# Synthesis and Evaluation of the Sensitivity and Vibrational Lifetimes of Thiocyanate and Selenocyanate Infrared Reporters

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## ELECTRONIC SUPPLEMENTARY INFORMATION

## Experimental

**General.** All reagents were commercially available and were used without further purification unless otherwise noted. All reactions were stirred using a magnetic stir bar. Reactions above room temperature were heated on a hot plate and reactions below room temperature were cooled in a Dewar flask containing ice and brine. Analytical thin layer chromatography (TLC) was conducted on Macherey-Nagel polygram SIL G/UV TLC-sheets, preparatory TLC was done on Analtech Silica Gel 20×20 cm plates, and column chromatography was done using 230-400 mesh silica gel. ATR-IR spectra for compound characterization were obtained on a thin film and the absorptions were reported in cm$^{-1}$. NMR spectra were obtained on a Varian INOVA 500 multinuclear Fourier transform NMR spectrometer. NMR spectra were recorded at 500 MHz for $^{1}$H and at 125 MHz for $^{13}$C NMR. NMR spectra were recorded using $CDCl_3$ as the solvent and were referenced to tetramethylsilane as an internal standard. All coupling constants (*J*) are reported in Hertz. Mass spectrometry data were collected using an Agilent 1100 Series Liquid Chromatograph Mass Spectrometer using electrospray ionization (ESI). *N*-(*tert*-Butoxycarbonyl)-4-iodo-L-phenylalanine was purchased from PepTech. Arabinosyl adenine (**1**, ara-A, vidarabine) was purchased from Chem-Impex. *N*-(*tert*-Butoxycarbonyl)-4-iodo-L-phenylalanine methyl ester (**4**) was prepared by the method of Miyake-Stoner et al.,[1] 9-[2-*O*-(Trifluoromethanesulfonyl)-3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanyl)-β-D-arabinofuranosyl] adenine was prepared by method of Puffer et al.[2] and Robins et al.[3]
**Abbreviations:** Attenuated total reflectance (ATR), electrospray ionization (ESI), N,N-dimethylformamide (DMF), ethyl acetate (EtOAc), deionized water (di. $H_2O$), petroleum ether (PE), tetrahydrofuran (THF), absolute ethanol (EtOH), 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TIPDSiCl_2$), trifluoromethanesulfonyl chloride (TfCl), palladium (II) acetate ($Pd(OAc)_2$) 1,3-bis(diphenylphosphino)propane (dppp), trioctylsilane ($Oct_3SiH$), triethylamine ($NEt_3$), methanol (MeOH).

**2'-Thiocyano-2'-deoxy-3',5'-*O*-(1,1,3,3-tetraisopropyl-1,3-disi-1oxanyl) adenosine, $Si_2$-dA-SCN**. 9-[2-*O*-(Trifluoromethanesulfonyl)-3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanyl)-β-D-arabinofuranosyl]adenine[3] (0.2620 g, 0.4082 mmol) was dissolved in DMF (3 mL) in a 4-dram vial with a magnetic stir bar and ammonium thiocyanate (0.1647 g, 2.164 mmol) was added. The reaction mixture was stirred under argon while the capped vial was heated at 50 °C. The reaction was monitored by TLC (1:1 EtOAc/PE) and was complete after 4 h. The reaction mixture was diluted with di. $H_2O$ and extracted with diethyl ether (6×). The combined organic phases were washed with brine, dried ($Na_2SO_4$), and concentrated under reduced pressure to yield 0.2089 g of a tan powder. The crude product was purified by preparatory TLC (3:1 EtOAc/PE) to yield 71.2 mg (32%) of **$Si_2$-dA-SCN** as a tan powder: $^{1}$H NMR δ 8.27 (s, 1H), 7.94 (s, 1H), 6.28 (d, *J* = 3.4, 1H), 6.15 (br s, 2H), 5.50 (t, *J* = 7.4, 1H), 5.05 (dd, *J* = 7.4, *J* = 3.4, 1H), 4.12 (m, 1H), 4.07 (d, *J* = 5.4, 2H), 1.15-0.99 (m, 28H) ppm; $^{13}$C NMR δ 12.68, 12.74, 13.08, 13.24, 16.84, 16.88, 17.00, 17.16, 17.25, 17.30, 17.32, 17.41, 54.33, 62.20, 71.51, 83.88, 89.64, 111.28, 120.60, 139.76, 149.17, 153.16, 155.84 ppm; IR (ATR) ν 3324.7 (w), 3176.7 (w), 2945.2 (m), 2867.2 (m), 2156.2 (w), 1649.9 (m), 1597.4 (m), 1464.3 (m), 1141.2 (s), 1090.0 (s), 1032.9 (vs), 884.3 (s), 857.0 (s), 694.6 (s) cm$^{-1}$; MS (ESI) 551.2 (M+1); HRMS (ESI) calcd. for $C_{23}H_{39}N_6O_4SSi_2$ [M + 1]$^+$ 551.2292; found 551.2285.

