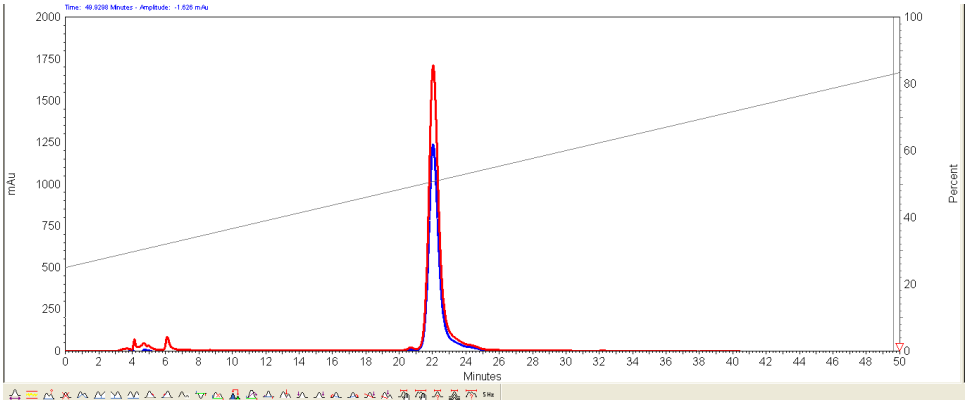
HPLC gradient: 50 mM TEAA buffer + 25 - 60 % MeCN in 60 minutes.
Blue trace: λ_{abs} = 494 nm; red trace: λ_{abs} = 260 nm.

Figure S8. Analytical HPLC of Purified green STAR SE.



HPLC gradient: 50 mM TEAA buffer + 10 – 55 % MeCN in 40 minutes.
Blue trace: λ_{abs} = 494 nm; red trace: λ_{abs} = 260 nm.

Figure S9. Analytical HPLC of Purified green STAR BHQ1 EC.



HPLC gradient: 50 mM TEAA buffer + 25 – 90 % MeCN in 60 minutes.
Blue trace: λ_{abs} = 494 nm; red trace: λ_{abs} = 260 nm.

^{1}H-NMR Spectra

Figure S10. ^{1}H-NMR spectrum of Methyl 4-[(1-azido-3-(3-(BHQ-1)-propionamido)propoxy)-methyl]benzoate (**4**); 500 MHz, $CDCl_3$.

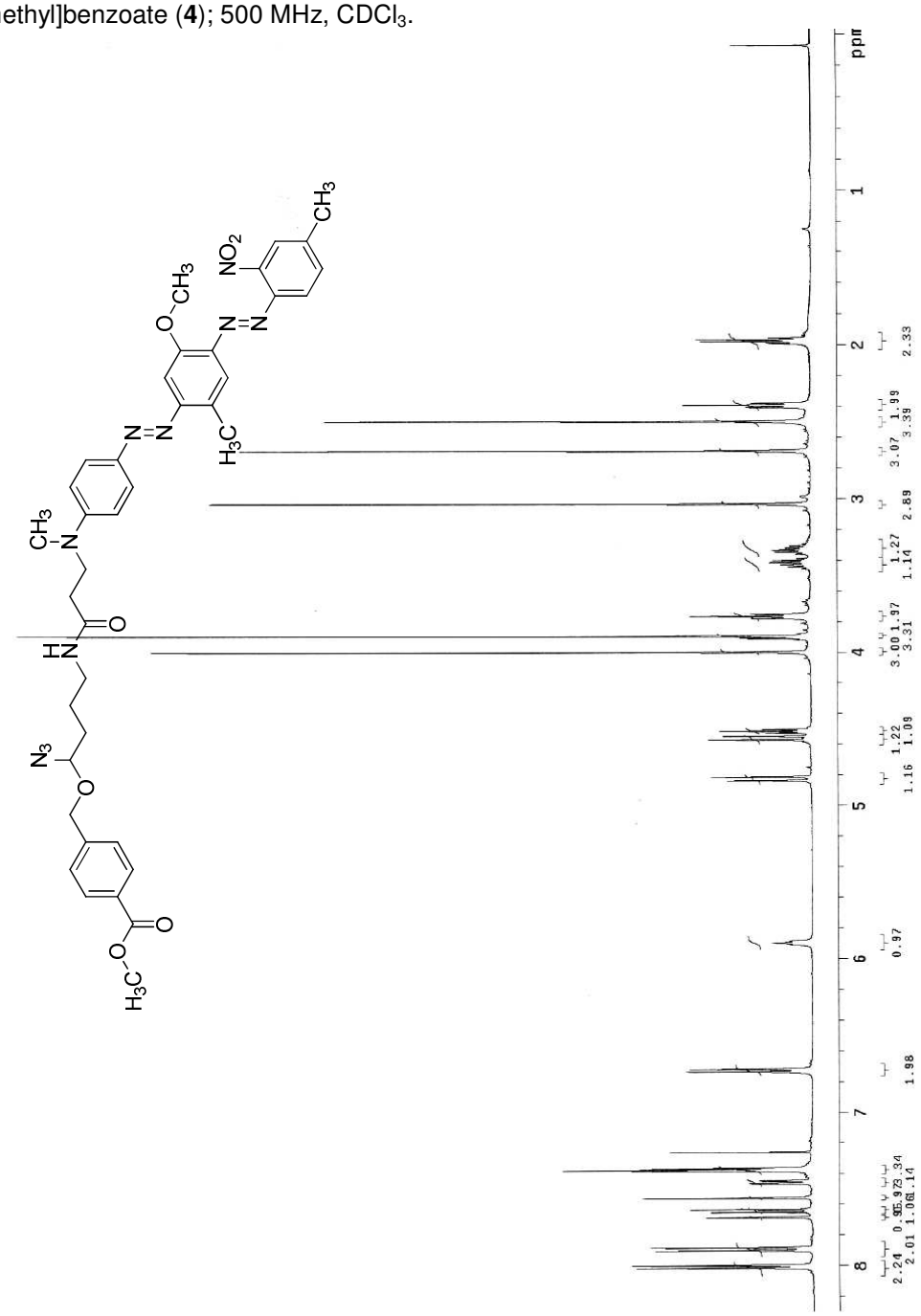


Figure S11. ^{1}H-NMR spectrum of 4-[(1-azido-3-(3-(BHQ-1)-propionamido)propoxy)-methyl]benzoaic acid (**2**). 500 MHz, [D_6]DMSO.

