SUPPORTING INFORMATION

Photo-Triggering of Caged Fluorescent Oligodeoxynucleotides

XinJing Tang and Ivan J. Dmochowski*

Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104-6323

CONTENTS:

Synthesis of photocleavable DABSYL compound:

5-bromomethyl-2-nitroacetophenone was converted to intermediate 5-methylammonium-2-nitroacetophenone chloride following literature procedures.[1] The intermediate was added to 10 ml DMF and filtered, the filtrate was transferred to a 50 ml flask, and 4-dimethylaminoazobenzene-4'-sulfonyl chloride (1.0 g, 3.09 mmol) in 10 ml DMF and triethylamine (0.85 ml, 6.2 mmol) were added. The reaction was stirred overnight. Chloroform (50 ml) was added, and the solution was washed with saturated sodium chloride. The aqueous layer was extracted with chloroform (40 ml) three times. The combined organic layer was dried with anhydrous sodium sulfate. Silica gel column chromatography with hexanes/ethyl acetate (2:1) gave a red solid 5-(4-dimethylaminoazobenzene-4'-sulfonylamidomethyl)-2-nitroacetophenone (0.76 g, yield 53%). ^{1}H NMR ($CDCl_3$, 250MHz) δ 2.49 (s, 3H), 3.13 (s, 6H), 4.30 (d, 2H), 5.04 (t, 1H), 6.78 (d, 2H), 7.28 (d, 2H), 7.50 (d, 1H), 7.8~8.1 (m, 6H); EI-MS, m/e 481.8 (M^+).

A solution of 5-(4-dimethylaminoazobenzene-4'-sulfonylamidomethyl)-2-nitroacetophenone (0.76 g, 1.58 mmol) in 15 ml methanol was cooled to 0 ^{o}C and $NaBH_4$ (0.247 g, 6.5 mmol) was added. The reaction was stirred at 0 ^{o}C for 1 hour and at room temperature for 2 hours. The reaction was quenched by addition of acetone. The solvent was removed by vacuum. The residue was neutralized with 0.1 M HCl and extracted with ethyl acetate and washed with saturated sodium chloride. The organic layer was dried by anhydrous sodium sulfate. Silica gel column chromatography with chloroform/ethyl acetate (4:1) gave a red solid 5-(4-dimethylaminoazobenzene-4'-sulfonylamidomethyl)-1-(2-nitrophenyl)ethanol (0.69 g, yield 90%). ^{1}H NMR ($CDCl_3$, 250MHz) δ 1.49 (d, 3H), 3.13 (s, 6H), 4.28 (d, 2H), 4.95 (t, 1H), 5.39 (q, 1H), 6.70 (d, 2H), 7.32 (d, 2H), 7.68 (s, 1H), 7.8~8.1 (m, 7H); EI-MS, m/e 483.8 (M^+).

To a solution of 5-(4-dimethylaminoazobenzene-4'-sulfonylamidomethyl)-1-(2-nitrophenyl)ethanol (0.49 g, 1.01 mmol) in 20 ml dry acetonitrile was added N,N'-disuccinimidyl carbonate (456 mg, 1.8 mmol) and triethylamine (500 μl, 3.6 mmol). The mixture was them stirred at room temperature for 6 hours under Ar. The solvent was removed, 40 ml ethyl acetate was added, and the organic layer was washed with 0.1 M aqueous sodium bicarbonate solution and dried by anhydrous sodium sulfate. Silica gel column chromatography with chloroform/ethyl acetate with a gradient of 10:1 to 4:1 gave a pure red solid, 5-(4-dimethylaminoazobenzene-4'-sulfonylamidomethyl)-1-(2-nitrophenyl)ethanol NHS ester (220 mg, yield 35%). ^{1}H NMR ($CDCl_3$, 250MHz) δ 1.68 (d, 3H), 3.13 (s, 6H), 4.30~4.37 (m, 2H), 5.86 (t, 1H), 6.28 (q, 1H), 6.76 (d, 2H), 7.32 (d, 2H), 7.50 (s, 1H), 7.8~8.0 (m, 7H); EI-MS, m/e 624.8 (M^+)(100%), 465.8 (60%).