**2’-Selenocyano-2’-deoxy-3’,5’-*O*-(1,1,3,3-tetraisopropyl-1,3-disi-1oxanyl) adenosine, $Si_2$-dA-SeCN**. 9-[2-*O*-(Trifluoromethanesulfonyl)-3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanyl)-β-D-arabinofuranosyl]adenine[3] (0.4914 g, 0.7656 mmol) was dissolved in DMF (8 mL) in a 4-dram vial and potassium selenocyanate (0.5633 g, 3.910 mmol) and 18-crown-6 (0.0242 g, 0.0916 mmol) were added. The reaction mixture was stirred under argon while the capped vial was heated at 50 °C. The reaction was monitored by TLC (3:1 EtOAc/PE) and was complete after ~4 h. The reaction mixture was diluted with di. $H_2O$ and extracted with diethyl ether (7×). The combined organic phases were washed with brine, dried ($Na_2SO_4$), and concentrated under reduced pressure to yield 0.2923 g (64%) of **$Si_2$-dA-SeCN** as a tan powder. A portion of **$Si_2$-dA-SeCN** was purified by preparatory TLC (3:1 EtOAc/PE) for characterization: $^1$H NMR δ 8.26 (s, 1H), 7.91 (s, 1H), 6.48 (d, *J* = 2.9, 1H), 6.14 (br s, 2H), 5.59 (t, *J* = 7.1, 1H), 5.32 (dd, *J* = 7.4, *J* = 3.0, 1 H), 4.19 (m, 1H), 4.07 (m, 2H), 1.21-1.00 (m, 28H) ppm; $^{13}$C NMR δ 12.72, 13.10, 13.24, 16.87, 16.91, 17.04, 17.19, 17.25, 17.34, 17.41, 52.78, 63.00, 72.86, 84.48, 90.51, 101.89, 120.66, 139.84, 148.99, 153.04, 155.85 ppm; IR (ATR) ν 3321.1 (w), 3176.5 (w), 2944.5 (m), 2866.9 (m), 2150.3 (w), 1650.6 (m), 1596.6 (m), 1463.9 (m), 1113.3 (m), 1090.9 (s), 1032.1 (vs), 883.8 (s), 852.2 (s), 693.3 (s) cm$^{-1}$; MS (ESI) 599.2 (M+1). HRMS (ESI) calcd. for $C_{23}H_{39}N_6O_4SeSi_2$ [M + 1]$^+$ 599.1737; found 599.1750.

***N*-(*tert*-Butoxycarbonyl)-4-hydroxymethyl-L-phenylalanine methyl ester (6).** Protected Phe **5** (5.0187 g, 12.385 mmol) was dissolved in DMF (60 mL) and potassium carbonate (4.2860 g, 31.011 mmol), palladium (II) acetate (225.0 mg, 1.002 mmol), and 1,3-bis(diphenylphosphino)propane (819.3 mg, 1.986 mmol) were added to form a heterogeneous mixture. The mixture was stirred and heated at 70 °C for 10 min and then the flask headspace was purged with carbon monoxide gas for 5 min via an inlet needle and a second exit needle. Trioctylsilane (11.2 mL, 24.9 mmol) was added via a syringe, and the exit needle was removed. The flask headspace was then purged with carbon monoxide gas for an additional 15 s. The reaction was monitored by TLC (4:6 EtOAc/hexane) and was complete after ~4 h. The reaction mixture became black over time, with a slight red color remaining towards the bottom of the flask. The reaction mixture was cooled to 0 °C, and sodium borohydride (564.0 mg, 14.91 mmol) and methanol (12 mL) were added. The mixture was stirred and allowed to return to room temperature, and TLC indicated it was complete after 45 min. The reaction was quenched with 10% aqueous acetic acid slowly until all excess sodium borohydride had reacted, and then further diluted with di. $H_2O$. The mixture was extracted with EtOAc (5×) and the combined organic phases were washed with brine, dried ($Na_2SO_4$), and concentrated under reduced pressure. The crude product was purified by column chromatography (40-60% EtOAc/hexane) to yield 1.0826 g (28%) of **6** as a yellow oil. The $^1$H NMR spectrum matched the literature.[4]

