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DNA-Compatible Copper/TEMPO Oxidation for DNA-Encoded Libraries

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Abstract

Aldehydes are important synthons for DNA-encoded library (DEL) construction, but the development of a DNA-compatible method for the oxidation of alcohols to aldehydes remains a significant challenge in the field of DEL chemistry. We report that a copper/TEMPO catalyst system enables the solution-phase, DNA-compatible oxidation of DNA-linked primary activated alcohols to aldehydes. The semi-aqueous, room-temperature reaction conditions afford oxidation of benzylic, heterobenzylic and allylic alcohols in high yield, with DNA compatibility verified by mass spectrometry, qPCR, Sanger sequencing, and ligation assays. Subsequent transformations of the resulting aldehydes demonstrate the potential of the method for robust library diversification.

Graphical Abstract

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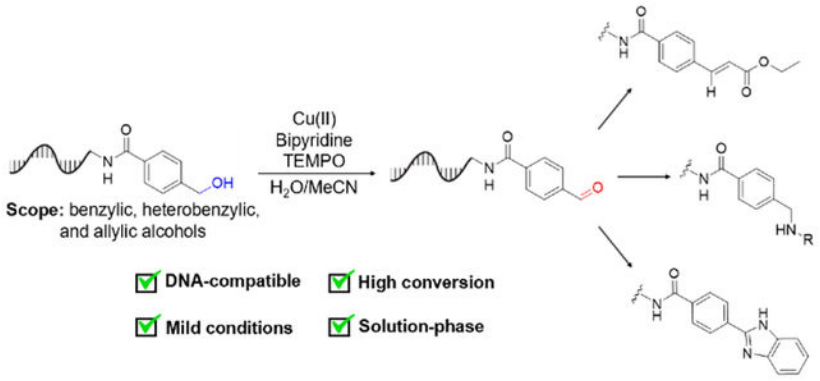
Supporting information: Experimental procedures, HPLC and ESI-MS of DNA conjugates, qPCR example calculation, supplementary experiments (damage analysis, aliphatic alcohol oxidation).

The authors declare the following competing financial interest(s): J.J.C. is a consultant for Thermo Fisher Scientific.



Keywords

DNA-encoded libraries; Oxidation; DNA-compatible; Solution-phase; Alcohols

DNA encoded libraries (DELs) are a powerful tool for drug discovery that have recently led to the identification of therapeutic precursors which have entered clinical trials[1,2,3,4,5,6]. A DEL is a library of DNA-linked small molecules in which each DNA sequence (built through the attachment of a series of DNA barcodes) describes the synthetic steps used to prepare each covalently linked, small molecule library member. This library can then be screened for bioactivity or affinity for a given target, and small molecules with promising activity can be identified through PCR amplification and DNA sequencing of the attached barcodes. DELs accelerate drug discovery by enabling simultaneous screening of large libraries, so designing a diverse library that covers a wide chemical space is critical to the success of the screen. Constructing diverse DELs requires DNA-compatible methods for the incorporation of diversity-enabling functional groups[7].

Aldehydes are important synthons for DEL diversification because they react with a wide variety of building blocks to form multiple functional groups using existing DNA-compatible methods[7]. This wide scope of reactivity has been demonstrated by Paciaroni et al. in an “explosion” strategy for DEL synthesis using DNA-linked aldehydes to access nine different diversification reactions[8]. Despite this utility, the use of aldehydes in DELs is limited by the accessibility of multifunctional aldehyde building blocks that can be attached to DNA. A commercial availability search revealed that there are 3,031 commercially available aldehydes bearing a carboxylic acid handle (for attachment to DNA) compared to 12,749 similarly functionalized primary alcohol derivatives (see Figure 1A). While bifunctional aldehyde building blocks could be pre-synthesized prior to attachment to DNA, the individual oxidation and purification of each compound creates a heavy synthetic burden. This burden could be bypassed with a DNA-compatible oxidation method allowing for simultaneous oxidation of all library members in a growing split-and-pool DEL.

