Supporting Information

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1. MATERIALS AND METHODS

1.1. General

DNA oligonucleotides were purchased from Integrated DNA Technologies, disuccinimidyl glutarate (DSG) from Thermo Fisher Scientific, succinimidyl 4-hydrazidoterephthalate hydrochloride (SHTH) and succinimidyl 6-hydrazinonicotinate acetone hydrazone (SANH) from SoluLink. The NVOC-protected *N*-hydroxysuccinimide esters of 4-aminomethylbenzoic acid (NVOC-SBA), 2-(aminooxy)acetic acid (NVOC-SAO), and 4-hydrazinobenzoic acid (NVOC-SHB) were synthesized as described in later section. HPLC grade acetonitrile was purchased from Thermo Fisher Scientific and 1,1,1,3,3,3-hexafluoroisopropanol, 99% was purchased from Apollo Scientific. 3-(2-Pyridyldithio)propionic acid *N*-hydroxysuccinimide ester (SPDP), and all other reagents and solvents were purchased from Sigma-Aldrich and used as received.

1.2. Synthesis of thioester- and nucleophile-modified oligonucleotides

1.2.1. Synthesis of thioesters T1-TAM, T1-PEG-TAM and T2-TAM.

A 50-fold excess of tris(2-carboxyethyl)phosphine hydrochloride (TCEP, typically 100 μL of a 10 mM solution) was added to equal volumes of 100 μM solutions of thiolated oligonucleotides S1, S2 and S3 in 100 mM sodium phosphate buffer, pH 7.5. The solutions were incubated at room temperature for 4-16 h. The reduced DNA samples were then combined with freshly dissolved 5-carboxy-tetramethylrhodamine N-succinimidyl ester (TAMRA, 200 μL, 50 mM in *N*,*N*-dimethylformamide – DMF) and incubated at room temperature overnight. Excess small-molecule reagents were removed using Illustra NAP-5 columns (GE Healthcare), and the recovered DNA samples were purified by HPLC chromatography. Fractions containing the

desired fluorescent thioester were collected and concentrated using a DNA120 Savant SpeedVac Concentrator. Modification yields calculated from areas of HPLC peaks in absorbance at 260 nm (A_{260}) are recorded in Table 1 of the main manuscript.

1.2.2. Synthesis of T1-HIS-TAM

DSG (100 μL, 200 μM) was combined with histamine (100 μL, 200 μM) in DMF and reacted for 10 minutes before adding this mixture to 200 μL of 100 μM oligonucleotide S4 in 100 mM sodium phosphate buffer, pH 7.5. The reaction mixture was left stirring overnight at room temperature. Excess DSG and histamine were removed with a NAP-5 column and two main oligonucleotide fractions were recovered after HPLC purification (fraction 1, 30.2% yield by HPLC peak area corresponds to the DSG modification alone, and fraction 2, 59.8% yield, corresponds to the DSG-histamine modification). The second fraction was concentrated and re-dissolved in 100 mM sodium phosphate buffer, pH 7.5. The TAMRA thioester was then prepared as described above.

1.2.3. Synthesis of nucleophile-modified oligonucleotides

Equal volumes of 100 μM *N*-hydroxysuccinimide esters SHTH, SANH, SPDP, NVOC-SBA, NVOC-SAO, or NVOC-SHB in DMF were combined with 100 μM solutions of N1 or N2 amino-modified oligonucleotides in 100 mM sodium phosphate buffer, pH 7.5. The mixtures were stirred overnight at room temperature wrapped in foil to provide protection from light. Excess small-molecule reagents were removed using NAP-5 columns and the oligonucleotide samples purified by HPLC. Fractions with the desired products (Scheme 12) were collected and concentrated. Product yields are recorded in Table 1 of the main manuscript.

To de-protect NVOC oligonucleotides (N3PG, N4PG and N6PG), samples were re-dissolved in Milli-Q H_2O, placed in glass vials, and exposed to UV_{363} light for approximately 6 h (UVP Mineralight lamp, model UVGL-58 254/363 nm, long wavelength setting). The resulting nucleophile-functionalized oligonucleotides N3 and N4 were purified by HPLC. Oligonucleotide N6, which is less stable in the free nucleophile form, was used in acyl transfer reactions immediately after photo-deprotection.

Oligonucleotide N7PG has an acetone protecting group which is spontaneously hydrolyzed in buffers pH ≤ 7.0 to give hydrazine oligonucleotide N7. This modification formed side products over time. Thiol oligonucleotide N8 was recovered after incubation of N8PG with 10 mM TCEP for at least 2 h (to ensure complete reduction of thiol residues) and used without further purification.

1.3. HPLC purification and analysis

HPLC analyses were performed on an Agilent 1200 system equipped with diode array absorbance and multisignal fluorescence detectors. Chromatography was performed on a Waters XBridge^{TM} OST C18 2.5 μm 4.6×50 mm column heated to 40 °C. Flow rate was set at 1 mL/min with a linear gradient of the following buffers: Buffer A, 0.1 M triethylammonium acetate, 5% acetonitrile, pH 7.0; buffer B, 0.1 M triethylammonium acetate, 70% acetonitrile, pH 7.0. Oligonucleotides were detected and quantified by measuring peaks at A_{260}. The TAMRA fluorophore was detected by measuring the fluorescence signal at wavelengths Ex554/Em583 nm.

1.4. PAGE analysis

Oligonucleotide samples were run on two-layered denaturing gels with a discontinuous Tris-glycine buffer system: stacking gel (5% acrylamide, 7 M urea, 25% formamide, 125 mM Tris, pH 6.8); separating gel (20% acrylamide, 7 M urea, 25% formamide, 400 mM Tris, pH 8.8); running buffer (2.5 mM Tris, 0.19 M glycine). Samples were combined in 1:1v/v ratio with a loading buffer consisting of 14 M urea, 50% formamide in Tris, pH 6.8 and heated to 95 °C for 5 min before loading. Gels were run at room temperature at 300V. DNA was visualized using a BioRad Pharos FX Plus Molecular Imager using wavelengths Ex532/Em605 nm and Ex488/Em530 nm before and after staining with SyBr Gold nucleic acid gel stain (Invitrogen). T1-TAM hydrolytic studies were performed in a universal buffer consisting of 10 mM citrate-phosphate-borate adjusted to the indicated pH with NaOH.

1.5. Mass spectrometry

Liquid chromatography-mass spectrometry (LC-MS) analysis was performed using an HP1050 LC coupled to a LCT Premier reflectron TOF mass spectrometer (Waters). Chromatography was carried out on a Waters XBridge OST C18 column (2.5 µm particle size, 4.6×50 mm) at a flow rate of 0.5 mL/min using a gradient of buffers C and D: Buffer C, 400 mM 1,1,1,3,3,3-hexafluoroisopropanol, 16.3 mM triethylamine, 5 % methanol); buffer D, 400 mM 1,1,1,3,3,3-hexafluoroisopropanol, 16.3 mM triethylamine, 60 % methanol. The eluent was directly injected into the mass spectrometer, and data were acquired in the negative-ion mode (mass range 505-3500) and analyzed using the manufacturer's software (MassLynx V4.1, Waters).

1.6. Acyl transfer reactions

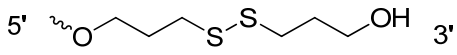
For experiments analyzed by PAGE, solutions were prepared with final oligonucleotide concentrations of 250 nM in the indicated buffers. Samples were annealed by heating to 95 °C and cooled to 20 °C over a period of approximately 10 min, then incubated at the indicated temperature for the indicated time using an Eppendorf Mastercycler or an Eppendorf Thermomixer before PAGE analysis.

