SUPPORTING INFORMATION

Sandwich Probes: Two Simultaneous Reactions for Templated Nucleic Acid Detection

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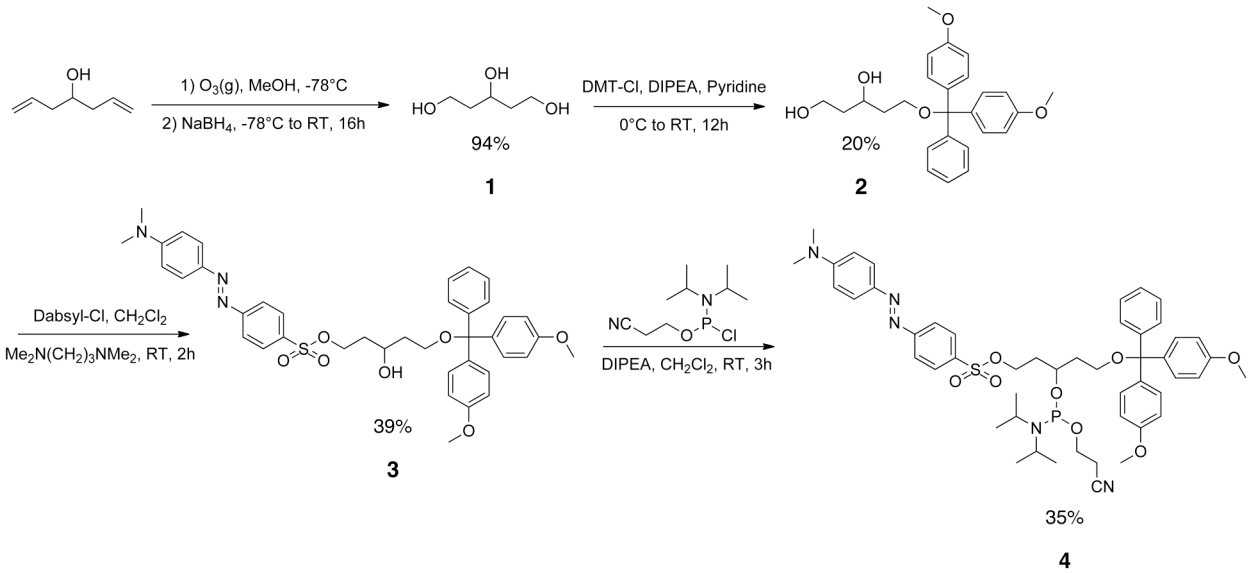


Scheme S1. Synthesis of 3'-Dabsyl linker phosphoramidite 4

Compound 2

1,3,5-pentanetriol (0.54 g, 4.5 mmol) was dissolved in 45 mL dry pyridine at 0°C. N,N-Diisopropylethylamine (2.3 mL, 13 mmol) and 4,4'-dimethoxytrityl chloride (0.92 g, 2.7 mmol) were sequentially added to the reaction mixture at 0°C and the reaction was stirred under argon for twelve hours, warming to room temperature over that time. The solvent was removed under vacuum and the crude mixture was purified by silica gel column chromatography (25% to 100% ethyl acetate in hexanes) to give **2** (0.38 g, 0.90 mmol) in 20% yield.

^{1}H-NMR ($CDCl_3$, 500 MHz) δ = 7.45 (d, 2H, ArH, *J*=7.9), 7.35 (d, 4H, ArH, *J*=8.6), 7.32 (d, 2H, ArH, *J*=7.2), 7.24 (t, 1H, ArH, *J*=7.0), 6.87 (d, 4H, ArH, *J*=8.8), 4.06 (m, 1H, C3-H), 3.86-3.78 (m, 8H, C1,6-H), 3.42 (m, 1H, C5-H_a), 3.28 (m, 1H, C5-H_b), 1.94-1.85 (m, 1H, C2,4-H), 1.76-1.64 (m, 3H, C2,4-H)

^{13}C-NMR ($CDCl_3$, 500 MHz) δ = 158.47, 144.68, 135.97, 135.81, 129.91, 127.97, 126.84, 113.18, 86.78, 71.62, 62.33, 61.36, 55.19, 38.44, 36.85

HRMS [+Scan]; calculated m/z for $C_{26}H_{30}O_5Na$ 445.1985; observed mass 445.1993

Compound 3

The protected diol **2** (0.15 g, 0.36 mmol) was dissolved in 3.5 mL dry dichloromethane at room temperature. N, N, N', N'-Tetramethyl-1,3-propanediamine[1] (20 μL, 0.11 mmol) and Dabsyl-Cl (0.036 g, 0.11 mmol) were sequentially added to the reaction mixture at room temperature and stirred for two hours. The solvent was removed under vacuum and the crude mixture was purified by silica gel column chromatography (0 to 10% ethyl acetate in dichloromethane) to give **3** (0.030 g, 0.043 mmol) in 39% yield.

