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RESEARCH ARTICLE

Micelle-Promoted Reductive Amination of DNA-Conjugated Amines for DNA-Encoded Library Synthesis

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Supporting information for this article is given via a link at the end of the document.

Abstract: DNA-encoded libraries (DELs) have become a leading technology for hit identification in drug discovery projects as large, diverse libraries can be generated. DELs are commonly synthesised via split-and-pool methodology; thus, chemical transformations utilised must be highly efficient, proceeding with high conversions. Reactions performed in DEL synthesis also require a broad substrate scope to produce diverse, drug-like libraries. Many pharmaceutical compounds incorporate multiple C-N bonds, over a quarter of which are synthesised via reductive aminations. However, few on-DNA reductive amination procedures have been developed. Herein is reported the application of the micelle-forming surfactant, TPGS-750-M, to the on-DNA reductive amination of DNA-conjugated amines, yielding highly efficient conversions with a broad range of aldehydes, including medicinally relevant heterocyclic and aliphatic substrates. The procedure is compatible with DNA amplification and sequencing, demonstrating its applicability to DEL synthesis.

Introduction

Over the past 30 years, DNA-encoded libraries (DELs) have emerged as a promising technology for the identification of hit molecules in both medicinal chemistry and chemical biology.[1–4] DELs comprise large collections of organic molecules attached to a complementary DNA sequence, which serves as an identifiable barcode unique to each compound. These encoded libraries are usually constructed using combinatorial split-and-pool methodology (Figure 1). This approach centres on the iterative conjugation of chemical building blocks onto a growing DNA tag, which are individually encoded through concomitant enzymatic ligations of corresponding DNA sequences.[5–7] Following multiple cycles of synthesis, large and diverse libraries can be prepared containing in excess of 10^9 library members. Libraries of such size are prohibitively difficult to achieve with traditional chemical synthesis. The resulting DELs can be screened against immobilised target proteins, typically through affinity selection, and the identity of hit compounds elucidated by polymerase chain reaction (PCR) amplification and sequencing of the DNA barcode. The potential of DEL technology for application to drug discovery has already been underlined through the progression of several DEL campaign derived candidates into clinical trials.[8–10]

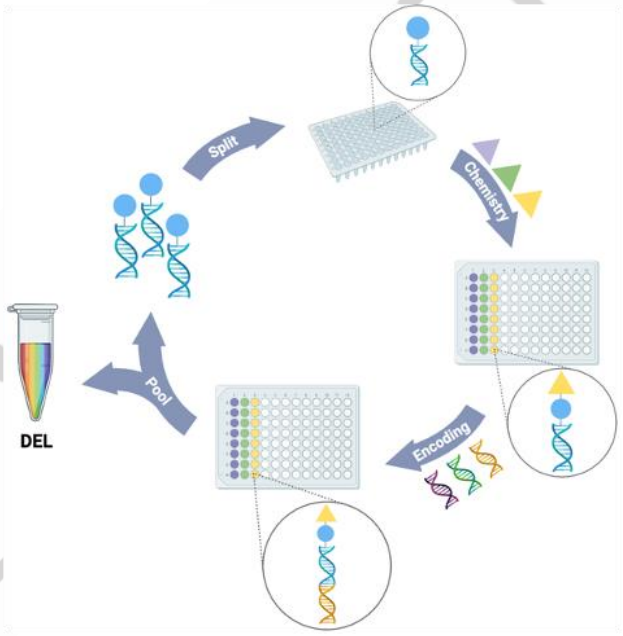


Figure 1. Schematic representation of the split-and-pool method of DEL synthesis, incorporating iterating chemical transformation (chemistry) and DNA ligation (encoding) steps. Created with BioRender.com.