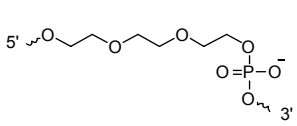
For experiments analyzed by HPLC, oligonucleotides were mixed to a final concentration of 5 μM, annealed, and incubated at 38 °C for the indicated time. Reactions were quenched by addition of 2 equivalents of displacer oligonucleotide S14 re-annealed, and kept at 4 °C until HPLC analysis. Typically, a 20 μL sample was injected for each reaction. Yields were calculated by measurement of the areas of A_{260} peaks corresponding to oligonucleotide-linked reactants and products

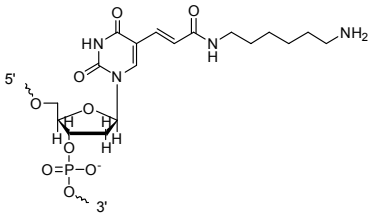
2. DNA sequences

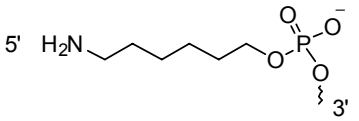
The following sequences were purchased from Integrated DNA Technologies (IDT). Strands with modifications (amine, thiol and triethylene glycol spacer) are shown below.

Strand	Sequence
S1	TCG AGG CAT ATC ACG GAC TGG CGT /3ThioMC3-D/
S2	CTG GTA CAC ACT AAG ACA ATC CCT /3ThioMC3-D/
S3	TCG AGG CAT ATC ACG GAC TGG CGT /iSp9//3ThioMC3-D/
S4	TCG AGG CAT ATC ACG GAC TGG CG/iAmMC6T/ /3ThioMC3-D/
S5	TAT TCG CAC ACT AAG ACA ATC CCT ACG CCA GTC CGT GAT ATG AGT TCT
S6	TAT TCG CAC ACT AAG ACA ATC CCT AAC GCC AGT CCG TGA TAT GAG TTC T
S7	TAT TCG CAC ACT AAG ACA ATC CCT TAA CGC CAG TCC GTG ATA TGA GTT CT
S8	TAT TCG CAC ACT AAG ACA ATC CCT TTA ACG CCA GTC CGT GAT ATG AGT TCT
S9	TAT TCG CAC ACT AAG ACA ATC CCT ATT AAC GCC AGT CCG TGA TAT GAG TTC T
S10	TAT TCG CAC ACT AAG ACA ATC CCT AAT TAA CGC CAG TCC GTG ATA TGA GTT CT
S11	TAT TCG CAC ACT AAG ACA ATC CCT AAA TTA ACG CCA GTC CGT GAT ATG AGT TCT
S12	TAT TCG CAC ACT AAG ACA ATC CCT TAA ATT AAC GCC AGT CCG TGA TAT GAG TTC T
S13	TAT TCG CAC ACT AAG ACA ATC CCT CTA AAT TAA CGC CAG TCC GTG ATA TGA GTT CT
S14	AGG GAT TGT CTT AGT GTG TAC CAG
N1	/5AmMC6/AGG GAT TGT CTT AGT GTG CGA ATA GGT AAC
N2	/5AmMC6//iSp9/AGG GAT TGT CTT AGT GTG CGA ATA GGT AAC

3ThioMC3-D= 

iSp9 = 

iAmMC6T = 

5AmMC6 = 

3. HPLC purification of adaptor oligonucleotides

Figure S-1 and Figure S-2 show the chromatograms for the synthesized thioester- and nucleophile-modified oligonucleotides. Coupling yields were calculated from peak areas of starting material, side products and desired product.

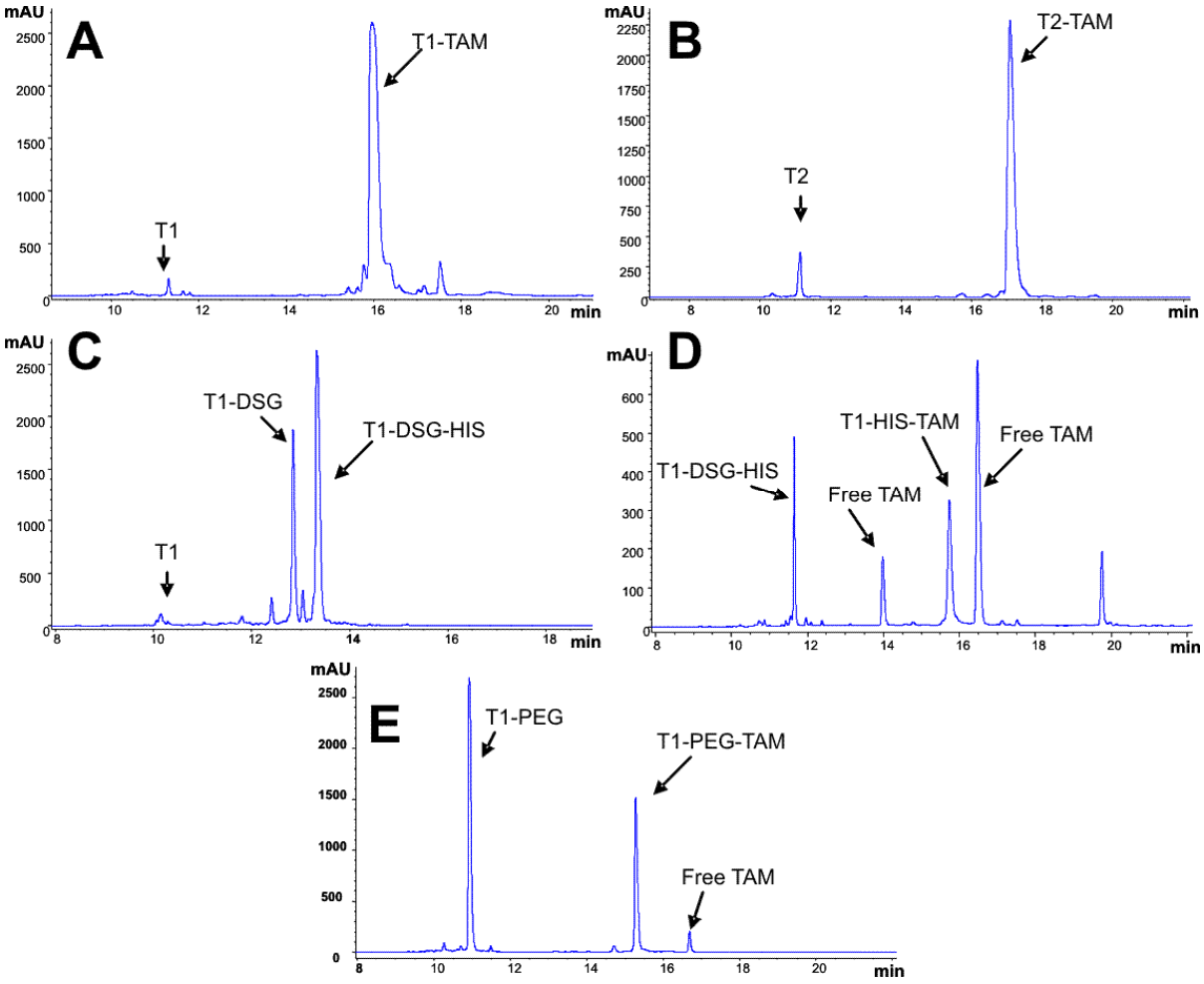


Figure S-1. HPLC traces of Thioester Adaptors. Synthesis and purification of oligonucleotide adaptors T1-TAM(**A**), T2-TAM(**B**), T1-DSG-HIS(**C**), T1-HIS-TAM(**D**), and T1-PEG-TAM(**E**)

