Supporting Information

DNA-Templated Functional Group Transformations Enable Sequence-Programmed Synthesis Using Small Molecule ~~Reactants~~Reagents

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General Experimental Methods

Oligonucleotides were synthesized on a Perseptive Biosystems Expedite 8090 DNA synthesizer using standard phosphoramidite protocols and purified using preparative scale reverse-phase HPLC. Reagents for automated solid-phase oligonucleotide synthesis were purchased from Glen Research. Functionalized DNA oligonucleotides were purified by analytical scale reverse-phase HPLC.

Concentrations of purified oligonucleotides in solution were determined based on their absorbance at 260 nm measured on a Hewlett-Packard 8453 UV-visible spectrophotometer (Agilent Technologies). Oligonucleotides stained with ethidium bromide were visualized and quantitated by UV transillumination and densitometry using an Eagle Eye II densitometer (Stratagene). Denaturing PAGE analysis was performed using 15 % polyacrylamide gel (TBE-urea).

MALDI-TOF mass spectrometry was performed on an Applied Biosystems Voyager-DE Pro Biospectrometry Workstation and processed with Voyager Data Explorer software. A mixture of nine parts hydroxypicolinic acid (HPA, 50 mg/mL in 50% MeCN/H_2O) and one part ammonium citrate (50 mg/mL in H_2O) was used as the matrix in all experiments.

Chemicals were purchased from Sigma-Aldrich unless otherwise noted.

Preparation of Functionalized DNA Oligonucleotides

Amine-Terminated and Biotinylated DNA Oligonucleotides

5' amino-modifier 5 (Glen Research) was used to prepare 5' amino-modified oligonucleotides.

3' amino-modifier C7 CPG (Glen Research) was used to prepare 3' amino-modified oligonucleotides.

Biotin TEG CPG (Glen Research) was used to prepare 3' biotin-labeled oligonucleotides.

5' Azide-Linked DNA Oligonucletodies.

The *N*-hydroxy succinimidyl (NHS) ester of an azido acid was prepared by mixing equal volumes of the azido acid (900 mM in DMF), EDC (900 mM in DMF) and NHS (900 mM in DMF) at 25 °C for 1 h. The crude NHS ester was added in two portions (50 μL each) to a solution of 5' amino-modified DNA oligonucleotide (50 μL, typically 300 μM) in 100 mM sodium phosphate buffer (pH 7.2, 350 μL). The coupling reaction was performed at 25 °C for 1 h. The reaction mixture was directly loaded onto a NAP-5 size exclusion column (Amersham Biosciences) to remove organic solvent, salts, and excess small molecules, and was further purified by analytical scale reverse-phase

HPLC (8–30% MeCN/0.1 M TEAA gradient). The desired oligonucleotide products were characterized by MALDI-TOF mass spectrometry.

*5' 2-azidomethylbenzoyl thio propionic acid thioester-linked DNA (**12** in Figure 1b)*

A solution of 2, 2'-dithiodipropionic acid in DMF (900 mM) was mixed with equal volumes of EDC (900 mM in DMF) and NHS (900 mM in DMF) at 25 °C for 1 h. The crude NHS ester (50 μL) was added to a solution of 5' amino-modified DNA oligonucleotide (50 μL, typically 300 μM) in 100 mM sodium phosphate buffer (pH 7.2, 350 μL). The coupling reaction was performed at 25 °C for 1 h. The reaction mixture was directly loaded onto a NAP-5 size exclusion column (Amersham Biosciences) and purified by analytical scale reverse-phase HPLC (8–30% MeCN/0.1 M TEAA gradient). The disulfide-linked oligonucleotide product was characterized by MALDI-TOF mass spectrometry. The 2-thiopropionic acid-linked oligonucleotide was prepared by treating the disulfide-linked oligonuleotide above (typically 10 μM) in 100 mM CAPS buffer (pH 8) with 20 mM DTT at 25 °C for 0.5 h. Excess DTT was removed by passing the reaction mixture through a gel filtration column. In parallel, the 2-azidomethylbenzoyl *N*-hydroxy succinimidyl (NHS) ester was prepared by mixing equal volumes of the azido acid (900 mM in DMF), EDC (900 mM in DMF) and NHS (900 mM in DMF) at 25 °C for 1 h. The crude NHS ester (100 μL) was added to a solution of 5' thiol-linked oligonucleotide (100 μL) in 100 mM sodium phosphate buffer (pH 7.2, 300 μL). The coupling reaction was performed at 25 °C for 1 h. The reaction mixture was directly loaded onto a NAP-5 size exclusion column and purified by analytical scale reverse-phase HPLC (8–30% MeCN/0.1 M TEAA gradient). The desired oligonucleotide product was characterized by MALDI-TOF mass spectrometry.