^{1}H-NMR ($CDCl_3$, 500 MHz) δ = 7.99 (d, 2H, ArH, *J*=8.5), 7.91 (d, 2H, ArH, *J*=8.8), 7.41 (d, 2H, ArH, *J*=8.1), 7.32-7.24 (m, 6H, ArH), 7.18 (d, 2H, ArH, *J*=9.4), 6.84 (d, 6H, ArH, *J*=8.5), 6.76 (d, 2H, ArH, *J*=9.4), 4.30-4.16 (m, 2H, C1-H), 3.92-3.86 (m, 1H, C3-H), 3.78 (s, 6H, C6-H), 3.38-3.31 (m, 1H, C5-H_a), 3.26-3.19 (m, 1H, C5-H_b), 3.12 (s, 6H, C7-H), 1.85-1.63 (m, 4H, C2,4-H)

^{13}C-NMR ($CDCl_3$, 500 MHz) δ = 158.59, 158.51, 153.27, 147.38, 144.64, 143.48, 135.79, 129.91, 128.94, 127.94, 126.89, 122.62, 113.23, 111.52, 86.84, 68.07, 67.46, 62.19, 60.43, 55.23, 40.33, 36.56, 36.30

HRMS [+Scan]; calculated m/z for $C_{40}H_{43}N_3O_7SNa$ 732.2714; observed mass 732.2715

Compound 4

Intermediate **3** (0.40 g, 0.57 mmol) was dissolved in 5.7 mL dry dichloromethane under argon. N,N-Diiso-propylethylamine (210 μL, 1.2 mmol) and 2-Cyanoethyl N,N-Diisopropylchloro-phosphoramidite (88 μL, 0.40 mmol) were sequentially added to the reaction mixture and stirred under argon for two hours at room temperature. The solvent was removed under vacuum and the crude mixture was purified by silica gel column chromatography (7:3:2% to 1:1:2%, hexanes:ethyl acetate:triethylamine) to give **4** (0.18 g, 0.20 mmol) in 35% yield.

^{1}H-NMR ($CDCl_3$, 400 MHz) δ = 8.00-7.88 (m, 8H, ArH), 7.42-7.38 (m, 2H, ArH), 7.31-7.25 (m, 6H, ArH), 7.22-7.16 (m, 2H, ArH), 6.84-6.78 (m, 4H, ArH), 6.77-6.73 (m, 2H, ArH), 4.28-4.04 (m, 3H, C1,3-H), 3.78 (s, 6H, C6-H), 3.64-3.40 (m, 4H, C5,8-H), 3.16-3.10 (m, 8H, C1,7-H), 2.60-2.54 (m, 2H, C10-H), 2.40 (t, 2H, C9-H, *J*=6.7), 1.96-1.72 (m, 4H, C2,4-H), 1.09 (dd, 6H, C11-H, *J*=2.7, 4.3), 1.01 (dd, 6H, C11-H, *J*=6.8, 7.4)

^{31}P-NMR ($CDCl_3$, 400 MHz) δ =149.16, 148.81

ESI-MS calculated m/z for $C_{49}H_{60}N_5O_8PS$ 910.07; observed mass 909.85

Materials and Methods

Preparation of 5'-Dabsyl Linker. 5'-Dabsyl Linker phosphoramidite was prepared and purified as previously described.[2]

Sandwich Probe Oligonucleotide Synthesis. The 3'-Dabsyl linker was first coupled to a 3'-Phosphate CPG column and then normal DNA synthesis was carried out using UltraMild phosphoramidites Pac-dA, iPr-dG, and Ac-dC (Glen Research). The fluorescein label was introduced with fluorescein-dT phosphoramidite (Glen Research). The 5'-Dabsyl linker was added as a final coupling step after the last base had been added. Deprotection and cleavage from the CPG support was carried out by incubation in 0.05 M K_2CO_3 in CH_3OH (Glen Research) for 4 hours at room temperature. The oligodeoxynucleotides were purified by reverse-phase HPLC (Prosphere C18 300Å 10u 250 mm, eluting with 0.1 M triethylammonium acetate, pH 7.0 and acetonitrile). Probe structure was confirmed by MALDI-TOF mass spectrometry (see Table S1).

Preparation of Single-Dabsyl Probes. Single-Dabsyl (butyl linker) probes were prepared and purified as previously described.[2]

Measuring Initial Background Fluorescence Levels. Fluorescence emission of each oligonucleotide was measured from 500 to 600 nm with an excitation of 494 nm in a Fluorolog 3 Jobin Yvon fluorophotospectrometer equipped with an external temperature controller. For each sample, 100 nM of the probe was dissolved in pH 7.0 buffer containing 70 mM PIPES, 10 mM $MgCl_2$, 50 μM DTT at 25°C.