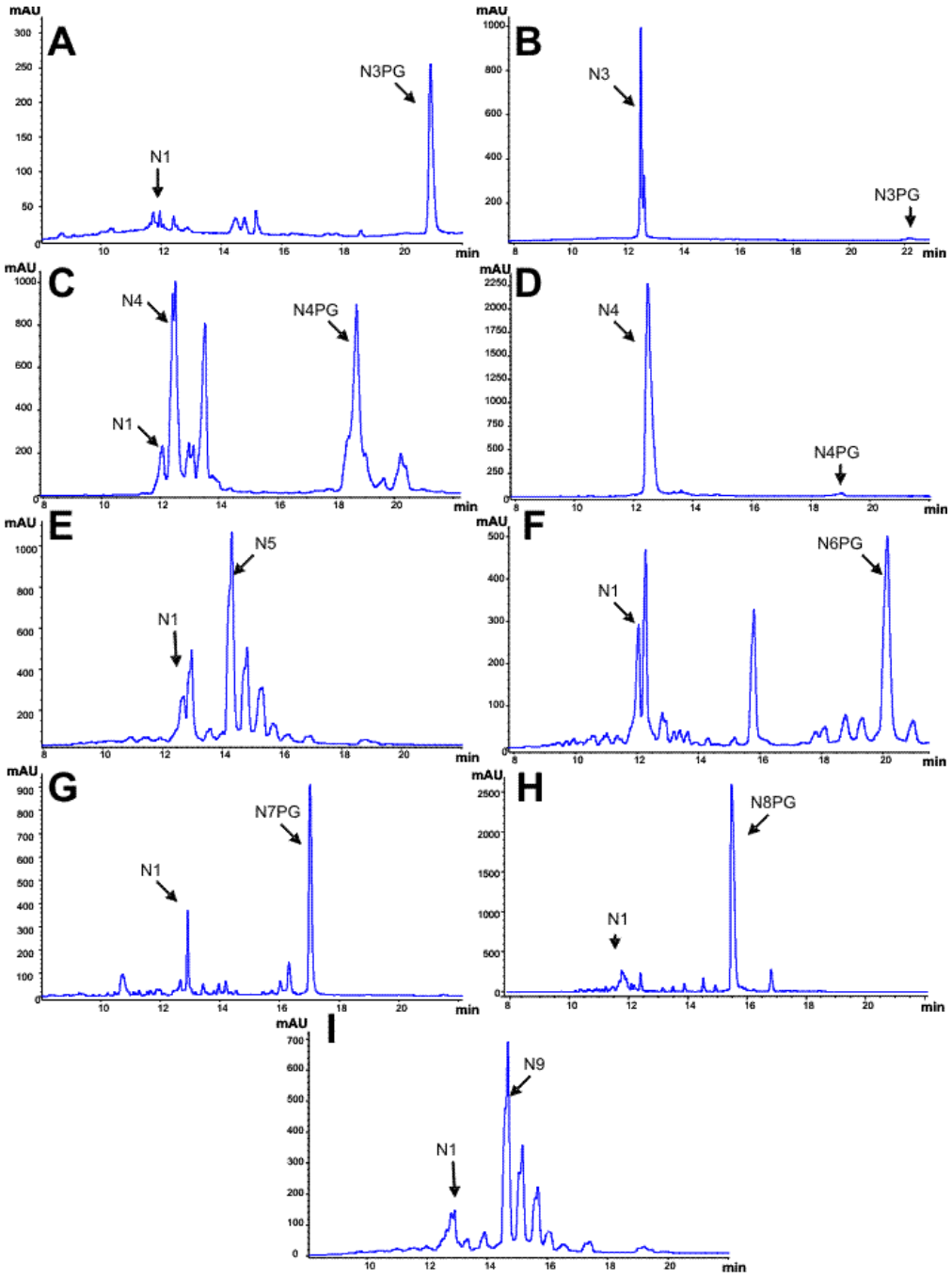


Figure S-2. HPLC traces of nucleophilic adaptors. (**A**) Synthesis of N3PG. (**B**) Photodeprotection of N3PG to obtain nucleophile oligonucleotide N3. (**C**) Synthesis of N4PG. (**D**) Photodeprotection of N4PG to obtain nucleophile oligonucleotide N4. (**E**) Synthesis of N5. (**F**) Synthesis of N6PG. (**G**) Synthesis of N7PG. (**H**) Synthesis of N8PG. (**I**) Synthesis of N9.

4. Hydrolytic stability of Thioesters

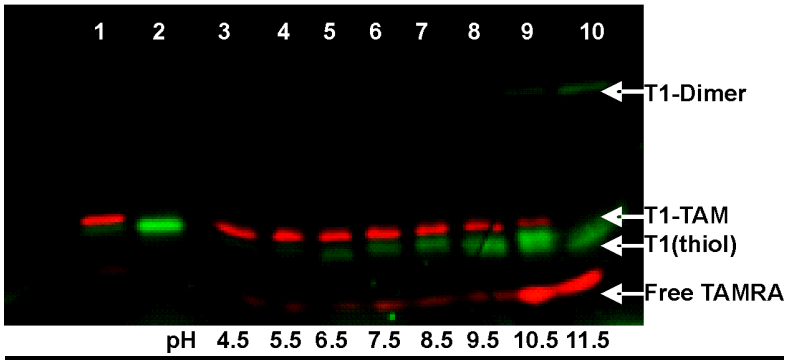


Figure S-3. Hydrolytic stability of the fluorescent TAMRA thioester. 20% denaturing PAGE gel of T1-TAM at different pHs. Red channel: TAMRA (Ex532/Em605 nm); green channel: SybrGold DNA stain (Ex488/Em530 nm). Lane 1: T1-TAM in H_2O; lane 2: T1 (thiol); lanes 3-10: T1-TAM after 48 h incubation at 38 °C in universal buffer at indicated pH.