Despite the advantages offered by DELs, specifically in facilitating more rapid and efficient synthesis and screening, limitations remain in the chemistries applicable to on-DNA synthesis. Traditionally, DNA-compatible reactions require the use of aqueous conditions at high dilutions and utilise milder reagents to limit damage to the DNA barcode. This has largely precluded the use of high temperatures, oxidants, and acids, all of which can result in a loss of DNA barcode integrity. Due to the limited purification methods applicable to DEL synthesis, organic transformations should ideally be high yielding across a broad range of substrates in order to maintain library fidelity.[10,11] In reality, many current methods afford only moderate conversions over a limited range of substrates. One approach to facilitating reactions under aqueous conditions is the application of micelle-forming surfactants.[12,13] Our group have previously reported the successful application of the surfactant TPGS-750-M in facilitating on-DNA amide couplings, Suzuki-Miyaura couplings, Buchwald-Hartwig aminations, Sonogashira couplings, and Heck reactions with high conversions and broad substrate scopes.[14–18]

Reductive aminations have long played an important role in medicinal chemistry due to their ability to synthesise diverse amines, a fundamental structural motif in pharmaceutical compounds that imparts desirable drug-like properties.[19,20] This versatile transformation typically involves the coupling of a carbonyl compound with an amine to form an intermediate hemiaminal, which undergoes reduction via various approaches; most commonly through the formation of a Schiff base (imine) or iminium ion, and subsequent reduction using either borohydride-based reducing agents ($NaBH_4$, $NaBH_3CN$, $NaBH(OAc)_3$) or catalytic hydrogenation.[20] Reductive aminations have found widespread applications in drug discovery due to their efficiency, cost-effectiveness, and ability to introduce complexity into molecular structures. Indeed, it has been reported that a quarter of the C-N bond forming reactions encountered in the pharmaceutical industry are performed using reductive aminations and numerous drugs, including the anticancer agent imatinib, asthma and chronic obstructive pulmonary disease (COPD) drug salbutamol, and the antidepressant sertraline have been synthesised via reductive aminations (Figure 2).[20–23]

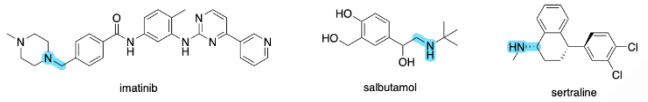


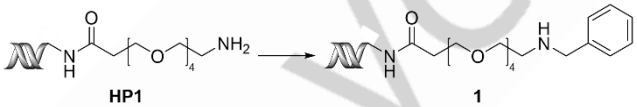
Figure 2. FDA-approved drugs synthesised using reductive aminations: imatinib, a tyrosine kinase inhibitor; salbutamol, a β_2 adrenergic receptor agonist; and sertraline, a selective serotonin uptake inhibitor. The bond formed via reductive amination is highlighted.

Despite the prominence of reductive aminations in medicinal chemistry, reports of on-DNA approaches have been very limited to date.[11,24–26] Typical approaches currently employ an on-DNA carbonyl substrate with excess amine in solution to shift the reaction equilibrium towards the intermediate imine or iminium ion. Procedures to couple DNA-tagged amines with carbonyl substrates have proven highly challenging, particularly with respect to maintaining control over single vs double alkylation in the presence of excess carbonyl reagent. Available literature has either been absent of any significant scope evaluation or has favoured double alkylation of the on-DNA amine. In a recent screen of on-DNA solution phase reductive aminations conducted by Pfizer, 218 different carbonyl compounds were assessed, and fewer than 50 provided greater than 50% yield.[27]

The most extensive report of an on-DNA reductive amination utilising a DNA-conjugated amine employed an approach termed reversible adsorption to solid support (RASS), in which the DNA is non-covalently adsorbed onto a solid support and subsequently eluted.[27] This approach provided an appreciable improvement for ketone substrates, with 14 examples reported proceeding with 16–82% yields. In contrast, aldehydes were significantly less efficient; of the 4 reported examples, yields of only 23–56% were achieved. An alternative approach conducted in free aqueous media (without RASS) was also reported, achieving conversions of 7-39% for the aldehyde substrates. This has implications for library development, as remaining starting materials or undesired side products remaining in the library can result in low overall fidelity and, consequently, less successful hit discovery programs. Hence, the development of on-DNA reductive amination conditions that proceed with high efficiency across a range of carbonyl substrates, with a particular focus on aldehydes is critical. Herein is reported the application of micellar catalysis to facilitate a reductive amination on DNA with broad aldehyde scope, including 55 aldehydes proceeding with greater than 70% conversion, representing a significant advance on existing methods.

Results and Discussion

Initial investigations focussed on assessing the efficiency of current literature procedures for a model system using a PEG_4-hexylamido-linked DNA-tagged amine, **HP1**, and benzaldehyde (Scheme 1).