Templated Double Displacement Reactions. Reactions were performed in 70 mM PIPES buffer (pH 7.0) containing 10 mM $MgCl_2$ and 50 μM dithiothreitol in a Fluorolog 3 Jobin Yvon fluorophotospectrometer equipped with an external temperature controller. Reaction mixtures contained 100 nM each of quencher probe and template and 120 nM of nucleophile DNA. Reactions were initiated by addition of the nucleophile DNA to wells containing the quencher probe and template DNA. The emission was measured in 2 min intervals at 522 nm with excitation at 494 nm over 4 hours. The control reactions were performed with the template omitted from the reaction mixture.

Denaturing Gel Electrophoresis. Reactions with 1 μM Quencher Probe, 1 μM template, and 1.2 μM nucleophile in 70 mM PIPES, 10 mM $MgCl_2$, 50 μM DTT were run for 4 h at 37 °C. The reaction mixtures were each desalted using Edge Bio Performa Spin Columns and then loaded onto a 10% TBE-Urea Gel (Invitrogen) and run for 45 minutes at 180 volts. The gel fluorescence was imaged on a Molecular Dynamics Storm 860 fluoroimager.

Intracellular rRNA Detection with Templated Probes. *E. coli* K12 cells were grown to log phase (OD600 = 0.4-0.6) in Luria-Bertani (LB) media at 37°C with rapid shaking. 100 μL aliquots of media were centrifuged for 5 min. The supernatant was removed and pellets were resuspended in 0.1 mL phosphate-buffered saline solution (pH 7.4). The cells were centrifuged again for five minutes and the supernatant was removed. The pellets were resuspended in 0.1 mL hybridization buffer: 0.9 M sodium chloride and 90 mM sodium citrate (6x SSC) and 0.05% SDS. Aliquots of bacteria suspended in the buffer were treated with quencher probe (200 nM), nucleophile probe (2 μM), and unlabeled helper probes* (3 μM each). The reaction mixtures were incubated in the dark at 37°C for two hours. Aliquots of incubated bacteria were gently mixed 1:1 with a 1% agarose solution and spotted on a glass slide without washing or fixation steps. 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.

*Helper probes, which are designed to bind adjacent to the reactive probes, have been shown to enhance RNA accessibility, as documented by Amann in standard fluorescence *in situ* hybridization experiments.[3]

Probe Sequences

Template DNAs

TAGTCCACGCCGTAAACGATGTC**G**ACTTGGAGGTTGTGCCCTTGA WT

TAGTCCACGCCGTAAACGATGTC**T**ACTTGGAGGTTGTGCCCTTGA mutT

TAGTCCACGCCGTAAACGATGTC**A**ACTTGGAGGTTGTGCCCTTGA mutA

TAGTCCACGCCGTAAACGATGTC**C**ACTTGGAGGTTGTGCCCTTGA mutC

Quenched Probes (D=dabsyl linker, f=fluorescein)

S 5′- ^{D}CAAGT^{f}CGACA^{D} -3′

C1 5′- ^{D}CAAGT^{f}CGACA -3′

C2 5′- CAAGT^{f}CGACA^{D} -3′

Nucleophile DNAs (PT = terminal phosphorothioate)

5-PT 5′ - ^{PT}TCG TTT ACG GCG TGG ACT - 3′

3-PT 5′ - CA AGG GCA CAA CCT C^{PT} - 3′

Helper DNAs

H1 5′ - TCC GGA AGC CAC GCC T – 3′

H2 5′ - TCG TTT ACG GCG TGG ACT -3′

Table S1. MALDI-TOF mass spectrometry data

Strand	Calculated mass	Observed mass
Sandwich Probe (S)	4589.462 m/z	4588.058 m/z
5'-Single Dabsyl Probe (C1)	3986.971 m/z	3987.083 m/z
3'-Single Dabsyl Probe (C2)	4136.013 m/z	4137.158 m/z
5'-Phosphorothioate (5-PT)	5617.616 m/z	5616.832 m/z
3'-Phosphorothioate (3-PT)	4626.008 m/z	4625.679 m/z