5. HPLC purification and analysis of acyl transfer products at different pH values

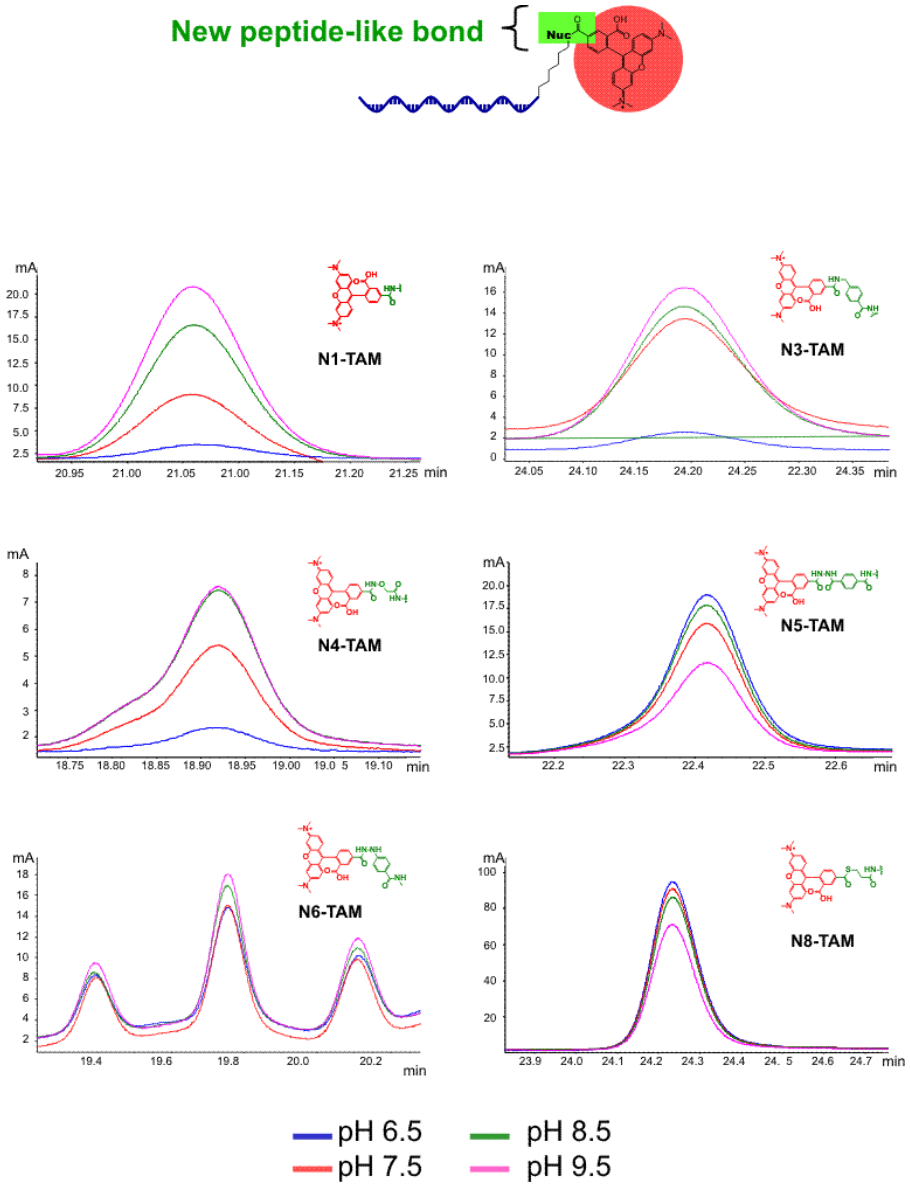


Figure S-4. Comparison of acylation products of nucleophiles N1-N6 and N8 with thioester T2-TAM characterized by HPLC. Oligonucleotides N1-N6 were incubated 38 ^{o}C in 100 mM phosphate buffer, 100 mM NaCl for 48 h in the presence of thioester T2-TAM at pH 6.5 (blue lines), 7.5 (red lines), 8.5 (green lines) or 9.5 (pink lines). Product peaks were identified by monitoring TAMRA fluorescence and DNA absorption, and yields were calculated from peak areas. Hydrazide oligos N5 and N6, and thiol oligo N8 showed significantly more product formation at lower pH values than the amine oligos N1, N3 and N4.

6. Mass Spec of characterized acyl transfer products

The new acyl transfer products were characterized by ESI-MS. Figure S-5 shows the mass spectra obtained from the samples shown in Figure S-4, as well as from acylations carried out using PEG oligos N2 and N9.

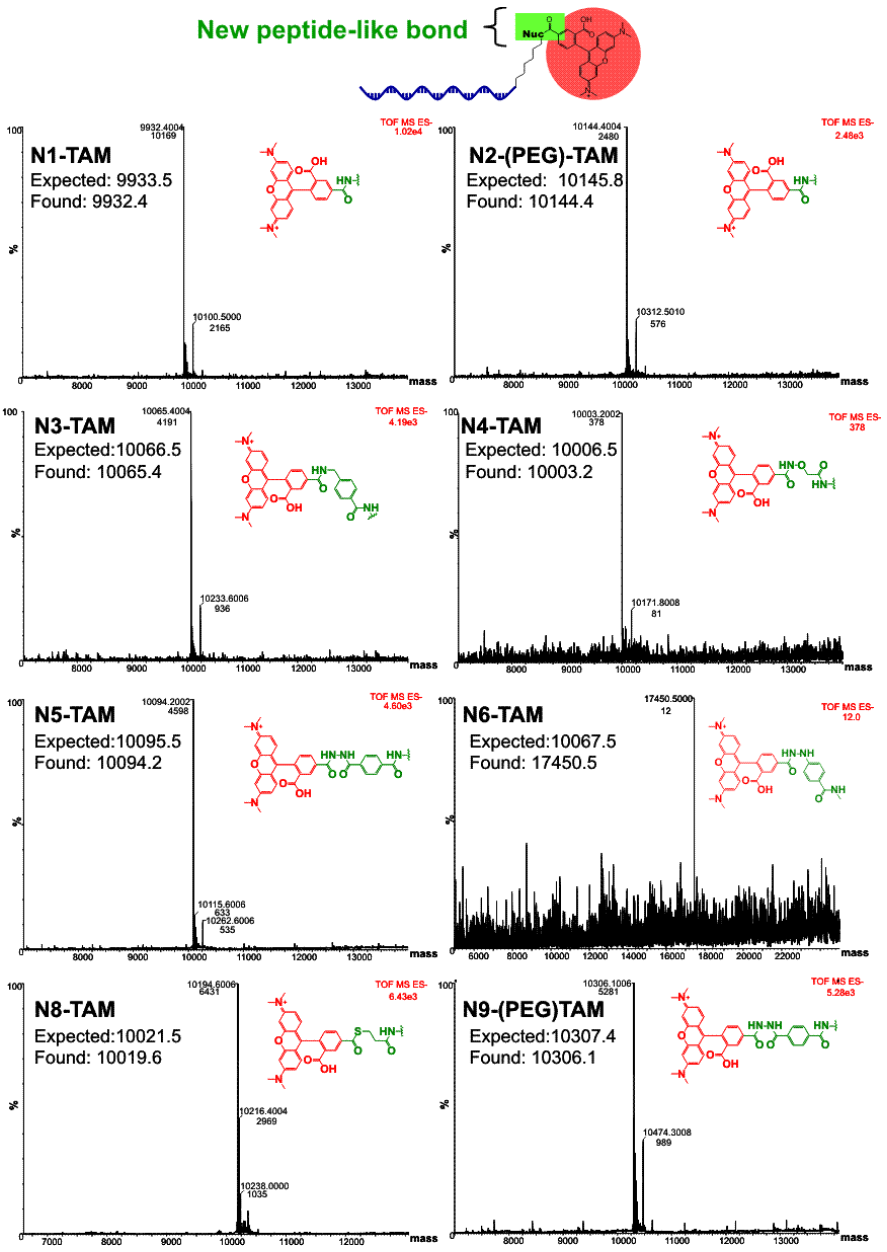


Figure S-5. ESI-MS spectra of acyl transfer products. All spectra display minor peaks with mass of +168 which correspond to the modified oligonucleotide-HFIP adducts from the mobile phase.