Oligonucleotide synthesis and purification:

Modified 5'-O-tritylated phosphoramidite cytidines were synthesized according to the procedure in Scheme S1, following standard protocols.[2-6] Oligodeoxynucleotides were synthesized on a 1.0 μmole scale on a PerSeptive Biosystems Expedite nucleic acid synthesis system using standard protocols in the "trityl off" mode with **6** and **Tg** or "trityl on" mode with oligonucleotides **2**, **4** and **5**, with extended coupling time (**X**, 5 min; **Y**, 15 min) for modified cytidine phosphoramidites. The coupling yields of modified cytidines were generally greater than 95% as determined by trityl cation monitoring.

Oligodeoxynucleotides **2** and **5** were treated with 10% DEA in acetonitrile for 5 minutes while still on the support, followed by a rinse with acetonitrile to remove all acrylonitrile. Oligonucleotides **2**, **4**, **5**, **6** and **Tg** were then cleaved and deprotected using concentrated ammonium hydroxide. The oligonucleotides **2**, **4**, and **5** with trityl still on were purified by reverse-phase HPLC (250 × 4.6 mm C18 column); eluents: 0.05 M triethylammonium acetate (A) and acetonitrile (B); gradient, 0-20 min, 5-60% B in A+B; flow rate, 1 ml/min.

Removal of the 4,4'-dimethoxytrityl group was performed by treating the purified oligonucleotides with 80% aq. acetic acid for 20 min at room temperature. After neutralization with triethylamine, followed by drying under vacuum, the oligonucleotides were again purified by HPLC: same C18 column, eluent gradient, 0-20 min, 5-60% B in A+B, 20-30 min 60% B in A+B; flow rate, 1 ml/min.

Oligonucleotides **1** and **3** were synthesized by the following procedure: To the solution of purified oligonucleotides **2** and **4** in 500 μl of 0.1 M sodium bicarbonate, pH 8.5, a total of 50- to 100-fold excess of the photocleavable DABSYL compound in 100 μl DMF was added in 10-μl aliquots at 20-min intervals. After stirring at 37 ^{o}C for 70 h, the solution was centrifuged to remove the particulate material and the supernatant was run through a gel filtration column to remove excess DABSYL. **1** and **3** were purified by HPLC: same C18 column, eluent gradient, 0-20 min, 5-60% B in A+B, 20-30 min 60% B in A+B; flow rate, 1 ml/min. The coupling yields were approximately 50% for transformation **2** → **1**, and 54 % for transformation **4** → **3**, according to the HPLC traces. All oligodeoxynucleotides were characterized by MALDI-TOF mass spectrometry (Table S1).

Table S1. Mass spectrometry data for all synthesized oligodeoxynucleotides

	Sequence (5'— 3')	Calculated	Observed
1	ATCCACAGCAGC**ZY**CTCCATCATCC	8404.1	8399.0*
2	ATCCACAGCAGC**XY**CTCCATCATCC	7896.3	7899.9
3	ATCCACAGCAGC**Z**CCTCCATCATCC	8002.8	7995.4*
4	ATCCACAGCAGC**Y**CCTCCATCATCC	7853.3	7856.4
5	ATCCACAGCAGC**X**CCTCCATCATCC	7494.0	7488.5
6	ATCCACAGCAGCCCCTCCATCATCC	7452.0	7451.5
Tg	GGATGATGGAGGGGCTGCTGTGGAT	7874.0	7856.7

Modified nucleotides in bold; **X**, aminolinked dC; **Y**, fluoresceinated aminolinked dC; **Z**, PC-DABSYL aminolinked dC.

*For both **1** and **3**, MALDI-TOF (λ_{ex} = 337 nm) gave the expected masses, as well as the photocleavage products, with masses corresponding to **2** and **4**. At this wavelength and laser power, an additional photo-product was observed that we assign to photochemical deazatization of the DABSYL group.[7,8] ESI mass spectrometry was performed to confirm the identities of **1** and **3**.

Thermal denaturation methods:

Solutions for the thermal denaturation studies contained a 1:1 ratio of one of six oligodeoxynucleotides (**1**-**6**) with the complementary target, **Tg**, in SSC buffer (pH 7). The solution was first heated to 90 °C for 5 min, then allowed to cool to room temperature for 2.5 h. Melting studies were carried out on an Agilent 8453 UV-Vis spectrophotometer equipped with a programmable Peltier temperature controller (Agilent 89090A). Samples were stirred and monitored at 260 nm while heating or cooling at a rate of 1.0 °C/min, with a 1 min hold per degree Celsius. Melting temperatures were determined from the peak of the first derivative plot of Abs_{260} vs. temperature. All the melting curves are shown in Figure S1.