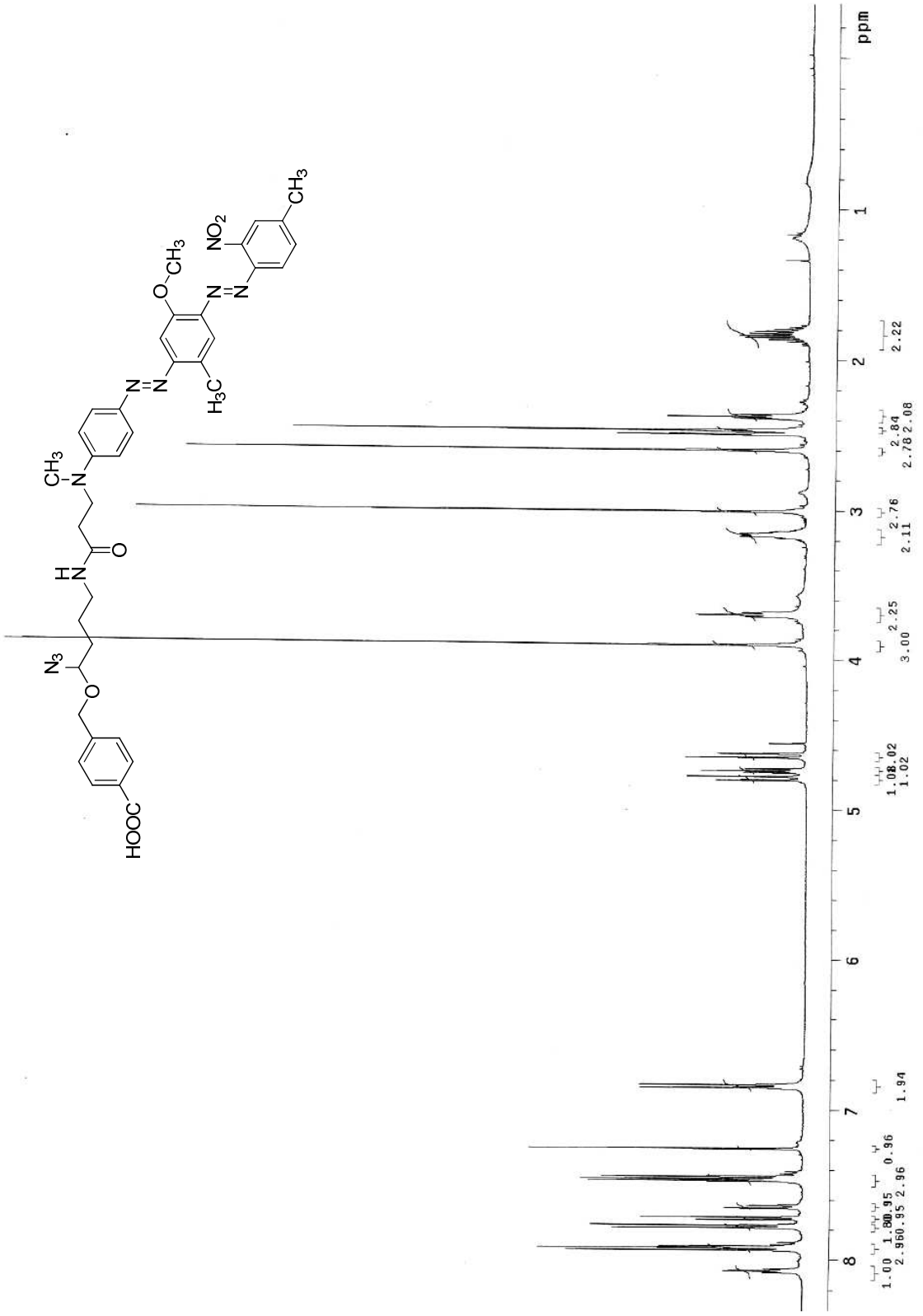


Figure S12. ^{1}H-NMR spectrum of *N*-(4-hydroxybutyl)trifluoroacetamide ***(5)***; 500 MHz, [D_6]DMSO.

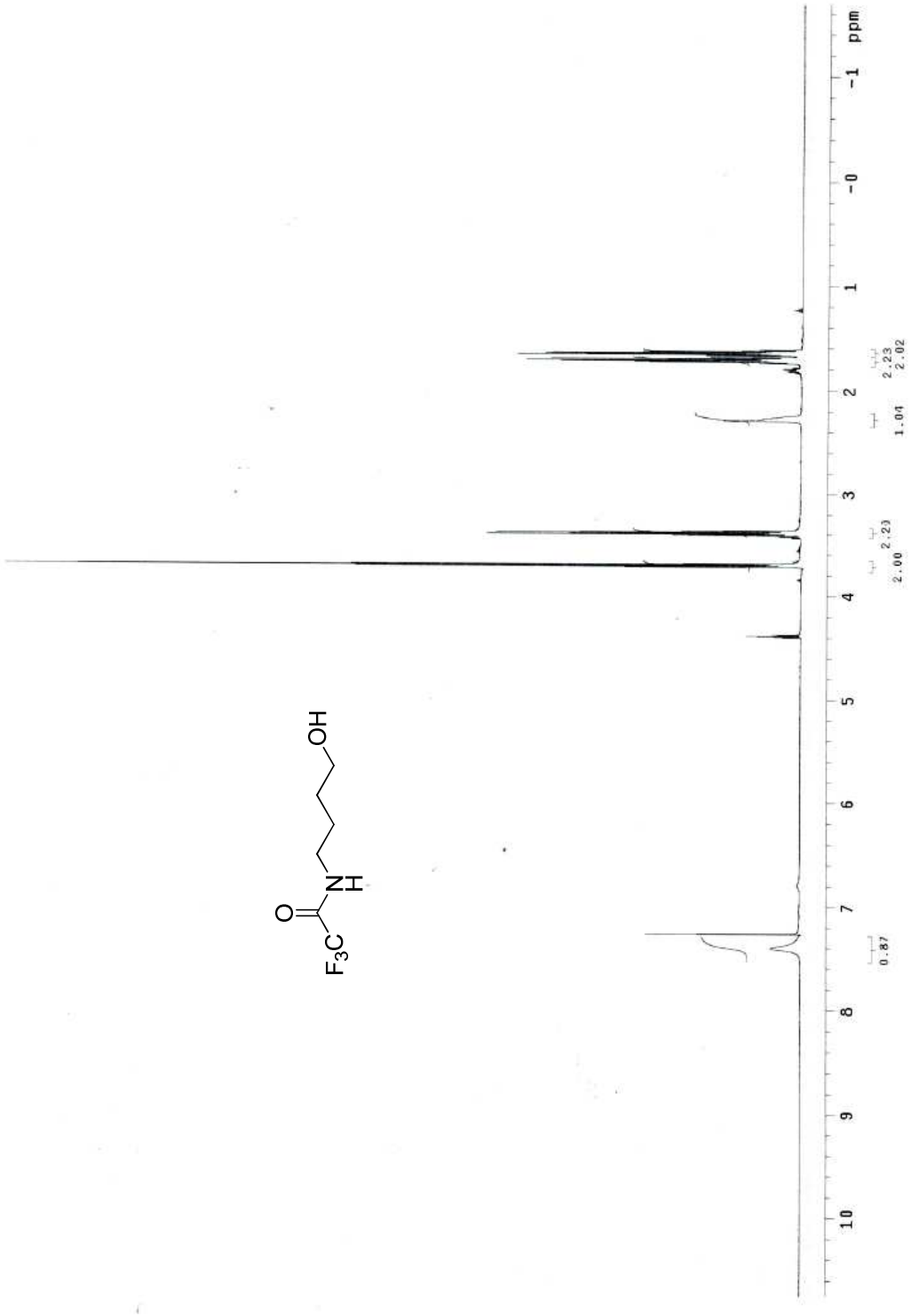


Figure S13. ^{1}H-NMR spectrum of *N*-(4-oxobutyl)trifluoroacetamide ***(6)*** ; 400 MHz, $CDCl_3$.

