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DNA Compatible Multistep Synthesis and Applications to DNA Encoded Libraries

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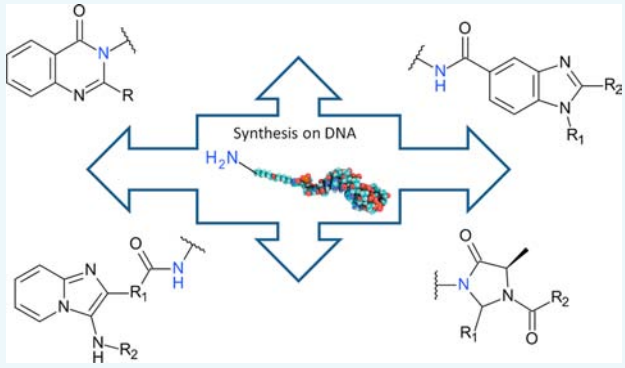
Supporting Information

ABSTRACT: Complex mixtures of DNA encoded small molecules may be readily interrogated via high-throughput sequencing. These DNA encoded libraries (DELs) are commonly used to discover molecules that interact with pharmaceutically relevant proteins. The chemical diversity displayed by the library is key to successful discovery of potent, novel, and drug-like chemical matter. The small molecule moieties of DELs are generally synthesized though a multistep process, and each chemical step is accomplished while it is simultaneously attached to an encoding DNA oligomer. Hence, library chemical diversity is often limited to DNA compatible synthetic reactions. Herein, protocols for 24 reactions are provided that have been optimized for high-throughput production of DELs. These protocols detail the multistep synthesis of benzimidazoles, imidazolidinones, quinazolinones, isoindolinones, thiazoles, and imidazopyridines. Additionally, protocols are provided for a diverse range of useful chemical reactions including BOC deprotection (under pH neutral conditions), carbamylation, and Sonogashira coupling. Last, step-by-step protocols for synthesizing functionalized DELs from trichloronitropyrimidine and trichloropyrimidine scaffolds are detailed.



INTRODUCTION

The recent invention of inexpensive high-throughput DNA sequencing has spurred interest in the synthesis of drug-like small molecule DNA conjugates.[1−5] Examples include the discovery of integrin lymphocyte function-associated antigen 1 (LFA-1) antagonists,[6] ADAMTS-5,[7] and RIP3 Kinase[8] inhibitors. The DNA moiety of the conjugate functions as a barcode, encoding the structure of the small molecule to which it is attached.[9] Complex mixtures of DNA encoded small molecules can be interrogated via sequencing and the relative concentrations of individual library members ascertained.[10,11] A protein may be (i) incubated with a complex mixture of DNA encoded small molecules, (ii) immobilized on a solid support, (iii) washed to remove unbound DNA conjugates, and (iv) denatured to allow for previously bound DNA conjugates to be eluted.[12] The collected sample, now enriched with protein binding library members, is then sequenced. Sequencing platforms are currently capable of correctly reading ∼10^8 sequences (barcodes) per run,[13] enough to appropriately interrogate the sample. The entire selection process takes 1 day and requires ∼10 μg of protein per condition.[12] In this manner, DNA encoded libraries (DELs) are used to discover new chemical starting points against pharmaceutically relevant targets.

The key to the ability of a library to provide useful chemical starting points is the structural diversity of the small molecules displayed by the DEL. Many methods for the production of small molecule DELs have been reported including split-and-pool,[14−18] template-directed synthesis,[19] and high-throughput parallel synthesis.[4,5,20] All these techniques allow for step-by-step synthesis of small molecules while they are simultaneously conjugated to their encoding DNA.[1,15,21] This differs from technologies such as phage display that are generally limited to peptides derived from naturally occurring amino acids.[22] However, the ability to employ traditional synthetic chemistry is complicated by the requirement of compatibility with the attached DNA oligomer.[23,24]

DNA conjugated chemistry lends itself to high-throughput multistep synthesis.[17,23,24] DNA conjugates readily precipitate from aqueous solution upon the addition of 2−3 volumes of ethanol. Precipitation allows a large excess of reagent to be removed and this technique is readily applied to a 96-well plate format. Due to its large size, the DNA oligomer dictates the molecule's HPLC retention time. Thus, a single HPLC assay protocol is applicable to all DNA conjugated synthetic transformations regardless of the physical properties of the desired small molecule product. The large mass of the DNA-conjugate is also readily distinguished from low-molecular-

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Table 1. Reactions Optimized for Use with ssDNA Conjugates and DEL Synthesis[a]