7. PAGE analysis of the influence of linker lengths

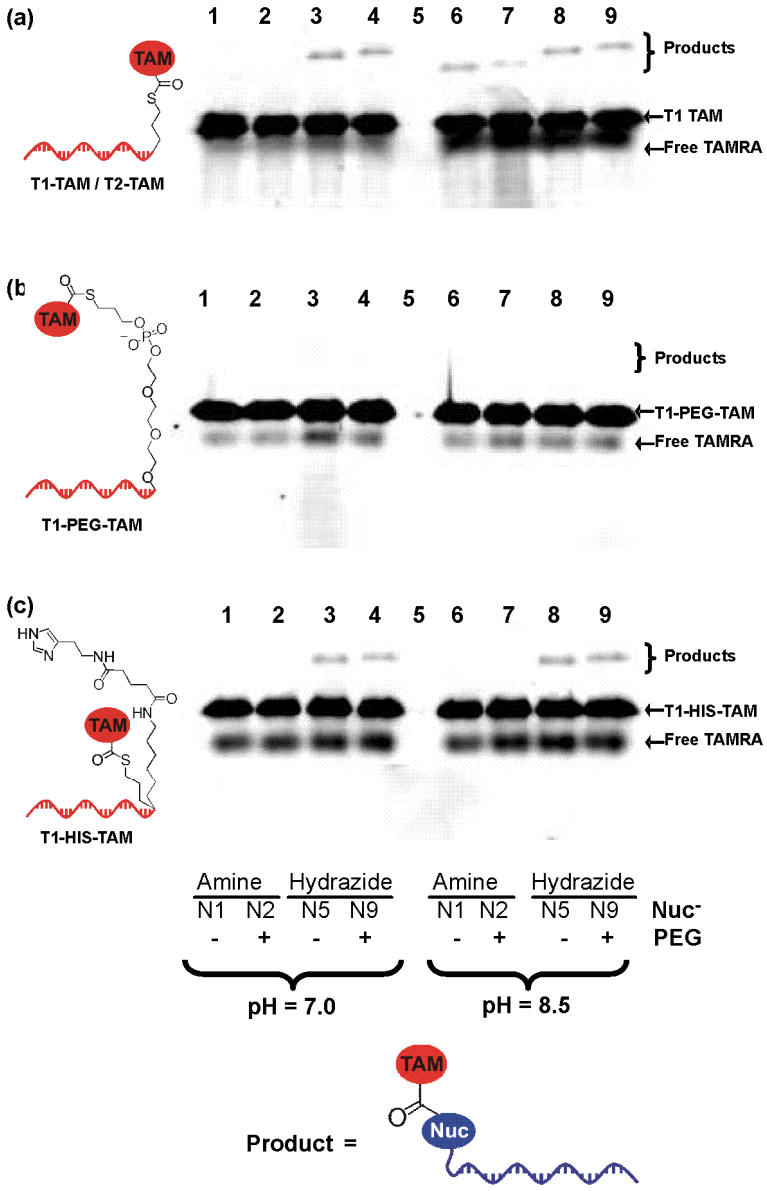


Figure S-6. Effect of linker flexibility and proximal histamine residue on acyl transfer efficiency. PAGE is used to compare the acyl transfer efficiency of various nucleophiles using thioester T1-TAM (a), T1-PEG-TAM (b), and T1-HIS-TAM (c). PAGE electrophoresis was performed after 24 h incubation of the respective thioester and nucleophile in the presence of template oligonucleotide S5 at room temperature in 100 mM phosphate buffer, pH 7.0 (lanes1-4) or pH 8.5 (lanes 6-8). Nucleophile used in lanes 1 and 6: N1; lanes 2 and 7: N2; Lane 3 and 8: N5; Lane 4and 9: N9.

8. PAGE analysis of the influence of temperature and added base

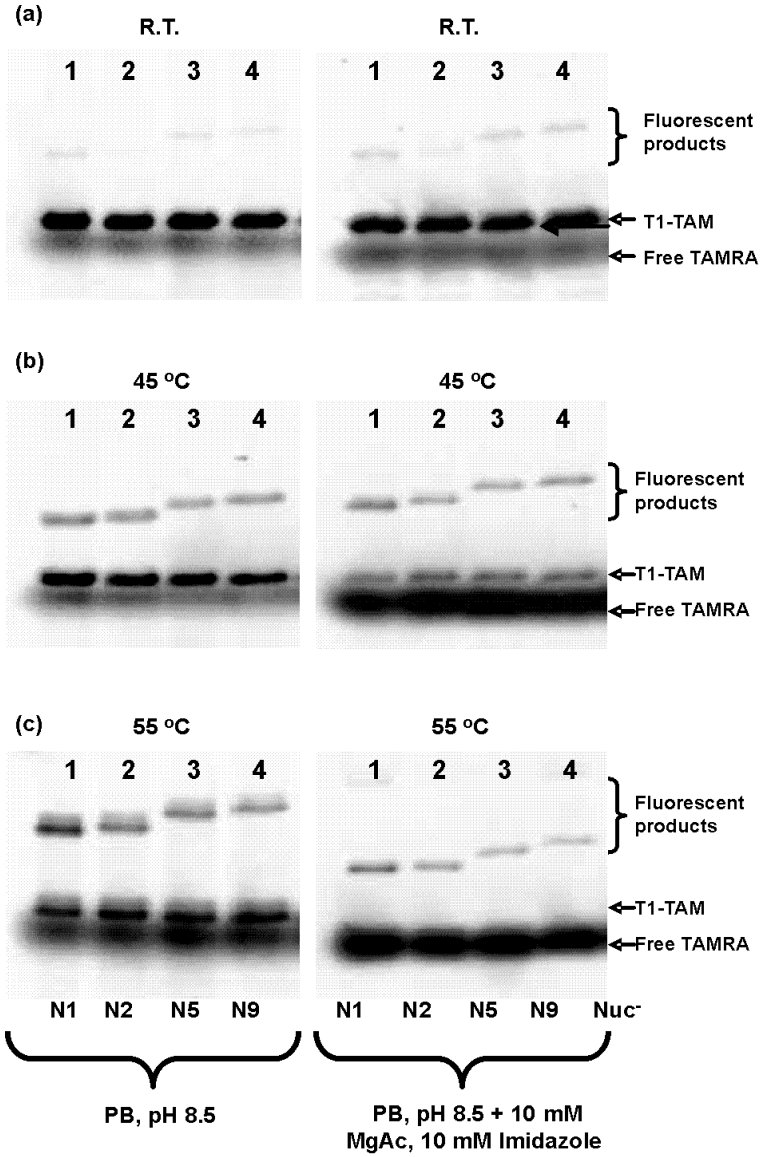


Figure S-7. Effect of temperature and added base (Mg^{2+}-imidazole). Acyl transfer reactions were carried out (after annealing) for 24 h in 100 mM phosphate buffer, pH 8.5 with and without 10 mM $Mg(OAc)_2$, 10 mM imidazole at room temperature (a), 45 °C (b), and 55 °C (c). Reactions contained T1-TAM, S4 and one of the following nucleophiles: Lane 1: N1; lane 2: N2; lane 3: N5; lane 4: N9.