***N*-(*tert*-Butoxycarbonyl)-4-thiocyanomethyl-L-phenylalanine methyl ester (Boc-Me-Phe$CH_2$SCN).** To a flame dried round bottom flask was added hydroxymethyl Phe **5** (1.0136 g, 3.2764 mmol), dichloromethane (6 mL), and triethylamine (0.690 mL, 4.95 mmol). The solution was stirred at 0 °C and methanesulfonyl chloride (0.330 mL, 4.26 mmol) was added slowly. The solution was allowed to warm passively to room temperature. The reaction was monitored by TLC (8:2 EtOAc/Hexane) and was complete after ~45 min. The mixture was diluted with di. $H_2O$ and extracted with dichloromethane (3×). The combined organic phases were washed with brine, dried ($Na_2SO_4$), and concentrated under reduced pressure to yield 1.1145 g (88%) of mesylate **6,** which was taken to the next step without purification.

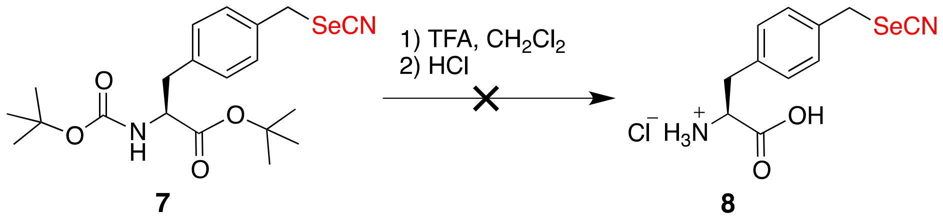
A portion of **6** (0.4150 g, 1.071 mmol) was dissolved in DMF (5 mL) in a 4-dram vial and sodium thiocyanate (0.2243 g, 2.768 mmol) and 15-crown-5 (33.2 µL, 0.168 mmol) were added. The mixture was stirred at 50 °C for 4 days and then diluted in di. $H_2O$ and extracted with EtOAc (2×). The combined organic layers were washed with saturated aqueous ammonium chloride, brine, dried ($Na_2SO_4$), and concentrated under reduced pressure. The crude material was purified by column chromatography (35% EtOAc/hexane) to yield 0.1452 g (39%) of **Boc-Me-PheCH$_2$SCN** as a white powder: $^1$H NMR δ 7.30 (d, *J* = 8.3, 2H, ArH), 7.16 (d, *J* = 8.3, 2H, ArH), 5.05 (d, *J* = 7.9, 1H, NH), 4.59 (m, 1H, $C_\alpha$-H), 4.14 (s, 2H, $CH_2SCN$), 3.71 (s, 3H, $OCH_3$), 3.13 (dd, *J* = 5.6, *J* = 13.8, 1H, $C_\beta$-$H_2$), 3.04 (dd, *J* = 6.4, *J* = 13.8, 1H, $C_\beta$-$H_1$), 1.42 (s, 9H, $C(CH_3)_3$) ppm; $^{13}$C NMR δ 28.28, 38.05, 38.14, 52.30, 54.32, 80.02, 110.70, 111.93, 129.16, 130.08, 133.07, 137.12, 155.02, 172.14 ppm; IR (ATR) ν 3369.1 (vs), 2978.0 (w), 2153.1 (w), 1697.4 (vs), 1508.3 (m), 1365.3 (m), 1162.7 (vs) cm$^{-1}$; MS (ESI) 373.1 (M+Na$^+$); HRMS (ESI) calcd. for $C_{17}H_{22}N_2O_4NaS$ $[M + Na]^+$ 373.1198; found 373.1189.

