

“Reaction Discovery Enabled by DNA-Templated Synthesis and In Vitro Selection”

Supplementary Information Index

Supplementary Methods for experiments described in the manuscript text, including protocols for the preparation of components of the reaction discovery system.

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Supplementary Tables S1-S6 (individually numbered and integrated into the above).

Supplementary Methods

General Experimental Methods

DNA oligonucleotides were synthesized using standard automated solid-phase phosphoramidite coupling methods on a PerSeptive Biosystems Expedite 8909 DNA synthesizer. All reagents and phosphoramidites for DNA synthesis were purchased from Glen Research. Oligonucleotides were purified by reverse-phase High Pressure Liquid Chromatography (HPLC) using a C18 stationary phase and an acetonitrile/100 mM triethyl ammonium acetate (TEAA) gradient and quantitated using UV spectroscopy. Labeled oligonucleotides were purified by HPLC and characterized by MALDI-TOF mass spectroscopy using a hydroxyypicolinic acid/ammonium citrate matrix. Observed masses were within 0.075% of expected masses. All H₂O used in the manipulations below was obtained from a Milli-Q purification system.

For purification and characterization of small-molecule products arising from non-DNA-templated reactions, analytical HPLC was performed on an Agilent 1100 Series instrument equipped with a Varian Pursuit C18 reverse-phase semi-preparative column. Preparative HPLC was performed on a Varian ProStar instrument equipped with a Varian Pursuit C18 1.0 gram-scale preparative reverse-phase column. Proton magnetic resonance (¹H-NMR), carbon nuclear magnetic resonance (¹³C-NMR), and COSY spectra were obtained using Varian Mercury 400 (400 MHz) and Varian INOVA500 (500 MHz) NMR spectrophotometers at 25°C. Small-molecule products were further analyzed by electrospray mass spectroscopy.

Preparation of Pool A DNA-linked Substrates

Pool A Oligonucleotide Sequence Design

Complete pool A templates contain (from 5' to 3'): a 15-base 5' PCR primer-binding site, a 15-base annealing region complementary to a pool B sequence, an 8-base constant region, a 12-base coding region used to identify the attached substrate, and a 15-base 3' PCR primer binding site (Fig. S1). Each pool A small-molecule substrate is attached to a pool A oligonucleotide through an amide linkage at the 5' end of the oligonucleotide.

Preparation of Component Oligonucleotides for the Modular Assembly of Pool A

Pool A DNA-linked substrates were assembled in a convergent, modular manner using four types of oligonucleotides: “coding”, “annealing”, “splint”, and “substrate-linked” (Fig. S1). The description and preparation of each type are detailed below.

Coding oligonucleotides are 35 bases long and consist of (5' to 3'): a 15-base PCR primer-binding site, a 12-base coding region, an 8-base constant region, and a 3'-modified group (arising from the C7 amino CPG). This 3' modification ensured that only the annealing oligonucleotides (which have natural 3'-OH groups,) were substrates for primer extension during pool A assembly (Fig. S1).

Annealing oligonucleotides are 28 bases long and contain (5' to 3'): a 5'-phosphate installed with Chemical Phosphorylating Reagent II, a 5-base portion of the 5'-PCR primer binding site, a unique 15-base annealing region, and an 8-base constant region complementary to the 8-base constant region present in the coding oligonucleotide. The 5'-phosphate group enabled these sequences to undergo a ligation reaction with the 3'-OH of the “substrate-labeled” oligonucleotides during pool A assembly (see below).

Splint oligonucleotides are 25-base sequences consisting of (5' to 3'): 15 bases complementary to an annealing region, 5 bases complementary to the 5' end of the annealing oligonucleotides, and 5 bases complementary to the 3' end of the substrate-linked oligonucleotides.

Substrate-linked oligonucleotides are 10-base sequences containing (5' to 3'): a 5' amino group (installed using the Amino-Modifier 5 phosphoramidite), and 10 bases representing the 5'-end of the 5'

PCR primer binding site. This oligonucleotide was labeled with 12 different carboxylic acids (or activated carboxylic acid derivatives) in separate reaction vessels. For a typical labeling reaction, a carboxylic acid was pre-activated as an NHS ester by combining 0.9 M solutions of the carboxylic acid, N-hydroxysuccinimide (NHS), and 1,3-dicyclohexyl carbodiimide (DCC) in a 1:1:1 ratio and letting the reaction proceed at 25° C for 40 min. The DCU precipitate in these reactions was pelleted by microcentrifugation and the supernatant was used directly in a labeling reaction. For those substrates that were commercially available as activated carboxylic acid derivatives, a 10 mg/mL stock solution in DMF was prepared and added directly to the labeling reaction. A typical labeling reaction consisted of 10 nmol of amino-terminated oligonucleotide in 100 μ L of 0.2 M phosphate buffer at pH 8.0, to which 45 μ L of the NHS ester solution was added. After 2 h at 25° C, labeling reactions were passed through a Nap-5 sephadex gel-filtration column (Amersham Biosciences) and the labeled oligonucleotide was purified by reverse-phase HPLC. See table below for MALDI characterization of each substrate-linked oligonucleotide used in the assembly of the pool A members.

The carboxylic acids (or carboxylic acid derivatives) used for the preparation of pool A were all obtained from commercial sources as follows: hexanoic acid (A1, Aldrich), cyclopropylacetic acid (A2, Oakwood Products), cyclobutanecarboxylic acid (A3, Aldrich), *trans*-3-hexenoic acid (A4, Aldrich), 6-heptynoic acid (A5, Aldrich), phenylacetic acid (A6, Aldrich), 4-iodobenzoic acid (A7, Aldrich), 3-(2-furyl)propanoic acid (A8, Acros), indole-5-carboxylic acid (A9, Aldrich), succinic anhydride (A10, Aldrich), methyl *N*-succinimidyl adipate (A11, Pierce), 6-oxoheptanoic acid (A12, Aldrich).

Modular Assembly of Pool A Members

The complete pool A DNA-linked substrates were assembled from the above components by primer extension and ligation (Fig. S1). A one-pot primer extension appended the coding sequence for a pool A member to 13 different annealing oligonucleotides and a subsequent ligation assisted by the splint oligonucleotides linked the pool A substrate encoded by the coding sequence to each of the 13 primer extension products. Primer extensions were performed on a 500 pmol scale (5 μ M coding sequence and 5 μ M total annealing sequences) in 100 μ L at 25° C for 3 h using Klenow *exo*⁻ (New England Biolabs). In addition to the coding and annealing sequences, 1 nmol of the splint oligonucleotides necessary for the ligation step was added to the primer extension step to minimize the formation of internal secondary structure within the annealing sequences.