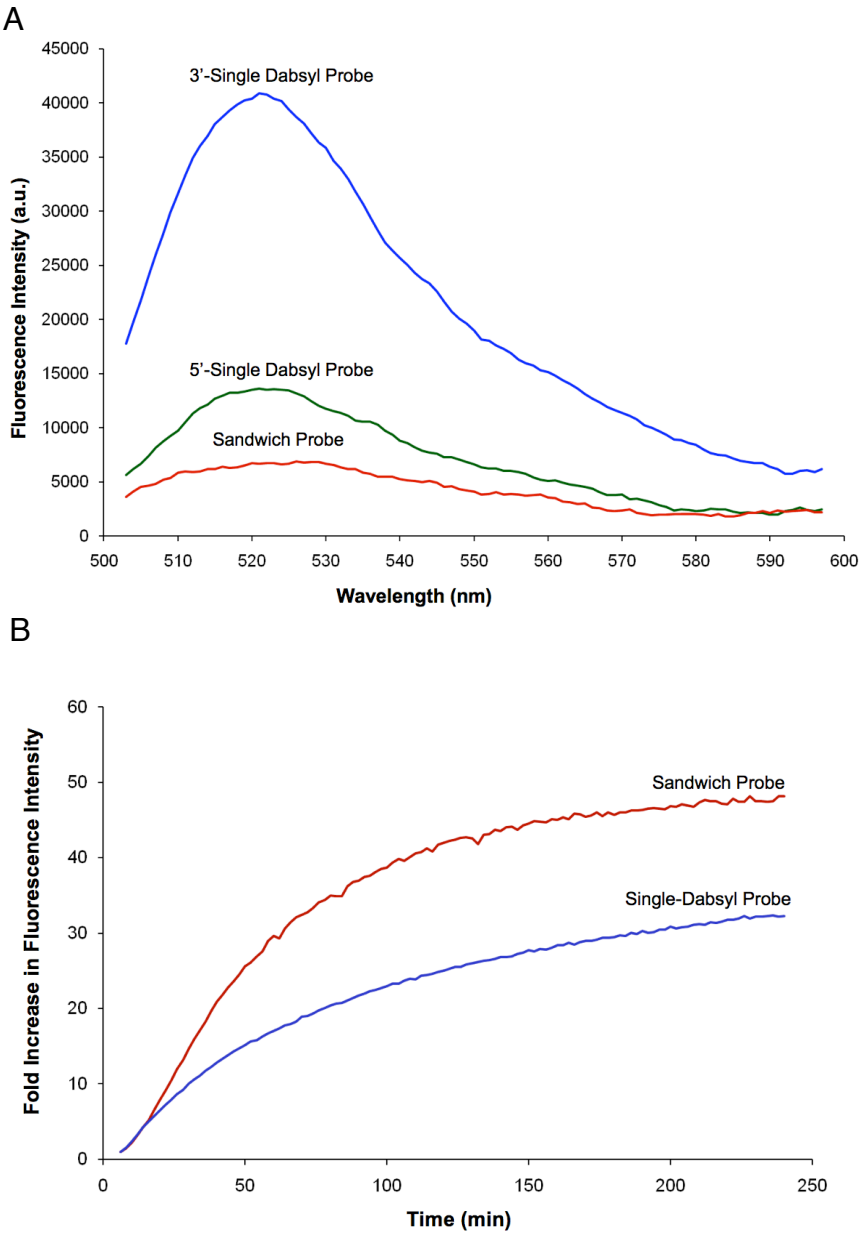


Figure S1. Quenching efficiency of Sandwich probes. (A) Relative initial quenching (residual fluorescence spectra) of different quencher-substituted probes, showing enhanced quenching of Sandwich probe. Conditions: 100 nM probe in pH 7.0 buffer containing 70 mM PIPES, 10 mM $MgCl_2$, 50 μM DTT at 25°C. Excitation was at 494 nm and the emission was measured from 500 to 600 nm. A 7-point running average was used to smooth the data. Interestingly, the 5' single dabsylate probe shows better quenching than the 3' single dabsylate probe, suggesting that the 5' dabsylate has a greater contribution to the Sandwich probe quenching than the 3' quencher. This is most likely due to differences in the distance between the quenchers and the fluorophore, and in flexibility of the two different dabsylate linkers. (B) Plot of fold increase in fluorescence in presence of template, showing advantage in light-up signal for Sandwich probe over prior single-dabsyl probe. Conditions are as in Fig. 2 (main text).