9. PAGE analysis of the influence of pH on amine and hydrazide reactivity

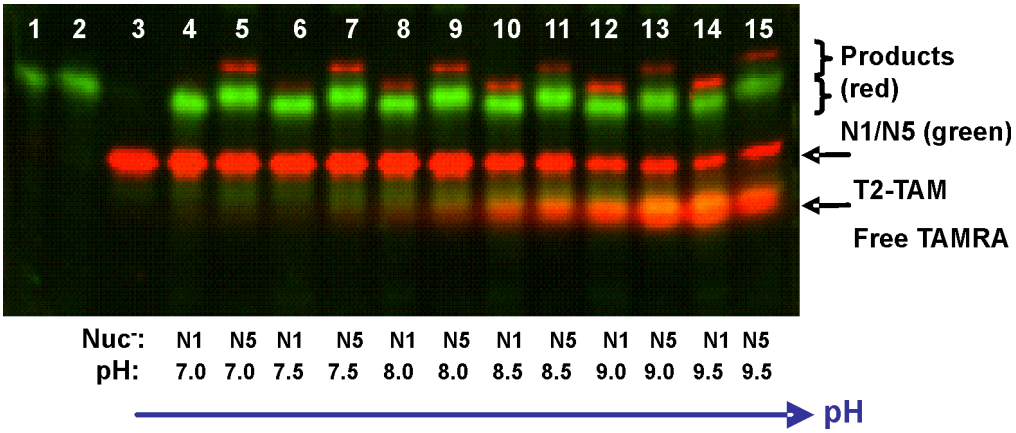
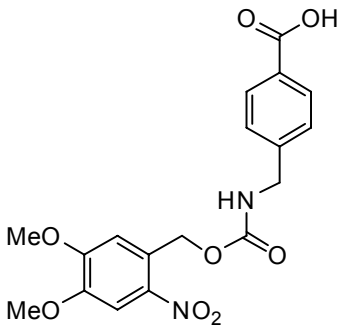


Figure S-8. Comparison between reactivities of amine- and hydrazide-modified oligonucleotides (N1 and N5) as functions of pH. PAGE analysis: Acyl transfer reactions were carried out in 100 mM phosphate buffer for 48 h at the indicated pH using N1 and N5 nucleophiles in the presence of the T2-TAM thioester at 38 ºC. Lanes 1-3: control lanes N1, N5 and free TAMRA; Lanes 4-15: acyl transfer reaction products at the indicated pH. Red channel: TAMRA (Ex532/Em605 nm); green channel: SybrGold DNA stain (Ex488/Em530 nm).

10. Synthesis of building blocks

10.1. 4-(((4,5-Dimethoxy-2-nitrobenzyloxy)carbonylamino)methyl)benzoic acid (S1)

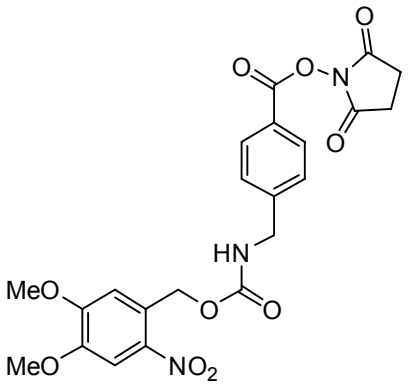


S1

To a cooled (0 °C) solution of 4-aminomethylbenzoic acid (200 mg, 1.32 mmol, 1.0 equivalents) in water (2 mL) was added 2.2 equivalents of sodium carbonate (308 mg, 2.9 mmoles). A solution of *o*-nitroveratryloxycarbonyl chloride (NVOC-Cl) (401 mg, 1.45 mmoles, 1.1 equivalents) in dioxane (2 mL) was then added dropwise at 0 °C. The resulting mixture was allowed to come slowly to room temperature over 15 hours. The reaction was quenched with water (10 mL), washed twice with ethyl acetate (2 x 10 mL), acidifed with 1M HCl (~20 mL), extracted twice with ethyl acetate (2 x 10 mL), dried over magnesium sulfate and concentrated *in vacuo*. A cream-coloured precipitate of **S1** was isolated in 36% yield (267 mg, 0.68 mmoles).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 12.91 (s, 1H), 8.11 (m, 1H), 7.90 (d, 2H, J = 10.1 Hz), 7.69 (s, 1H), 7.39 (d, 2H, J = 10.1 Hz), 7.18 (s, 1H), 5.38 (s, 2H), 4.30 (d, 2H, J = 6.7 Hz), 3.86 (s, 3H), 3.30 (s, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$): 166.0, 154.3, 151.9, 146.4, 143.3, 138.0, 128.0, 126.3, 125.7, 109.4, 107.0, 61.2, 54.9, 54.7, 42.1. FTIR (film, cm^{-1}): 3343, 1702, 1679, 1613, 1579, 1505. MS: HRMS (ES+): calcd for $C_{18}H_{18}N_2O_8Na$ [M+Na^+]: 413.0974; found: 413.0976

10.2. 2,5-Dioxopyrrolidin-1-yl 4-(((4,5-dimethoxy-2-nitrobenzyloxy)carbonylamino)methyl) benzoate (S2, NVOC-SBA)

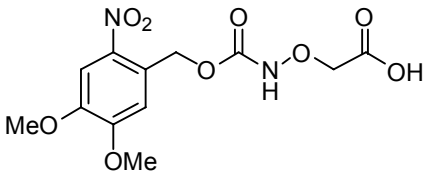


S2

To a solution of **S1** (204 mg, 0.5 mmoles, 1.0 equivalents) in acetonitrile (5 mL) was added ~0.7 mL diisopropylethylamine (DIEA) to adjust pH to >7. O-(N-Succinimidyl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TSTU) (166 mg, 0.55 mmoles, 1.1 equivalents) was then added and the resulting mixture was allowed to stir at room temperature for 15 hours. The solvent was then removed *in vacuo*, and the residue was partitioned between 1M HCl (10 mL) and dichloromethane (10 mL). The organic layer was isolated and washed ten times with 10 mL water, dried over magnesium sulfate and concentrated *in vacuo*. The desired product **S2** was isolated as a tan precipitate in 74% yield (181 mg, 0.37 mmoles).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.02 (d, 2H, J = 10.1 Hz), 7.12 (s, 1H), 7.38 (d, 2H, J = 10.1 Hz), 6.98 (s, 1H), 5.49 (s, 2H), 5.21 (m, 1H), 4.42 (d, 2H, J = 6.7 Hz), 3.86 (s, 6H), 2.82 (s, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$): 169.5, 166.0, 154.3, 151.9, 146.4, 143.3, 138.0, 128.0, 126.3, 125.7, 109.4, 107.0, 61.2, 54.9, 54.7, 42.1, 26.0. FTIR (film, cm^{-1}): 2945, 1771, 1732, 1703, 1611, 1579, 1505. MS: HRMS (ES+): calcd for $C_{22}H_{21}N_3O_{10}Na$ [M+Na^+]: 510.1138, found: 510.1134

10.3. 2-((4,5-dimethoxy-2-nitrobenzyloxy)carbonylaminooxy)acetic acid (S3)

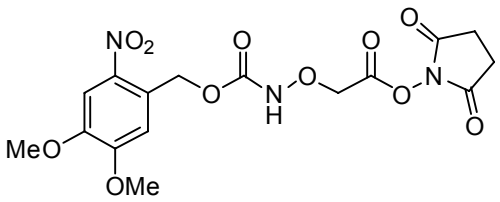


S3

To a solution of 2-(aminooxy)acetic acid (200 mg, 1.8 mmol, 1.2 equivalents) in dimethylformamide (DMF) (5 mL) was added 1.0 equivalent of NVOC-Cl (400 mg, 1.45 mmoles, 1.0 equivalents) and the resulting mixture was allowed to stir for 15 hours. The reaction was quenched with 4% aqueous lithium bromide (10 mL), washed twice with ethyl acetate (2 x 10 mL), dried over magnesium sulfate and concentrated *in vacuo*. A creamy yellow precipitate of **S3** was produced in 68% yield (238 mg, 0.68 mmoles).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 12.82-13.00 (br s, 1H), 10.74-10.88 (br s, 1H*)*, 7.72 (s, 1H), 7.21 (s, 1H), 5.94 (s, 2H), 4.33 (s, 2H), 3.92 (s, 3H), 3.88 (s, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$): 170.3, 157.0, 154.5, 148.1, 139.9, 127.2, 111.0, 108.6, 72.5, 63.4, 56.7, 56.5. FTIR (film, cm^{-1}): 3322, 2957, 1733, 1578, 1525. MS: HRMS (ES+): calcd for $C_{12}H_{14}N_2O_9Na$ [M+Na^+]: 353.0589, found: 353.0591