3' Triphenylphosphine-Linked DNA

Attachment of the triphenylphosphine group was performed on 3' amino-modified oligonucleotides linked to CPG resin. The Fmoc group on 3' FMOC-NH-oligonucleotides was removed by three cycles of: (*i*) treatment with 20% piperidine in DMF for 10 min; (*ii*) washing with DMF; and (*iii*) washing with MeCN. The resin was dried under a stream of nitrogen gas. A solution of 4-diphenylphosphino benzoic acid (30.6 mg, 100 μmol), EDC (19.1 mg, 100 μmol), *N,N*-diisopropylethylamine (36.8 μL, 211 μmol) in DMF (0.6 mL) was added to the resin and the mixture was incubated at 37 °C for 2 h. The resin was washed with DMF (2x) and with MeCN (2x), then dried under nitrogen. The derivatized oligonucleotide was cleaved from the CPG resin by incubation in 1:1 ammonium hydroxide:methyl amine (AMA) with tris(2-carboxyethyl)phosphine hydrochloride (TCEP-HCl, 1 mg) at 55 °C for 45 min. The cleavage solution was filtered and purified by analytical scale reverse-phase HPLC (8–30% MeCN/0.1 M TEAA gradient). The desired oligonucleotide products were characterized by MALDI-TOF mass spectrometry.

3' Bromoacetate-Linked DNA

The NHS ester of bromoacetic acid was prepared by mixing equal volumes of 900 mM bromoacetic acid in DMF, 900 mM EDC in DMF, and 900 mM NHS in DMF at 25 °C for 1 h. The crude NHS ester (100 μL) was added to a solution of 5' amino-modified DNA oligonucleotide (50 μL, typically 300 μM) in 100 mM sodium phosphate

buffer (pH 7.2, 350 μL). The coupling reaction was allowed to proceed at 25 °C for 1 h. The reaction mixture was directly loaded onto a NAP-5 size exclusion column to remove organic solvent, salts, and excess small molecules and was further purified by analytical scale reverse-phase HPLC (8–30% MeCN/0.1 M TEAA gradient). The desired oligonucleotide products were characterized by MALDI-TOF mass spectrometry.

3' 4-Formylbenzoate-Linked DNA

The 4-formylbenzoate linked 20-mer DNA was prepared following the protocol for bromoacetate-linked DNA using 4-formyl benzoic acid instead of bromoacetic acid.

3' Succinic Acid Monoester-Linked DNA

Succinic anhydride (22 mg, 0.1 mmol) was activated with NHS (10 mg, 0.1 mmol) in DMF (200 μL) at 25 °C for 15 min. 100 μL of the mixture was added to the 3' amino modified template (50 μL, typically 300 μM) in 100 mM HEPES buffer (pH 8.5; 850 μL) and was incubated at 37 °C for 16 h. The reaction mixture was desalted by NAP-5 size exclusion column and further purified by analytical scale HPLC (8–30% MeCN/0.1 M TEAA gradient). The desired oligonucleotides products were characterized by MALDI-TOF mass spectrometry.

Synthesis of Azido Acids

Azido substrates for the synthesis of **1**-**12** were prepared from the corresponding carboxylic acid precursors as follows.

4-Azidomethylbenzoic Acid

Sodium azide (1.3 g, 20 mmol) and 18-crown-6 ether (0.2 mL, 1 mmol) were dissolved in DMSO (4 mL). To the resulting solution was added 4-chloromethyl benzoic acid (1.71g, 10mmol) and the reaction mixture was stirred 12 h at 25 °C. The reaction was diluted in EtOAc, washed with 0.1 N HCl (2x), then washed with brine. The organic layer was dried with Na_2SO_4 and concentrated to provide a white solid (1.75 g, quant.). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (2H, d, J = 8.4 Hz) 7.45 (2H, d, J = 8.4 Hz) (1H, s); ESMS calculated for $C_8H_6N_3O_2$: 176.0460; observed: 176.0461.

The following azido acids were synthesized according to the method described for the synthesis of azido acids by diazo transfer in Lundquist, IV, J. T., Pelletier, J. C., *Org Lett*, **2001**, *3*, 781.

1-Azidocyclohexyl Carboxylic acid

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.86 (4H, m) 1.63 (4H, m) 1.36 (2H, m); CIMS calculated for $C_7H_7N_3O_2$ ($M+NH_4^+$): 1187.1195; observed: 1187.1188.

Azidoisoglutamic acid

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.43 (2H, d, J = 17.4 Hz) 3.14 (1H, dd, J = 14.1 Hz, J = 6.9 Hz) 2.54 (2H, t J = 7.5 Hz) 2.23 (2H, dd, J = 13.6 Hz J = 6.6 Hz); CIMS calculated for $C_7H_7N_3O_2$ ($M+NH_4^+$): 190.0931; observed: 190.040.

4-Azidobenzyl-Cyclohexyl Dicarboxylic Acid Monoester

Trans-cyclohexyl dicarboxylic acid (200 mg, 1.16 mmol), EDC (223 mg, 2.32 mmol), and N,N-diisopropylethylamine (0.4 mL, 2.32 mmol) were dissolved in CH_2Cl_2 (4 mL) and stirred for 30 min at 25 °C. To this mixture was added 4-azido benzyl alcohol (86.6 mg, 0.58 mmol). The reaction was stirred 12 h at 25 °C. The reaction mixture was concentrated and purified by flash chromatography (30% EtOAc/hexanes). The desired ester was obtained as a yellow solid (18.2 mg, 5%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (2H, d, J = 8.4 Hz) 7.03 (2H, d, J = 8.4 Hz) 5.08 (1H, s) 2.33 (2H, m) 2.09(4H, d, J = 9.3 Hz) 1.47 (4H, t, J = 9.9 Hz); ESMS calculated for $C_{15}H_{17}N_3O_4$ ($M+HCO_2^-$): 348.1196; observed: 348.1195

4-Azidobenzyl-Succinic Acid Monoester.