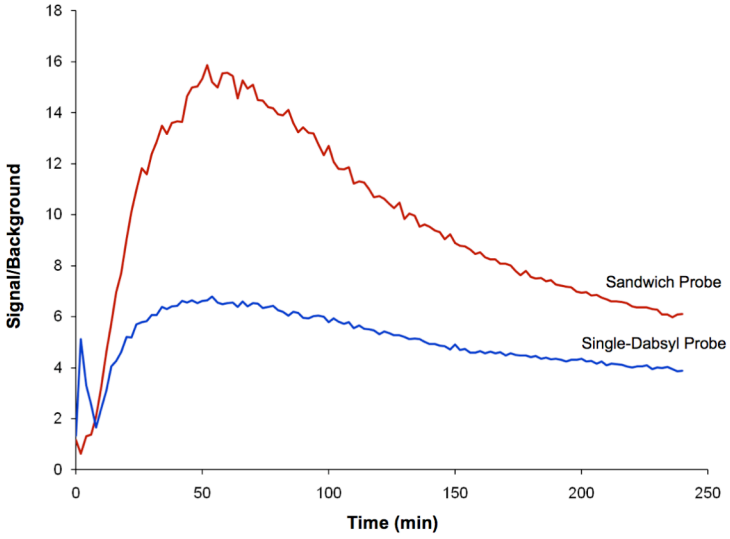
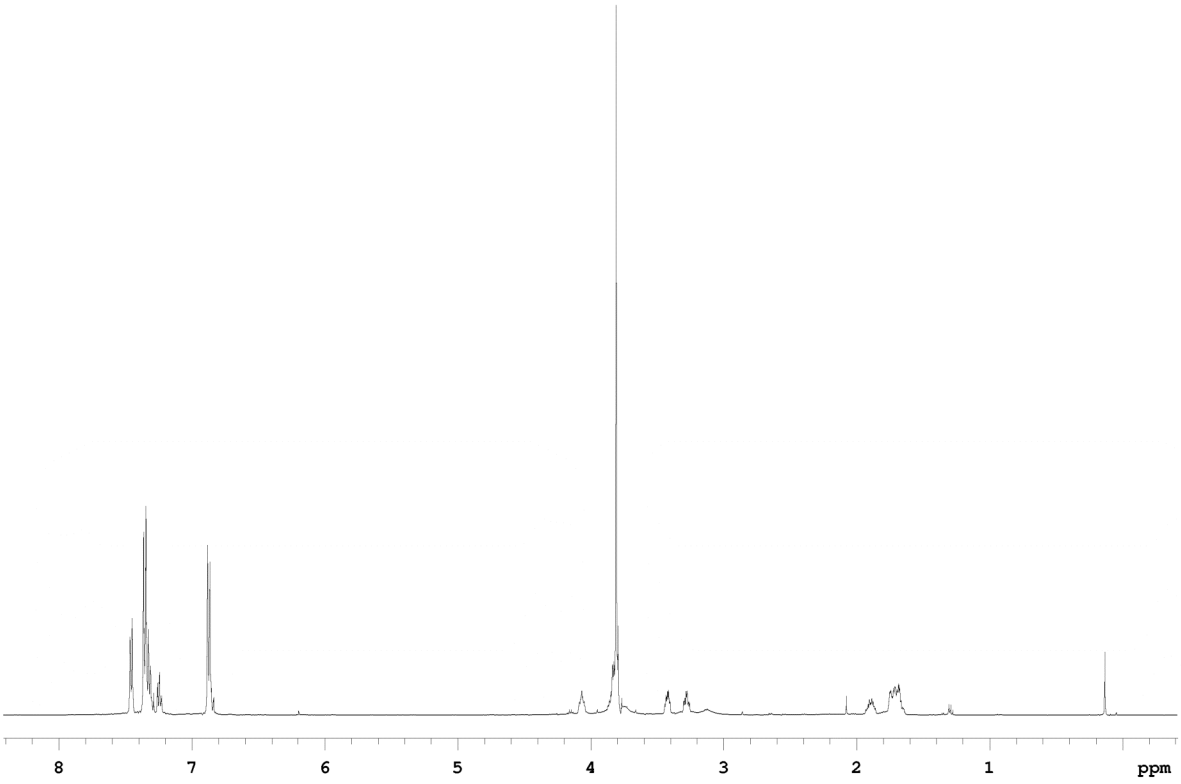
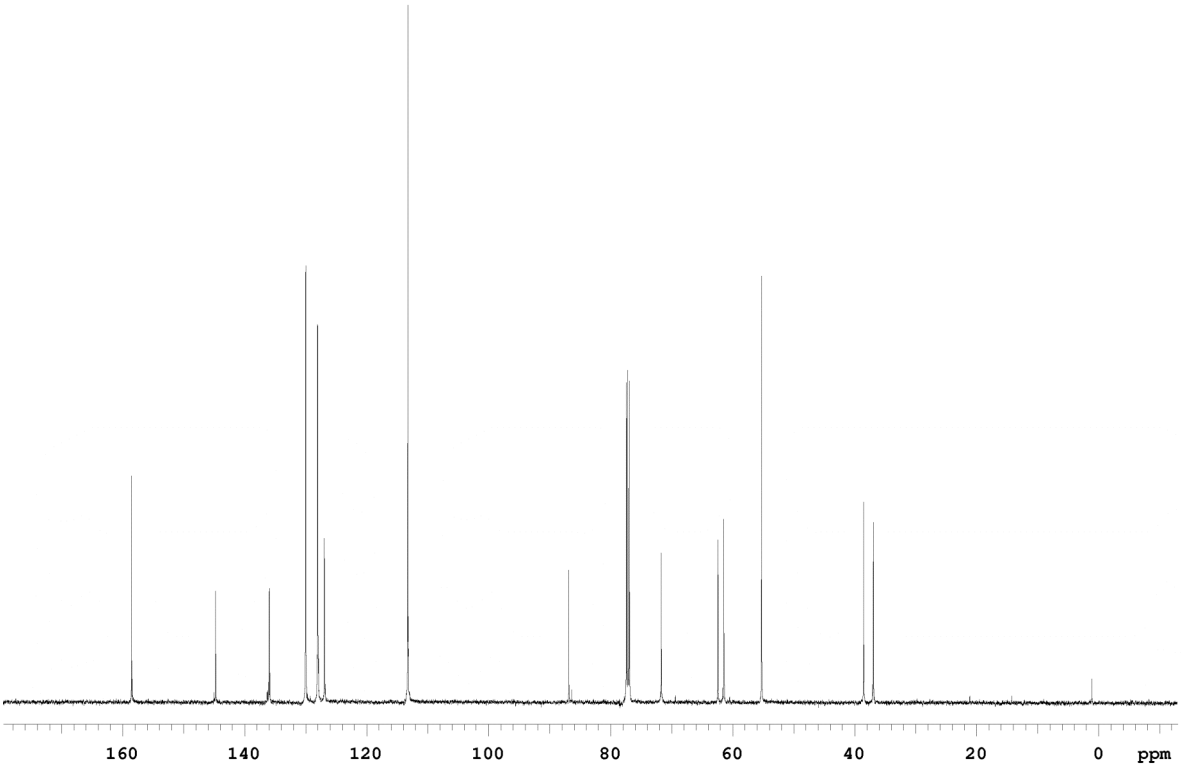