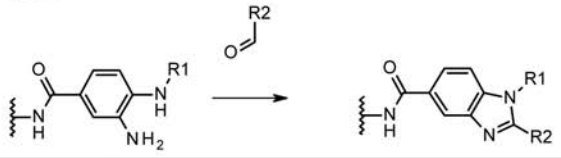
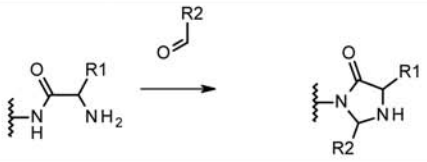
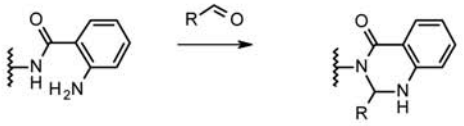
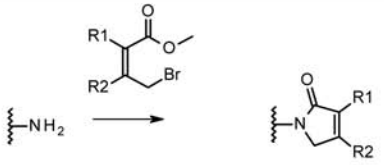
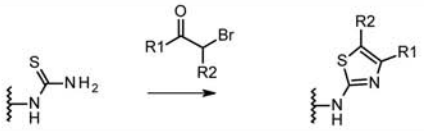
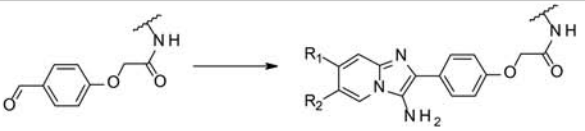
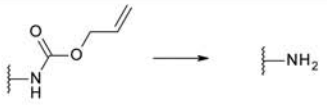
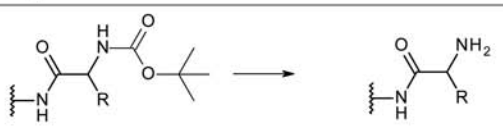
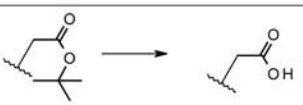
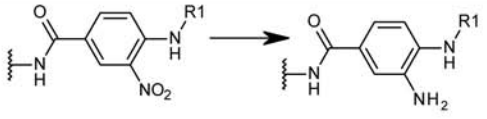
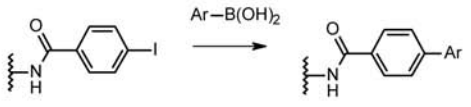
	Description	Scheme	Method [b,c]
1	Benzimidazole [8,27,28] Table S1		1mM DNA (in buffer[b]), 60 equiv aldehyde (200 mM DMA stock), 60°C, 18 h.
2	Imidazolidinone [29-31] Table S2		1mM DNA (1:3 MeOH:buffer[b]), 60 equiv aldehyde (200 mM DMA stock), 60°C, 18 h.
3	Quinazolinone[32] Table S3		1mM DNA (buffer[b]), 200 equiv NaOH (1N aq), 90°C, 14 h.
4	Isoindolinone[33] Table S4		1mM DNA (buffer[b]), 20 equiv benzylbromide (200 mM DMA stock), 60°C, 2 h.
5	Thiazole[34] Table S5		1 mM DNA (buffer[b]), 50 equiv bromoketone (200 mM DMA stock), rt, 24 h.
6	Imidazopyridine [35,36] Table S6		1mM DNA (buffer[b]), 50 equiv 2-aminopyridine (200 mM DMA stock), 2500 equiv NaCN (1M aq stock), 90°C, 10 h.
7	Alloc deprotection[37]		1mM DNA (buffer[b]), 10 equiv $Pd(PPh_3)_4$ (10 mM DMA stock), 10 equiv $NaBH_4$ (200 mM ACN stock), rt, 2 h.
8	BOC deprotection[38] Table S7		0.5 mM DNA (buffer[b]), 90°C, 16 h.
9	*t*-Butyl ester hydrolysis[38]		1mM DNA (buffer[b]), 80°C, 2 h.
10	Methyl/ethyl ester hydrolysis[38]		1mM DNA (buffer[b]), 100 equiv NaOH, 60°C, 2 h.
11	Nitro reduction with hydrazine and Raney Ni[39]		200 µl DNA (1 mM aq), 20 µl Raney Ni (aq slurry), 20 µl hydrazine (0.4 M aq), rt, 2-24 h w/shaking.
12	Suzuki[27,40,41] Table S8		**Step 1**: 1 mM DNA (aq), 50 equiv boronic acid (200 mM DMA stock), 300 equiv Na_2CO_3 (200 mM aq stock), 0.8 equiv $Pd(OAc)_2$ (10 mM DMA stock) premixed with 20 equiv TPPTS (100 mM aq stock), 65 °C, 1h. Precipitate. **Step 2**: 1 mM DNA (buffer[b]), 120 equiv Na_2S (400 mM aq stock), 65 °C, 1 h.

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Table 1. continued

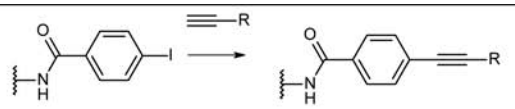
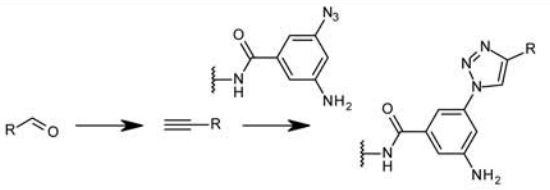
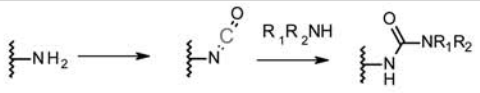
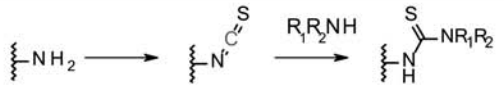
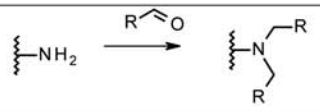
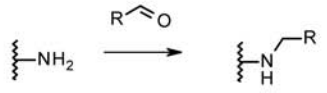
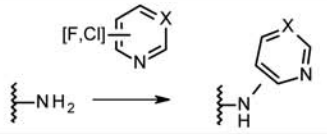
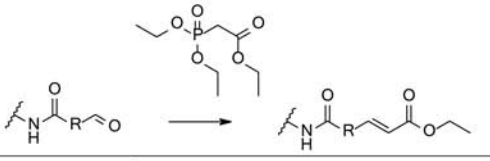
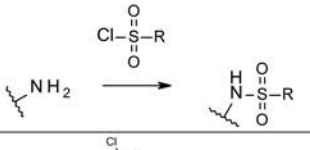
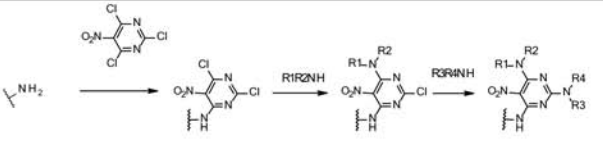
13	Sonogashira[42-45] Table S9		1 mM DNA (aq), 100 equiv alkyne (200 mM DMA stock), 300 equiv pyrrolidine (200 mM DMA stock), 0.4 equiv $Pd(OAc)_2$ (10 mM DMA stock), 20 equiv TPPTS (100 mM aq stock), 65°C, 2 h.
14	Triazole via *in situ* formation of alkynes[47,48,49] Table S10a		**Step 1.** To 12 µl aldehyde (200 mM in DMA, 2.4 µmol) was added 7.2 µL 1-(dimethoxyphosphoryl)-2-oxopropane-1-diazonium (400 mM in DMA, 2.88 µmol, 1.2 equiv) and 12 µL potassium carbonate (400 mM in water, 4.8 µmol, 2 equiv), rt, 16 h. **Step2.** To crude reaction mixture from above was added 20 µl DNA (1mM aq, 0.02 µmol), and a mixture of copper (I) bromide 1.6 µL (200 mM in DMA, 0.32 µmol) and 3.2 µL TBTA (200 mM in DMA, 0.64 µmol), rt, 16 h.
15	Carbamylation via isocyanate intermediate[38,50] Table S11		**Step 1.** 1 mM DNA (aq), 1:4 v/v TEA (neat), 50 equiv di-2-pyridyl carbonate (200 mM DMA stock), rt, 2 h. **Step2.** Then add 40 equiv amine (200 mM DMA stock), rt, 1-2 h.
16	Thiourea[51] Table S12		**Step 1.** 1mM DNA (buffer[b]), 20 equiv di-2-pyridyl thionocarbonate (200 mM DMA stock), rt, 30 min. **Step 2.** Then add 40 equiv amine (200 mM DMA), rt-60°C, 1-18 h.
17	Reductive alkylation of amines[23,38]		1mM DNA (buffer[b]), 50 equiv aldehyde (200mM DMA), 60°C, 4 h.
18	Reductive mono-alkylation of primary amines[52]		**Step 1.** 1mM DNA (buffer[b]), 40 equiv aldehyde (200 mM DMA), rt, 1h. **Step 2.** Then add 40 equiv $NaBH_4$ (200 mM ACN stock), rt, 1 h.
19	S_NAr with hetarylhalide building blocks[38] Table S13		1 mM DNA (buffer[b]), 60 equiv hetarylhalide (200 mM DMA stock), 60°C, 12 h.
20	Reaction of amines with bromoacetamide[53] Table S14		1 mM DNA (aq), 40 equiv amine (200 mM DMA stock), rt, 1 h.
21	Horner-Wadsworth-Emmons[1] Table S15		1 mM DNA (buffer[b]), 50 equiv ethyl 2-(diethoxyphosphoryl)acetate (200 mM DMA stock), 50 equiv cesium carbonate (200 mM aq stock), rt, 16 h.
22	Sulfonamides[38] Table S16		1 mM DNA (buffer[b]), 40 equiv sulfonylchloride (200 mM DMA stock), rt, 16 h.
23	Trichloronitropyr-imidine (TCNP)[58] Table S17		**Step 1.** 1 mM DNA (buffer[b]), 20 equiv TCNP (200 mM DMA stock), 5°C-rt, 1 h. Precipitate. **Step 2.** 1 mM DNA (buffer[b]), 40 equiv amine (200 mM DMA stock), 100 equiv TEA (neat) or $KHCO_3$ (1 M aq stock), rt, 2 h. Precipitate. **Step 3.** 1 mM DNA (buffer[b]), 50 equiv amine (200 mM DMA stock), 100 equiv TEA (neat) or $KHCO_3$ (1 M aq stock), rt, 24 h.