4-Azidobenzyl alcohol (100 mg, 0.67 mmol), succinic anhydride (134 mg, 1.37 mmol), and N,N-dimethylaminopyridine (3.7 mg, 30 μmol) were dissolved in DMF (1 mL) and stirred 12 h at 25 °C. The reaction mixture was concentrated and purified by flash chromatography (30% EtOAc/hexanes). The desired ester was obtained as yellow solid (75.9 mg, 45%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (2H, d, J = 7.8 Hz) 6.96 (2H, d, J = 7.8 Hz), 5.05 (2H, s), 2.63 (4H, m); ESMS calculated for $C_7H_6N_3O_2$: 248.0672; observed: 248.0660.

4-Azidobenzyl-Diphenicacid Monoester.

4-Azidobenzyl alcohol (112 mg, 0.5 mmol) and diphenic acid anhydride (74.5 mg, 0.5 mmol) were dissolved in pyridine (1 mL) and stirred 12 h at 25 °C. The reaction mixture was diluted in EtOAc, washed with phosphate buffer (pH 6.0, 2x), then washed with brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash chromatography (25% EtOAc/hexanes). The desired ester was obtained as yellow solid (193 mg, 99%.). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.87 (1H, s) 7.92 (1H, dd, J = 7.2 Hz, J = 1.2 Hz) 7.86 (1H, dd, J = 7.8 Hz, J = 1.2 Hz) 7.43 (2H, dd, J = 5.7 Hz J = 1.5 Hz) 7.38 (2H, dd, J = 5.7 Hz, J = 1.2 Hz) 7.32 (2H, dd, J = 7.5 Hz, J = 1.2 Hz) 7.28 (2H, dd, J = 7.3 Hz , J = 1.2 Hz) 6.98 (2H, J = 8.4 Hz) 6.85 (2H, J = 8.4 Hz) 4.91(2H, J = 2.7 Hz); ESMS calculated for $C_{21}H_{16}N_3O_4$ ($M+H^+$): 374.1141; observed: 374.1149.

1-Azidomethylbenzoyl Thioacetic Acid Thioester.

2-Azidomethylbenzoyl acid (40 mg, 0.23 mmol) was mixed with EDC (64.9 mg, 0.34 mmol) and NHS (39.1 mg, 0.34 mmol) in CH_2Cl_2 at 25 °C for 2 h. The reaction mixture was washed with $NaHCO_3$ (2x), then washed with brine. The organic layer was concentrated and the crude product was directly used in the next step without further purification. *N*-hydroxylsuccinimidyl 2-azidomethyl benzoate ester (16.4 mg, 47 μmol) and thioacetic acid (3.2 μL) in DMF (250 μL) were allowed to react at 25 °C for 24 h. The reaction mixture was diluted in EtOAc and washed with $NaHCO_3$ (2x), then washed with brine. The organic layer was dried with Na_2SO_4 and concentrated in vacuo. The crude mixture was purified by flash chromatography (30% EtOAc/hexanes) to provide the thioester (9.8 mg, 83%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, 1H, J = 7.8 Hz) 7.56 (t, 1H, J = 7.8 Hz) 7.50 (d, 1H, J = 6.6 Hz) 7.41 (t, 1H, J = 7.8 Hz) 4.64 (s, 2H) 3.88 (s, 2H); ESMS calculated for $C_7H_6N_3O_2$: 250.0287; observed: 250.0284.