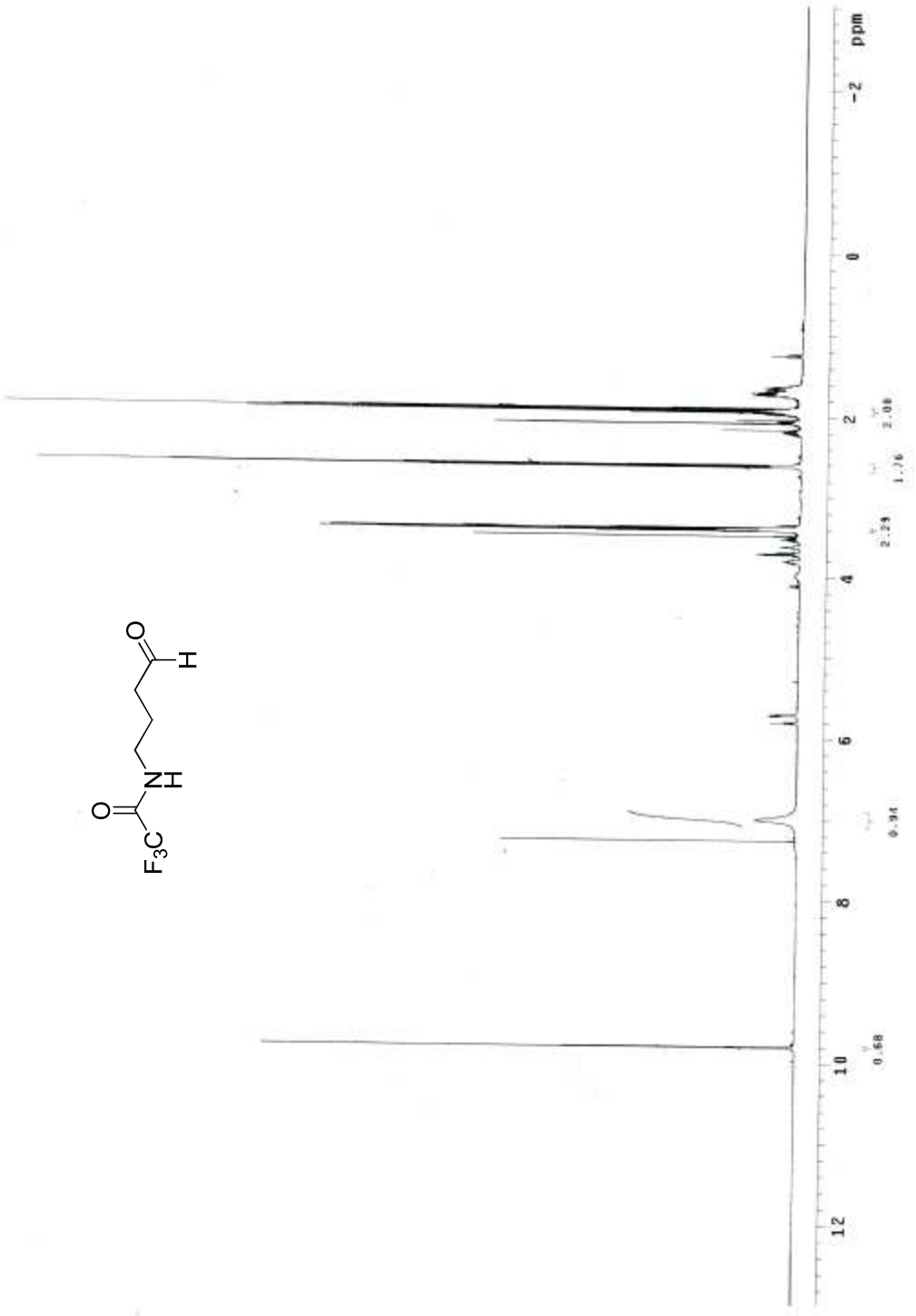


Figure S14. ^{1}H-NMR spectrum of Methyl 4-[(1-azido-4-trifluoroacetamidobutoxy)methyl]-benzoate *(**7**)*; 500 MHz, $CDCl_3$.