The formation of aldehydes on DNA from alcohol precursors poses a substantial challenge because DNA is notoriously sensitive to oxidation,[9–13] and established oxidation reactions lead to significant oxidative damage[14,15] interfering with the PCR amplification[16] and the

sequencing readout[17] of a DEL. The susceptibility of DNA to damage severely limits the scope of oxidation reactions that can be used in a DEL[14,15].

To date, only two methods for alcohol oxidation in a DEL context have been developed. In the first demonstration of biocatalysis for DEL synthesis, Thomas et al. used galactose oxidase variants to oxidize the C6 alcohols of DNA-linked hexoses (Figure 1B)[18]. However, the substrate specificity of enzymes is problematic in the context of most DELs where maximizing substrate diversity is critical. Škopić et al. explored the use of Cu/TEMPO alcohol oxidation reaction for three DNA-linked alcohols but were only able to achieve DNA-compatible oxidation in the presence of a block-copolymer micelle, introducing procedural complexity and deviating from the solution-phase chemistry commonly used in split-and-pool DEL synthesis[19]. Thus, there is a need for a more robust and procedurally simple DNA-compatible transformation of alcohols to aldehydes.

Copper/TEMPO alcohol oxidations are exceptionally mild and allow alcohol oxidation without intermediacy of highly oxidizing oxoammonium intermediates commonly invoked in TEMPO-catalyzed oxidations[20,21]. Although traditionally run under anhydrous conditions, the reaction works in semi-aqueous conditions and in the presence of DNA[22,23,24]. While DNA is known to be damaged under oxidizing conditions and in the presence of copper[25], copper is commonly used for DNA-compatible click reactions[26,27] and oxidative DEL methods involving copper have been reported for inverse-electron-demand Diels-Alder reactions and oxidative amidation methods[28,29], with reports of minimal DNA damage. Motivated by this literature precedent and our experience with Cu/TEMPO oxidations in the presence of DNA[24], we sought to identify high-yielding, solution phase, DNA-compatible conditions for alcohol oxidation for use in DELs.

Here we report a mild, solution-phase Cu/TEMPO oxidation of benzylic, hetero-benzylic, and allylic alcohols to aldehydes (Figure 1C), demonstrate its DNA-compatibility by qPCR and high-resolution and accurate-mass mass spectrometry, and explore its utility for DEL synthesis in a model workflow by introducing diversity elements through solution-phase, DNA-compatible reductive amination, olefination, and heterocyclization.

To determine the optimal conditions for alcohol oxidation on DNA, we assessed a variety of reaction conditions using a model benzylic alcohol substrate covalently linked to a 26-nucleotide single-stranded DNA oligomer (without HPLC purification following bioconjugation). We evaluated reaction outcomes using two criteria: reaction yield (determined by ion-pair reversed-phase-HPLC, with formation of the intended product confirmed via high-resolution mass spectrometry; see SI) and DNA-compatibility (determined by qPCR measurement of DNA amplifiability after reaction, see SI). Yields were calculated using peak integration of starting material and product peaks on HPLC chromatograms (excluding impurities from bioconjugation reactions) (See S3). Impurities were identified via HPLC and characterized via high-resolution MS. (See S35). We began by investigating catalyst concentration and found that with 500 μM catalyst loading (500 μM $Cu(ClO_4)_2$, 500 μM TEMPO, and 500 μM bipyridine, condition 5), the aldehyde product formed in 90% yield after 12 hours of reaction. A 2.02 Da mass decrease was observed via mass spectrometry, indicating the formation of the aldehyde (Figure 2c.-2d.).