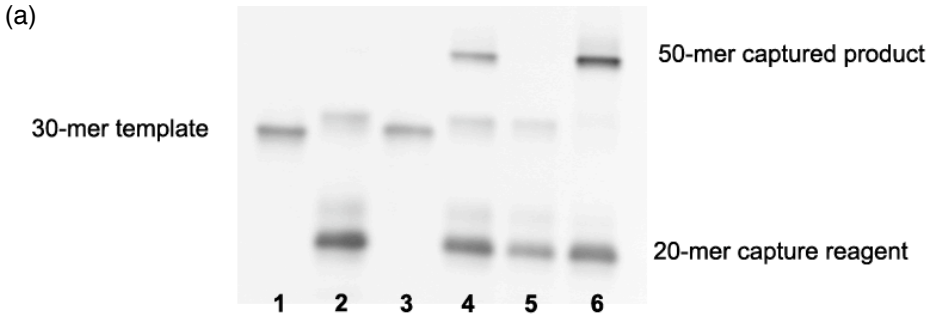
DNA-Templated Syntheses

DNA-templated azide-to-amine functional group transformations were performed by mixing a 30-mer 5' azide-linked template (12 pmol) and 10-mer 3' triphenylphosphine-linked reagent (24 pmol) in a total volume of 200 μL of 100 mM CAPS buffer (pH 10) containing 500 mM NaCl at 25 °C for 16 h. For substrates **4** and **5**, 1 M NaCl and the addition of 0.5 mM DTT to inhibit phosphine oxidation was found to increase yields. To capture amine products, 20-mer 3' carboxylic acid-linked reagent (24 pmol) was added to the reaction mixture with EDC (30 mM) and sulfo-NHS (15 mM) in MES buffer (pH 6.5). For substrate **7**, the product was captured with a 20-mer 3' aldehyde-linked reagent in the presence of $NaBH_3CN$ (3 mM) in MES buffer (pH 6.5). Reaction yields were quantitated by denaturing PAGE followed by ethidium bromide staining, UV visualization and CCD-based densitometry of product and template bands. Yield calculations assumed that templates and products in denaturing gels stained with equal intensity per base. In cases where products are partially double-stranded during quantitation, changes in staining intensity may result in higher apparent yields. Typical results by denaturing PAGE analysis are shown in Figures S1a and S1b (represented are the cases using **3** from Figure 1b for the amine acylation capture method, and **7** for the reductive amination capture method).

DNA-templated azide-to-carboxylic acid transformations were performed as above, except that the buffer contained 0.1 M MES pH 6.0 and 1 M NaCl. To capture carboxylic acid products, 20-mer 3' amine-linked reagent was added to the reaction mixture with EDC (30 mM) and sulfo-NHS (15 mM) in MES buffer (pH 6.5). Typical results are shown in Figure S1c (represented is the case using **8** from Figure 1b).

DNA-templated azide-to-thiol transformations were performed as above, except that the buffer contained either 0.1 M MES pH 6.0 (for substrate **11**) or MOPS pH 7.5 (for substrate **12**) and 1 M NaCl. To capture thiol products, 20-mer 3' alkyl bromide-linked reagent was added to the reaction mixture and incubated at 37 °C for 6 h. Typical results are shown in Figure S1d (represented is the case using **11** from Figure 1b).

Following product capture, the DNA-linked species were precipitated with NaOAc (pH 5), ethanol, and glycogen. The resulting pellet was dissolved in denaturing gel-loading buffer and was subjected to denaturing PAGE analysis.