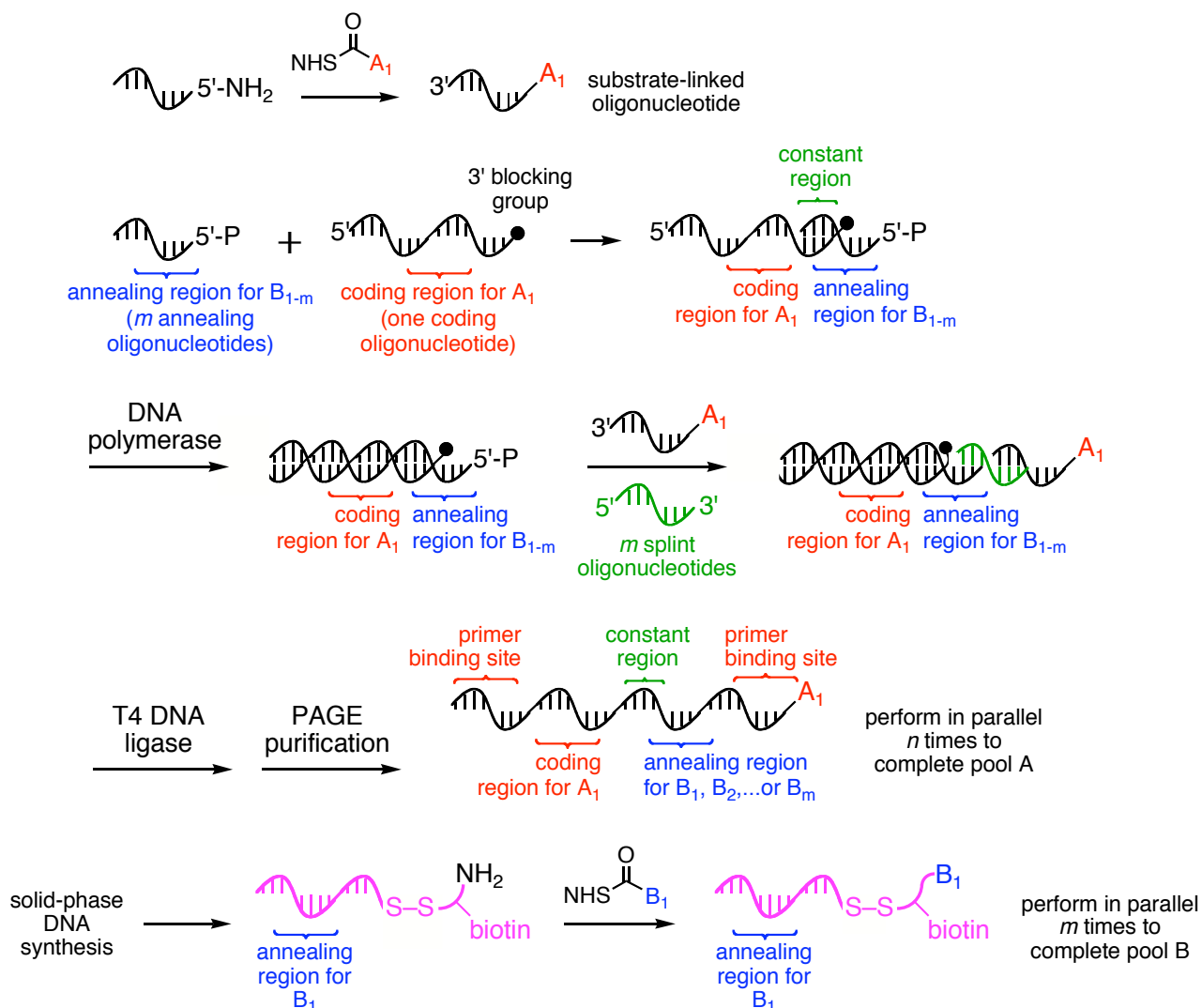


Fig. S1. Assembly of pool A and pool B.

Ligations were performed directly following buffer exchange of the primer extension reactions. One nmol of the appropriate 5'-pool A substrate-linked oligonucleotide (two equiv. with respect to the primer extension products, one equiv. with respect to the splint oligonucleotides) was added to the buffer-exchanged reaction and the ligation was performed overnight at 16° C using T4 DNA ligase. Ligation reactions were precipitated with ethanol and full-length single-stranded pool A DNA-linked substrates were purified by denaturing PAGE. Typically, a 500 pmol-scale assembly of a pool A DNA-linked substrate yielded ≥ 100 pmol of purified full-length templates. The mass spectroscopic analysis of the substrate-linked oligonucleotides used in the assembly of pool A is shown below in Table S1.

substrate	expected mass of DNA-linked substrate	observed mass
hexanoic acid (A1)	3281.7	3281 $\pm$ 3
cyclopropylacetic acid (A2)	3265.6	3267 $\pm$ 3
cyclobutane carboxylic acid	3265.6	3267 $\pm$ 3

(A3)		
3-hexenoic acid (A4)	3279.6	3280 $\pm$ 3
6-heptynoic acid (A5)	3291.6	3292 $\pm$ 3
phenylacetic acid (A6)	3301.6	3304 $\pm$ 3
4-iodobenzoic acid (A7)	3413.5	3414 $\pm$ 3
3-(2-furyl)propanoic acid (A8)	3305.6	3306 $\pm$ 3
indole-5-carboxylic acid (A9)	3326.6	3327 $\pm$ 3
succinic acid (A10)	3283.6	3385 $\pm$ 3
adipic acid monomethyl ester (A11)	3325.6	3326 $\pm$ 3
6-oxoheptanoic acid (A12)	3309.6	3310 $\pm$ 3

Table S1. MALDI-TOF characterization of pool A substrate-linked oligonucleotides.**Preparation of Pool A DNA-linked Substrates for the Detection of Pool B Homocoupling**

In addition to the 12 pool A members described above (each containing 13 different sequences), 12 individual pool A sequences were prepared, each linked to a different pool B substrate. These DNA-linked substrates were designed to enable detection of homocoupling of any of the pool B substrates. Each of these DNA-linked substrates was prepared in a manner analogous to the preparation of pool A members described above except that only one annealing oligonucleotide and only one splint oligonucleotide were used in the primer-extension and ligation steps. Additionally, all of these DNA-linked substrates were prepared using the same coding oligonucleotide (rather than coding for a substrate, this coding sequence encoded for homocoupling). Mass spectroscopic characterization of the substrate-linked oligonucleotides is provided in Table S2.

substrate	expected mass of DNA-linked substrate	observed mass
5-chlorovaleric acid (B1)	3301.6	3303 $\pm$ 3
<i>p</i> -formylbenzoic acid (B2)	3315.6	3317 $\pm$ 3
acrylic acid (B3)	3237.6	3240 $\pm$ 3
5-oxiranyl pentanoic acid (B4)	3309.6	3313 $\pm$ 3
6-heptenoic acid (B5)	3293.7	3293 $\pm$ 3
3-(4-hydroxy-phenyl)propionic acid (B6)	3331.6	3332 $\pm$ 3
5-methoxycarbonyl-4-oxopentanoic acid (B7)	3339.6	3342 $\pm$ 3
4-(2-carboxyethyl)benzeneboronic acid (B8)	3515.8 (citric acid adduct)	3516 $\pm$ 3
4-azidobutyric acid (B9)	3294.6	3296 $\pm$ 3
8-cyano-octanoic acid (B10)	3334.7	3336 $\pm$ 3
6-hydroxycaproic acid (B11)	3297.6	3298 $\pm$ 3
unlabeled amine (no carboxylic acid) (B12)	3183.6	3185 $\pm$ 3

Table S2. MALDI-TOF characterization of pool A substrate-linked oligonucleotides for the detection of pool B homocoupling.**Preparation of Pool B**

Pool B DNA-linked substrates are 16 bases in length and contain (5' to 3'): a 15-base annealing region complementary to one of the annealing regions in pool A, a single C, a disulfide linker, a biotin

group, and an amide-linked substrate (Fig. S1). The modified groups were introduced using the 3' PT Amino-Modifier C6 CPG, the biotin phosphoramidite, and the thiol modifier C6 S-S. The 3' amine-terminated oligonucleotides were labeled with the appropriate activated carboxylic acid using the protocol described in the preparation of pool A. The purified labeled oligonucleotides were characterized by mass spectroscopy as is shown below. In all, 24 DNA-linked substrates were prepared and characterized in this manner comprising 12 DNA-linked pool B substrates and 12 additional DNA-linked substrates for detection of homocoupling of any of the pool A substrates.