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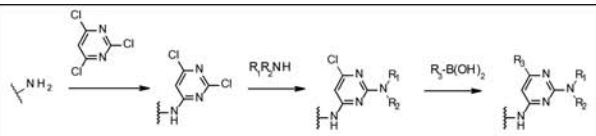
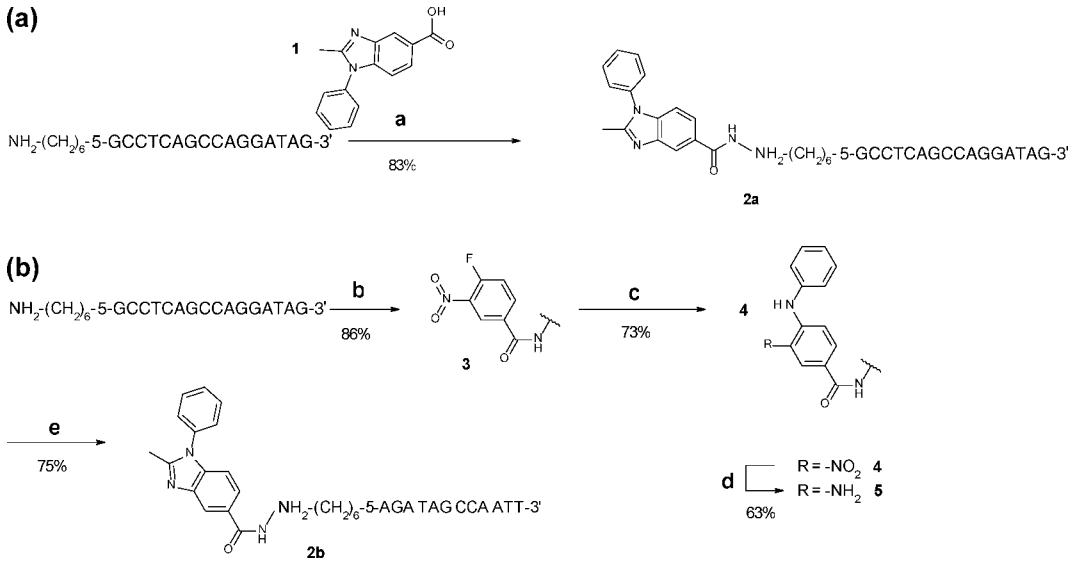
Table 1. continued

24	Trichloro-pyrimidine[59] Table S18-19		**Step 1.** 1mM DNA (buffer[b]), 50 equiv 2,4,6-trichloropyrimidine (200 mM DMA stock), rt, 3.5 h. Precipitate. **Step 2.** 1mM DNA (buffer[b]), 40 equiv amine (200 mM ACN stock), 60-80°C, 16h. Precipitate. **Step 3.** 1mM DNA (buffer[b]), 60 equiv boronic acid (200 mM DMA stock); 200 equiv NaOH (500 mM aq stock), 2 equiv $Pd(OAc)_2$ (10 mM DMA stock) and 20 equiv TPPTS (100 mM aq stock) premixed, 75°C, 3 h. Precipitate. **Step 4:** 1 mM DNA (aq), 120 equiv Na_2S (400 mM water), 65°C, 1 h.