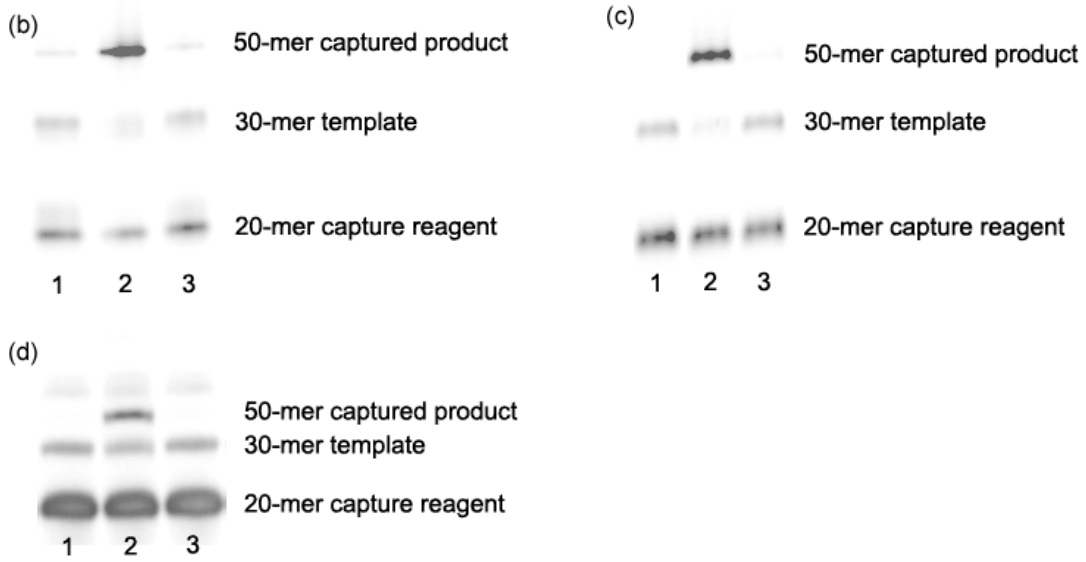


Figure S1: (a)Denaturing PAGE analysis of a DNA-templated azide-to-amine transformation for **3** (Figure 1b). Lane 1: azide-linked 30-mer template. Lane 2: azide-linked 30-mer template + carboxylic acid-linked 20-mer capture reagent + 30 mM EDC + 15 mM sulfo-NHS (showing no product formation). Lane 3: azide-linked 30-mer template + phosphine-linked 10-mer reagent (10-mer not visible). Lane 4: azide-linked 30-mer template + phosphine-linked 10-mer reagent + carboxylic acid-linked 20-mer capture reagent + 30 mM EDC + 15 mM sulfo-NHS; the 50-mer secondary product arising from azide-to-amine transformation following by DNA-templated amine acylation is visible. Lane 5: azide-linked 30-mer template + phosphine-linked 10-mer reagent containing a mismatched sequence + carboxylic acid-linked 20-mer capture reagent + 30 mM EDC + 15 mM sulfo-NHS. Lane 6: azide-linked 30-mer template + 5 mM TCEP-HCl + carboxylic acid-linked 20-mer capture reagent + 30 mM EDC + 15 mM sulfo-NHS (positive control in which the azide is reduced *in situ* by TCEP). Incomplete denaturing of the duplex between the 30-mer template and 20-mer capture reagent at the onset of electrophoresis results in band blurring (lane 2, 4-6). (b) Denaturing PAGE analysis of a DNA-templated azide-to-amine transformation for **7** (Figure 1b). Lane 1: azide-linked 30-mer template + aldehyde-linked 20-mer capture reagent + 3 mM $NaBH_3CN$. Lane 2: azide-linked 30-mer template + phosphine-linked 10-mer reagent + aldehyde-linked 20-mer capture reagent + 3 mM $NaBH_3CN$. Lane 3: azide-linked 30-mer template + phosphine-linked 10-mer reagent containing a mismatched sequence + aldehyde-linked 20-mer capture reagent + 3 mM $NaBH_3CN$. Slight 50-mer captured product formation is observed in lane 1 and 3, which arises from slow spontaneous reduction of the phenyl azide during the preparation of a substrate-linked template and during the DNA-templated reactions. The background reactivity observed in lane 1 and 3 (<13 %) is subtracted to determine the reported yield for lane 2. (c) Denaturing PAGE analysis of a DNA-templated azide-to-carboxylic acid transformation for **8** (Figure 1b). Lane 1: azide-linked 30-mer template + amine-linked 20-mer capture reagent + 30 mM EDC + 15 mM sulfo-NHS. Lane 2: azide-linked 30-mer template + phosphine-linked 10-mer reagent + amine-linked 20-mer capture reagent + 30 mM EDC + 15 mM sulfo-NHS. Lane 3: azide-linked 30-mer template + phosphine-linked 10-mer reagent containing a mismatched sequence + amine-linked 20-mer capture reagent + 30 mM EDC + 15 mM sulfo-NHS. (d) Denaturing PAGE analysis of a DNA-templated azide-to-thiol transformation for **11** (Figure 1b) using a 10 % polyacrylamide gel. Lane 1: azide-linked 30-mer template + alkyl bromide-linked 20-mer capture reagent. Lane 2: azide-linked 30-mer template + phosphine-linked 10-mer reagent + alkyl bromide-linked 20-mer capture reagent. Lane 3: azide-linked 30-mer template + phosphine-linked 10-mer reagent containing a mismatched sequence + alkyl bromide-linked 20-mer capture reagent.

MALDI-TOF Mass Spectrometric Analysis of DNA-Templated Functional Group Transformations

To a solution of 10-mer 5'-azide-linked, 3'-biotinylated template (12 pmol) in 100 mM CAPS buffer (pH 10) with 500 mM NaCl was added a complementary DNA-linked phosphine reagent (24 pmol). The mixture was agitated at 25 °C for 0.5 h then at 37 °C for 12 h. The biotinylated products and unreacted templates were purified by treating the reaction mixture with streptavidin-linked magnetic particles (Roche) and eluted following the manufacturer's protocol. DNA in the eluant was precipitated with ethanol and glycogen. Substrates **11-12** were subjected directly to the subsequent mass spectroscopic analysis. Samples for MALDI-TOF analysis were prepared by desalting the pellets dissolved in the matrix solution using a ZipTip C18 column (Millipore). Entry numbers in Table S1 correspond to the azide substrates numbered in Figure 1b. Due to the instability under the conditions for MALDI-TOF experiments, thiol-linked products (**11-12**) were captured as alkyl thioether adducts by treating with iodoacetamide (5 mM) following the DNA-templated Staudinger reaction (The MALDI-TOF data for **11-12** in Table S1 are of captured thioether adducts). A representative spectrum is shown in Figure S2.

Table S1

entry	expected mass	observed mass
1	5866.05	5868.02 ± 9
2	5922.16	5926.50 ± 9
3	5942.15	5945.18 ± 9
4	5937.13	5940.98 ± 9
5	5934.17	5934.61 ± 9
6	5942.15	5944.46 ± 9
7	5928.12	5930.91 ± 9
8	5963.16	5968.42 ± 9
9	5909.07	5915.97 ± 9
10	6032.23	6038.24 ± 9
11	5941.16	5944.61 ± 9
12	5955.19	5957.01 ± 9

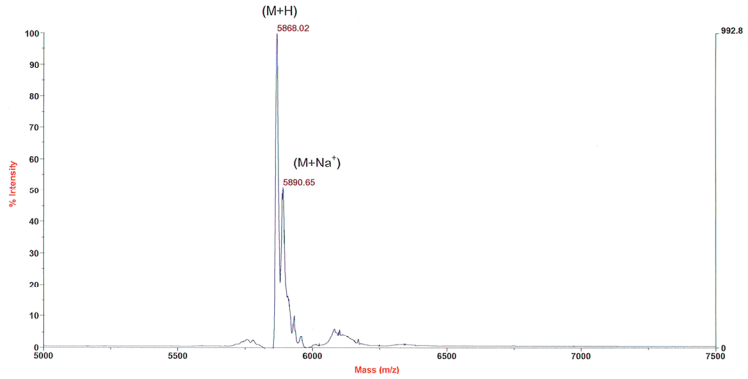


Figure S2: Representative MALDI-TOF spectrum from a DNA-templated functional group transformation (in this case, the amine product arising from **1**).