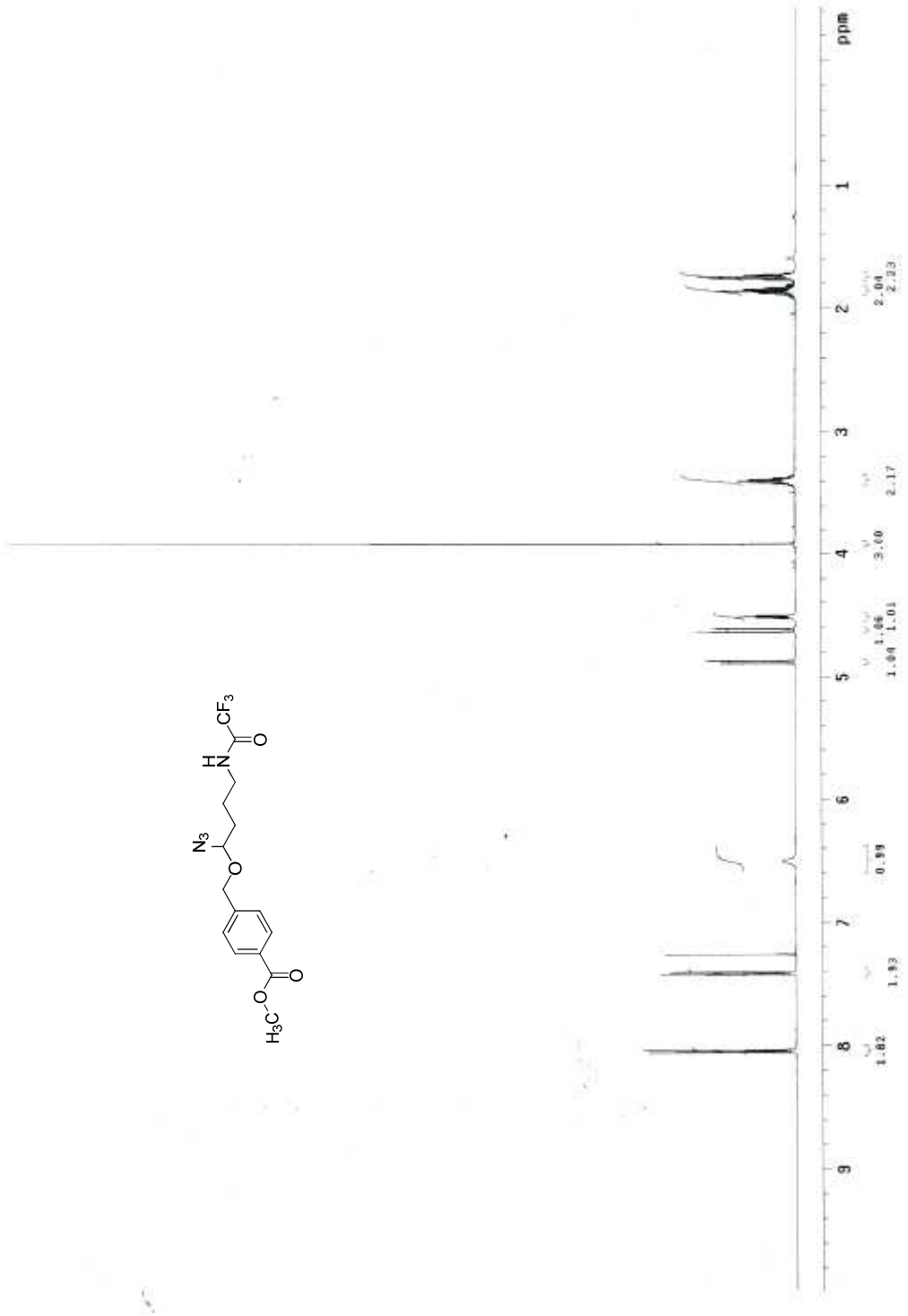


Figure S15. ^{1}H-NMR spectrum of Methyl 4-[(4-amino-1-azidobutoxy)methyl]benzoate *(**8**)*; 500 MHz, $CDCl_3$.

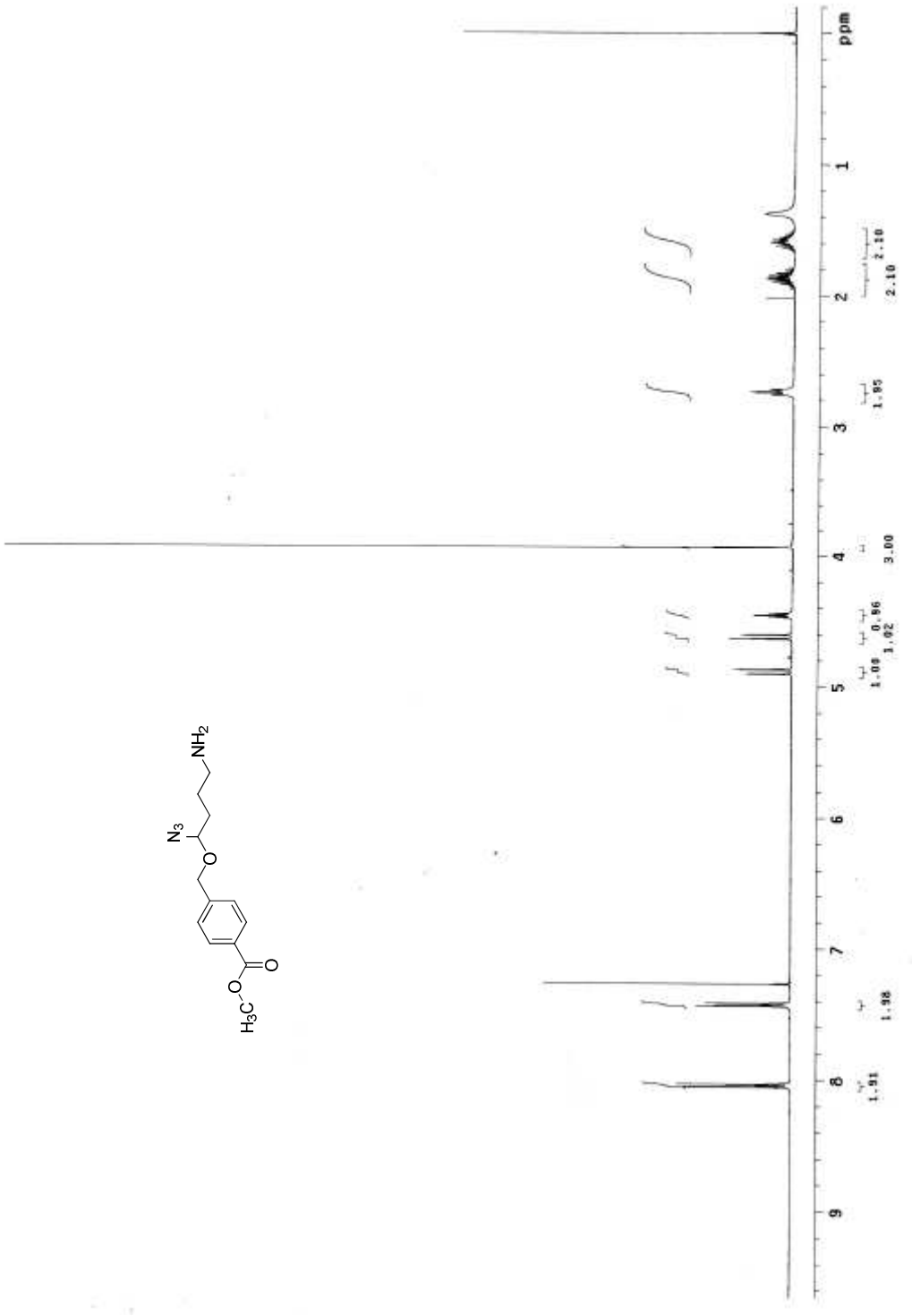


Figure S16. ^{1}H-NMR spectrum of Methyl 4-[(1-azido-4-(3-(BHQ-2)-propionamido)butoxy)methyl]-benzoate *(**9**)*; 500 MHz, $CDCl_3$.