**N-(tert-Butoxycarbonyl)-4-selenocyanomethyl-L-phenylalanine methyl ester (Boc-Me-PheCH$_2$SeCN).** Mesylate **6** (0.5245 g, 1.354 mmol) from above was dissolved in DMF (10 mL) in a 4-dram vial and potassium selenocyanate (0.5935 g, 4.119 mmol) and 18-crown-6 (35.6 mg, 0.135 mmol) were added. The mixture was stirred at 50 °C for 4 days and then diluted in di. $H_2O$ and extracted with EtOAc (2×). The combined organic layers were washed with saturated aqueous ammonium chloride, brine, dried ($Na_2SO_4$), and concentrated under reduced pressure. The crude material was purified by column chromatography (35% EtOAc/hexane) and then further purified by preparatory TLC (5% EtOAc/$CH_2Cl_2$) to yield 53.0 mg (9.9%) of **Boc-Me-PheCH$_2$SeCN** as a yellow solid: $^1$H NMR δ 7.30 (d, *J* = 8.3, 2H, ArH), 7.14 (d, *J* = 7.8, 2H, ArH), 5.01 (d, *J* = 7.8, 1H, NH), 4.59 (m, 1H, $C_\alpha$-H), 4.28 (s, 2H, $CH_2SeCN$), 3.71 (s, 3H, $OCH_3$), 3.13 (dd, *J* = 5.7, *J* = 13.9, 1H, $C_\beta$-$H_2$), 3.03 (dd, *J* = 6.3, *J* = 13.9, 1H, $C_\beta$-$H_1$), 1.42 (s, 9H, $C(CH_3)_3$) ppm; $^{13}$C NMR δ 28.29, 32.50, 38.17, 52.33, 54.31, 80.05, 101.83, 129.18, 130.09, 134.10, 136.89, 155.01, 172.14 ppm; IR (ATR) ν 3367.4 (m), 2977.8 (w), 2149.9 (w), 1738.8 (s), 1694.1 (vs), 1511.5 (s), 1436.6 (m), 1365.1 (s), 1160.9 (vs) cm$^{-1}$; MS (ESI) 420.9 (M+Na$^+$); HRMS (ESI) calcd. for $C_{17}H_{22}N_2O_4NaSe$ $[M + Na]^+$ 421.0642; found 421.0634.

**Deprotection Attempts.** Attempts to deprotect Si$_2$-dA-SCN, Si$_2$-dA-SeCN, Boc-Me-PheCH$_2$SCN, and Boc-Me-PheCH$_2$SeCN under basic and acidic conditions were unsuccessful. Preliminary results suggest that brief treatment under slightly acidic conditions may be successful in deprotecting the Phe analogues (data not shown). However, prolonged exposure of the *t*-butyl ester selenocyanate analogue Boc-*t*Bu-PheCH$_2$SeCN (**7**) [prepared in an analogous manner to the methyl ester analogue] to trifluoroacetic acid did not result in the desired deprotected product PheCH$_2$SeCN (**8**), Scheme S1. Instead, mass spectral analysis indicated that diselenide **9** was formed as the major product instead (Figures S1, S2).

**Formation of Diselenide 9.** Phe analogue **7** (2.125 g, 4.828 mmoles) was dissolved in a minimal amount of $CH_2Cl_2$ (15 mL) at 0 ˚C and then trifluoroacetic acid (TFA; 3.70 mL, 0.0483 moles) was added. The mixture was stirred at room temperature for 40 h and additional TFA (1.30 mL, 0.170 moles) was added. After 36 h, $CH_2Cl_2$ (15 mL) was added and the solution was concentrated under reduced pressure. This process (addition and concentration) was repeated three more times to provide a yellow oil. The oil was dissolved in 30 mL of 0.1 M HCl solution and the solution was lyophilized for 72 h to provide 0.6468 g of a dark red solid which contained **9** as the major product. MS (ESI) 517 Da (M+1), see Figure S2.