Codon Generation for the Experiment in Figure 2

Coding sequences used in Figure 2 were designed by computational screening to (*i*) ensure that at least 6 non-complementary base pairs existed between any two different codons, (*ii*) maintain a constant %GC per codon in order to minimize differences in

melting temperatures between reagents, and (*iii*) vary in mass such that the molecular weights of the 16 theoretical small-molecule coupling products are distinct and identifiable by MALDI-TOF mass spectrometry.

HPLC Analysis of Transformations of Amine-Linked Templates Using Small-Molecule Reagents

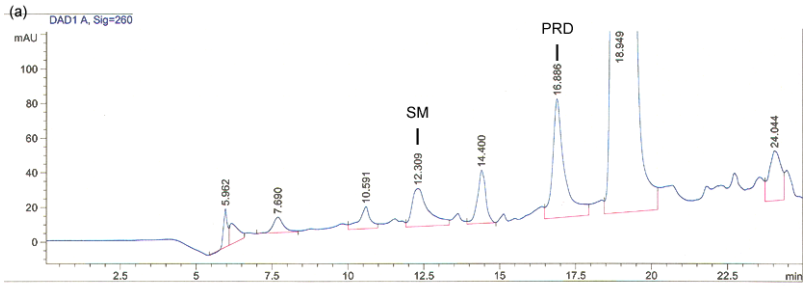
Amine-linked templates were prepared by treating azide-linked templates (**13**-**16**) with 5 mM TCEP-HCl in 100 mM MOPS buffer (pH 7.5) at 25 °C for 3 h and purified by HPLC.

Amine-linked template **13** (400 pmol) in 100 μL of 100 mM aqueous $NaHCO_3$ (pH 9.0) was mixed with 20 mM dansyl chloride **21** in 100 μL DMF and agitated at 37 °C for 1 h. The reaction mixture was diluted in 200 μL 0.1 M TEAA and passed through a NAP-5 size exclusion column. The eluant in 1 mL 0.1 M TEAA was analyzed by analytical scale reverse phase HPLC (8-30 % MeCN/ 0.1 M TEAA gradient). Product yield was calculated based on the integrated peak areas (based on UV absorbance at 260 nm) of the starting material, the product, and any side products. A representative chromatogram is shown in Figure S3a.

Amine-linked template **14** (400 pmol) in 100 μL of 200 mM aqueous $NaHCO_3$ (pH 9.0) was mixed with 40 mM ethyl chloroformate **22** in 100 μL DMF and agitated at 37 °C for 1 h. The reaction was quenched by addition of glycogen in NaOAc buffer (pH 5.0) followed by ethanol precipitation. The pellet was dissolved in 0.1 M TEAA and analyzed by analytical scale reverse phase HPLC (8-30 % MeCN/ 0.1 M TEAA gradient). A representative chromatogram is shown in Figure S3b.

Amine-linked template **15** (400 pmol) in 100 μL of 500 mM aqueous triethylamine (pH 10) was mixed with 20 mM 4-methoxyphenylisocyanate **23** in 100 μL DMF and agitated at 37 °C for 1 h. The reaction mixture was quenched and analyzed as described above. A representative chromatogram is shown in Figure S3c.

Amine-linked template **16** (400 pmol) in 100 μL of 500 mM aqueous triethylamine (pH 10) was mixed with 20 mM and was allowed to react with 20 mM 6-morpholino isothiocyanate **24** in 100 μL DMF and agitated at 37 °C for 1 h. The reaction mixture was quenched and analyzed as described above. A representative chromatogram is shown in Figure S3d.