[a]Reactions were run using the amine functionalized ssDNA conjugate NH_2-$(CH_2)_6$-5′-GCCTCAGCCAGGATAG-3′ (**DNA-1**, MW 5070) or NH_2-$(CH_2)_6$-5′-AGATAGCCAATT-3′ (**DNA-2**, MW 3832). [b]Buffer is pH ∼9.4 250 mM borate. [c]Procedures for spin filtering, precipitating, determining percent conversion, and general handling of DNA conjugates provided in Supporting Information section S1.

Scheme 1. (a) Synthesis of DNA-Conjugated Benzimidazole 2a via Acylation of DNA-1 with Characterized Reagent 1; (b) Multistep DNA-Compatible Synthesis of Benzimidazole 2b[a]



[a]Reagents and conditions: (a) 20 μL **DNA-1** (1 mM in borate buffer pH 9.4, 0.02 μmol), 5 μL **1** (200 mM in DMA, 1 μmol, 50 equiv), 5 μL DMT-MM (200 mM aq, 1 μmol, 50 equiv), rt, 16 h; (b) 100 μL **DNA-1** (1 mM in borate buffer pH 9.4, 0.1 μmol), 25 μL 4-fluoro-3-nitrobenzoic acid (200 mM in DMA, 5 μmol, 50 equiv), 25 μL DMT-MM (200 mM aq, 5 μmol, 50 equiv), rt, 16 h; (c) 90 μL **3** (1 mM in borate buffer pH 9.4, 0.09 μmol), 22 μL aniline (200 mM in DMA, 4.5 μmol, 50 equiv), 80°C, 16 h; (d) 80 μL **4** (1 mM in borate buffer pH 9.4, 0.08 μmol), 6.4 μL hydrazine (1 M aq, 6.4 μmol, 80 equiv), 16 μL Raney nickel (50% aq slurry, 0.16 μmol, 2 equiv), rt, 7 h; (e) 20 μL **5** (1 mM in borate buffer pH 9.4, 0.02 μmol), 6 μL acetaldehyde (200 mM in DMA, 1.2 μmol, 60 equiv), 60°C, 16 h; DMT-MM = 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride.

weight reagents by both mass spectroscopy and usually UV absorbance. Reaction progress of crude DNA conjugates may be monitored with little or no sample preparation. Solubility of DNA conjugated products is also predictable. DNA conjugates always dissolve in aqueous solutions and precipitate upon addition of ethanol. However, significant limitations in the application of common synthetic transformations to DNA conjugates exist. DNA conjugates require aqueous solutions, generally greater than 50% water, to avoid precipitation. Also, reagents must not cause degradation of the DNA oligomer. Deleterious conditions include exposure to acids and oxidants. These strengths and weaknesses are unique to DNA conjugate chemistry and differ from related technologies, for instance, encoding with PNA.[25]

The ability to synthesize structurally diverse DNA encoded libraries requires DNA compatible synthetic transformations.[24] However, only approximately 30 different DNA compatible chemistries have been reported in the literature.[23] Reported reactions provide access to a limited number of heterocyclic structures and often result in products with limited appeal to the medicinal chemistry community due to high molecular weight and lipophilicity.[24,26] With the goal of accessing new drug-like structures, a variety of known organic reactions are modified to be compatible with DNA-conjugated high-throughput synthesis. Table 1 lists reactions that (i) have never been previously accomplished in the presence of DNA, (ii) are mentioned in the patent literature but lack experimental details to be reproducible, or (iii) have been previously reported for use in template-directed DEL synthesis, and

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required modification to be compatible with split-and-pool or high-throughput parallel synthesis.

RESULTS

Access to highly functionalized heterocyclic rings is desirable. Table 1 details DNA compatible methods for forming benzimidazoles, imidazolidinones, quinazolinones, isoindolinones, thiazoles, and imidazopyridines. DNA compatible benzimidazole formation has been previously suggested but no methods or experimental details provided.[8,27] The synthesis of benzimidazole small molecules in aqueous solution has been shown to not require the presence of added oxidizing agent.[28] We serendipitously observed benzimidazole formation when an aqueous solution of 5-nitropyrimidin-4-amine, Raney nickel, hydrogen gas, and DMA were left standing overnight (Figure S1). An optimized method for DNA-conjugated multistep synthesis of benzimidazoles is shown in Scheme 1 and Table 1 Entry 1. Cyclized benzimidazole product was characterized according to Scheme 1a. Benzimidazole **1** was synthesized, characterized, and then conjugated to a DNA oligomer to yield DNA-conjugate **2a** (see Figure S2a for LCMS trace). DNA-conjugate **2b** was synthesized according to the DNA compatible methods detailed in Scheme 1b (see Figure S2b for LCMS trace). Analytical HPLC coinjection was then used to ascertain that **2a** and **2b** are most likely identical (Figure 1). Benzimidazole formation was further investigated using 12 aldehyde reagents (Table S1). Purity of the final product crude mixtures, following four steps of chemistry, was determined by LCMS total ion count peak area of crude product samples following ethanol precipitation. Seven of the twelve desired DNA-conjugated benzimidazole products were observed with an overall conversion of >50%. General methods for analyzing and purifying DNA-conjugates are provided in the Supporting Information section S1.

Imidazolidinones are known drug-like scaffolds and are accessible through parallel synthesis.[29−31] DNA-conjugated

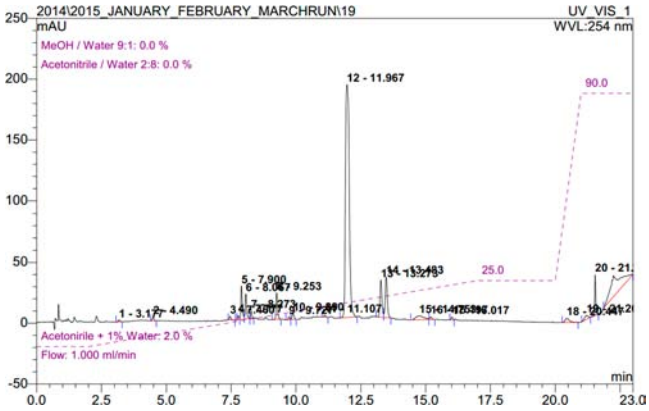

Figure 1. HPLC of coinjected DNA-conjugates **2a** and **2b**.