Scheme 1. Schematic representation of initial on-DNA reductive amination attempt using benzaldehyde. Optimal conditions: **HP1** (5 nmol), borate buffer (250 mM, pH = 9.4), benzaldehyde (200 mM in MeCN), rt, 800 rpm, 0.5 h; then $NaBH_4$ (200 mM in MeCN/H_2O), rt, 800 rpm, 16 h.

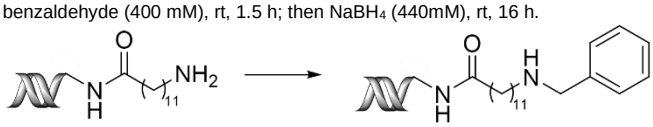
This afforded varying outcomes, including both single and double alkylation products, with moderate conversions to the desired single alkylation product. The most promising conditions, utilising a pH 9.4 borate buffer and $NaBH_4$ reducing agent, proceeded with 68% conversion to the desired product, alongside high control over single vs double alkylation when performed in two steps. The aldehyde and reducing agent were also both dissolved in an acetonitrile based cosolvent system (100% MeCN and 1:1 MeCN/H_2O, respectively) and administered as a stock solution to aid reaction feasibility.

Initial optimisation of these conditions identified modifications to reaction time and reagent concentration that yielded improvements. Both increased time for the imine formation step and increased benzaldehyde concentration appeared to positively affect the imine-aldehyde equilibrium, providing an optimised conversion of 85%. However, attempted application of micellar catalysis to this system, via the addition of TPGS-750-M, gave no appreciable improvement, with conversion decreasing to 81%. It was hypothesised the use of an alternative DNA headpiece, containing a C_{11} alkyl linker, **HP2**, could be beneficial through providing more favourable interactions with the surfactant molecules, consequently providing an increased effective concentration of aldehyde and decreased amount of water proximal to the DNA-conjugated amine, and thus increased conversion.

An investigation into this alternative DNA headpiece construct followed, with the previously optimised conditions offering a starting point for further optimisation. Exploration of the reducing agent was initially conducted, with higher conversion afforded using $NaBH_4$ (73%) relative to $NaBH_3CN$ (56%) (Table 1). The impact of co-solvents in on-DNA reactions has also been widely reported, hence the choice of $NaBH_4$ stock solution cosolvent was investigated. The aforementioned 1:1 mixture of

MeCN/H_2O provided optimal conversion at 73% and neat water, MeCN, or DMF performed slightly less well (Table 2). Analogously, the aldehyde cosolvent was also assessed. Optimal conversion was again afforded using the previously employed 100% MeCN stock solution, with a significant decrease in conversion with water alone (Table 3).

Table 1. Coupling of **HP2** with benzaldehyde employing different reducing agents. Conditions: **HP2** (1 nmol), borate buffer (250 mM, pH = 9.4), benzaldehyde (400 mM), rt, 1.5 h; then $NaBH_4$ (440mM), rt, 16 h.



HP2 **2**

Reducing agent	Product (%)
$NaBH_4$	73
$NaBH_3CN$	56

Table 2. Coupling of **HP2** with benzaldehyde with different solvents for the reducing agent. Conditions: **HP2** (1 nmol), borate buffer (250 mM, pH = 9.4), benzaldehyde (400 mM), rt, 1.5 h; then $NaBH_4$ (440mM), rt, 16 h.

$NaBH_4$ solvent	Product (%)
MeCN	65
H_2O	69
DMF	63
MeCN/H_2O (1:1)	73

Table 3. Coupling of **HP2** with benzaldehyde dissolved in different solvents. Conditions: **HP2** (1 nmol), borate buffer (250 mM, pH = 9.4), benzaldehyde (400 mM), rt, 1.5 h; then $NaBH_4$ (440mM), rt, 16 h.

Aldehyde solvent	Product (%)
DMF	65
MeCN	73
H_2O	25

With optimal reducing agent and solvent combinations identified, the role of the borate buffer in the reaction was investigated. Equivalent conditions exchanging the borate buffer for both water and the surfactant TPGS-750-M yielded significant reductions in conversion. Despite this, the potential benefit of micellar catalysis in this approach was evident, with higher conversion in the presence of TPGS-750-M compared to the equivalent non-micellar aqueous conditions. Additionally, to assess whether the impact of the borate buffer is solely pH driven, a pH 10 adjusted aqueous NaOH solution was used in place of the buffer, providing a similarly basic reaction environment, however, conversion was further decreased to 1% (Table 4). This underlined the importance of the borate buffer for reactivity.