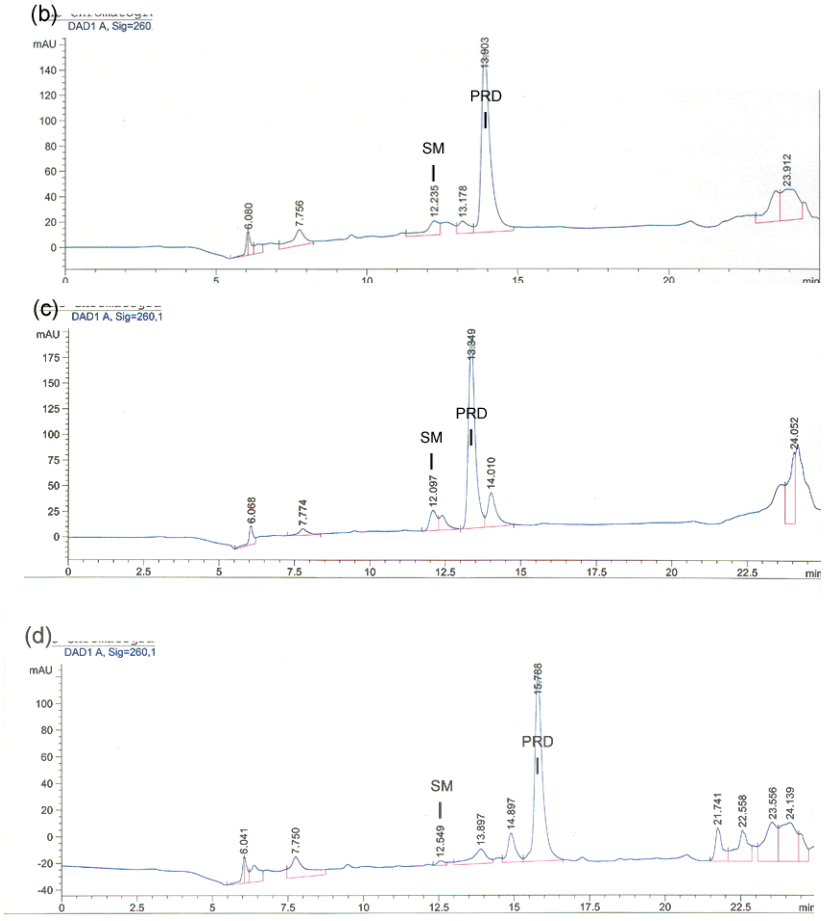


Figure S3: HPLC analysis (monitored at 260 nm) of reactions of amine-linked templates with small-molecule reagents. SM indicates unreacted starting material amine-linked template peaks. PRD indicates derivitized products. Unless otherwise noted, peaks other than those labeled as SM or PRD do not correspond to DNA-linked speices as judged by UV absorption at 230 nm and by MALDI-TOF analysis. (a) Reaction of amine-linked template **13** with **21**. (b) Reaction of amine-linked template **14** with **22**. (c) Reaction of amine-linked template **15** with **23**. (d) Reaction of amine-linked template **16** with **24**.

Sequence-Specific Transformation of Four Azide-Linked Templates into Four Amine Derivatives (Figure 2)

To a single solution of four 5' azide-linked templates (**13-16**, 100 nM for each template) in 100 mM CAPS buffer (pH 10) and 500 mM NaCl was added 3' triphenylphosphine-linked oligonucleotide **17** (8 equiv.) to effect azide-to-amine transformation. The mixture was incubated at 25 °C for 0.5 h then 37 °C for 12 h. The oligonucleotides were precipitated by the addition of glycogen in NaOAc buffer (pH 5.0) and ethanol. The pellet was dissolved in 100 μL of 100 mM $NaHCO_3$ and was allowed to react with dansyl chloride **21** in 100 μL of DMF (20 mM) at 37 °C for 1 h. The reaction mixture was desalted by ethanol precipitation.

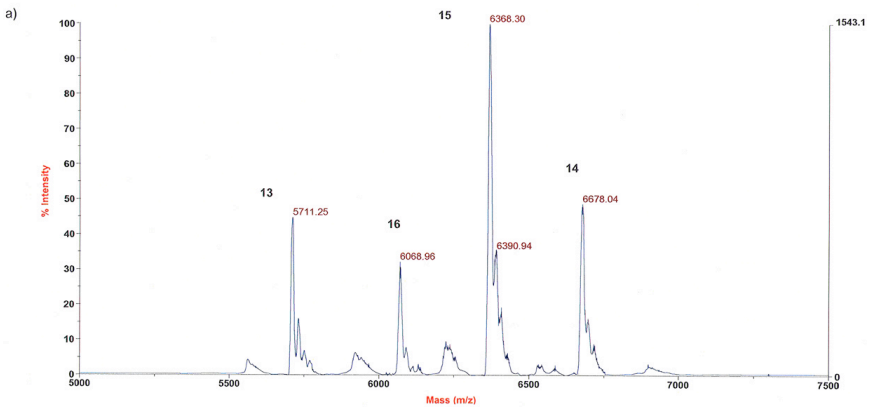
The DNA-templated azide-to-amine transformation above was repeated using a phosphine-linked oligonucleotide **18**. The pellet was dissolved in 100 μL of 200 mM $NaHCO_3$ and was allowed to react with ethyl chloroformate **22** in 100 μL of DMF (40

mM) at 37 °C for 1 h. The reaction mixture was desalted by ethanol precipitation and dried.

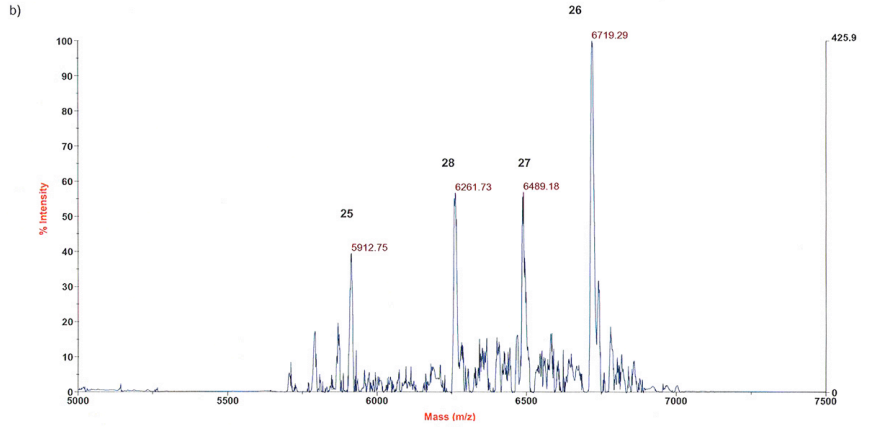
The DNA-templated azide-to-amine transformation above was repeated using phosphine-linked oligonucleotide **19**. The pellet was dissolved in 100 μL of 500 mM aqueous triethylamine solution and was allowed to react with 4-methoxyphenylisocyanate **23** in 100 μL of DMF (20 mM) at 37 °C for 1 h. The reaction mixture was desalted by ethanol precipitation and dried.