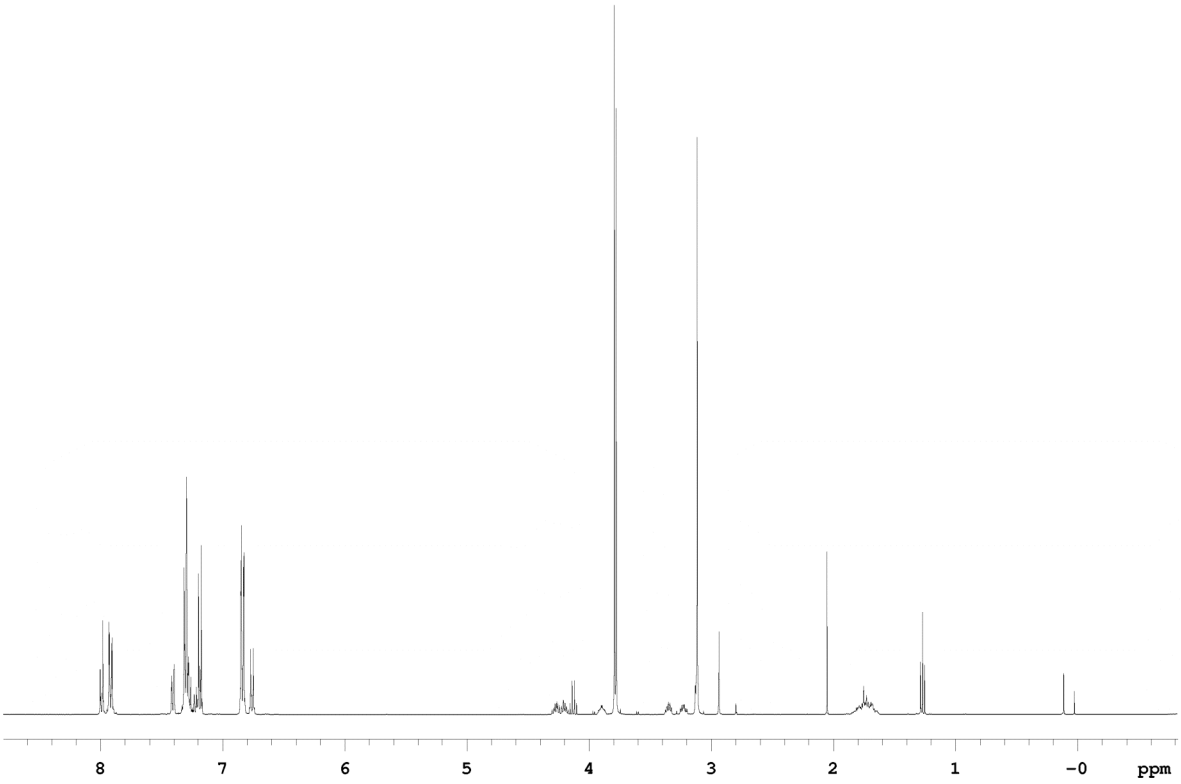
Figure S2. Signal/background of Sandwich probes. Shown is a plot of signal/background for Sandwich probes during templated reaction, with comparison to prior 5'-single-dabsyl probes. Signal/background data were obtained by dividing raw fluorescence signal intensity of templated reactions by the intensity for identical reaction mixtures in the absence of a template. Reactions were run in 70 mM PIPES buffer with 10 mM $MgCl_2$ and 50 μM DTT for 4 h at 37 °C with 494 nm excitation and monitoring emission every two minutes at 522 nm. Quencher probes were added to reaction mixture after four minutes and nucleophile probes were added four minutes after that to initiate reaction.