Table 4. Coupling of **HP2** with benzaldehyde in different solvent systems. Conditions: **HP2** (1 nmol), solvent, benzaldehyde (400 mM), rt, 1.5 h; then $NaBH_4$ (440mM), rt, 16 h.

Medium	Product (%)
Borate Buffer[a]	73
Water	9
TPGS	18
aq. NaOH (pH 10 adjusted)	1

[a] pH 9.4, 250 mM

Given the clear importance of the borate buffer, attempts were subsequently made to develop an optimised buffer system. Increasing the volume of borate buffer had a detrimental impact on reactivity, with a 30 μL increase leading to a significant fall in conversion (Table 5). The borate buffer pH was also optimised. A general trend of increasing conversion with increasing pH was observed; when using a pH 10.1 borate buffer, an optimal balance between maximising conversion and remaining within the buffering capacity (pH ~8-10) was achieved, affording 85% conversion to the desired product (Table 6). The impact of micellar catalysis was also investigated at this point, however, as addition of TPGS-750-M leads to dilution of the reaction mixture, this in turn decreases the effective overall reaction pH. Since pH has a significant impact on conversion in this reaction, a higher pH borate buffer (pH 10.8) was used to ensure the final reaction was at the optimal pH 10. Addition of surfactant had a minimal impact on the reaction at this stage, with conversion approximately maintained at 83%.

Table 5. Coupling of **HP2** with benzaldehyde in different total volumes. Conditions: **HP2** (1 nmol), borate buffer (250 mM, pH = 9.4), benzaldehyde (400 mM), rt, 1.5 h; then $NaBH_4$ (440mM), rt, 16 h.

Total volume	Product (%)
21	73
31	61
51	34

The impact of borate buffer concentration was additionally assessed, varying the boric acid concentration from 8 to 1000 mM. A reduction in conversion resulted from both extreme concentration points (8 mM and 1000 mM), with optimal conversion of 93% achieved at 350 mM (Table 7). To investigate the breadth of the impact of micellar catalysis, five aldehydes, including benzaldehyde, were coupled with the DNA-tagged amine, **HP2,** in the presence and absence of TPGS-750-M. For all five aldehydes, conversion was higher when TPGS-750-M was

added, with an average 9% increase in conversion observed across the entire aldehyde series (Table 8). Micellar catalysis therefore appears to have a significant impact on the efficiency of this reaction, which is crucial in the synthesis of DELs.

Table 6. Coupling of **HP2** with benzaldehyde at varied pH values. Conditions: **HP2** (1 nmol), borate buffer (250 mM), benzaldehyde (400 mM), rt, 1.5 h; then $NaBH_4$ (440mM), rt, 16 h.

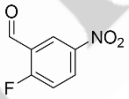
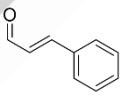
Borate Buffer pH	Product (%)
8.2	53
8.8	47
9.4	73
10.1	85

Table 7. Coupling of **HP2** with benzaldehyde at a range of borate buffer concentrations. Conditions: **HP2** (1 nmol), borate buffer (pH = 10.8), 5% TPGS-750-M, benzaldehyde (400 mM), rt, 1.5 h; then $NaBH_4$ (440mM), rt, 16 h.

Buffer Concentration	Product (%)
8	78
150	80
250	83
300	91
350	93
400	90
500	77
1000	73