Deviating from this loading led to poor reaction outcomes: increasing catalyst concentration 10-fold (condition 1) led to extensive damage and loss of DNA, while lowering the catalyst concentration 10-fold (condition 11) decreased the yield to 5%. In assessing DNA-compatibility, the 5 mM catalyst loading led to <1% amplifiability after reaction, while the 50 μM and 500 μM loadings led to greater than 60% amplifiability (exceeding the 30% benchmark for DNA-compatibility proposed by Malone and Paegel)[16]. We next explored the effect of changing the solvent composition. Decreasing the water content below 50 % or increasing the water content above 70 % decreased the yield, while decreasing water content below 50% led to significant decreases in amplifiability. Taking both the yield and DNA-compatibility into account, we determined 500 μM catalyst loading in a 70% aqueous solvent system to be the optimal conditions for this reaction. Comparison of our optimized conditions with stoichiometric oxoammonium salt oxidations (using TEMPO+ and Bobbitt's Salt) confirmed that the co-catalytic copper/TEMPO system is milder: both oxoammonium salts led to lower DNA amplifiability after reaction. To further ensure that our conditions would be acceptable for a DEL, we demonstrated the retention of a 5′ phosphoryl group, successful ligation, and sequence integrity post-oxidation (S38–S41).

With optimized conditions in hand, we then investigated the substrate scope of activated primary alcohol oxidation. Formation of desired products was confirmed via high-resolution mass spectrometry (see SI). We successfully demonstrated the oxidation of a variety of DNA-linked primary benzylic alcohols containing halogens, electron-donating groups, and electron-withdrawing groups. Notably, the nitro-functionalized alcohol had a lower conversion compared to non-functionalized and differently functionalized alcohols. To demonstrate that the reaction is applicable to substrates beyond benzylic alcohols, we exemplified the high-yielding oxidation of several heterobenzylic alcohols (including a pyridine-containing substrate) and an allylic alcohol. Several of these substrates also showed further reactivity: oxidation of an allylic alcohol under these conditions resulted in addition of water (presumably at the beta position, Figure 3 Panel b). Furthermore, while overoxidation to the carboxylic acid was generally not observed, the carboxylic acid was observed to be a minor product for the 3-hydroxymethyl pyridine substrate (Figure 3 Panel c).

Copper/TEMPO catalyst systems have been successfully used for oxidation of primary aliphatic alcohols. However, when we exposed a DNA-linked primary aliphatic alcohol to our optimized reaction conditions, we did not observe the oxidation product. Replacing TEMPO with a less-hindered nitroxyl radical co-catalyst (ABNO) known to accelerate primary aliphatic alcohol oxidations[30] led to oxidation of the DNA-linked primary alcohol (to the carboxylic acid) as well as the unprotected primary alcohol on the 5′ end of the oligonucleotide (S44). Control experiments using unmodified and 5′-phosphorylated oligonucleotides showed that the presence of a 5′-phosphate group can prevent Cu/ABNO-catalyzed oxidation of the 5′-alcohol (S44). These results suggest that it may be possible in the future to develop alternative copper/nitroxyl radical catalyst systems that enable aliphatic alcohol oxidations in DEL workflows.

To demonstrate that this alcohol oxidation could fit into a synthetic sequence with known DEL chemistry, we converted a DNA-linked benzyl alcohol to a variety of amines using

a two-step sequence: our optimized alcohol oxidation followed by a DNA-compatible reductive amination[31]. We observed moderate to excellent reductive amination yields using a variety of amines, including primary and secondary amines, aliphatic and aromatic amines, and amines bearing secondary synthons such as another benzylic alcohol and an aryl chloride (Figure 4.). Formation of desired products was confirmed via high-resolution mass spectrometry (see SI).

We envisioned that DNA-appended aldehydes generated through alcohol oxidation could act as a starting point for an “explosive” diversification of a DNA-encoded library using solution-phase methods compatible with standard split-and-pool library construction. Towards this end, we adapted on-bead reactions[8] into solution-phase conditions for the Horner-Wadsworth-Emmons olefination[32 33] and benzimidazole heterocyclization of DNA-linked aldehydes. We performed each reaction in sequence with our optimized copper/TEMPO-catalyzed alcohol oxidation reaction (see Scheme 1). The reactions were both high-yielding, and the heterocyclization afforded the benzimidazole adduct without need for a photosensitizer[34]. Thus, in addition to demonstrating reductive amination with a variety of amines, we realized high-yielding solution-phase olefin formation and benzimidazole formation from aldehydes produced from on-DNA alcohol oxidation.