**Scheme S1**



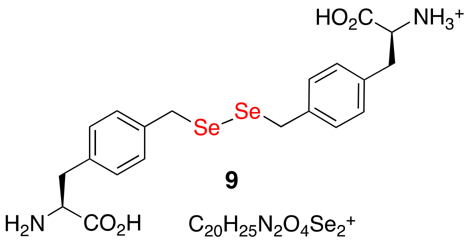


**Figure S1.** Chemical structure of diselenide product formed

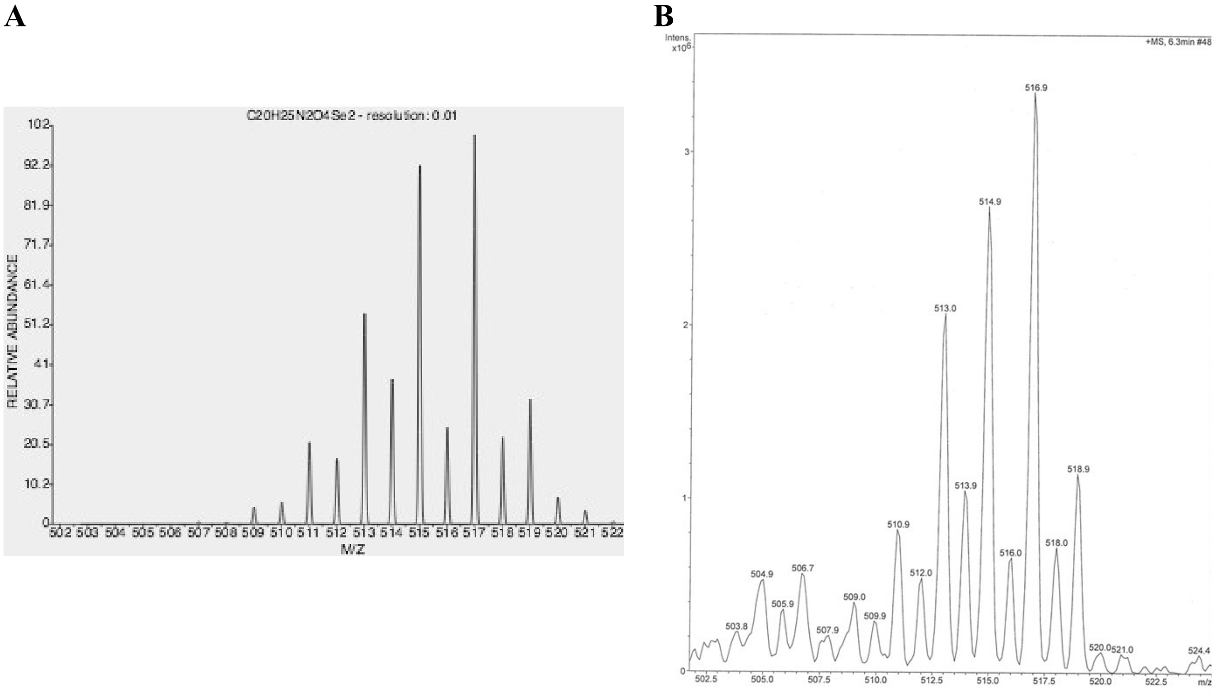


**Figure S2.** (A) Calculated[5] mass spectral isotope pattern for $C_{20}H_{25}N_2O_4Se_2^+$.
(B) Experimental mass spectrum of the product.

## Equilibrium FTIR Measurements

Transmission FTIR absorbance spectra were obtained using a Bruker Vertex 70 FTIR spectrometer at a resolution of 1.0 $cm^{-1}$ with a globar source, potassium bromide beamsplitter, and liquid nitrogen cooled mercury-cadmium-telluride detector. Each spectra was the result of 256 scans in a dual-compartment temperature-controlled transmission cell composed of calcium fluoride windows with a path length of ~125 μm. The sample temperature was maintained at 25 °C with a Thermo Haake K10/DC30 oil bath. All spectra were baseline corrected and normalized. Samples were prepared at 30-40 mM using THF (Fisher Scientific, 99.9%) or THF and 18 MΩ-cm water mixtures prepared by volume.

## Vibrational Lifetime Measurements

To measure the vibrational population relaxation, an infrared pump-probe experiment was employed. The sample concentrations were ~400–500 mM. A $CaF_2$ sample cell was used with a path length of 56 μm. Fourier-transform limited 80-fs pulse with center frequencies of 2150 and 2154 $cm^{-1}$ were used for SeCN and SCN samples in THF, respectively. The mid-IR pulse is then passed through a ZnSe beamsplitter to generate the pump (2 μJ) and probe (1 μJ) beams. The beamsize of the IR probe pulse was ~75% of the pump pulse. The pump and probe pulses of the same polarization are collected by a parabola prior to the sample ensuring a minimal crossing angle between the pump and probe pulses. A chopper was placed within the path of the pump beam in an attempt to remove scatter from the signal. The spatial overlap of the beams (~166 μm at focal point) is achieved by first using 200 μm pinhole at the sample holder, followed by maximizing the transient absorption signal of a sodium azide sample solution in $D_2O$. The temporal overlap was determined by scanning the time domain spectrum of the transient absorption of the sodium azide sample. The transmitted probe pulse is focused onto the focal plane of a monochromator equipped with a 64 element mercury-cadmium-telluride array detector (InfraRed Associates, Stuart, FL). Each detector element is 200 μm in width and 1 mm in height. The monochromator (focal length 270 mm) used a 75 lines per mm groove grating. The time dependent spectra were obtained by sampling the frequency domain spectra (averaged over 49 scans, 900 shots per scan) at various time points from 0 to 80 ps. A motorized translational stage with a temporal range of 120 ps was used to control the time delay between the two pulses. Since each scan included times prior to zero time to assure proper data collection, only 80 ps were available for data collection. A kinetic trace of $Si_2$-dA-SCN is shown starting ~50 ps before t = 0 to illustrate that no signal is observed and capture the signal to noise ratio, Figure S3. A filter was applied during data collection on a shot-to-shot basis to eliminate any data not within two standard deviations from the mean. Following Park et al.[6], the kinetic traces were fit to a single exponential function of the $S(t) = Ae^{-t/T}$, where T is the vibrational lifetime and A is the amplitude. Although the amplitude was varied during the fit, both the fit and the data were normalized with respect to that amplitude. To account for the total bandwidth associated with the transition, a global analysis approach was taken. Multiple kinetic traces for different vibrational frequencies within the transient absorption band were fitted and determined to have the same decay. In Park, et al. the heating contribution from water exists due to the OD vibrational relaxation, which was removed from the decay rate.[6] Since no significant contributions are expected for THF, an exponential decay is sufficient.