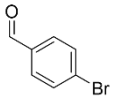
With a set of optimised conditions in hand, these were assessed for the reactivity of **HP2** with a series of 66 diverse aldehydes (Table 9). A range of benzylic, heteroaromatic (including five- and six-membered systems), and aliphatic aldehydes were evaluated. 53 of the aldehydes reacted with greater than 70% conversion to the desired product, a further 10 afforded greater than 55% conversion, while only 3 examples yielded less than 55% conversion. Aldehyde compatibility generally correlated with the electronic nature of the aromatic systems; electron-deficient benzylic systems typically conferred high conversions, including 4-nitro- (entry 19, 95%), 4-trifluoromethyl- (entry 21, 93%) and 3-cyano- (entry 7, 98%) phenyl substituents. Reduced conversions were largely restricted to electron-donating substituents, such as 4-hydroxy- (entry 12, 29%), 4-isopropyl- (entry 19, 56%) and 4-pyrrolidinyl- (entry 20, 61%) substituted benzaldehydes. In contrast, electron-rich systems, including 4-methoxy- (entry 16, 78%) and 4-methyl- (entry 17, 84%) substituted benzaldehydes displayed remarkably high conversions. This trend was additionally observed across the heteroaromatic series, with electron-deficient systems including isonicotinaldehyde (entry 41, 97%), pyrazine-2-carbaldehyde (entry 51, 97%), and thiazole-5-carbaldehyde (entry 57, 95%) proceeding with high efficiency. Electron-rich heterocycles, such as 1*H*-indole-3-carbaldehyde (entry 63, 82%) and thiophene-2-carbaldehyde (entry 55, 87%), proceeded with high conversions. Both cyclic (4-formyltetrahydropyran, entry 35, 90%) and acyclic (2-methyl butanal, entry 31, 88%) aliphatic aldehydes reacted successfully. α,β-Unsaturated aldehydes (entries 30 and 37) also reacted well. A wide range of functional groups were tolerated, including esters, nitriles, and alkenes.

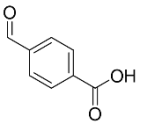
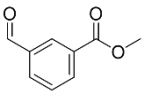
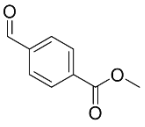
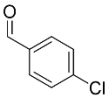
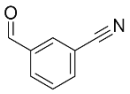
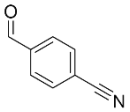
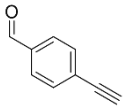
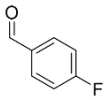
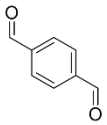
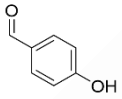
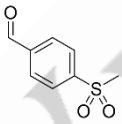
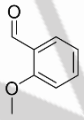
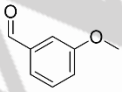
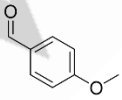
Table 8. Comparison of coupling of **HP2** with a selection of aldehydes in the presence of absence of TPGS-750-M. Conditions: **HP2** (1 nmol), borate buffer (350 mM, pH = 10.8), 5% TPGS-750-M / water, aldehyde (400 mM), rt, 1.5 h; then $NaBH_4$ (440mM), rt, 16 h.

Aldehyde	Product (%)	
	TPGS	Water
	93	81
	73	61
	98	97
	96	83
	95	86

The highlighted reaction conditions show superior conversion when compared to the existing literature procedures.[19] 2-Chloro-4-iodonicotinaldehyde and quinoline-3-carbaldehyde proceeded with 97% and 98% conversion to desired product, respectively, compared to 40% and 23% previously reported (Table 9, entries 44 and 49).

Table 9. Substrate scope of reductive amination between **HP2** and a variety of aldehydes. Conditions: **HP2** (1 nmol), borate buffer (350 mM, pH = 10.8), 5% TPGS-750-M, aldehyde (400 mM), rt, 1.5 h; then $NaBH_4$ (440mM), rt, 16 h.

Entry	Aldehyde	Conversion (%)	Product (%)
1		94	93
2		87	87

3		2	2
4		96	89
5		97	92
6		85	85
7		98	98
8		97	97
9		93	93
10		91	89
11		94	91[a]
12		40	29
13		96	93
14		93	91
15		88	88
16		79	78
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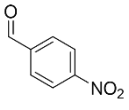
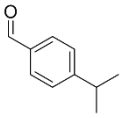
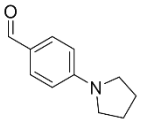
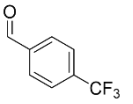
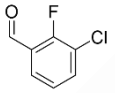
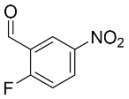
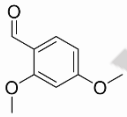
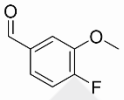
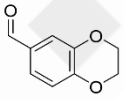
18		95	95
19		56	56
20		61	61
21		93	93
22		100	100
23		99	99
24		81	77
25		96	96
26		84	84
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29		76	63
30		100	80
31		88	88
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34		79	79
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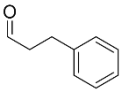
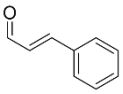
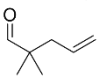
36		58	58
37		96	96
38		74	74
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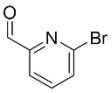
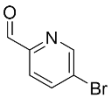
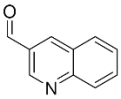
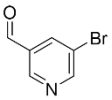
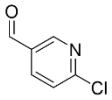
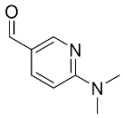
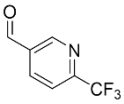
42		97	95
43		95	93
44		98	98
45		98	98
46		97	97
47		73	73
48		100	98
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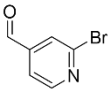
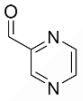
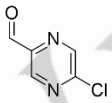
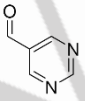
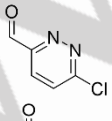
50		97	96
51		97	97
52		98	98
53		93	83
54		96	96
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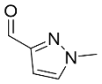
59		92	90
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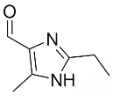
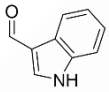
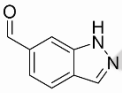
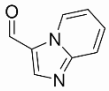
62		83	61
63		82	82
64		97	97
65		68	68
66		92	92