Photolysis and fluorescence experiments:

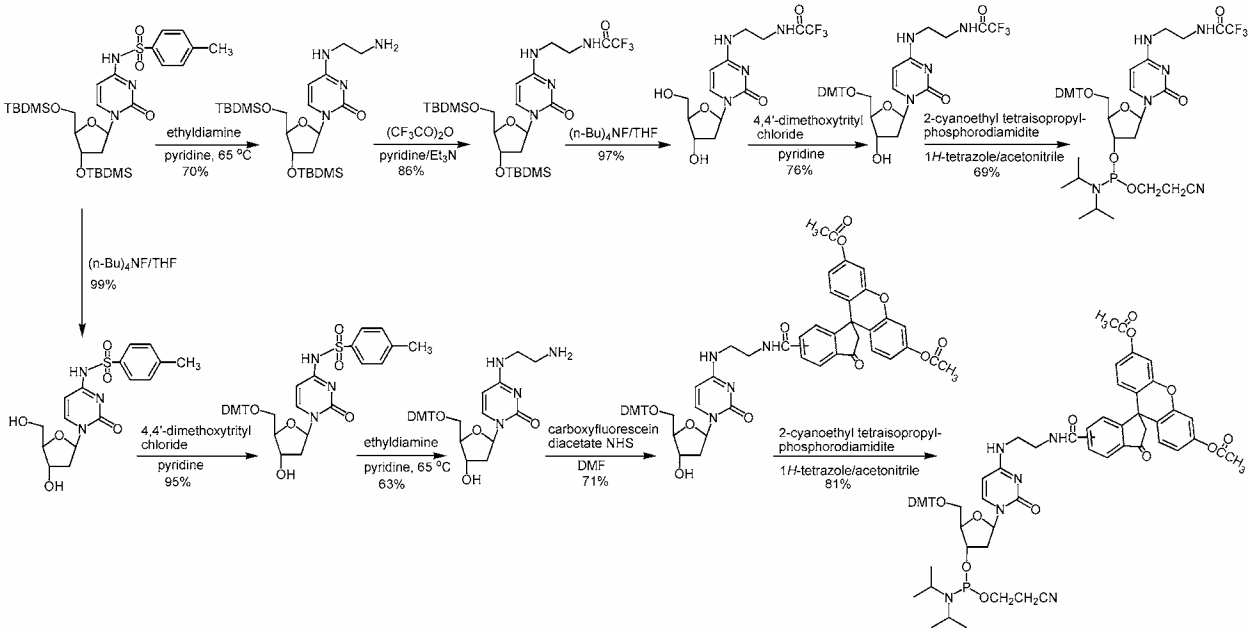
Photolysis and fluorescence measurements were carried out on a Spex Fluorolog-3 spectrofluorometer (supplied by Horiba, manufactured by Jobin Yvon). Solutions for photolysis and fluorescence experiments were prepared in SSC buffer (pH 7) and degassed with Ar for 15 min. The solution was scanned from 500-650 nm (λ_{ex} = 491 nm) before irradiation and after every minute of irradiation (λ_{irr} = 355 nm, 36mW/cm^2), to monitor the progress of the photocleavage reaction.

Confocal imaging experiments:

Oligodeoxynucleotide **1** was microinjected into one-cell zebrafish embryos using a Zeiss Stemi SV 11 light microscope, Harvard Apparatus microinjector, and Narashige micromanipulator. The injected embryos were cultured 24 hours in the dark in a 28 °C incubator, and then irradiated for 2 hours with 365 nm light from a hand-held light source (~0.5 mW/cm^2) at 28 °C. The image and spectra (Figure 3 in the manuscript) were collected with a Zeiss LSM-510 Meta confocal microscope using a 40x, 1.2 N.A. water immersion lens. The embryos were irradiated at 488 nm with an Ar-ion laser, and fluorescence spectra were collected between 500 and 610 nm, in order to distinguish fluorescein emission from background autofluorescence.