10.4. 2,5-dioxopyrrolidin-1-yl 2-((4,5-dimethoxy-2-nitrobenzyloxy)carbonylaminooxy)acetate (S4, NVOC-SAO)



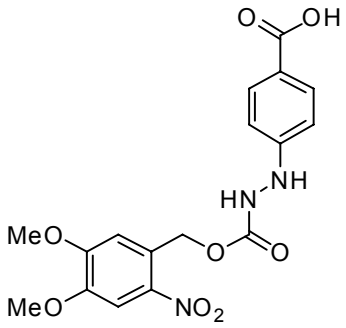
S4

To a solution of **S3** (150 mg, 0.43 mmoles, 1.0 equivalents) in acetonitrile (10 mL) was added ~0.8 mL DIEA to adjust pH to >7. TSTU (143 mg, 0.47 mmoles, 1.1 equivalents) was then added and the resulting mixture was allowed to stir at room temperature for 15 hours. The solvent was then removed *in vacuo*, and the residue was partitioned between 1M HCl (10 mL) and dichloromethane (10 mL). The organic layer was isolated and washed ten times with 10 mL

water, dried over magnesium sulfate and concentrated *in vacuo*. The desired product **S4** was isolated as an orange precipitate in 44% yield (82 mg, 0.19 mmoles).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.65 (s, 1H), 7.45 (m, 1H), 6.92 (s, 1H), 5.51 (s, 2H), 4.38 (s, 2H),3.92 (s, 3H), 3.88 (s, 3H), 2.94 (s, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$): 169.5, 166.0, 154.3, 151.9, 146.4, 143.3, 138.0, 128.0, 126.3, 125.7, 109.4, 107.0, 61.2, 54.9, 54.7, 42.1, 26.5. FTIR (film, cm^{-1}): 2922, 1734, 1586, 1523. MS: HRMS (ES+): calcd for $C_{18}H_{18}N_2O_8$ [M+H]: 428.1791, found: 428.1795.

10.5. 4-(2-((4,5-dimethoxy-2-nitrobenzyloxy)carbonyl)hydrazinyl)benzoic acid (S5)

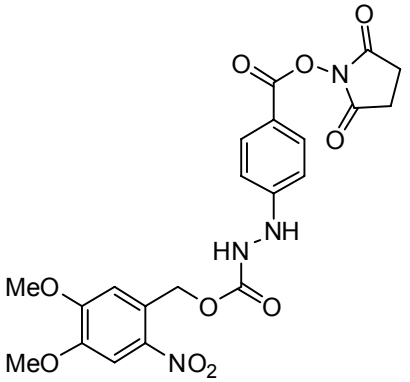


S5

To a solution of 4-hydrazinylbenzoic acid (100 mg, 0.66 mmol, 1.2 equivalents) in DMF (5 mL) was added 1.0 equivalent of NVOC-Cl (151 mg, 0.54 mmoles, 1.0 equivalents) and the resulting mixture was allowed to stir for 15 hours. The reaction was quenched with 4% aqueous lithium bromide (10 mL), washed twice with ethyl acetate (2 x 10 mL), dried over magnesium sulfate and concentrated *in vacuo*. A precipitate of **S5** was produced in 47% yield (120 mg, 31 mmoles).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 12.91 (s, 1H), 7.92 (d, 2H, J = 10.3 Hz), 7.62 (m, 2H), 7.17 (s, 1H), 6.78 (d, 2H, J = 10.3 Hz), 6.25 (br s, 1H), 5.48 (s, 2H), 3.91-3.89 (2 x s, 6H). ^{13}C-NMR (125 MHz, $CDCl_3$): 166.0, 154.3, 151.9, 146.4, 143.3, 138.0, 128.0, 126.3, 125.7, 109.4, 107.0, 61.2, 54.9, 54.7. FTIR (film, cm^{-1}): 3302, 2970, 1710, 1674, 1603, 1581, 1519, 1507. MS: HRMS (ES+): calcd for $C_{17}H_{17}N_3O_8Na$ [M+Na^+]: 414.0927, found: 414.0929

10.6. 2,5-Dioxopyrrolidin-1-yl 4-(((4,5-dimethoxy-2-nitrobenzyloxy)carbonyl)hydrazinyl)benzoate (S6, NVOC-SHB)



S6

To a solution of **S5** (67 mg, 0.19 mmoles, 1.0 equivalents) in acetonitrile (5 mL) was added ~0.5 mL DIEA to adjust pH to >7. TSTU (64 mg, 0.21 mmoles, 1.1 equivalents) was then added and the resulting mixture was allowed to stir at room temperature for 15 hours. The solvent was then removed *in vacuo*, and the residue was partitioned between 1M HCl (10 mL) and dichloromethane (10 mL). The organic layer was isolated and washed ten times with 10 mL water, dried over magnesium sulfate and concentrated *in vacuo*. The desired product **S6** was isolated as a reddish precipitate in 56% yield (52 mg, 0.11 mmoles).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.92 (d, 2H, J = 10.3 Hz), 7.62 (m, 2H), 7.17 (s, 1H), 6.78 (d, 2H, J = 10.3 Hz), 6.25 (br s, 1H), 5.48 (s, 2H), 3.91-3.89 (2 x s, 6H). 2.83 (s, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$): 169.5, 166.0, 154.3, 151.9, 146.4, 143.3, 138.0, 128.0, 126.3, 125.7, 109.4, 107.0, 61.2, 54.9, 54.7, 26.4. FTIR (film, cm^{-1}): 3031, 1734, 1605, 1581, 1521. MS: HRMS (ES+): calcd for $C_{21}H_{20}N_4O_{10}Na$ [$M+Na^+$]: 511.1077, found: 511.1079

11. Cosolvent effects tested using model activated thioesters

The effect of cosolvents was explored using activated thioester small molecule reagents and benzylamine in 100 mM phosphate buffer. The percentage conversion and hydrolysis were determined from HPLC traces in the presence and absence of the cosolvents. Addition of 10% THF improved the overall acylation efficiency and minimized the rate of hydrolysis of the thioester.

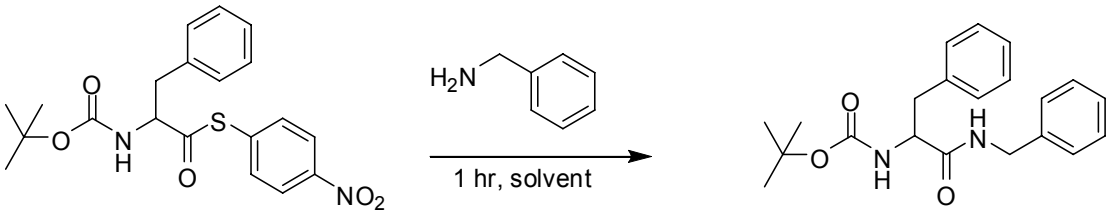


Table S- 1

Conditions	**Conversion (%)***	**Hydrolysis (%)***
pH 8, Amine	55	6
pH8, Amine, 10% THF	83	1
pH8 only, no amine present	NA	4

*Values determined from HPLC traces.