The following carboxylic acids or carboxylic acid derivatives used in the preparation of pool B were available from commercial sources: 5-chlorovaleric acid (Aldrich, B1), *p*-formylbenzoic acid N-hydroxysuccinimide ester (Aldrich, B2), acrylic acid (Aldrich, B3), 6-heptenoic acid (Aldrich, B5), 3-(4-hydroxy-phenyl)propionic acid N-hydroxysuccinimide ester (Aldrich, B6), 4-(2-carboxyethyl)benzeneboronic acid (Lancaster, B8), and 6-hydroxycaproic acid (Aldrich, B11). 5-methoxycarbonyl-4-oxopentanoic acid (B7) was prepared according to a literature procedure (Oku, A., Numata, M. *J. Org. Chem.* **65** 1899-1906 (2000)). 4-azidobutyric acid (B9) was prepared according to a literature procedure (Kusumoto, S., Sakurai, K., Shiba, T. *Bull. Chem. Soc. Jpn.* **59**, 1296-1298 (1986)). 5-oxiranyl pentanoic acid (B4) was prepared from 6-heptenoic acid using the epoxidation procedure described in Corey, P. F., Ward, F. E. *J. Org. Chem.* **51**, 1925-1926 (1986). ¹H NMR (CDCl₃): δ = 1.50-1.61 ppm (m, 4H), 1.67-1.74 (m, 2H), 2.38 (t, 2H), 2.48 (dd 1H), 2.76 (t, 1H), 2.92 (m, 1H). HRMS: calculated = 162.1130 (NH₄⁺); observed = 162.1125. ¹³C NMR (CDCl₃): δ = 24.63, 25.66, 32.28, 34.06, 47.29, 52.34, 179.66.

Preparation of 8-Cyano-octanoic Acid (Substrate B10):

The preparation of the cyano-carboxylic acid representing substrate B10 was adapted from a procedure for the preparation of 4-(2-cyanoethyl)benzoic acid (Delia, T. J., Baumann, M., Bunker, A. *Heterocycles*, **35**, 1397-1410 (1993)). To 501 mg (2.25 mmol) of 8-bromooctanoic acid in 7.0 mL of methanol was added 520 mg NaCN (10.6 mmol) in 2.0 mL H₂O. The solution was heated at reflux for 2.5 h. After cooling to 25 °C, the solution was acidified to pH ~1.5 with concentrated HCl (*caution*: HCN gas evolves with the addition of HCl) and diluted with 40 mL EtOAc. The resulting suspension was washed twice with 15 mL H₂O. The organic layer was dried by filtration through MgSO₄, and the solvent was removed *in vacuo* to afford 282 mg (74%) of a light brown oil. The ¹H NMR spectrum agreed with previously reported data in Cotarca, L., Delogu, P., Maggioni, P., Nardelli, A., Bianchini, R., Sguassero, S. *Synthesis* **1997**, 328-332.

Mass spectroscopic characterization of pool B members are shown in Tables S3 and S4.

substrate	expected mass of DNA-linked substrate	observed mass
5-chlorovaleric acid (B1)	5922.3	5922 ± 5
4-formylbenzoic acid (B2)	5896.2	5895 ± 5
acrylic acid (B3)	5778.2	5778 ± 5
5-oxiranyl pentanoic acid (B4)	5952.3	5952 ± 5
6-heptenoic acid (B5)	5874.3	5874 ± 5
3-(4-hydroxy-phenyl)propionic acid (B6)	5823.2	5823 ± 5
5-methoxycarbonyl-4-oxopentanoic acid (B7)	5960.3	5960 ± 5
4-(2-carboxyethyl)benzeneboronic acid (B8)	6145.3 (citric acid adduct)	6145 ± 5
4-azidobutyric acid (B9)	5755.2	5754 ± 5

8-cyano-octanoic acid (B10)	5906.3	5907 $\pm$ 5
6-hydroxycaproic acid (B11)	5869.3	5869 $\pm$ 5
unlabeled amine (no RCOOH) (B12)	5775.3	5775 $\pm$ 5

Table S3. MALDI-TOF characterization of pool B members.

Hexanoic acid (A1)	5835.3	5835 $\pm$ 5
Cyclopropylacetic acid (A2)	5855.3	5854 $\pm$ 5
Cyclobutanecarboxylic acid (A3)	5917.3	5917 $\pm$ 5
3-hexenoic acid (A4)	5891.3	5891 $\pm$ 5
6-heptynoic acid (A5)	5845.2	5843 $\pm$ 5
Phenylacetic acid (A6)	5873.3	5873 $\pm$ 5
4-iodobenzoic acid (A7)	6003.2	6003 $\pm$ 5
3-(2-furyl)propionic acid (A8)	5877.3	5877 $\pm$ 5
Indole-5-carboxylic acid (A9)	5876.3	5875 $\pm$ 5
Succinic acid (A10)	5815.2	5814 $\pm$ 5
Adipic acid monomethylester (A11)	5906.3	5906 $\pm$ 5
6-oxoheptanoic acid (A12)	5819.3	5818 $\pm$ 5

Table S4. MALDI-TOF characterization of pool B members for the detection of pool A homocoupling**Preparation of DNA Microarrays**

Commercial 5'-amino-modified oligonucleotides (Illumina) were printed on aldehyde-silane glass slides (Schott-Nexterion) using a robotic microarrayer (GeneMachines). Stock solutions for printing were 20 μ M in 3X SSC buffer. Fifteen copies of the microarray were printed on each slide, in 5 columns of 3 copies each. This enabled each analyte sample to be hybridized to three different microarrays per experiment. In all cases, the three replicate microarrays per experiment gave virtually indistinguishable signals. Printed oligonucleotides were immobilized and unreacted aldehydes on the slides were blocked according to the manufacturer's protocol with one 15 s concentrated NH_4OH wash followed by a 1 min H_2O wash added to the end of the blocking procedure. After blocking, slides were stored in a dessicator covered with aluminum foil and used within two weeks.

Each printed oligonucleotide representing a unique combination of substrates was a 35-mer consisting of (5' to 3') one of the 15-base annealing regions, an 8-base constant region, and one of the 12-base coding regions from the pool A sequences. The 169th oligonucleotide used to detect the internal standard (see Selections for Bond Formation) shared only the constant region with any of the other 168 sequences and was printed in the lower right corner of each array.

Selections for Bond-Formation*Internal Standard Oligonucleotide:*

To compare fluorescence intensity ratios between arrays, a biotinylated oligonucleotide was included in each selection at an amount equal to 1/10th of the amount of an individual pool A sequence encoding a unique substrate combination. This oligonucleotide served as an internal standard representing ~10% DNA-templated reactivity in a selection. The reference oligonucleotide was synthesized with a 5' biotin group using the 5' Biotin Phosphoramidite and purified by Oligonucleotide Purification Cartridge (OPC, Applied Biosystems, Inc.)

Universal Clamp Oligonucleotide

To minimize secondary structure in the pool A oligonucleotides, a “universal clamp” oligonucleotide was included in each selection for bond formation. This oligonucleotide consisted of (5' to 3') 15 bases complementary to the 3' primer binding site of the pool A sequences and 8 bases complementary to the constant region of the pool A sequences.

Selection Components

A typical selection for bond-formation used five components: pool A, pool B, a “universal clamp”, the biotinylated internal standard, and the metal complex or reagents being tested. For selections, stock solutions were prepared for each pool. The stock solution for pool A was 1 μ M in total DNA and contained equal amounts of each of the 168 different oligonucleotides (encoding the 144 heterocoupling and 24 homocoupling substrate combinations) in addition to 1/10 of an equivalent of the biotinylated internal standard oligonucleotide. The stock solution for pool B was 2 μ M in total DNA and contained equal amounts of each of the 12 pool B oligonucleotides and 1/12 of one equivalent of each of the “self-A” homocoupling-encoding oligonucleotides.