imidazolidinones were synthesized as shown in Scheme 2 and Table 1 Entry 2. The imidazolidinone **6** was fully characterized prior to DNA conjugation to yield **7a**. DNA-conjugate **7b** was synthesized according to the protocols provided in Scheme 2. Comparison of LCMS traces of **7a** and **7b** suggest that the two molecules are the same (Figures S3−S4). Imidazolidinones such as **9**, prior to acylation of the nucleophilic cyclic amine, are generally stable in aqueous solution even upon heating at 80 °C for 20 min (data not shown). The choice of amino acid influences cyclization yield. Alanine gives high conversions to the desired imidazolidinone, while glycine results in low conversions to product. Amino acids with bulkier side chains such as phenylalanine also give high conversions to product (data not shown). However, these sterically hindered imidazolidinones are difficult to further functionalize via acylation (data not shown). β-Alanine provides no desired 6-membered-ring product. Solvent also influences cyclization and the addition of methanol as a cosolvent is required. Imidazolidinone formation with six different aldehydes are exemplified in Table S2.

Intramolecular cyclization of ortho-acetamidobenzamides under basic conditions to yield quinazolinones has been reported.[32] DNA-conjugated ortho-aminobenzamides may be acylated with carboxylic acid building blocks to yield ortho-acetamidobenzamide intermediates such as **14**, which may then be cyclized as described in Scheme 3 and Table 1 Entry 3. In general, DNA oligomers are found to be stable under strongly basic conditions with heating (data not shown). DNA-conjugate **11a** was synthesized from the fully characterized quinazolinone **10**, while **11b** was synthesized stepwise as a DNA-conjugate (Scheme 3). LCMS coinjection suggests that DNA-conjugates **11a** and **11b** are identical (Figures S5−7). Quinazolinone cyclization with five different carboxylic acids are exemplified in Table S3.

Reaction of ortho-bromomethylbenzoates with primary amines to form isoindolinones under basic conditions is known and appeared potentially applicable to DNA-conjugated chemistry.[33] Optimization yielded the method provided in Table 1, Entry 4. Ortho-bromomethylbenzoates readily undergo S_N2 reaction with amine conjugated **DNA-2**. Bis-alkylation of **DNA-2** was not observed as the monoalkylated intermediate readily cyclizes to provide the desired isoindolinone. Unlike the previously discussed examples of DNA-conjugated cyclizations, this reaction can be monitored by LCMS, and loss of bromide and methanol readily observed (Figure S8). Isoindolinone cyclization was investigated using 12 ortho-bromomethyl benzoates and heterocyclic analogs. Benzoate and pyridine-caboxylate derivatives provide high conversions to desired product as judged by LCMS TIC peak area (see Table S4, entries 1−8). Five-membered-ring systems such as 4-(bromomethyl)oxazole-5-carboxylate provide little or no desired product (Table S4, entries 9−12).

Halo-ketones are known to react with thioureas under mild conditions to provide thiazoles.[34] 2-Haloketones may be combined with DNA-conjugated thiourea in pH 9.4 buffer at rt to provide the corresponding thiazoles in high conversions (Table 1 Entry 5, and Figure S9). Two examples are provided in Table S5. Synthesis of the precursor thiourea is described in the Supporting Information (Table S5).

Imidazopyridines may be formed by combination of 2-aminopyridine, sodium cyanide, and arylcarboxaldehyde.[35,36] Formation of the desired imidazopyridine ring was investigated as outlined in Scheme 4. Imidazopyridine **15** was synthesized, characterized, and acylated with **DNA-1** to yield DNA-conjugate **16a**. DNA-conjugate **16b** was synthesized according to the DNA compatible methods detailed in Table 1 Entry 6 and Scheme 4b. LCMS analysis was then used to ascertain that **16a** and **16b** are likely identical (Figures S10−S12). Table S6 provides examples of imidazopyridine formation employing different substituted 2-aminopyridine reagents. The resulting DNA-conjugated imidazopyridin-3-amine products may be further functionalized via reductive alkylation of the exocyclic

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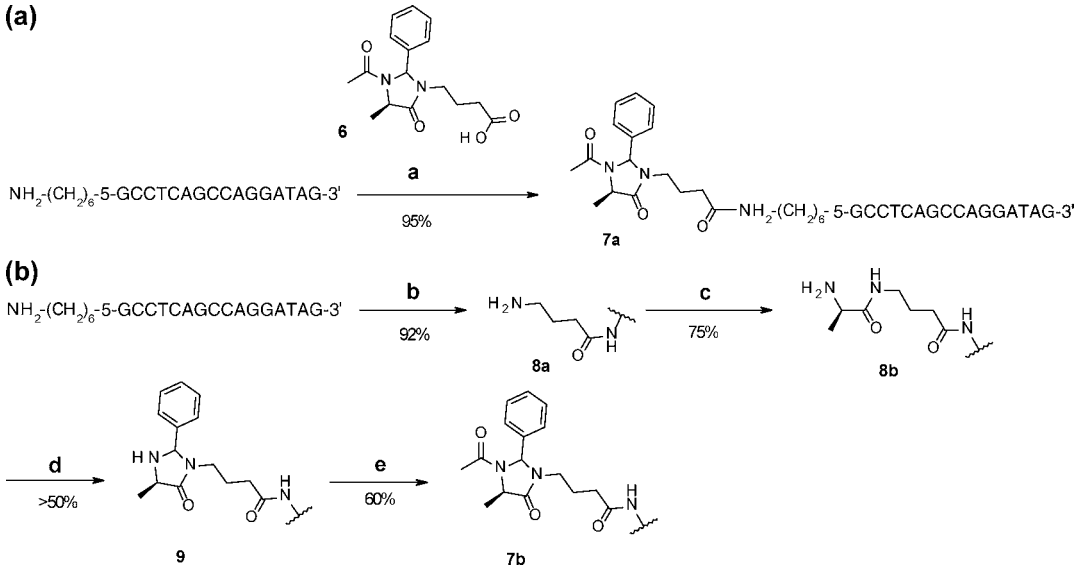
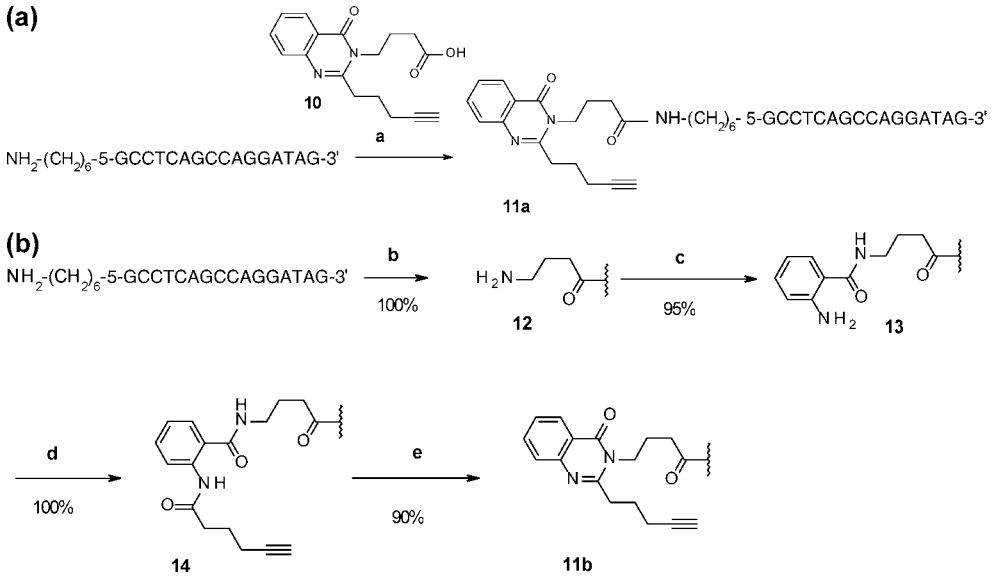
Scheme 2. (a) Synthesis of DNA-Conjugated Imidazolidinone 7a via Acylation of DNA-1 with Characterized Reagent 6;[a] (b) Multistep DNA-Compatible Synthesis of Imidazolidinone 7b[b]