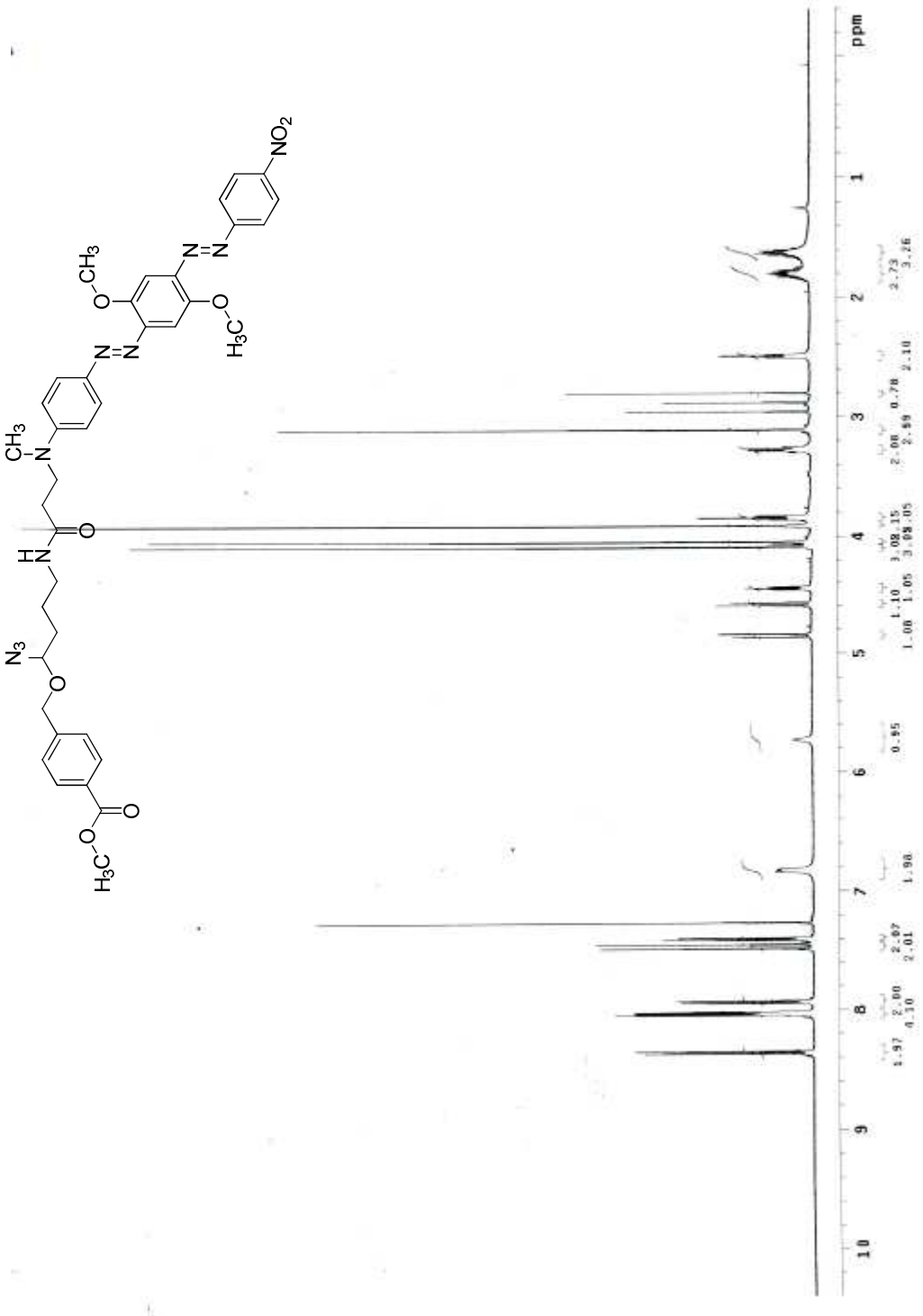
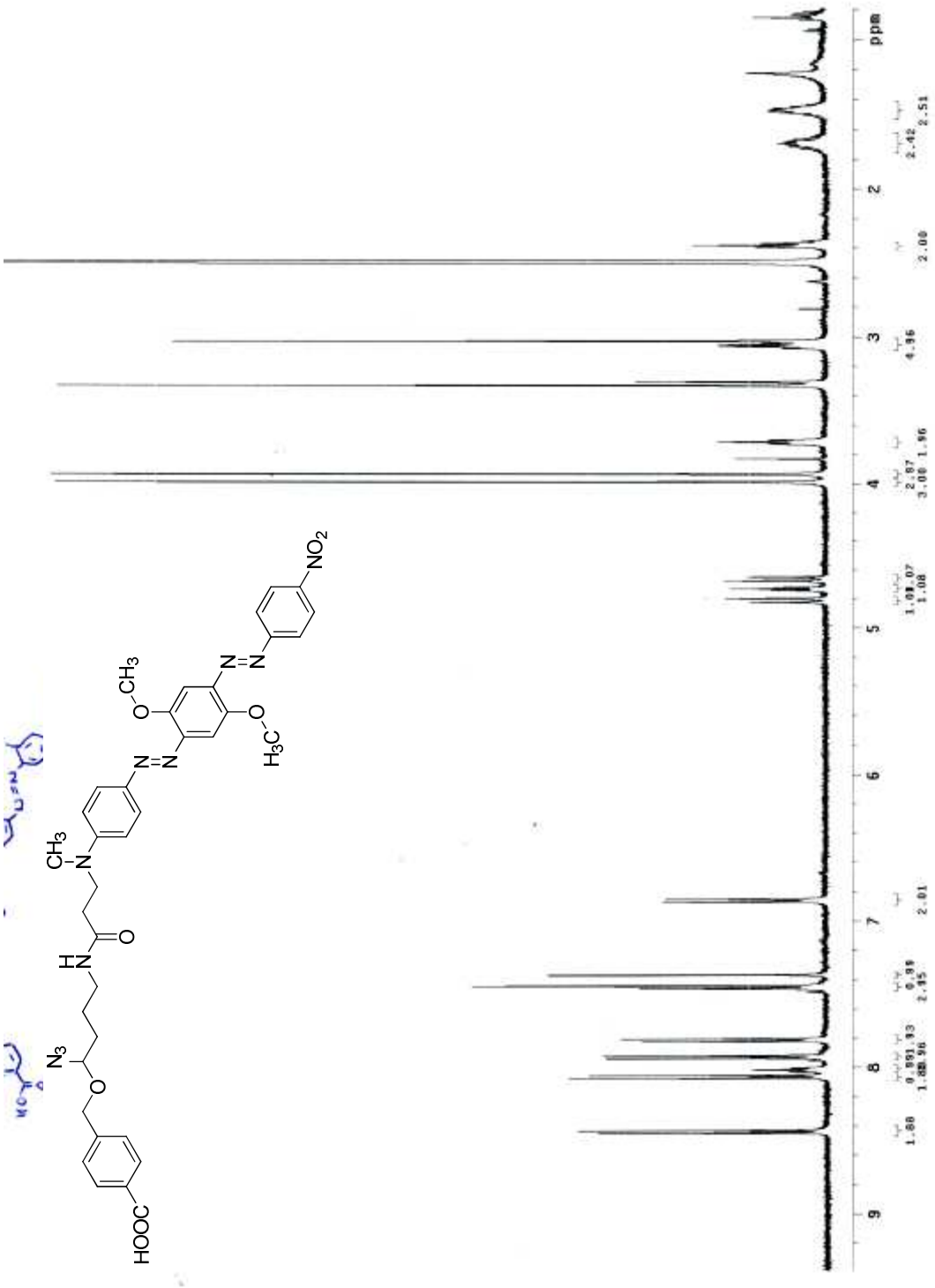


Figure S17. ^{1}H-NMR spectrum of 4-[(1-azido-4-(3-(BHQ-2)-propionamido)butoxy)methyl]-benzoaic acid (**3**); 500 MHz, [D_6]DMSO.