Injected zebrafish embryos containing CFO **1** were alternatively irradiated directly with an onboard UV ion laser (Coherent II, 351 nm, 80 mW) and imaged with the Zeiss LSM-510 Meta confocal microscope, as described above. This procedure led to observed emission of the fluoresceinated oligonucleotide within milliseconds, followed by virtually instantaneous bleaching of the fluorescent signal. Protocols to optimize photoactivation of these compounds are under development.

Scheme S1. Synthesis of modified 5'-O-tritylated phosphoramidite cytidines



Scheme S2. Synthesis of CFO **1**, modified with fluorescein and photocleavable DABSYL

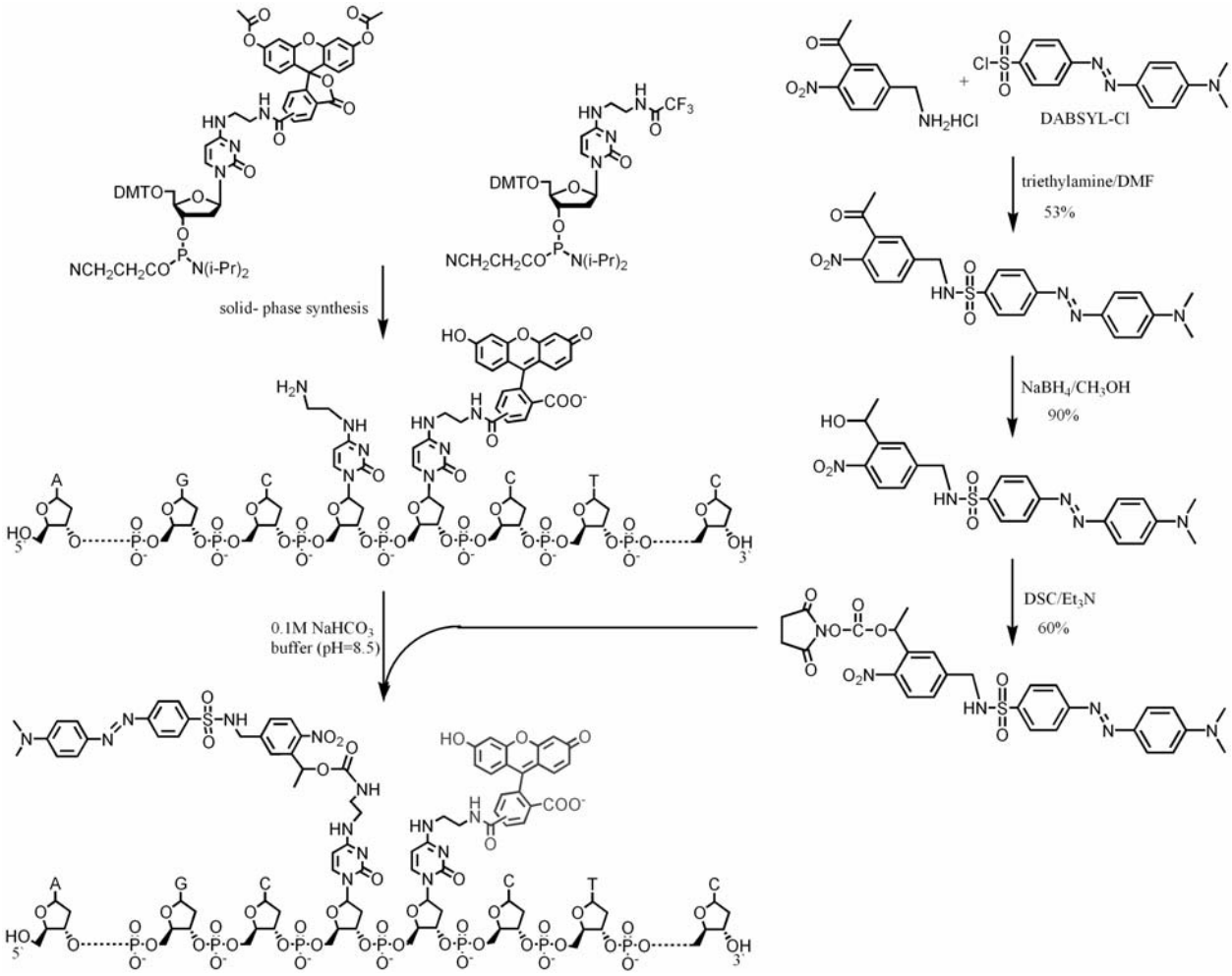
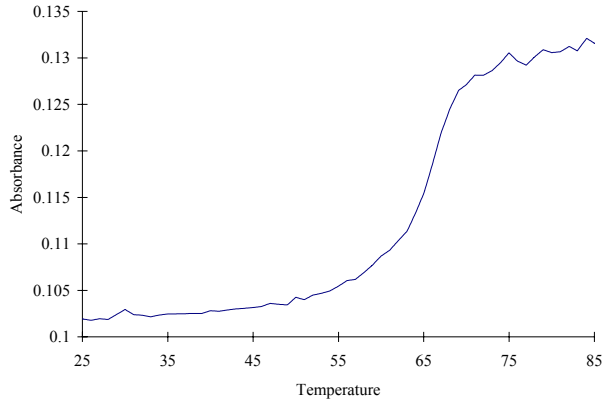
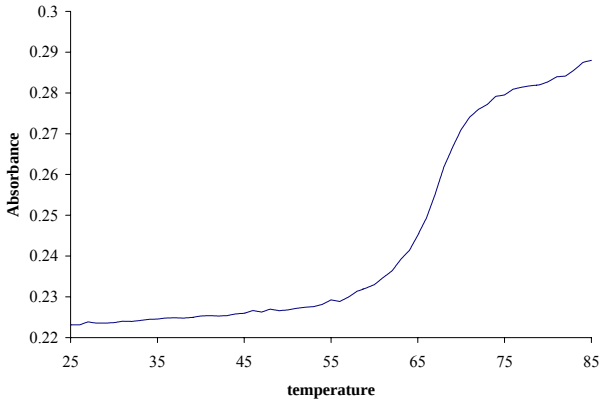