The DNA-templated azide-to-amine transformation above was repeated using phosphine-linked oligonucleotide **20**. The pellet was dissolved in 100 μL of 500 mM aqueous triethylamine solution and was allowed to react with 6-morpholino-3-pyridinylisothiocyanate **24** in 100 μL of DMF (20 mM) at 37 °C for 1 h. The reaction mixture was desalted by ethanol precipitation and dried. The pellet was dissolved in 100 mM MES buffer (pH 6), first treated with TCEP-HCl (5 mM) at 25 °C for 2 h then with NHS activated resin (Amersham Biosciences; 5 μL resin solution for 100 pmol template) for another 2 h. The resin was removed by filtration and washed three times with 0.1 M TEAA.

The final reaction mixture was treated with streptavidin-linked magnetic particles to capture the biotin-linked products. The captured olignucleotides were eluted from the particles following the manufacturer's protocol. The DNA in the eluant was precipitated with NaOAc (pH 5.0), glycogen, and ethanol. DNA recovery was determined spectrometrically by monitoring UV absorbance for the starting material pool and the final product pool at 260 nm. The concentration for a mixture containing equal amounts of **25**-**28** with a UV absorbance of 1.0 at 260 nm was estimated to be 5.5 μM. Samples for MALDI-TOF analysis were prepared as described above. Figure S4 shows representative spectra of starting materials and products.



expected mass	observed mass
13: 5705.55	5711.25 ± 9
14: 6672.18	6678.04 ± 10
15: 6363.06	6368.30 ± 9
16: 6065.81	6068.96 ± 9



expected mass	observed mass
25: 5912.61	5912.75 ± 9
26: 6718.21	6719.29 ± 10
27: 6262.54	6261.73 ± 9
28: 6486.12	6489.18 ± 9

Figure S4: A MALDI-TOF spectra of four azide starting materials in one solution (a; **13-16**) and their sequence-specific transformation into four products (b; **25-28**).

DNA Oligonucleotide Sequences and Structures

30-mer template (for Figure 1, **1-12**): 5'$NH_2(C_2H_4O)_2$-PO_3H-GGT ACG AAT TGC ACT CGG GAA ATC CAC CTT.

10-mer reagent (Figure 1): 5' AAT TCG TAC C-OPO_3H-$CH_2CH(CH_2OH)(CH_2)_4NHCOC_6H_4PPh_2$.

10-mer reagent containing a three-base mismatch: 5' AAT ACA TCC C-OPO_3H-$CH_2CH(CH_2OH)(CH_2)_4NHCOC_6H_4PPh_2$.

20-mer secondary reagent (Figure 1): 5' TCC CGA GTG CAA TTC GTA CC-OPO_3H-$CH_2CH(CH_2OH)(CH_2)_4NH_2$.

10-mer 3' biotinylated template (for MALDI-TOF analysis): 5' $NH_2(C_2H_4O)_2$-PO_3H-GGT ACG AAT TGC ACT CGG GAA ATC CAC CTT-OPO_3H-$CH(CH_2OH)CH_2(OC_2H_4)_4CH_2NHCO$-biotin.

10-mer reagent (for MALDI-TOF analysis): 5’ CGA GTA GGA A- OPO_3H-$CH_2CH(CH_2OH)(CH_2)_4NHCOC_6H_4PPh_2$.

Template (for Figure 2, **13**-**16**): 5’ $NH_2(C_2H_4O)_2$-PO_3H-TT-(codon)-GTA_n-OPO_3H-$CH(CH_2OH)CH_2(OC_2H_4)_4CH_2NHCO$-biotin.

Codon for **13**: GTG CAA CGT CAT, n = 0
Codon for **14**: CCT AGT CGT CAT, n = 3
Codon for **15**: TAA GCC CGT CAT, n = 2
Codon for **16**: AGC TTG CGT CAT, n = 1

Reagent **17** (Figure 2): 5’ CGT TGC ACA A- OPO_3H-$CH_2CH(CH_2OH)(CH_2)_4NHCOC_6H_4PPh_2$.

Reagent **18** (Figure 2): CGA CTA GGA A- OPO_3H-$CH_2CH(CH_2OH)(CH_2)_4NHCOC_6H_4PPh_2$.

Reagent **19** (Figure 2): 5’ 5’ CGG GCT TAA A- OPO_3H-$CH_2CH(CH_2OH)(CH_2)_4NHCOC_6H_4PPh_2$.

Reagent **20** (Figure 2): 5’ CGC AAG CTA A- OPO_3H-$CH_2CH(CH_2OH)(CH_2)_4NHCOC_6H_4PPh_2$.