From the pump-probe spectra, Figure S4 (and Figure 5 of the manuscript) two distinct peaks representing the ground ($\nu = 0 \rightarrow 1$) transition and an anharmonically shifted excited state ($\nu = 1 \rightarrow 2$) transition are present. Each ground state transition at t = 0 is similar to the frequency as seen in the linear IR. A slight change in the energy is observed at t = 80 ps for the Boc-Me-

PheCH$_2$SCN. Due to its larger transition dipole, the IR absorption could be contributing to the broadening of the peak of the $\nu = 0 \rightarrow 1$ transition at later time. The average value of the anharmonicity for all four samples was determined to be 21 cm$^{-1}$. A baseline spectrum was measured at $t < 0$ (before $t = 0$) for each sample for comparison of the signal-to-noise ratios. The observed signal-to-noise ratio is the lowest for the Boc-Me-PheCH$_2$SeCN measurements and the highest for the Boc-Me-PheCH$_2$SCN measurements.

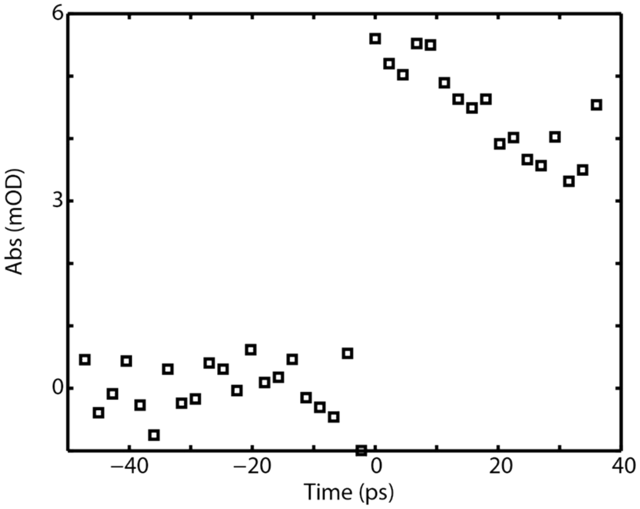


**Figure S3.** Kinetic trace of excited state absorption for Si$_2$-dA-SCN starting ~50 ps before the observed signal.

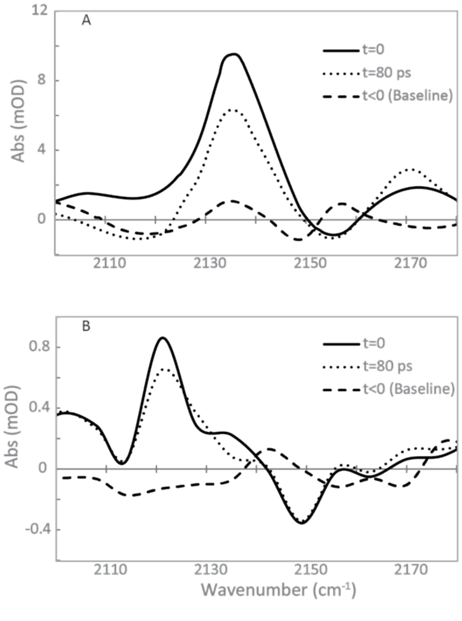


**Figure S4.** Transient absorption spectra of (A) Si$_2$-dA-SCN and (B) Boc-Me-PheCH$_2$SeCN at t = 0, t = 80 ps, and t < 0.

## References

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