[a]Reagents and conditions: (a) 20 μL **DNA-1** (1 mM in borate buffer pH 9.4, 0.02 μmol), 5 μL **6** (200 mM in DMA, 1 μmol, 50 equiv), DMT-MM (200 mM in borate buffer, 5 μL, 1 μmol, 50 equiv), 22°C, 4 h; (b) 200 μL **DNA-1** (1 mM in borate buffer pH 9.4, 200 μL, 0.2 μmol), 50 μL Fmoc-GABA (200 mM in DMA, 10 μmol, 50 equiv), 50 μL DMT-MM (200 mM in water, 10 μmol, 50 equiv) rt, 16 h; (c) 200 μL **8a** (1 mM in borate buffer pH 9.4, 0.1 μmol), 50 μL FMOC-Ala-OH (200 mM in DMA, 10 μmol, 50 equiv), 50 μL DMT-MM (200 mM in borate buffer pH 9.4, 10 μmol, 50 equiv), 22°C, 16 h; (d) 20 μL **8b** (1 mM in borate buffer 250 mM pH 9.4, 0.02 μmol), 5 μL aldehyde 200 mM in DMA (1 μmol, 50 equiv), 20 μL MeOH, 80°C, 16 h; (e) 5 μL **9** (1 mM in water, 0.005 μmol), 2.5 μL acetic acid (200 mM in DMA, 0.5 μmol, 100 equiv), 2.5 μL DMT-MM (200 mM in borate buffer 250 mM pH 9.4, 0.5 μmol, 100 equiv), 22°C, 16 h; DMT-MM = 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride. [b]Percent purity of crude reaction mixtures following ethanol precipitation, as judged by LCMS TIC peak area, are provided.

Scheme 3. (a) Synthesis of DNA-Conjugated Quinazolinone 11a via aAcylation of DNA-1 with Characterized Reagent 10; (b) Multistep DNA-Compatible Synthesis of Quinazolinone 11b[a]



[a]Reagents and conditions: 20 μL **DNA-1** (1 mM in borate buffer pH 9.4, 0.02 μmol), 5 μL **15** (200 mM in DMA, 1 μmol, 50 equiv), 5 μL DMT-MM (200 mM in water, 1 μmol, 50 equiv), rt, 2 h; (b) 20 μL **DNA-1** (1 mM in borate buffer pH 9.4, 0.02 μmol), 5 μL Fmoc-GABA (200 mM in DMA, 50 equiv), rt, 18 h, then 2.5 μL piperidine (10% v/v), rt, 1 h; (c) 20 μL DNA-conjugate **12** (1 mM in borate buffer pH 9.4, 0.02 μmol), isatoic anhydride (200 mM DMA, 50 equiv), rt, 2.5 h; (d) 20 μL DNA-conjugate **13** (1 mM in borate buffer pH 9.4, 0.02 μmol), 1.5-hexynoic acid(200 mM in DMA, 50 equiv), DMT-MM (200 mM aq, 50 equiv); (e) 20 μL DNA-conjugate **14** (1 mM in borate buffer pH 9.4, 0.02 μmol), NaOH (1 N, 200 equiv), 90°C, 14 h.

amine using the procedure provided in Table 1 Entry 17 (data not shown). Note that aryl primary amines reductively alkylated using the procedure provided in Table 1 Entry 17 are generally mono- and not dialkylated (data not shown).