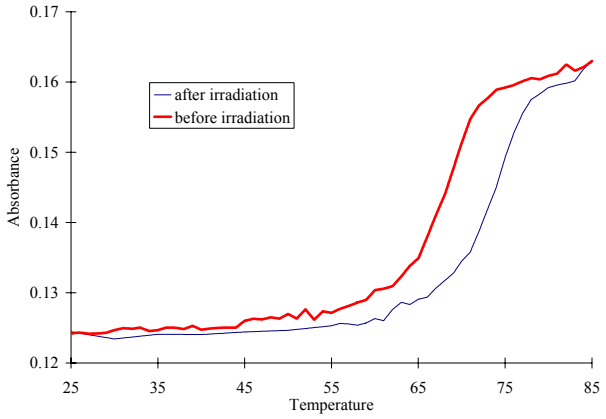
Figure S1. Melting curves of oligodeoxynucleotides **1-6** with target, **Tg**



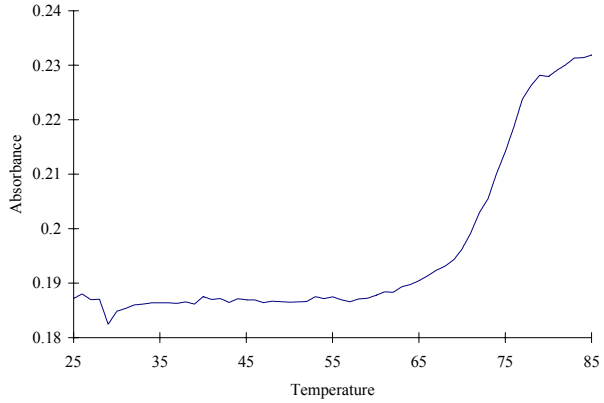
Oligo **1** and Target before irradiation