In summary, we have addressed the outstanding challenge of alcohol oxidation in the DEL chemical space through the development of a mild, DNA-compatible method for the oxidation of activated alcohols to aldehydes using a copper/TEMPO catalyst system. These conditions successfully oxidized activated alcohols including benzylic, heterobenzylic, and allylic alcohols, and tolerated both electron-donating and -withdrawing substituents. This preliminary scope demonstrates the possible application of this method for the oxidation of a wide scope of activated alcohols. We also obtained promising results suggesting that, in the future, related catalyst systems may enable the challenging oxidation of aliphatic alcohols in a DEL context.

The DNA-compatibility of the reaction was demonstrated extensively through qPCR, Sanger sequencing, ligation assays, and mass spectrometry. These results confirm that alcohol oxidations can be DNA-compatible and open the door for further exploration of oxidative DEL methods. Furthermore, the mildness of these conditions suggests that these oxidations may find broader applications in biomolecular modification methods.

Finally, we explored how this alcohol oxidation could be incorporated into an “explosion” strategy for DEL diversification by performing the reaction in sequence with three different solution-phase coupling reactions. This showcases the key role that alcohol oxidation can play as an intermediate step in DEL synthesis, and we hope that this method will find ready application in pharmaceutical and agrochemical discovery chemistry.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

TEMPO	2,2,6,6-tetramethyl-1-piperidyloxy radical
TEMPO BF_4	2,2,6,6-tetramethyl-1-piperidyloxy tetrafluoroborate
DEL	DNA Encoded Library
DNA	Deoxyribose Nucleic Acid
ABNO-9	Azabicyclo[3.3.1] nonane N-oxyl radical
qPCR	Quantitative polymerase chain reaction
$NaBH_3CN$	Sodium Cyanoborohydride
MeCN	Acetonitrile
NMP	N-Methylpyrrolidine
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DMA	Dimethylacetamide
$Cu(ClO_4)_2$	Copper Perchlorate
GalOx	Galactose Oxidase
HRP	Horse Radish Peroxidase
HWE Olefination	Horner-Wadsworth-Emmons

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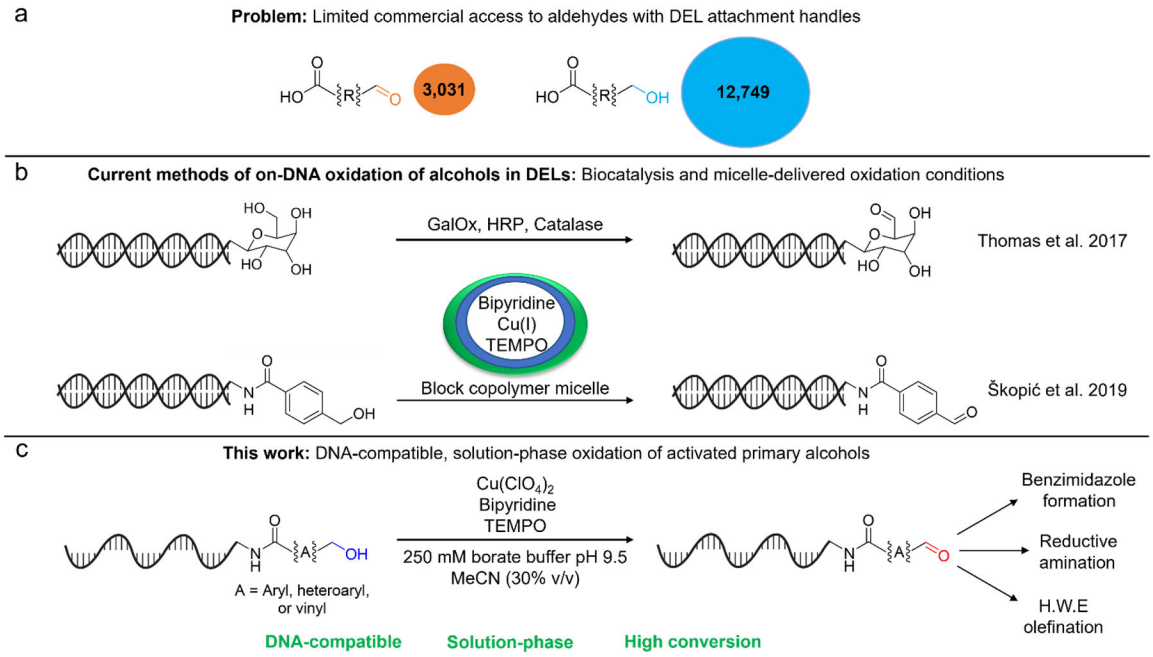
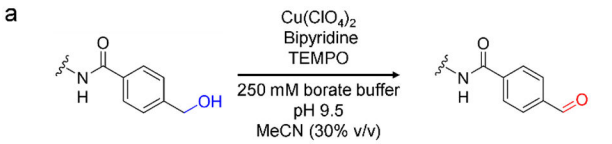