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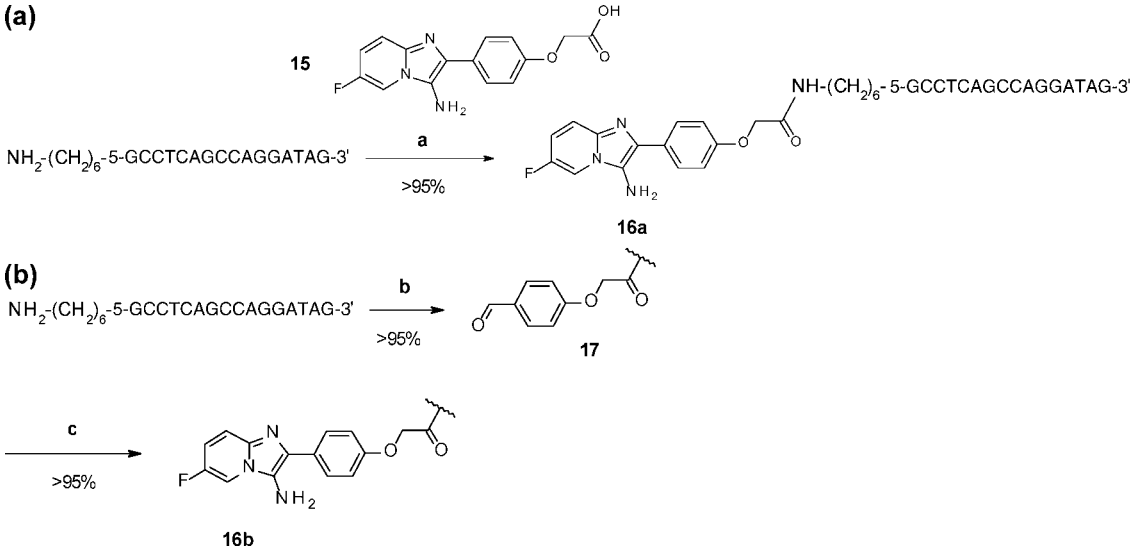
Scheme 4. (a) Synthesis of DNA-Conjugated Imidazopyridine 16a via Acylation of DNA-1 with Characterized Reagent 15; (b) Multistep DNA-Compatible Synthesis of Imidazopyridine 16b[a]



[a]Reagents and conditions: (a) 20 μL **DNA-1** (1 mM in borate buffer pH 9.4, 0.02 μmol), 5 μL **15** (200 mM in DMA, 1 μmol, 50 equiv), 5 μL DMT-MM (200 mM in water, 1 μmol, 50 equiv), rt, 2 h; (b) 200 μL **DNA-1** (1 mM in borate buffer pH 9.4, 0.2 μmol), 50 μL 2-(4-formylphenoxy)acetic acid (200 mM in DMA, 10 μmol, 50 equiv), rt, 24 h; (c) 10 μL DNA-conjugate **17** (1 mM in borate buffer pH 9.4, 0.01 μmol), 2.5 μL 2-amino-5-fluoropyridine (200 mM in DMA, 0.5 μmol, 50 equiv), 2.5 μL NaCN(1 M aq, 25 μmol, 2500 equiv), 90°C, 10 h. DMT-MM = *N*-(3,5-dimethoxytrizin-1-yl)-*N*-methylmorpholinium chloride.

Orthogonal protecting groups are often required to synthesize desired small molecules. Removal of FMOC has been previously detailed.[15] Other DNA compatible protecting groups are listed in Table 1, entries 7−11. Alloc deprotection is detailed in Figure S13 and Table 1 Entry 7.[37] Conditions for removal of BOC in the presence of DNA have been previously reported; however, no examples, yields, or product characterization is provided.[38] An exemplified method is shown in Table 1 Entry 8 and examples provided in Table S7. This method for BOC removal requires heating for 16 h but avoids the customary use of strong acids. Conditions for hydrolysis of *t*-butyl and methyl/ethyl esters have been suggested but details not provided.[38] A detailed method is provided in Table 1, entries 9 and 10 (data not shown). Aryl nitro groups may be reduced in the presence of DNA employing hydrogen gas and Raney nickel catalyst (data not shown). However, hydrogen gas is not readily compatible with 96-well high-throughput synthesis. An alternative is to use hydrazine in place of hydrogen gas[39] (Table 1 Entry 11, and Scheme 1b step d).

Table 1 entries 12−22 provide methods to couple reagents with DNA conjugated functional groups. DNA compatible Suzuki coupling has been previously reported by Omumi et al.,[40] Gouliaev et al.,[27] and Ding and Clark.[41] An alternative method is provided in Table 1 Entry 12, and detailed in Table S8, and Figures S14−S15. The method provided herein is also applicable to chloropyrimidine derivatives (see Table 1 Entry 24). It is also possible to use bromoaryl instead of iodoaryl DNA-conjugates; however, the number of boronic acid/ester equivalents must be increased (data not shown).

Sonogashira coupling between an alkyne and an arylhalide has not, to the best of our knowledge, been reported in the presence of DNA. Starting from examples of reported aqueous Sonogashira chemistry,[42−45] we developed an optimized procedure for this coupling reaction. Detailed results are provided in Table S9.

The in situ formation of building blocks for use in DNA compatible chemistry has been previously reported by Buller et al.[46] Therein it was reported that 54 maleimides were prepared and used for Diels−Alder cycloaddition without purification. Table 1 Entry 14 provides a one-pot method for conversion of aldehydes to alkynes (using the reagent dimethyl-1-diazo-2-oxopropylphosphonate as described by Muller et al.[47]) and modifies the procedure to be compatible with a 96-well plate. Methods for DNA compatible triazole cycloaddition directly from purified alkyne have been previously reported by Rasmussen[48] and Chen et al.,[49] although our method differs (see Table S10b for procedure). The results of adding crude alkyne products derived from aldehydes to a DNA-conjugated arylazide are summarized in Table S10a. Observed conversion to triazole product is comparable to that achieved by direct reaction with commercially available alkynes (see Table S10a,b). The benefit of in situ generation of alkynes is that a large and diverse set of aldehydes are available from commercial sources.[24]

The reaction of isocyanate building blocks with an amine functionalized DNA oligomer has been previously reported by Franch et al.[38] However, no examples or percent conversions are provided. Additionally, the reported method requires 1500 equiv of isocyanate building block. We find that this reaction generally proceeds well with 40 equiv of isocyanate reagent in pH 9.4 buffer at rt in 1−18 h (data not shown). However, the availability of isocyanates is limited and they tend to degrade quickly. The use of phosgene or its equivalents including nitrophenylchloroformate, disuccinimidyl carbonate, carbonyl dimidazole, and pentafluorophenyl dicarbonate to form symmetrical ureas from amine starting materials is known. The formation of nonsymmetrical ureas is less common due to competition with the formation of undesired symmetrical byproducts.[50] However, DNA-conjugate chemistry allows for the use of a large excess of reagent and low concentrations of

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DNA. Excess phosgene-like reagent (20 equiv) may be added to a DNA-conjugated amine to give complete conversion to the transient DNA-conjugated isocyanate (Table 1, Entry 15). No DNA dimer is observed. The DNA-conjugated isocyanate hydrolyzes to starting material over the course of ∼1 h (data not shown). Alternatively, the DNA-conjugated isocyanate may be trapped by the addition of excess amine (40 equiv), yielding the desired nonsymmetrical urea product. The method is limited to cases where a primary amine is conjugated to the DNA oligomer as the required isocyanate intermediate cannot form upon reaction with a DNA-conjugated secondary amine. Details are provided in Table S11 and Figure S17. Small molecule thioureas may be formed in a manner similar to that of the above-discussed ureas.[51] An optimized procedure for the formation of DNA-conjugated thioureas is provided in Table 1 Entry 16. One minor difference is that the isothiocyanate is generally less reactive than the corresponding isocyanate and may require heating to form the desired thiourea product. One discrete example of thiourea formation is provided in Table S5.