Reaction Discovery Experiments and Selection Procedure

Each reaction discovery experiment was performed in 12 μ L total solution containing 12 fmol of material representing each unique substrate combination. Two pmol of total pool A material, 2 pmol of total pool B material, 8 pmol of the universal clamp, and 1.2 fmol of the biotinylated internal standard were combined in buffer and allowed to hybridize at 25 °C for 30 min. Reactions were initiated by adding 1 μ L of a solution containing the metal or non-DNA-linked reagents. The detailed conditions for each experiment shown in Fig. 2b are as follows:

- No metal: 50 mM MOPS, 0.5 M NaCl, pH 7.0, 37 °C, 1h.
- Cu(I): 500 μ M CuSO₄, 500 μ M sodium ascorbate, 50 mM MOPS, 0.1 M Na₂SO₄, pH 7.0, 25 °C, 10 min.
- EDC + sNHS: 20 mM EDC, 15 mM sNHS, 50 mM MES, 0.5 M NaCl, pH 6.0, 25°C, 1h.
- Pd(II), 37 °C: 500 μ M Na₂PdCl₄, 50 mM MOPS, 0.5 M NaCl, pH 7.0, 1h.
- Pd(II), 25 °C: 500 μ M Na₂PdCl₄, 50 mM MOPS, 0.5 M NaCl, pH 7.0, 20 min.

After the reaction times indicated above, the solution was diluted with 20 μ L of H₂O and passed through a gel filtration microcentrifuge column (Princeton Separations) to remove small-molecule reactants. To the eluant was added 150 μ L of a 0.1 M aqueous solution of *tris*(2-carboxyethyl)phosphine hydrochloride in 1.0 M sodium phosphate, pH 8.0 to effect disulfide cleavage. After 1 h at 25 °C, this solution was added to a 20 pmol binding-capacity aliquot of streptavidin-linked magnetic particles (Roche Biosciences) suspended in 300 μ L of 0.1 M NaCl, 10 mM Tris, 1 mM EDTA, pH 7.5 (“loading buffer”). The supernatant was removed after 15 min. The particles were rinsed once with 200 μ L H₂O, and once with 200 μ L 1 M NaCl, 10 mM Tris, 1 mM EDTA, pH 7.5 (“washing buffer”). The particles were then resuspended in 20 μ L of 95% formamide-5% 10 mM EDTA and incubated at 65 °C for 10 min. Fifteen μ L of this eluant was diluted with 60 μ L H₂O and added to a fresh aliquot of streptavidin particles suspended in 350 μ L of loading buffer. After 15 min, the supernatant was removed and the particles were rinsed with H₂O and washing buffer as above. The particles were resuspended in 15 μ L of 95% formamide-5% 10 mM EDTA and incubated at 90 °C for 10 min. Ten μ L of this eluant was diluted with 35 μ L H₂O and passed through a gel filtration spin-column (Princeton Separations). This solution was used directly in PCR reactions for microarray analysis.

Microarray Analysis of Selections***Preparation of PCR Primers***

Primer 1 was synthesized using standard automated methods and purified using an OPC column to avoid trace contaminants from HPLC purification that could be amplified in a PCR reaction. Primer 2 was synthesized with the C6 Amino-dT phosphoramidite in place of the dT monomer and also purified by OPC. (See the DNA Oligonucleotide Sequences section for the primer sequences). A solution of 0.5 μ mol primer 2 in 200 μ L of 0.1 M sodium carbonate pH 9.3 was added to a Cy-3 Mono-Reactive Dye Pack (Amersham Biosciences) and the solution was agitated in the dark at 25 °C for 1h before being passed through a gel filtration spin-column and a Nap-5 gel filtration column. The Nap-5 eluant was quantitated by UV and stored at –80 °C until it was used in a PCR reaction. A 0.5 μ mol aliquot of primer 2 was labeled with Cy-5 using an identical procedure with the Cy-5 Mono-Reactive Dye Pack.

Blocking Oligonucleotides

Twenty-three-base oligonucleotides complementary to each annealing region + constant region and 20-base oligonucleotides complementary to each constant region + coding region present on the array were purchased from Illumina. These oligonucleotides were included in the hybridization solution to maximize annealing fidelity.

PCR Amplification of Pre- and Post-Selection Sequences and Microarray Analysis

Ten μ L aliquots of selected sequences (see Selections for Bond Formation) were added to a 250 μ L PCR reaction containing 2.5 mM MgCl₂, 0.2 mM dNTPs, 40 nM primer 1, and 400 nM primer 2 labeled with Cy-3. The sequences were amplified with 35 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 20 s. Forty fmol of an equimolar mixture of the 169 pool A sequences (including the internal standard sequence) was amplified by PCR under identical conditions using primer 2 labeled with Cy-5. The PCR reactions were extracted with phenol-chloroform and precipitated with EtOH. A pre-selection pellet (Cy-5-labeled) and post-selection pellet (Cy-3-labeled) were resuspended together in 24 μ L of 20 mM HEPES pH 7.5, 1X SSC containing 10 μ g BSA and 10 μ M blocking oligonucleotides. The solution was filtered using a 0.45 μ M microcentrifuge filter (Millipore). Six μ L of formamide and 0.45 μ L of 10% SDS were added to the filtrate and the solution was heated to 100 °C for 2 min. After cooling to room temperature, this solution was hybridized at 65 °C for 12 h to the DNA microarray described above. After hybridization, the slides were washed with 0.025% SDS, 0.6X SSC (5 min) and 0.05X SSC (5min) and scanned immediately using an Axon GenePix 4000B scanner (Axon Instruments).

DNA Oligonucleotide Sequences and Linker Structures

Pool A oligonucleotide sequence:

5'-CGTTGATATCCGCAGXXXXXXXXXXXXXXXXXXCACACACC
 YYYYYYYYYYYYGCCAGCTGCTAGCTT-3'

where XXXXXXXXXXXXXXXX (the annealing region) is one of the following:

- | | |
|--------------------|---------------------|
| 1) AACTTCCTCTCGGGA | 8) CTTAGTTTCGCGCAC |
| 2) ACGCGATGTTTCGAC | 9) GAGGGTGATGCATGT |
| 3) AGCGTTATGGTCCGA | 10) GCTGGACTAGCTACA |
| 4) ACATGAGCCCCACTA | 11) TAGACACGCATGTGC |
| 5) CCACTGTTACTAGGG | 12) TCGGACTTTGATGGC |
| 6) CGTGCTTGAGGAGAA | 13) ACCGAAGGGCAATAC |
| 7) AGGCCTCTTTAGACC | 14) GTCAGCCCTTGGTAT |

15) CACCTACCGGTAATC
16) CAGTCCGGTACCTAA
17) CCTGAGAAAGAACCG
18) TTGATGACCAGGCCA
19) TGCGCAACTGGTCTT

20) TGGCAAATCCAGCGT
21) GACCTTGGCGTTTAG
22) TCCAGGGGATGCATA
23) GCACGTACATTGCTG
24) GGTGTATACGTGGCT

and **YYYYYYYYYYYY** (the coding region) is one of the following:

1) AAGCTGATCACG (**A1**)
2) TAGTGCCGTTGA (**A2**)
3) ACCATATCCCCT (**A3**)
4) ACGTATAGCGGT (**A4**)
5) AGAAAGTGTGCG (**A5**)
6) ATAGTTGGCTCC (**A6**)
7) ATCTGGAACCTC (**A7**)

8) ATTCAACCGTCTG (**A8**)
9) CACCGAAGTAAC (**A9**)
10) TTGGAGCCTGAT (**A10**)
11) CCCTCCTTATCA (**A11**)
12) TGTCGGGACAAT (**A12**)
13) TGCTAATGACGC (SELF-B)

Annealing and coding sequences were generated by computer algorithms to minimize undesired reactivity arising from the mismatched hybridization of a pool B member with a non-complementary pool A member. The overall GC/AT ratio of these sequences was kept constant to minimize differences in melting temperatures between different annealing and coding sequences.