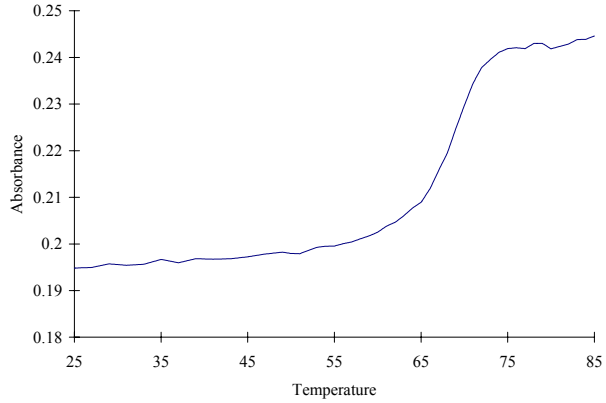
Oligo **2** and Target



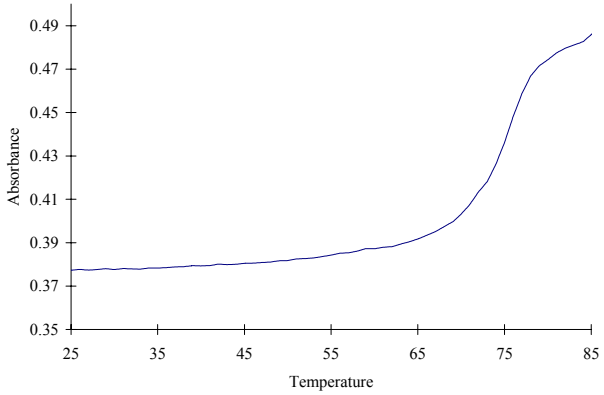
Oligo **3** and Target before and after irradiation



Oligo **5** and Target

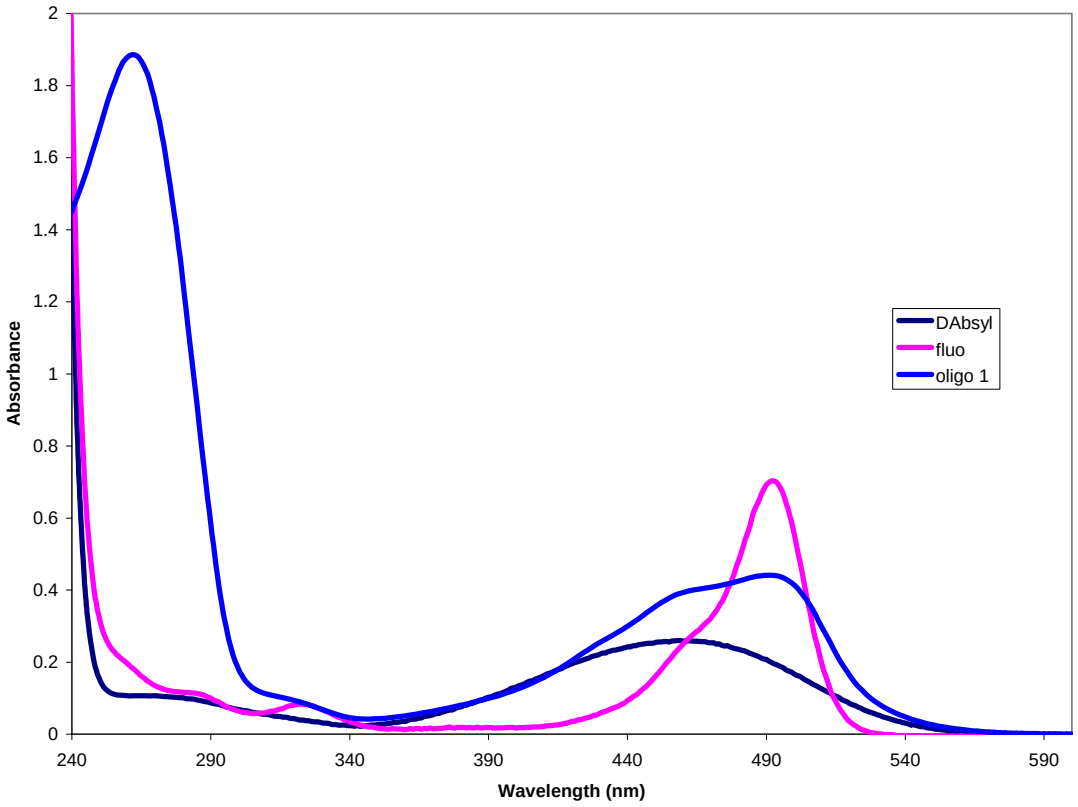


Oligo **4** and Target



Oligo **6** and Target

Figure S2. UV-Vis data for CFO **1,** fluorescein, and DABSYL



References

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