Templated and nontemplated methods for DNA compatible reductive alkylation have been reported.[23,38] Both reported methods employ $NaCNBH_3$ as the reducing agent with mildly acidic buffers. The nontemplated method reported by Franch et al.[38] lacks yields and other details. Table 1 Entry 17 provides a method for the alkylation of secondary amines, and the bis-alkylation of aliphatic primary amines (data not shown). Monoalkylation of a primary amine DNA-conjugate as shown in Table 1 Entry 18 is useful as the resulting secondary amine may then be further functionalized. The small molecule procedure reported by Abdel-Magid et al.[52] details this stepwise approach of first preforming the imine and then quenching with reducing agent to achieve selective monoalkylation. Table S12 provides percent conversions for six aldehydes. The reaction works best on arylcarboxaldehydes, although even in these cases the percent conversions are <70%. This reaction may not be a good choice for DEL synthesis due to relatively low yields. However, it may provide value in the multistep synthesis of specific DNA-conjugated intermediates or control molecules.

The use of hetarylhalides as building blocks that can be reacted with an amine functionalized DNA-conjugate has been previously reported by Franch et al.[38] However, no scope or product yields are provided. Table S13 and Figure S18 provide some insight into which hetarylhalides are compatible with DNA-conjugated S_NAr reactions. In general, only the most reactive reagents provide high conversion to product. Increased temperatures or reaction times do not appear to improve yields (data not shown). Optimized reaction conditions are provided in Table 1 Entry 19.

Table 1 Entry 20 provides a method for reacting amines with a bromoacetamide functionalized DNA-conjugate. Franzinin et al.[53] have recently reported a similar reaction using chloroacetamide functionalized DNA. Table S14 and Figures S19−20 provide some examples. Methods for DNA-templated Wittig reactions have been previously reported.[1] Table 1 Entry 21 provides a method for a DNA-conjugated HWE reaction which may be applied to split-and-pool or high-throughput DEL synthesis. Table S15 provides ten examples employing different DNA-conjugated aldehydes. This reaction potentially could be used to synthesize interesting DNA-conjugates, for instance, to access ring systems such as pyrazol-3-ones.[54]

Protocol for the reaction of an aryl sulfonyl chloride with an amine DNA-conjugate has been previously reported by Franch et al.;[38] however, no examples or details were provided. An optimized method is provided in Table 1 Entry 22, and examples shown in Table S16. In general, this reaction provides aryl sulfonamides in high yields. However, the commercial availability of sulfonyl chlorides, relative to other building block sets such as amines and carboxylic acids, is limited.[24] Additionally, the use of aryl sulfonyl chlorides in library synthesis requires caution as their stock solutions tend to degrade rapidly.

Table 1 entries 23 and 24 provide examples of the synthesis of functionalized DNA-conjugated scaffolds.[58,16] 2,4,6-Trichloro-5-nitro-pyrimidine (Table 1, Entry 23) and trichloropyrimidine (Table 1, Entry 24) provide methods for the production of trifunctionalized pyrimidine DELs. Example reactions and percent conversions to product are provided in Tables S17−S19 and Figures S21−22. DNA-conjugated trichloropyrimidine is less reactive than its nitro-substituted analog (Table 1 Entry 23) and substitution is generally limited to strong nucleophilic cyclic amines.

SUMMARY

High interest in DNA encoded combinatorial chemistry has made acute the lack of reported organic transformations compatible with DNA oligomers. Luk and Satz[23] may be referenced for a list of all previously reported DNA compatible reactions. Table 1 lists the reactions recently developed or optimized herein. The reactions listed in Table 1 were developed with the intent of synthesizing diverse libraries of drug-like small molecules. An emphasis was placed on the synthesis of highly functionalized heterocyclic rings and products comprising diverse sets of building blocks including amines, carboxylic acids, aldehydes, alkynes, and boronic acids. Diverse sets of building blocks inevitably provide more diverse final products.[24]

Products with lower molecular weights and lipophilicity are also desired. Combinatorial chemistry historically has generated large numbers of molecules through numerous cycles of split-and-pool.[55] However, this strategy conflicts with the goal of producing lower-molecular-weight products. Library schemes employing fewer (2−3) chemistry cycles in combination with extremely large split sizes helps to overcome this issue.[24] For instance, a library with two cycles of chemistry, where each cycle possesses 5000 building blocks, yields 25 million final products, and many of these products will have reasonable molecular weights.

The ability to successfully employ and tag extremely large split sizes, and later interrogate the resulting complex mixtures with high throughput sequencing, distinguishes current DNA encoded technology from previous iterations of combinatorial chemistry.[15] However, this building block intensive approach requires robust chemistry employing the most diverse reagent sets. The methods listed in Table 1 provide the ability to access a number of DELs which meet this criterion. Indeed, the most common reactions used by medicinal chemists are shown herein to be applicable to DNA conjugates.[58] Still, DNA compatible chemistry is limited and access to many desirable heterocyclic ring systems does not exist. Continued efforts developing DNA compatible synthetic transformations are necessary to improve the properties and diversity of future DELs.

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■ ASSOCIATED CONTENT

Supporting Information

General procedures, preparation of starting materials, exemplar LCMS traces, and tables of example DNA conjugate reactions. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.bioconjchem.5b00239.

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Notes

The authors declare no competing financial interest.

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