Internal standard oligonucleotide sequence:

5'CGTTGATATCCGCAGGTGCATTAGCACGCACACACCTCTAACACAGCCGCCAGCTGC
TAGCTT-3'

Pool B oligonucleotide sequences are as follows:

1) TCCCGAGAGGAAGTTC (**B1**)
2) GTCGAAACATCGCGTC (**B2**)
3) TCGGACCATAACGCTC (**B3**)
4) TAGTGGGGCTCATGTC (**B4**)
5) CCCTAGTAACAGTGGC (**B5**)
6) TTCTCCTCAAGCACGC (**B6**)
7) GGTCTAAAGAGGCCTC (**B7**)
8) GTGCGCGAACTAAGC (**B8**)
9) ACATGCATCACCTCC (**B9**)
10) TGTAGCTAGTCCAGC (**B10**)
11) GCACATGCGTGTCTAC (**B11**)
12) GCCATCAAAGTCCGAC (**B12**)

13) GTATTGCCCTTCGGTC (SELF A1)
14) ATACCAAGGGCTGACC (SELF A2)
15) GATTACCGGTAGGTGC (SELF A3)
16) TTAGGTACCGGACTGC (SELF A4)
17) CGGTTCTTTCTCAGGC (SELF A5)
18) TGGCCTGGTCATCAAC (SELF A6)
19) AAGACCAGTTGCGCAC (SELF A7)
20) ACGCTGGATTTGCCAC (SELF A8)
21) CTAAACGCCAAGGTCC (SELF A9)
22) TATGCATCCCCTGGAC (SELF A10)
23) CAGCAATGTACGTGCC (SELF A11)
24) AGCCACGTATACACCC (SELF A12)

Sequences used in DNA-templated characterization of array positives:

30-base oligonucleotide: 5'- CGTTGATATCCGCAGACGCGATGTTTCGAC

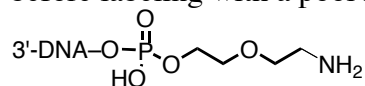
16-base oligonucleotide: 5'- GTCGAAACATCGCGTC

Sequences used in MALDI-TOF analysis of DNA-templated reactions:

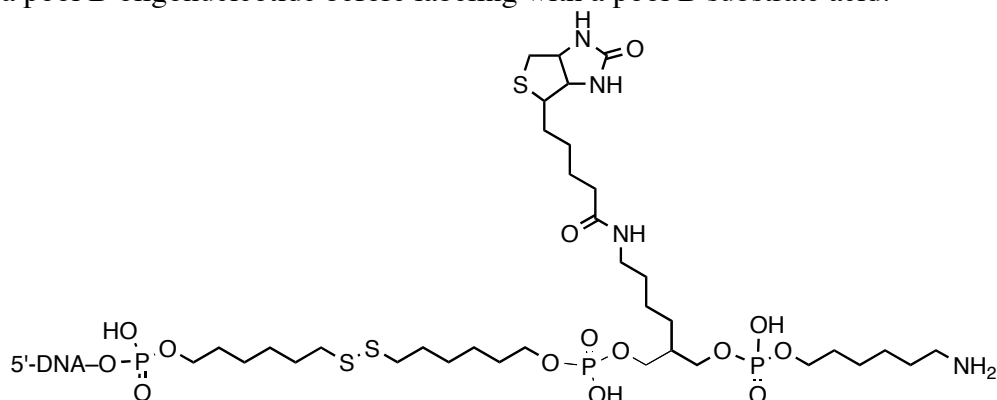
20-base oligonucleotide: 5'- CATTACCATGTACATACCAG

15-base oligonucleotide: 5'- CTGGTATGTACATGG

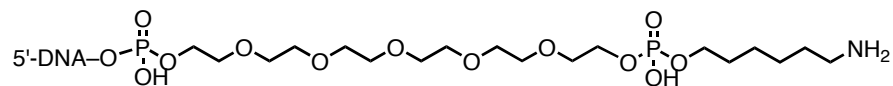
Structure of a pool A oligonucleotide before labeling with a pool A substrate acid:



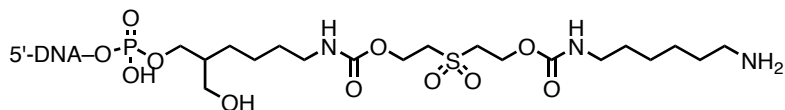
Structure of a pool B oligonucleotide before labeling with a pool B substrate acid:



Structure of an unlabeled 16-base oligonucleotide used in the DNA-templated characterization of array positives:



Structure of an unlabeled 15-base oligonucleotide with a cleavable sulfone linker used in the MALDI-TOF characterization of DNA-templated reactions:



Supplementary Data

Quantitative Microarray Data

The ratio of median green:red fluorescence intensities of the spots on the arrays were calculated using the GenePix Pro 5.0 software. The values for the spots with the highest green:red ratios of median fluorescence in each of the arrays in Fig. 2B are as follows:

- Cu(I): A5+B9 = 8.5; standard = 2.8
- EDC + sNHS: standard = 15.6; A10+B12 = 6.9
- Pd(II), 37 °C: A7+B3 = 3.6; A5+B3 = 3.5; A4+B8 = 3.0; standard = 2.9; A5+B5 = 2.7; A5+B13 = 2.6; A9+B3 = 1.9; A8+B8 = 1.8; A8+B3 = 1.8; A5+B8 = 1.6; A5+B9 = 1.6
- Pd(II), 25 °C: A5+B5 = 3.7; standard = 3.5; A5+B3 = 3.1; A4+B8 = 2.8; A5+B13 = 2.7; A5+B8 = 1.9; A5+B9 = 1.7

Characterization of Putative Reactions in a DNA-Templated Format

Reactions corresponding to array positives from the Pd(II) selections were performed in a DNA-templated format on a 25 pmol scale and analyzed by denaturing PAGE. Two oligonucleotides were used for these experiments: a 30-base oligonucleotide labeled with a substrate at the 5' end and a 16-base oligonucleotide (complementary to a region of the 30-base oligonucleotide) labeled with a substrate at the 3' end. The 30-base oligonucleotide was synthesized with a 5' amine using the 5'-Amino-Modifier 5 phosphoramidite. The 16-base oligonucleotide was synthesized with a 3' amine using the 3'-PT Amino-Modifier C6 CPG and the Spacer 18 phosphoramidite (see Supplementary Methods for the complete structure). The Spacer 18 phosphoramidite enabled the number of bonds between the substrate and the 3' base of the 16-base oligonucleotide to match the number of bonds between the substrate and the 3' base in a pool B DNA-linked substrate. Both oligonucleotides were labeled with appropriate substrates as described in the preparation of the substrate-linked oligonucleotides in pool A. Labeled oligonucleotides were characterized by MALDI-TOF mass spectroscopy (Table S5).

substrate	expected mass of labeled 30-base oligonucleotide	observed mass
3-hexenoic acid (A4)	9456.6	9457 ± 9
6-heptynoic acid (A5)	9468.6	9468 ± 9
4-iodobenzoic acid (A7)	9590.5	9591 ± 9
3-(2-furyl)propionic acid (A8)	9482.6	9481 ± 9
indole-5-carboxylic acid (A9)	9503.6	9502 ± 9
hexanoic acid (negative control)	9458.7	9457 ± 9

substrate	expected mass of labeled 16-base oligonucleotide	observed mass
acrylic acid (B3)	5441.05	5441 ± 5
6-heptenoic acid (B5)	5497.12	5497 ± 5
4-(2-carboxyethyl)benzeneboronic acid (B8)	5719.11 (citric acid adduct)	5717 ± 5
6-heptynoic acid (A5)	5495.10	5494 ± 5
hexanoic acid (negative control)	5485.10	5484 ± 5

Table S5. MALDI-TOF characterization of DNA-linked substrates for evaluating array positives in a DNA-templated format.