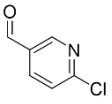
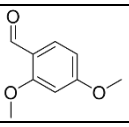
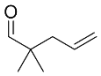
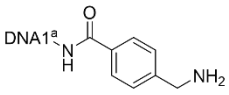
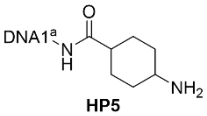
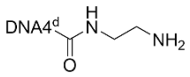
[a] Product is benzyl alcohol.

For certain heterocyclic aldehydes, such as 1*H*-imidazole-4-carbaldehyde and 1-methyl-1*H*-imidazole-2-carbaldehyde (Table 9, entries 60 and 61), lower conversion was attributed to low solubility in MeCN. While these aldehydes are soluble in water, as previously noted, lower conversion was observed with aldehyde as an aqueous solution. When dissolved in 5:4 MeCN/H_2O they exhibited improved solubility, and consequently improved conversions (73% from 29% and 86% from 63%, respectively) were achieved (Table 10).

Table 10. Comparison of coupling of **HP2** with aldehydes either dissolved in MeCN, or H_2O/MeCN. Conditions: **HP2** (1 nmol), borate buffer (350 mM, pH = 10.8), 5% TPGS-750-M, aldehyde (400 mM), rt, 1.5 h; then $NaBH_4$ (440mM), rt, 16 h.

Aldehyde	Product (%)	
	MeCN	Water/MeCN
	29	73
	63	86

Table 11. % Product from the application of optimized reductive amination conditions, utilising a selection of aldehydes, to a variety of DNA headpieces. Conditions: **HP1–6** (1 nmol), borate buffer (350 mM, pH = 10.8), 5% TPGS-750-M, aldehyde (400 mM), rt, 1.5 h; then $NaBH_4$ (440mM), rt, 16 h.

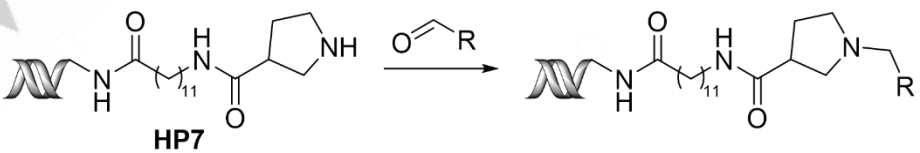
						
 **HP2**	93	92	88	97	77	74
 **HP3**	84	79	76	97	65	58
 **HP1**	90	85	85	96	72	65
 **HP4**	93	90	89	100	76	80
 **HP5**	81	82	71	92	57	37
 **HP6**	98	91	88	96	90	79

[a] 12-amino-*N*-hexyldodecanamide-dsDNA (Figure S1). [b] 1-amino-*N*-hexyl-3,6,9,12-tetraoxapentadecan-15-amide-hairpinDNA (Figure S69). [c] 1-amino-*N*-hexyl-3,6,9,12-tetraoxapentadecan-15-amide-dsDNA (Figure S70). [d] 15-carboxy-*N*-hexylpentadecanamide-dsDNA (Figure S73).