^{13}C-NMR Spectra

Figure S18. ^{13}C-NMR spectrum of Methyl 4-[(1-azido-3-(3-(BHQ-1)-propionamido)propoxy)-methyl]benzoate (**4**); 500 MHz, $CDCl_3$.

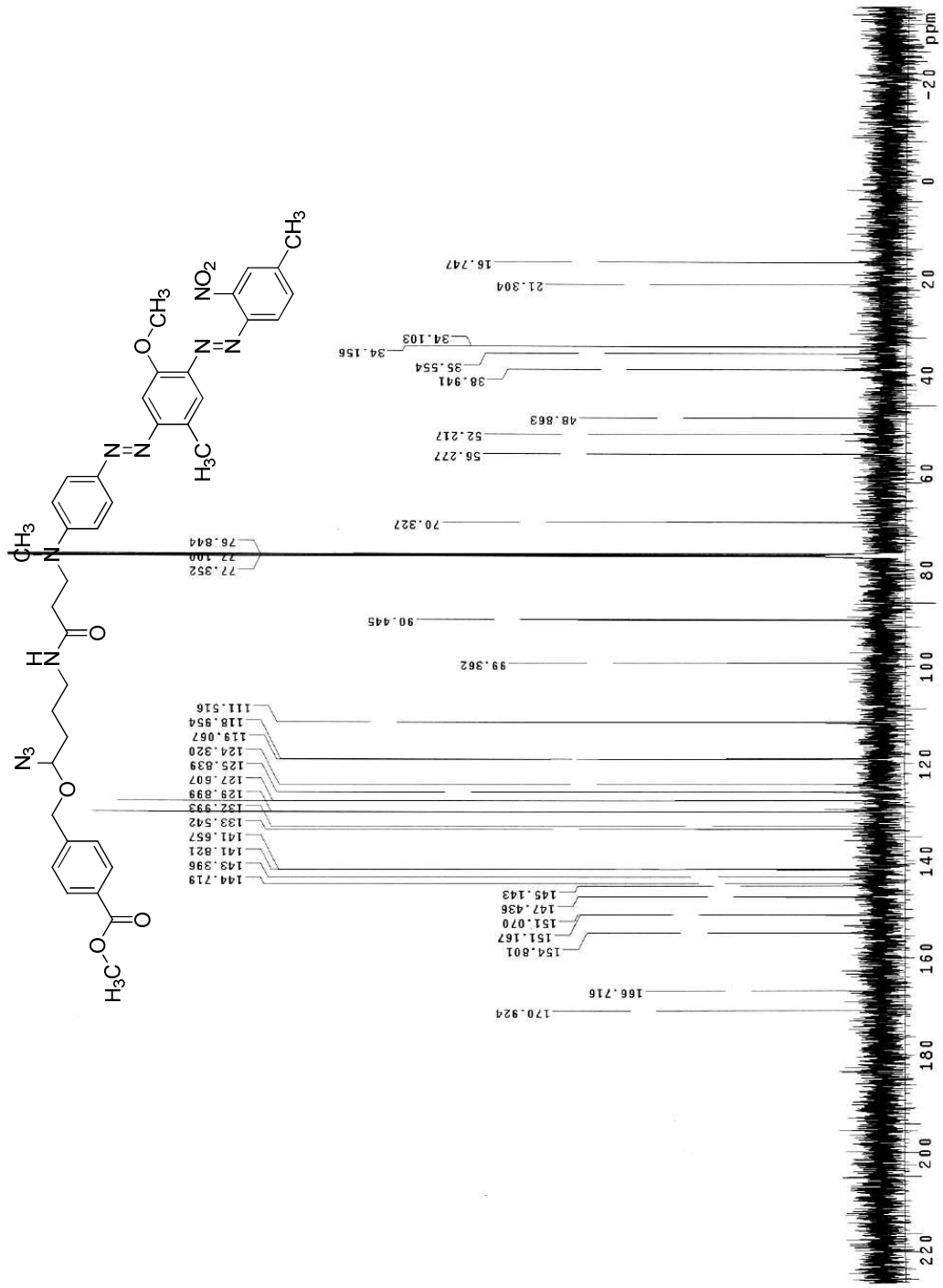


Figure S19. ^{13}C-NMR spectrum of *N*-(4-hydroxybutyl)trifluoroacetamide ***(5)***; 500 MHz, $CDCl_3$.