To perform a DNA-templated reaction, 25 pmol of substrate-linked 30-mer was combined with 37.5 pmol of substrate-linked 16-mer in 360 μL of 0.1 M MOPS, 1 M NaCl, pH 7.0 and allowed to hybridize for at least 20 min. Reactions were initiated by adding 40 μL of a 5 mM solution of Na_2PdCl_4 in H_2O and allowed to react for either 20 min at 25 $^\circ\text{C}$ or 1 h at 37 $^\circ\text{C}$ before the Pd was quenched with 4 μL of 2 M β -mercaptoethanol and the reactions were EtOH precipitated. Ten pmol aliquots of the reactions were electrophoresed on denaturing polyacrylamide gels and the efficiencies of the reactions were determined by quantitation of ethidium bromide staining. Control reactions in which Pd was omitted showed no product formation. Control reactions with Pd in which one of the substrates was replaced with an alkane also showed no product formation, although occasionally we observed faint diffuse higher molecular weight bands that we attribute to Pd-complexed species. In the case of the reaction discovery selections, cleavage of the disulfide linker prevents Pd complexation from generating false positives. Reaction yields were quantitated by UV visualization of stained gels and densitometry of product and starting material bands using a Stratagene Eagle Eye II densitometer. Yield calculations assumed that species in denaturing gels stain with comparable intensity per nucleotide. The DNA-templated yields of the nine positives arising from the 37 $^\circ\text{C}$ Pd(II) array shown in Fig. 2b are shown in Fig. S2.

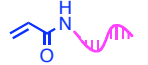
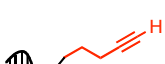
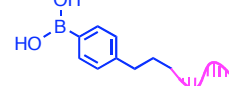
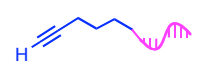
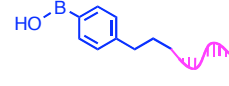
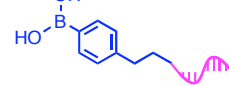
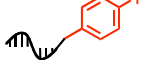
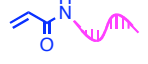
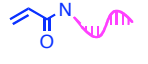
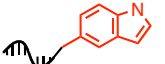
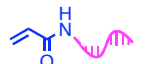
substrates		DNA-templated yields	
		37 $^\circ\text{C}$	25 $^\circ\text{C}$
		35	31
		28	20
		36	34
		45	42
		57	39
		30	<10
		39	14
		19	<10
		<10	<10

Fig. S2. DNA-templated yields of reactions corresponding to array positives.

MALDI-TOF Analysis of DNA-Templated Reaction Products

The products arising from reactions validated in a DNA-templated format by gel electrophoresis were further characterized by MALDI-TOF mass spectrometry. Two oligonucleotides were used for these experiments: a 20-base oligonucleotide labeled with a substrate at the 5' end and a 15-base oligonucleotide (complementary to a 15-base region of the 20-mer) with a 5' biotin group and a substrate attached to the 3' end through a base-labile sulfone linker. The 20-base oligonucleotide was synthesized with a 5' amine using the 5' Amino-Modifier 5 phosphoramidite and labeled using the procedures described in the preparation of substrate-linked oligonucleotides for pool A.

The 15-base oligonucleotide was synthesized with a 3' amine and a 5' biotin using the 3' Amino-Modifier C7 CPG and the 5' Biotin phosphoramidite. The oligonucleotide was labeled with a substrate via a cleavable linker in a two step procedure. In the first step, 5-10 nmol of oligonucleotide in 100 μ L of 0.2 M phosphate pH 8.0 was combined with 10 μ L of a 0.1 M solution of *bis*[2-(succinimidylloxycarbonyloxy)-ethyl]sulfone (BSOCOES, Pierce) in DMF and vortexed for 90 s. Immediately after vortexing, 9 μ L of a 0.2 M solution of hexanediamine dihydrochloride salt in H₂O was added and the solution was agitated at 25 °C for 1 h. The solution was then passed through a Nap-5 gel filtration column (Amersham) and the hexanediamine-labeled oligonucleotide was purified by reverse-phase HPLC. The purified oligonucleotide was then labeled with the appropriate carboxylic acid using the same procedures described above.

Substrate-linked 20-base and 15-base oligonucleotides used in these experiments were characterized by MALDI-TOF mass spectroscopy as shown in Table S6.

substrate	expected mass of labeled 20-base oligonucleotide	observed mass
6-heptynoic acid	6317.15	6315 $\pm$ 5
4-iodobenzoic acid	6439.02	6440 $\pm$ 5
3-(2-furyl)propionic acid	6331.13	6330 $\pm$ 5
4-(2-carboxyethyl)benzeneboronic acid	(citric acid adduct) 6541.2	6539

substrate	expected mass of labeled 15-base oligonucleotide	observed mass
acrylic acid	5611.2	5610 $\pm$ 5
6-heptenoic acid	5667.2	5668 $\pm$ 5
4-(2-carboxyethyl)benzeneboronic acid	(citric acid adduct) 5889.2	5888 $\pm$ 5
6-heptynoic acid	5665.2	5664 $\pm$ 5
3-hexenoic acid	5653.2	5656 $\pm$ 5

Table S6. MALDI-TOF characterization of DNA-linked substrates for characterizing reaction products in a DNA-templated format.

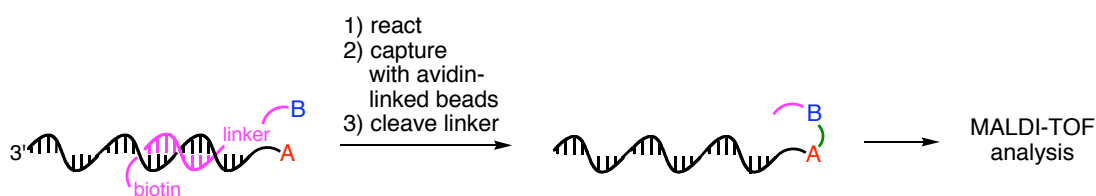


Fig. S3. Strategy used to characterize products of DTS reactions by MALDI-TOF mass spectrometry.

To obtain a product sample suitable for MALDI-TOF analysis (Fig. S3), 50 pmol of substrate-labeled 20-mer was combined with 75 pmol of substrate-labeled 15-mer in 450 μL of 0.1 M MOPS, 1 M NaCl, pH 7.0 and allowed to hybridize for at least 20 min at 25 $^{\circ}\text{C}$. Reactions were initiated by adding 50 μL of 5 mM Na_2PdCl_4 in H_2O and allowed to react for either 20 min at 25 $^{\circ}\text{C}$ or 1 h at 37 $^{\circ}\text{C}$ before the Pd was quenched with 5 μL 2 M β -mercaptoethanol. The DNA-linked material was precipitated with EtOH and the pellets were resuspended in 300 μL 10 mM Tris, 0.1 M NaCl, 1 mM EDTA, pH 8.2 and added to an aliquot of streptavidin magnetic particles (Roche) representing 80 pmol of biotinylated oligonucleotide binding capacity. After 15 min, the supernatant was removed and the particles were washed twice with 150 μL Milli-Q H_2O . The particles were then resuspended in 0.1 M CAPS, pH 11 and agitated for 15 min to effect sulfone linker cleavage. The supernatant was spin-filtered and subjected to EtOH precipitation. The pellet was resuspended in 0.1 M TEAA, desalted using a Zip-Tip cartridge (Millipore), and subjected to MALDI-TOF analysis, yielding the results in Fig. S4.