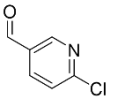
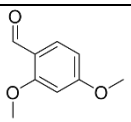
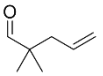
Following confirmation of the broad aldehyde substrate scope, the amine compatibility was investigated. A series of six aldehydes were selected from the established aldehyde scope with a range of conversions on the exemplified C_{11} alkyl DNA **HP2**. Each aldehyde was assessed on five alternative DNA-linked amines (Table 11). The optimised conditions proved to be transferable across a range of alternative primary amines, with high conversions consistently observed across the series. Benzaldehyde, 3,5-dimethylisoxazol-4-ylcarbaldehyde, 2-methylbutyraldehyde, and 4-chloronitcotinaldehyde gave high conversions (>70%) with all six amine headpieces, while 2,4-dimethoxybenzaldehyde and 2,2-dimethylpent-4-enal were more variable, particularly on the cyclohexylamine headpiece **HP5**, with conversions of 57% and 37% respectively. Gratifyingly, DNA-conjugated aminobenzyl and aminoethyl groups, **HP4** and **HP6** respectively, both gave excellent conversions, comparable to the exemplar C_{11} DNA **HP2**. A DNA-conjugated aniline was also trialled, however, conversions of <5% were observed. Overall, good amine transferability has been demonstrated for a series of primary amines, with equivalent conversions and control over single alkylation achieved across this series.

DNA-tagged secondary amines proved more challenging. Coupling of a pyrrolidine functionalised DNA **HP7** with the six exemplar aldehydes highlighted above resulted in little to no conversion to desired product. Because secondary amines are unable to form an intermediate imine, the two-step approach is ineffective. An alternative, one-step, Borch reductive amination was therefore explored, in which the intermediate iminium ion is reduced immediately upon formation. $NaBH_3CN$ was used as the reducing agent in place of $NaBH_4$ due to its reduced reactivity, and increased aqueous stability relative to $NaBH(OAc)_3$.[28] Increased conversion was achieved in all six examples, with an average conversion of 92% and no example below 80% (Table 12). A further assessment of this approach was carried out by coupling 2-methylbutyraldehyde with three alternative secondary amine-based DNA HPs, **HP8–10**, containing azetidine, piperazine, and piperidine groups. Conversion with all three amines was above 90%, demonstrating high transferability across differing DNA-conjugated secondary amines (Table 13).

Table 12. % Product form the application of one-step reductive amination conditions to **HP7**, utilising a selection of aldehydes. Conditions: **HP7** (1 nmol), borate buffer (350 mM, pH = 10.8), 5% TPGS-750-M, aldehyde (400 mM), $NaBH_3CN$ (440mM), rt, 17.5 h.



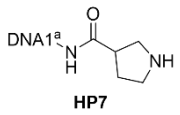
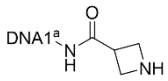
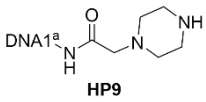
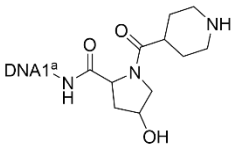
HP7

Stepwise with $NaBH_4$	0	0	7	11[a]	5	0
One-step with $NaBH_3CN$	100	87	100	82	93	89

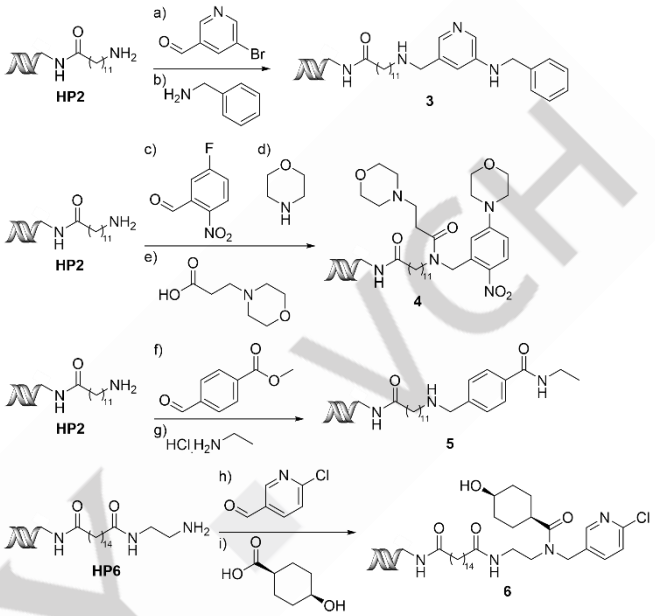
[a] Product mass is equivalent to either hydrolysed product, or reduced S_NAr product.