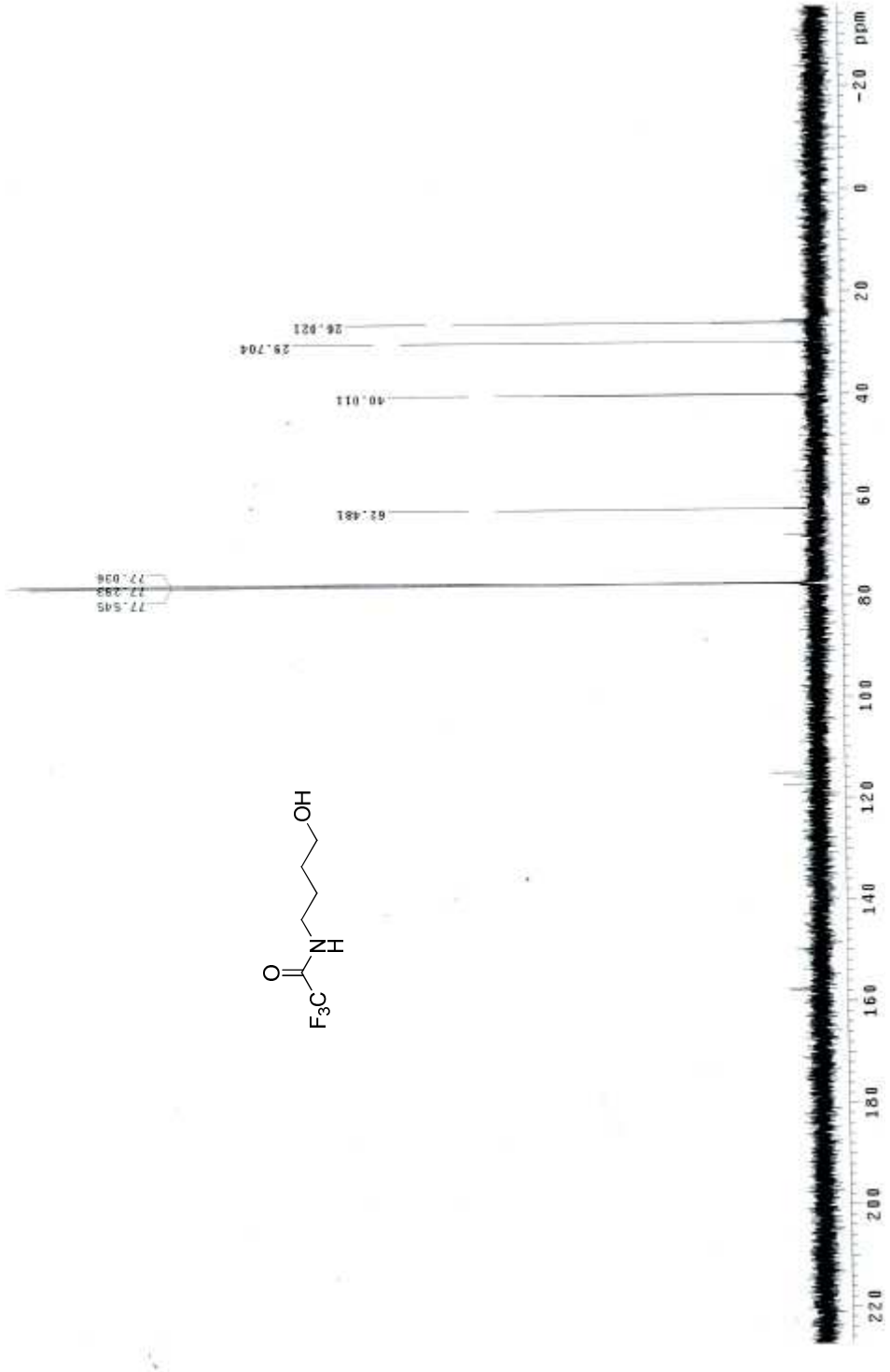


Figure S20. ^{13}C-NMR spectrum of Methyl 4-[(1-azido-4-trifluoroacetamidobutoxy)methyl]-benzoate *(**7**)*; 500 MHz, $CDCl_3$.

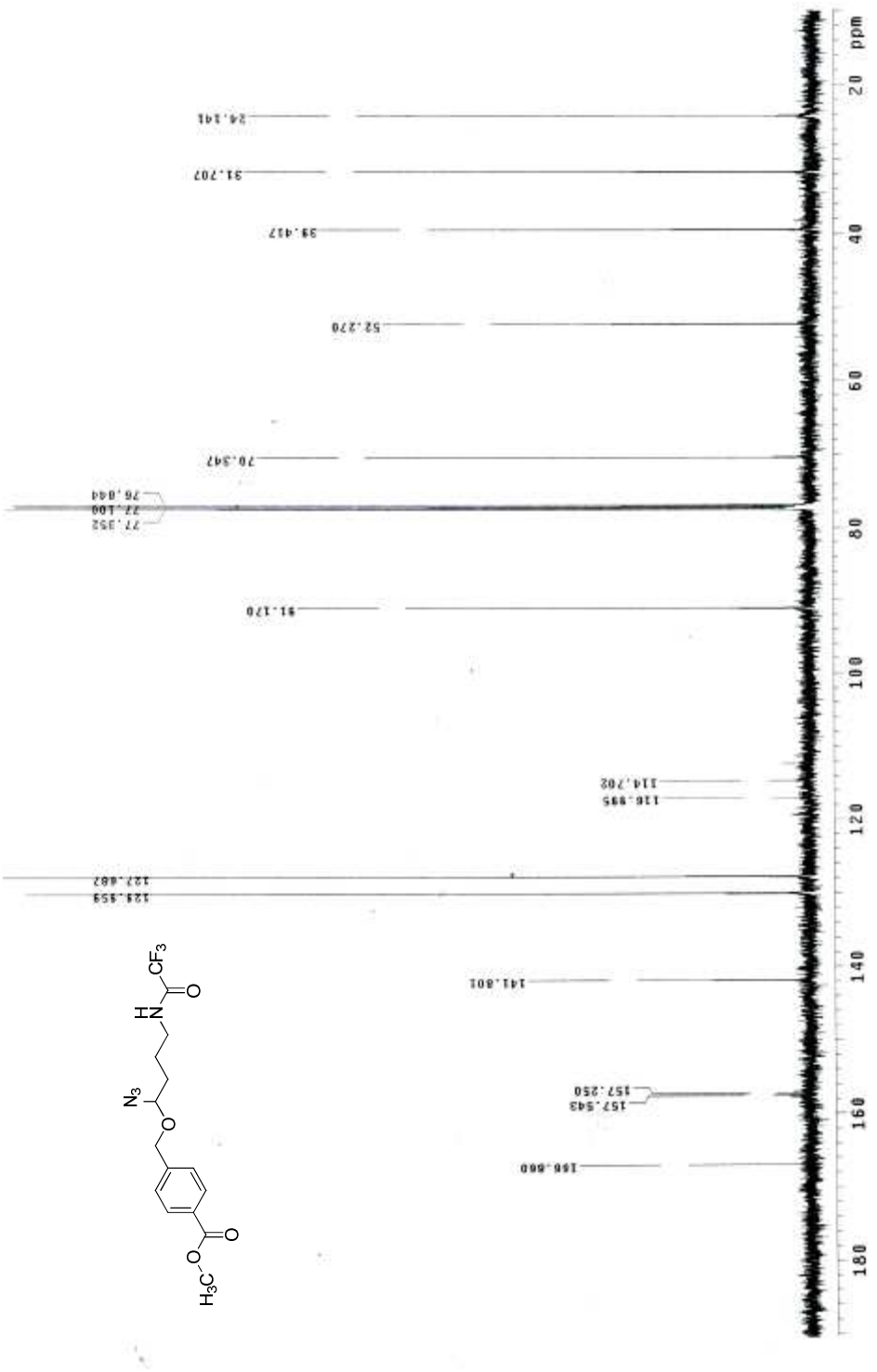


Figure 21. ^{13}C-NMR spectrum of Methyl 4-[(4-amino-1-azidobutoxy)methyl]benzoate *(**8**)*; 500 MHz, $CDCl_3$.