Figure 1a.

Comparison of commercial availability of carboxylic acid bearing bifunctional alcohols and aldehydes. b. Current literature methods for DNA-compatible oxidation of alcohols in DNA-encoded libraries (DELs). c. New DNA-compatible method for oxidation of primary activated alcohols to aldehydes as a precursor to DNA-compatible aldehyde transformations (Benzimidazole formation, Reductive amination, Horner-Wadsworth-Emmons Olefination).



Condition	Catalyst Concentration	Aqueous: MeCN (% v/v)	Yield (%)
1	5 mM	70:30	ND
2	500 μM	100:0	81
3	500 μM	90:10	83
4	500 μM	80:20	86
5	**500 μM**	**70:30**	**91**
6	500 μM	60:40	74
7	500 μM	50:50	67
8	500 μM	40:60	36
9	500 μM	30:70	10
10	500 μM	20:80	5
11	50 μM	70:30	5
12*	Bobbitt's Salt	70:30	35
13†	$TEMPO^+$ $BF4^-$	70:30	26

Figure 2a.

Optimization table for oxidation of DNA-linked benzylic alcohol using Cu/TEMPO/bipyridine catalyst system. Standard Reaction Conditions: 100 equiv. $Cu(ClO_4)_2$, 2,2′-bipyridine, TEMPO (20 μL, 5 mM stock in MeCN), 1 equiv. DNA conjugate (1 nmol), 250 mM borate buffer pH 9.5 (16 μL, 16.5% v/v), nuclease-free water (52.5% v/v). *100 equiv. Bobbitt's salt (10 μL, 5 mM in MeCN), MeCN (20 μL, 20% v/v), 250 mM borate buffer pH 9.5 (16 μL, 16.5% v/v), nuclease-free water (51 μL, 52.5% v/v). †100 equiv $TEMPO^+$ BF_4^- salt (10 μL, 5 mM in MeCN), MeCN (20 μL, 20% v/v), 250 mM borate buffer pH 9.5 (16 μL, 16.5% volume), nuclease-free water (51 μL, 52.5% v/v). 2b. qPCR-determined amplifiability of an unmodified DNA oligo after exposure to varied oxidation conditions. 2c. Partial ESI-MS of DNA1A showing predominant species; full spectra in SI. 2d. Partial ESI-MS of DNA2A showing predominant species; full spectra in SI