12. Degradation of hydrazine adaptors

Hydrazine adaptors N6 and N7 formed degradation products over time when the protecting group was removed. In the case of N6, this occurred after exposure to UV_{363}, and for N7 when the strand was dissolved in acidic buffers or close to neutrality. Figure S-9 shows typical chromatograms obtained with oligonucleotide N7 after the protecting group was removed simply by dissolving the adaptor in water. After 3 h multiple oxidized products were observed. It was not possible to characterize the deprotected products N6 and N7 by MS (only the masses of oxidation products were observed), and therefore only the masses of the protected groups are shown in the main text.

To avoid degradation, the protecting groups were removed immediately before carrying out the acyl transfer reactions. Oligo N7PG was kept at -20 ^{o}C in 100 mM sodium carbonate buffer, pH 11.0 until it was diluted with the thioester oligo at the indicated pH values. In the case of N6PG, UV deprotection was performed after annealing the adaptor to the thioester strand.

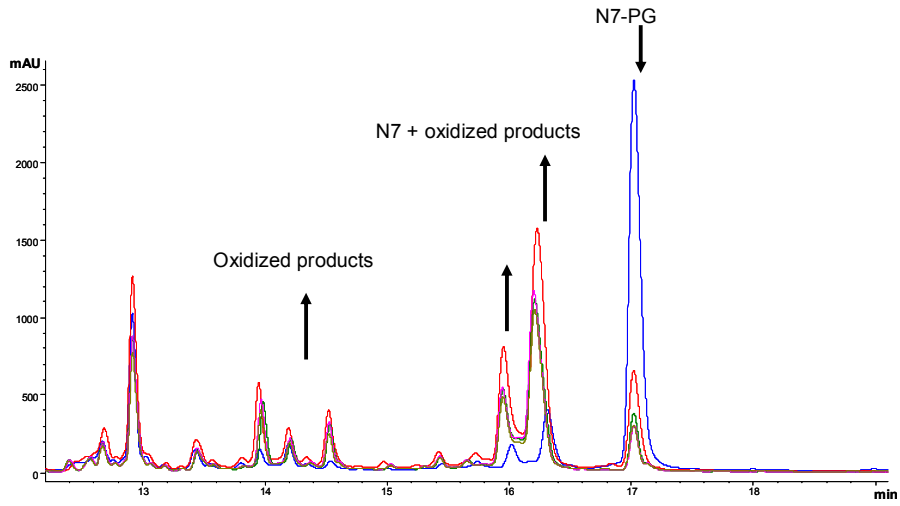


Figure S-9. Degradation of hydrazine adaptor during HPLC purification. Blue- injection immediately after NAP-5 column purification. Red- 5th injection of identical material after approximately 3 h.

Degradation of hydrazine-modified oligonucleotides: hydrazinopyridine and phenylhydrazine moieties result in the formation of aniline, pyridine-2-amine, benzene and pyridine side products with release of nitrogen. The masses observed for the degradation products of the hydrazine-modified adaptors correspond to possible oxidation of the adapters.

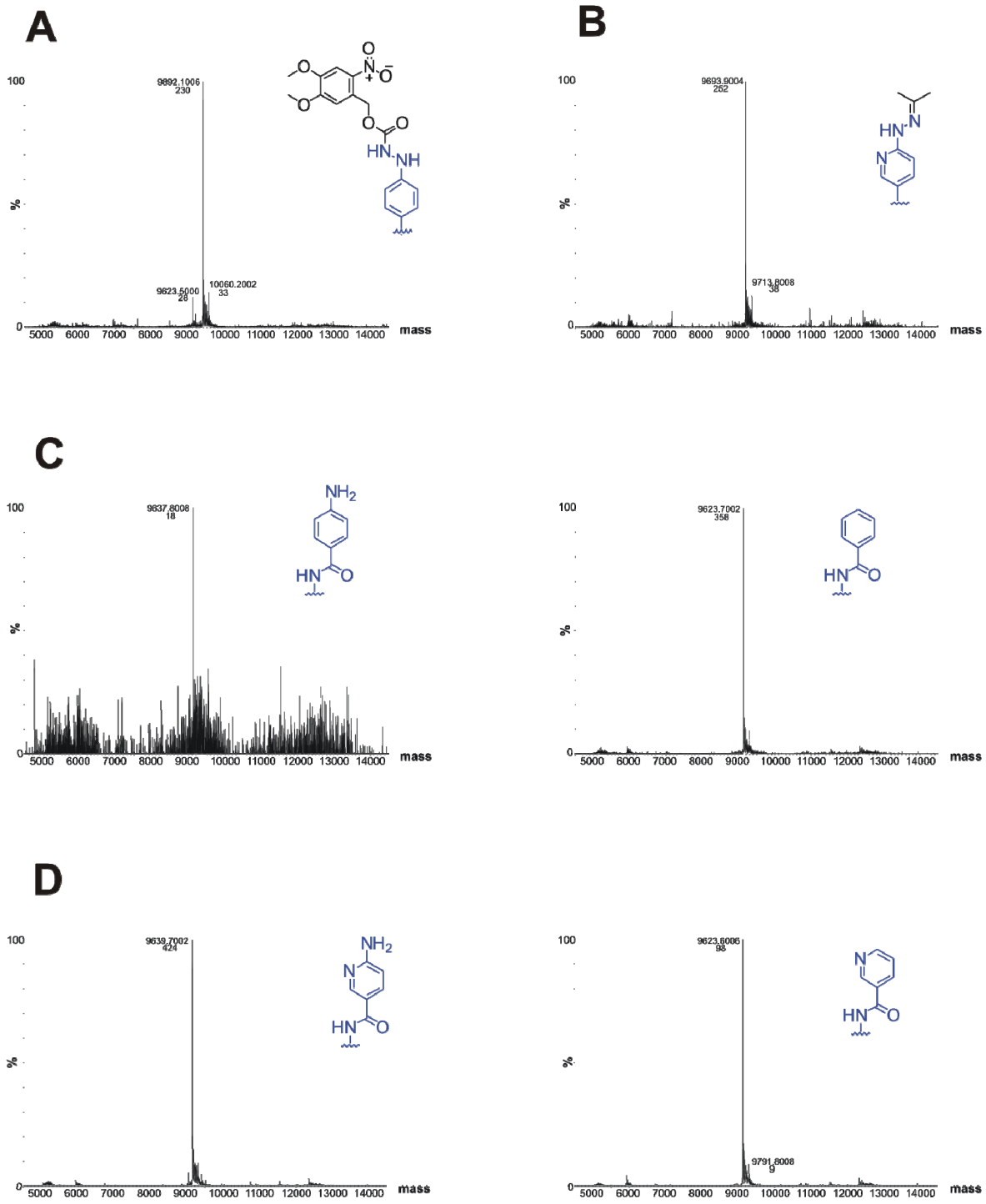


Figure S-10. Degradation of hydrazine adaptors monitored by ESI-MS. Deconvoluted mass spectra of adaptor N6 before (**A**) and after (**B**) NVOC deprotection by UV_{350}. Deconvoluted mass spectra of Adaptor N7 before (**C**) and after deprotection (**D**) by changing the pH from 11 to 7.