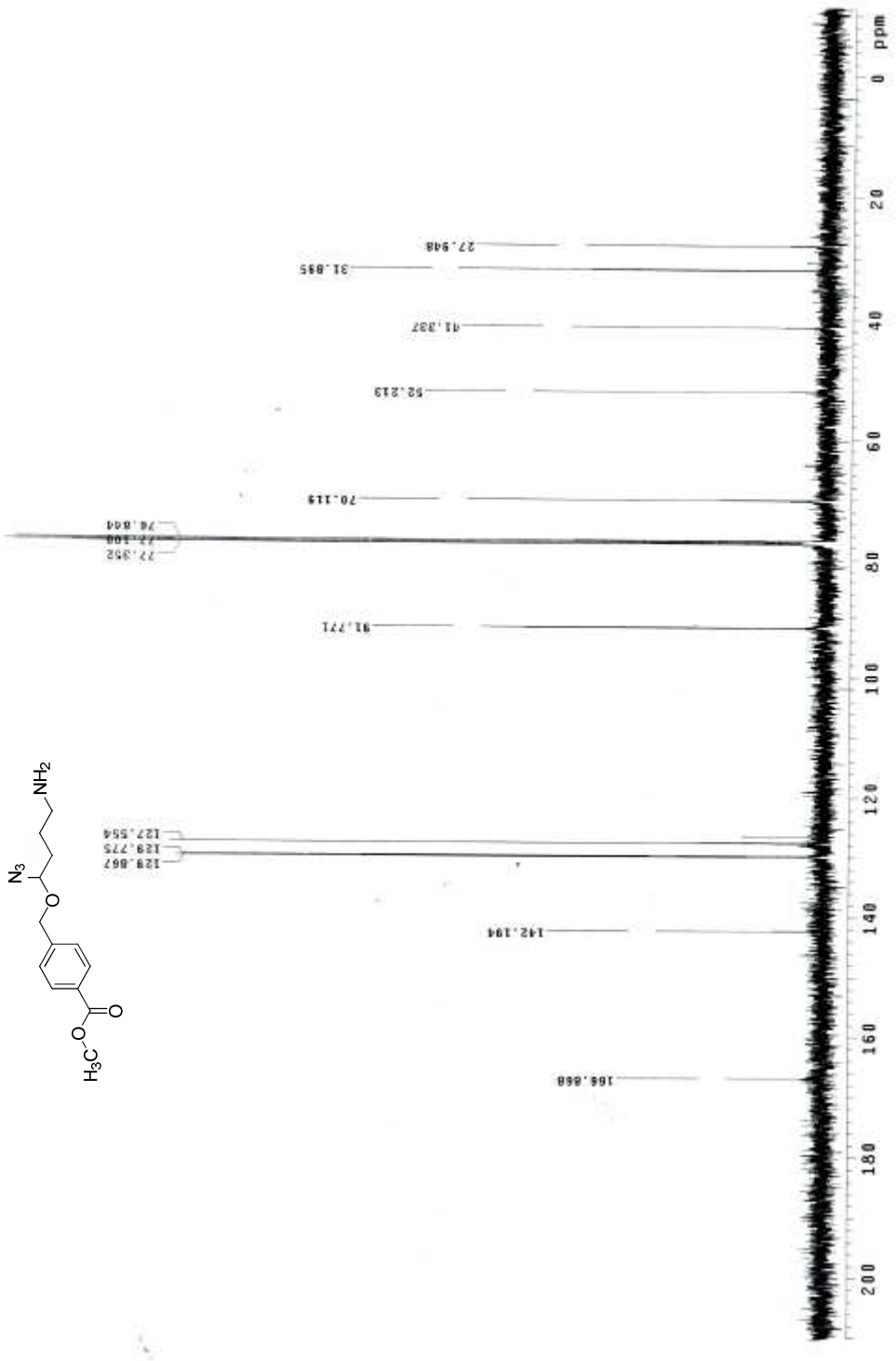
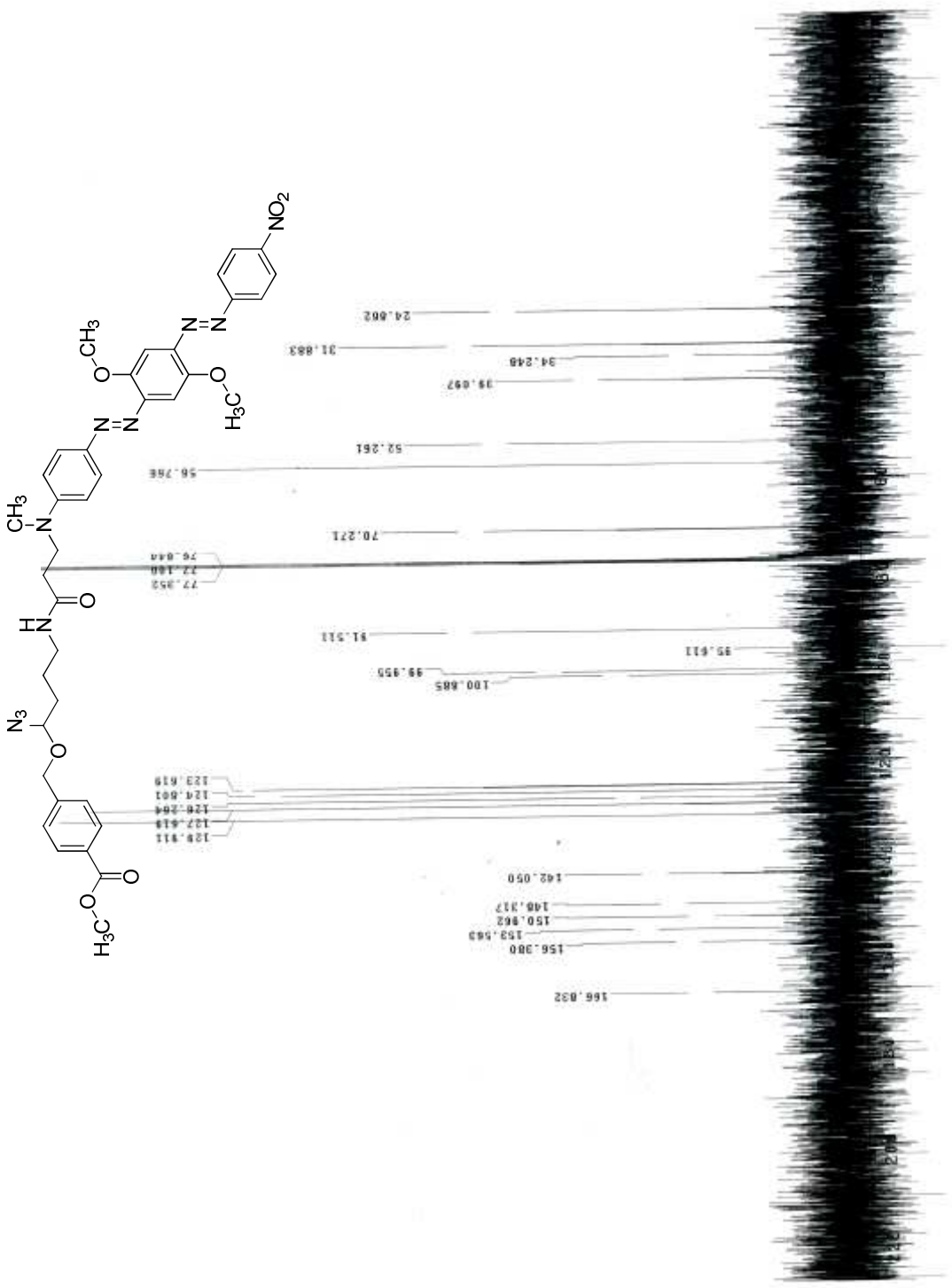


Figure S22. ^{13}C-NMR spectrum of Methyl 4-[(1-azido-4-(3-(BHQ-2)-propionamido)butoxy)methyl]-benzoate *(**9**)*; 500 MHz, $CDCl_3$.



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[1] R. M. Franzini, E. T. Kool *J. Am. Chem. Soc.* **2009**, *J. Am. Chem. Soc. 131*, 16021.

[2] C. Quinet, L. Sampoux, I. E. Marko *Eur. J. Org. Chem.* **2009**, *2009*, 1806-1811.