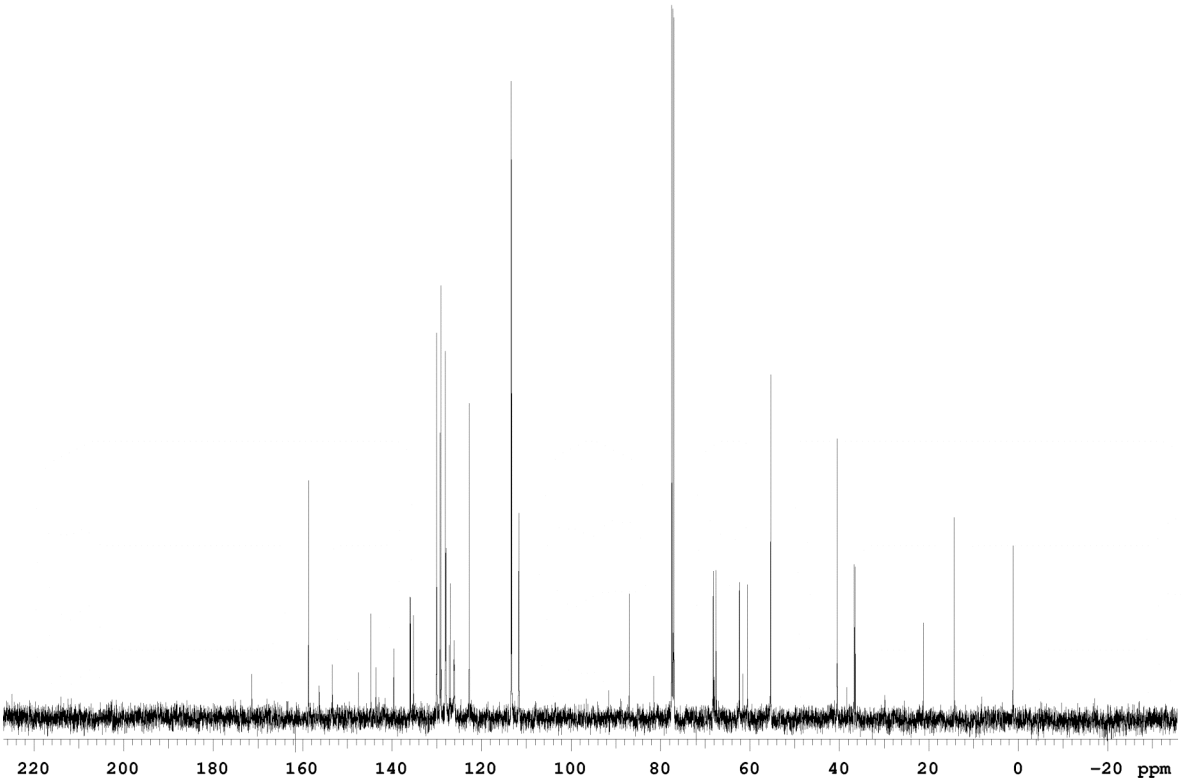
^{1}H, ^{13}C NMR spectra of Compound **2**