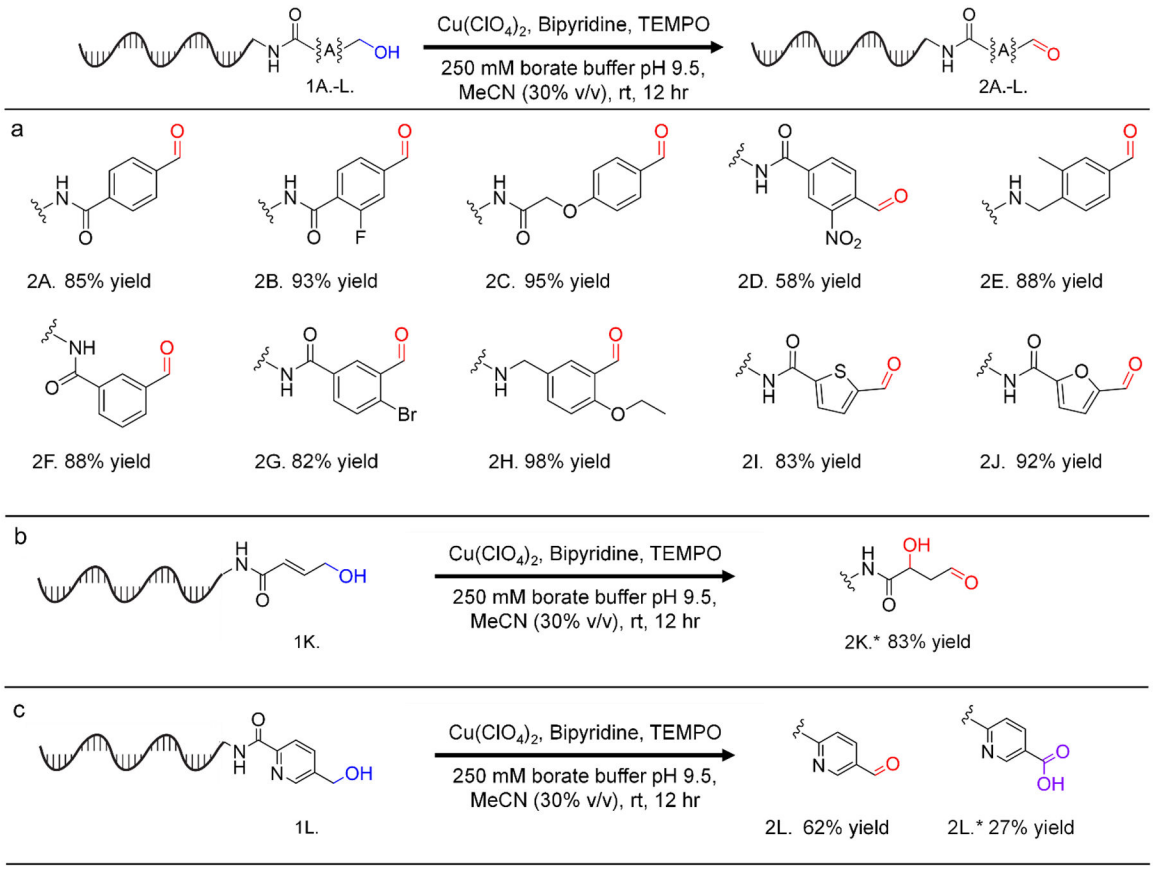


Figure 3.

Oxidation of diverse, DNA-linked, primary activated alcohols to the corresponding aldehydes. Reaction conditions: DNA (1 equiv, 1 nmol), $Cu(ClO_4)_2$ (100 equiv, 20 μL of 5 mM stock in MeCN), TEMPO (100 equiv, 20 μL of 5 mM stock in MeCN), bipyridine (100 equiv, 20 μL of 5 mM in MeCN), H_2O/250 mM borate buffer pH 9.5/MeCN (v/v/v 52.5/16.5/31 −194 μL total volume), rt, 12 hours.