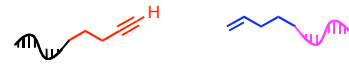
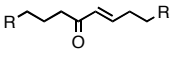
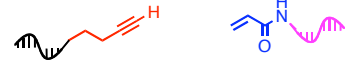
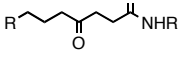
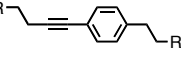
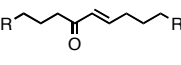
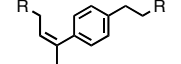
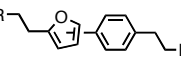
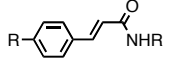
substrates	possible product	expected mass	observed mass
		6559.4	6557 $\pm$ 5
		6505.3	6501 $\pm$ 5
		6563.3	6563 $\pm$ 5
		6559.4	6557 $\pm$ 5
		6551.3	6548 $\pm$ 5
		6577.3	6579 $\pm$ 5
		6481.3	6479 $\pm$ 5

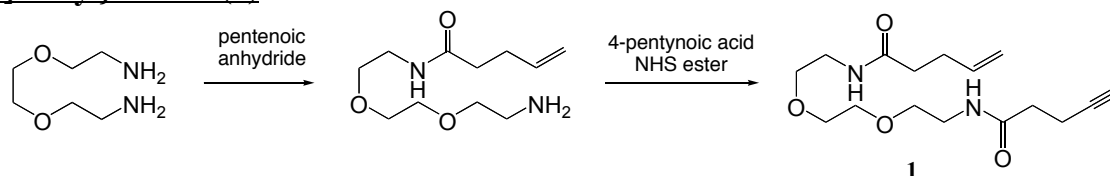
Fig. S4. MALDI-TOF characterization of products from DNA-templated reactions.

Additional relevant reaction possibilities tested in a DNA-templated format in the presence of Pd(II) include:

- Ketone (A12) + alkyne (A5): yields no product
- Alkyne (A5) + azide (B9), a borderline positive by green:red fluorescence ratio quantitation: yields only very weak product formation.
- Benzeneboronic acid (B8) homocoupling: yields product consistent with biphenyl formation (expected mass = 6587.3; observed mass = 6585 $\pm$ 5).

Copper(I)-Mediated Degradation of DNA

To assess the effects of the Cu(I)-containing conditions used above on DNA stability, we exposed 17 pmol of a DNA 30-mer in 100 μL to 500 μM CuSO_4 , 500 μM sodium ascorbate, 50 mM MOPS, 0.1 M Na_2SO_4 , pH 7.0, at 25 $^\circ\text{C}$ for 10 min. The solution was subjected to gel filtration to remove small molecules (including Cu(I)) and then analyzed by denaturing PAGE. For comparison, identical reactions were also performed in the absence of CuSO_4 and sodium ascorbate. DNA staining with ethidium bromide and densitometry were used to quantitate the amount of full-length DNA remaining in each sample. The sample exposed to Cu(I) was observed to contain 52% of the full-length DNA present in the no-Cu control sample.

Synthesis and Characterization of Pent-4-enoic Acid {2-[2-(2-Pent-4-Ynoylamino-Ethoxy)-Ethoxy]-Ethyl}-Amide (1)

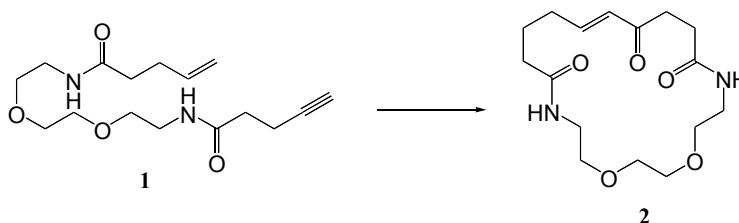
Pentenoic anhydride (789 mg, 4.3 mmol, 1.0 equiv.) in acetonitrile (10 mL) was added dropwise over the course of 30 min to a stirring solution of 2,2'-(ethylenedioxy)bis(ethylamine) (640 mg, 4.3 mmol, 1.0 equiv.) in 20 mL acetonitrile at 25 $^\circ\text{C}$. At the conclusion of addition the reaction solution was dried *in vacuo*. The dried material was dissolved in 50 mM acetic acid in H_2O (20 mL) and purified by preparative HPLC (acetonitrile/50 mM aqueous acetic acid gradient) followed by lyophilization to afford the acetate salt of the monoacylated material (560 mg, 45%) as a pale yellow oil. To the monoacylated material (300 mg, 1.0 mmol, 1.0 equiv.) in acetonitrile (20 mL) was added *N*-hydroxysuccinimide activated 4-pentynoic acid (220 mg, 1.1 mmol, 1.1 equiv.) and the resulting clear solution was allowed to stir under N_2 atmosphere at 25 $^\circ\text{C}$ for 20 min. The reaction was dried *in vacuo*, and the material was resuspended in H_2O (20 mL) and purified via preparative HPLC (acetonitrile/water gradient). Lyophilization afforded **1** (230 mg, 74%, 33% overall) as an off-white crystalline solid.

^1H -NMR (500 MHz, CDCl_3): δ 6.20 (br, s, 1H, NHCO), δ 6.01 (br, s, 1H, NHCO), δ 5.86-5.78 (m, 1H, CHCH_2), δ 5.06 (dd, 1H, $J = 1.5, 17.0$ Hz, CHCH_2), δ 4.99 (dd, 1H, $J = 1.5, 10.0$ Hz, CHCH_2), δ 3.60 (s, 4H, $\text{OCH}_2\text{CH}_2\text{O}$), δ 3.55 (m, 4H, CHOCH_2), δ 3.47 (m, 4H, CH_2NH), δ 2.52 (m, 2H, CH_2CCH), δ 2.44-2.36 (m, 4H, CH_2CHCH_2 , NHCOCH_2), δ 2.28 (t, 2H, NHCOCH_2), δ 2.00 (t, 1H, CCH).

^{13}C -NMR (100 MHz, CDCl_3): δ 175, 174, 137.3, 115.8, 83.2, 70.5, 70.4, 70.2, 70.1, 69.5, 39.5, 39.4, 36.0, 35.5, 29.8, 15.1.

HRMS (TOF ES^+) calculated for $\text{C}_{16}\text{H}_{26}\text{N}_2\text{O}_4$ ($\text{M}+\text{H}$) $^+$ = 311.1972; observed = 311.1989.

Synthesis and Characterization of 1,4-Dioxo-7,18-Diaza-Cycloicos-12-Ene-8,11,17-Trione (2)



In a typical experiment, a 6 mM solution of **1** was added over the times listed in Fig. 4 to a stirring aqueous solution containing Na_2PdCl_4 and CuCl_2 in the stoichiometries listed in Fig. 4. The final concentration of **1** was typically 1.5 mM. After completion of the addition, analytical HPLC typically revealed quantitative conversion of **1** to a single product. To purify the product, the reaction was subjected to preparative reverse-phase HPLC purification with an acetonitrile/water gradient. Lyophilization of the purified material provided macrocyclic enone **2** as a yellow oil in the isolated yields reported in Fig. 4.

Figure 4, Entry a

To a solution of Na_2PdCl_4 (9.4 mg, 32 μmol , 1.0 equiv.) in 1 M NaCl in water (17 mL) stirring at 25 °C open to the air was added, at a rate of 0.34 mL/h, a solution of **1** (10 mg, 32 μmol , 1.0 equiv.) in water (5.0 mL). At the completion of addition, analytical HPLC of 1.0 mL of the reaction showed quantitative conversion of starting material to a single product. To isolate the product, 20 mL of the 22 mL total reaction volume was subjected to preparative reverse-phase HPLC purification with an acetonitrile/water gradient. Lyophilization of purified material provided macrocyclic enone **2** (8.2 mg, 86%) as a yellow oil.