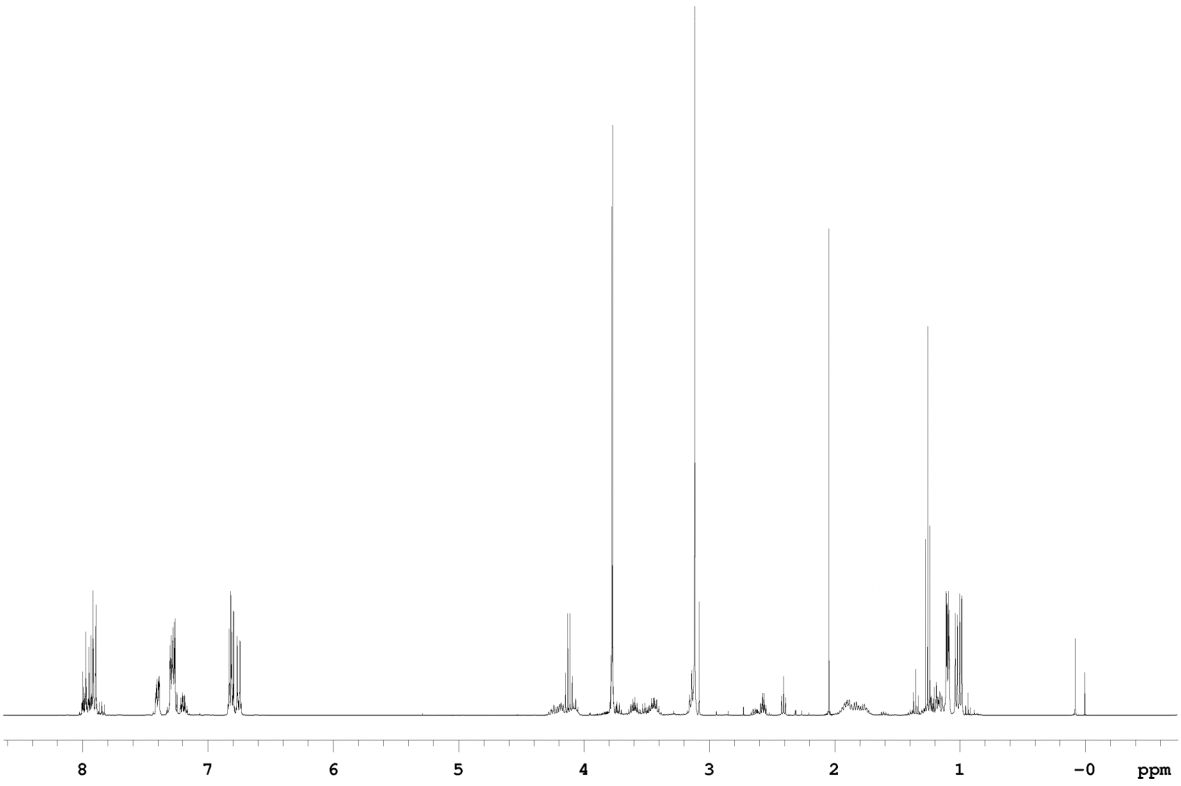


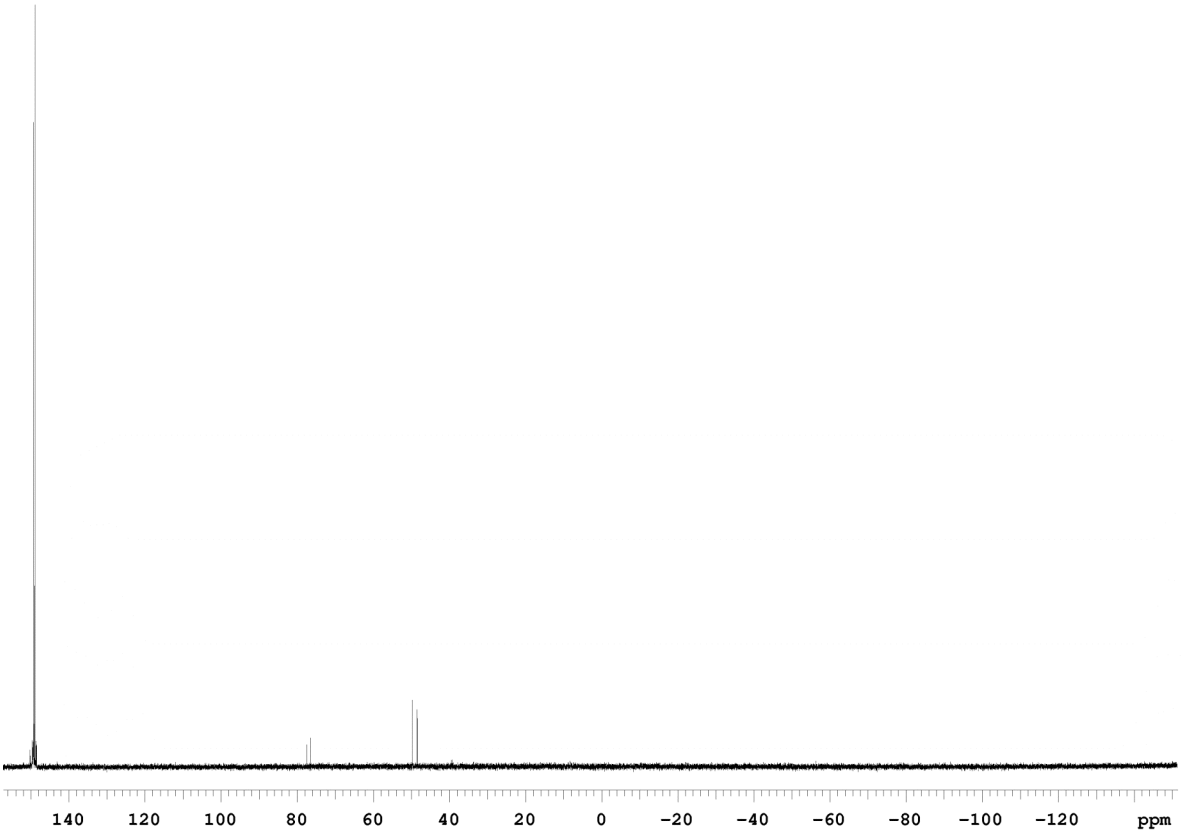
^{1}H, ^{13}C NMR spectra of Compound **3**





^{1}H, ^{13}C NMR spectra of Compound **4**





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