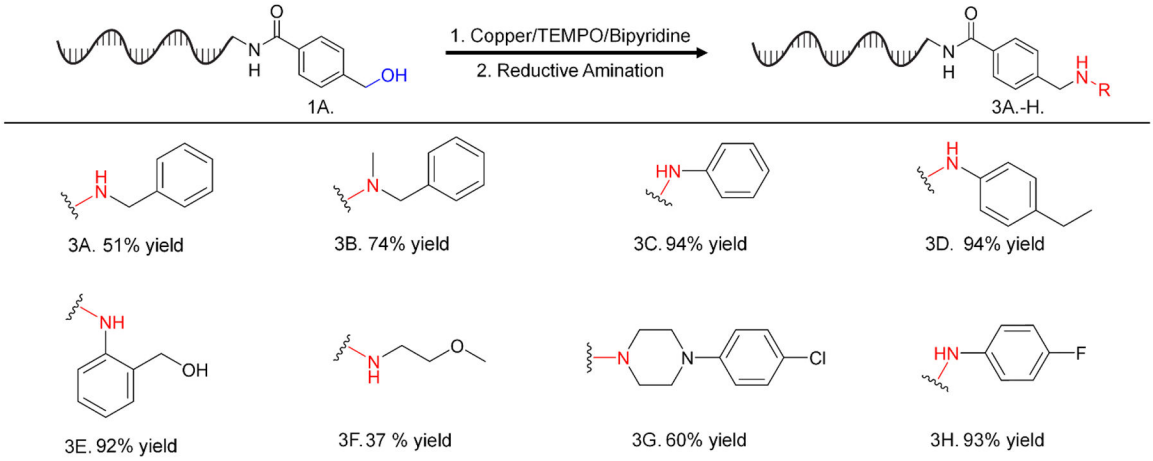
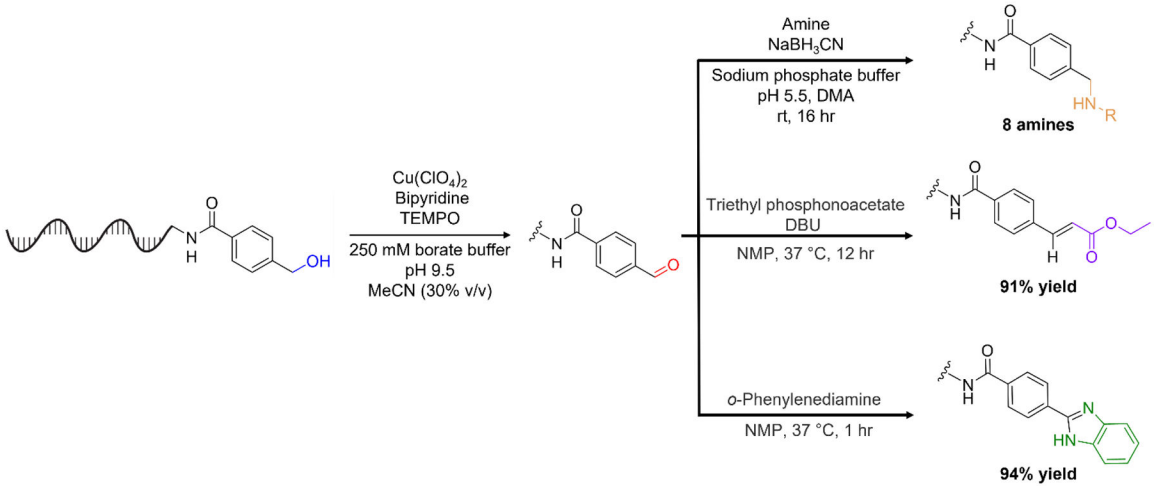


Figure 4.

Reductive amination using a DNA-linked aldehyde produced using our optimized oxidation conditions. Reaction conditions: DNA (1 equiv, 1 nmol), 250 mM sodium phosphate buffer pH 5.5 (25 μL, 33% v/v), amine (5,000 equiv, 25 μL of 200 mM stock in DMA (33% v/v), sodium cyanoborohydride (5,000 equiv, 25 μL of 200 mM stock in MeCN (33% v/v), rt, 15 hr. Yields reported above are for the reductive amination step.



Scheme 1.

Oxidation of an activated alcohol to an aldehyde followed by solution phase reductive amination, Horner-Wadsworth-Emmons olefination, and benzimidazole formation.