Figure 4, Entry b

To a solution of Na_2PdCl_4 (0.49 mg, 1.6 μmol , 0.05 equiv.) in 100 mM NaCl in water (15 mL) was added CuCl_2 (5.4 mg, 32 μmol , 1.0 equiv.). A 6 mM solution of **1** (10 mg, 32 μmol , 1.0 equiv.) in water (5 mL) was added at 4.1 mL/h. The reaction mixture was allowed to stir at 25 °C open to the air for 1 h. Analytical HPLC of 500 μL of the reaction showed quantitative conversion of the starting material to a single product. The product was purified as described above to afford **2** (9.0 mg, 90%) as a clear oil.

Figure 4, Entry c

To a solution of Na_2PdCl_4 (0.49 mg, 1.6 μmol , 0.05 equiv.) in 6.5:1 tetrahydrofuran:water (15 mL) was added CuCl_2 (5.4 mg, 32 μmol , 1.0 equiv.). A 6 mM solution of **1** (10 mg, 32 μmol , 1.0 equiv.) in tetrahydrofuran (5 mL) was added at 2.7 mL/h. The reaction mixture was allowed to stir at 25 °C open to the air for 2 h. Analytical HPLC of 500 μL of the reaction showed quantitative conversion of the starting material to a single product. Tetrahydrofuran was removed *in vacuo*, and the product was purified as described above to afford **2** (9.0 mg, 91%) as a yellow oil.

Figure 4, Entry d

To a solution of Na_2PdCl_4 (1.4 mg, 4.8 μmol , 0.15 equiv.) in 9:1 tetrahydrofuran:water (40 mL) was added **1** (10 mg, 32 μmol , 1.0 equiv.). The reaction mixture was allowed to stir at 25 °C under 1 atm O_2 for 14 h. Following analytical HPLC of 1.0 mL of the reaction, the product was purified as described above to afford **2** (7.5 mg, 73%) as a yellow oil.

Figure 4, Entry e

To a solution of CuCl₂ (2.7 mg, 16 μmol, 1.0 equiv.) in 100 mM NaCl in water (10 mL) was added **1**. The reaction was allowed to stir at 25 °C open to the air for 4 h at which point 1.0 mL of the reaction mixture was subjected to analytical HPLC. No conversion of **1** to any product was observed.

Figure 4, Entry f

To a suspension of CuCl (1.6 mg, 16 μmol, 1.0 equiv.) in 100 mM NaCl in water (10 mL) was added **1**. The reaction was allowed to stir at 25 °C open to the air for 4 h at which point 1.0 mL of the reaction mixture was subjected to analytical HPLC. No conversion of **1** to any product was observed.

UV: 230 nm, 310 nm

HRMS (TOF ES⁺) calculated for C₁₆H₂₆N₂O₅ (M+H)⁺ = 327.1921; found = 327.1914.

¹H-NMR (400MHz, CD₃OD): δ 6.94-6.86 (dt, 1H, J = 7.2, 16 Hz, CHCHCO) δ 6.08 (dt, 1H, J = 1.2, 16 Hz, CHCHCO), δ 3.62 (s, 4H, OCH₂CH₂O), δ 3.56 (t, 2H, OCH₂CH₂), δ 3.54 (t, 2H, OCH₂CH₂), δ 3.38-3.35 (m, 4H, CH₂NH), δ 2.92 (t, 2H, CHCOCH₂), δ 2.41 (t, 2H, CH₂CONH), δ 2.30 (m, 2H, CH₂CH₂CH), δ 2.24 (t, 2H, CH₂CH₂CONH), δ 1.91- 1.82 (m, 2H, CH₂CH₂CH₂).

¹H-NMR (500MHz, CDCl₃): δ 6.83-6.77 (dt, 1H, J = 7.2, 16 Hz, CHCHCO), δ 6.51 (bs, 1H, NHCO), δ 6.12-6.07 (dt, 1H, J = 1.2, 16 Hz, CHCHCO), δ 6.10 (bs, 1H, NHCO), δ 3.60 (s, 4H, OCH₂CH₂O), δ 3.56 (t, 2H, OCH₂CH₂), δ 3.53 (t, 2H, OCH₂CH₂), δ 3.47-3.42 (m, 4H, CH₂NH), δ 2.88 (t, 2H, CHCOCH₂), δ 2.57 (t, 2H, CH₂CONH), δ 2.32 (m, 2H, CH₂CH₂CH), δ 2.18 (t, 2H, CH₂CH₂CONH), δ 1.91 (m, 2H, CH₂CH₂CH₂).

¹³C-NMR (100 MHz, CDCl₃): δ 199.9, 173.0, 172.4, 148.0, 132.6, 70.8, 70.6, 70.4, 69.8, 39.5, 39.2, 35.0, 34.2, 31.6, 31.0, 23.2.

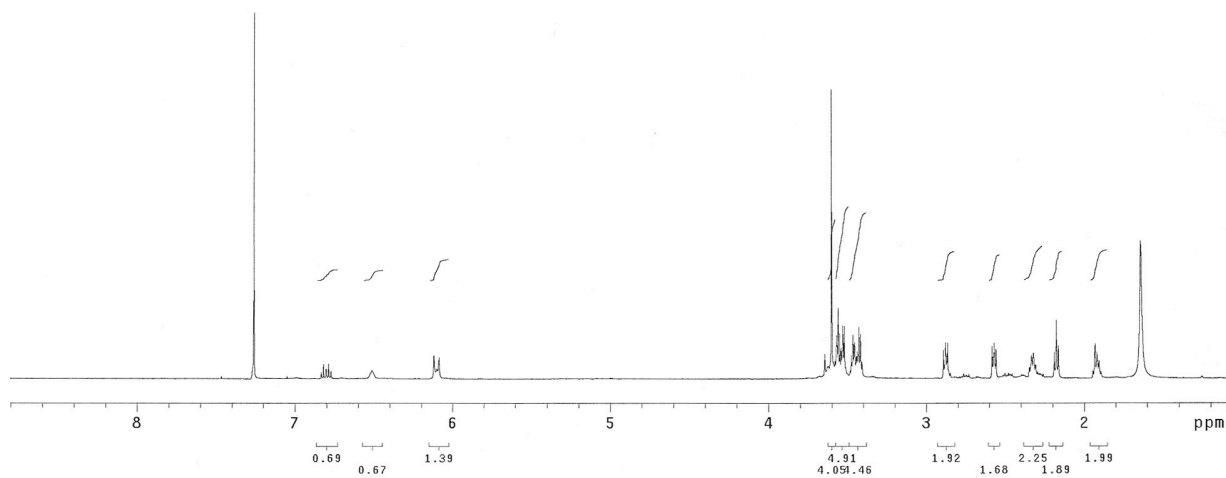
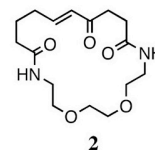
NMR spectra of **2** are shown below:

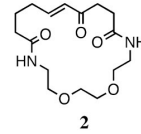
¹H-NMR spectrum:

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Pulse 74.0 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.7479203 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 0 min, 40 sec





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STANDARD PROTON PARAMETERS
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Solvent: COC13
Temp. 23.0 C / 296.1 K
INNOVA-500 "innova500"

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Acq. time 0.128 sec
Width 8000.0 Hz
2D width 8000.0 Hz
Single scan
128 increments
OBSERVE H1, 499.7479203 MHz
DATA PROCESSING
Sg. sine bell 0.064 sec
F1 DATA PROCESSING
Sg. sine bell 0.016 sec
FT size 2048 x 2048
Total time 2 min, 45 sec

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