Table 13. % Product from the coupling of **HP7–10** with 2-methylbutyraldehyde at a range of borate buffer concentrations. Conditions: DNA **HP** (1 nmol), borate buffer (350 mM. pH = 10.8), 5% TPGS-750-M, 2-methylbutyraldehyde (400 mM), rt, 1.5 h; then $NaBH_4$ (440mM), rt, 16 h.

	
 **HP7**	100
 **HP8**	92
 **HP9**	100
 **HP10**	98

[a] 12-amino-*N*-hexyldodecanamide-dsDNA (Figure S1).

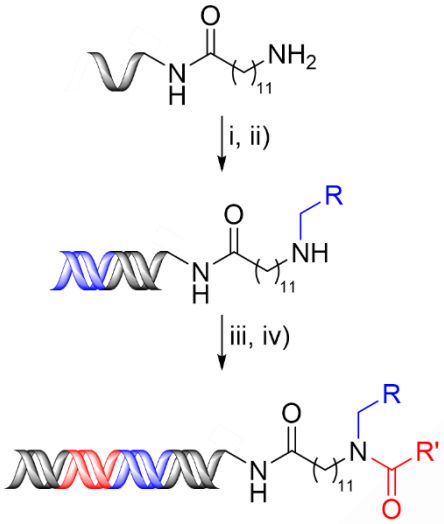
For widespread application in DEL synthesis, chemical transformations should be transferable across a range of DNA concentrations to enable efficient library synthesis. It has been demonstrated that these optimised reductive amination conditions are tolerable to changes in overall DNA concentration, with reactions using 0.5 nmol and 5 nmol DNA maintaining comparable conversions (91% and 92% respectively). Additionally, during library design it is imperative that the DNA-tagged organic molecule can undergo further chemical transformations with high efficiency. A series of previously validated on-DNA reactions were attempted on the reductive amination products of DNA **HP2** with a range of aldehydes displaying varied functionality for further reactions. Buchwald-Hartwig coupling, nucleophilic aromatic substitution (S_NAr), ester hydrolysis followed by amide coupling with ethylamine, and amide couplings with acid coupling partners have all been exemplified, with high conversions of >90% achieved in all cases (Scheme 2).



Scheme 2. Further reactions on reductive amination products with key building blocks shown. Conditions: a) **HP2** (1 nmol), borate buffer (350 mM. pH = 10.8), 5% TPGS-750-M, 5-bromonicotinaldehyde (400 mM), rt, 1.5 h; then $NaBH_4$ (440mM), rt, 16 h; b) DNA, benzylamine, 5% TPGS-750-M, K_3PO_4 (5 M), $[(Crotyl)PdCl]_2$ (80 mM), tBuXPhos (160 mM), 70 °C, 1 h; c) **HP2** (1 nmol), borate buffer (350 mM. pH = 10.8), 5% TPGS-750-M, 5-fluoro-2-nitrobenzaldehyde (400 mM), rt, 1.5 h; then $NaBH_4$ (440mM), rt, 16 h; d) DNA, morpholine, THF, H_2O, 60 °C, 24 h; e) DNA, borate buffer (150 mM, pH = 9.3), 3-morpholinopropanoic acid (150 mM), DMT-MM (250 mM), rt, 16 h; f) **HP2** (1 nmol), borate buffer (350 mM. pH = 10.8), 5% TPGS-750-M, methyl 4-formylbenzoate (400 mM), rt, 1.5 h; then $NaBH_4$ (440mM), rt, 16 h; g) DNA, ethylamine hydrochloride, 5% TPGS-750-M, HOAt, 2,6-Lutidine (1.2 M), DIC, 45 °C, 3 h; h) **HP6** (1 nmol), borate buffer (350 mM. pH = 10.8), 5% TPGS-750-M, 6-chloronicotinaldehyde (400 mM), rt, 1.5 h; then $NaBH_4$ (440mM), rt, 16 h; i) DNA, borate buffer (150 mM, pH = 9.3), (1*S*,4*S*)-4-hydroxycyclohexane-1-carboxylic acid (150 mM), DMT-MM (250 mM), rt, 16 h.

To demonstrate the applicability of this transformation to library production, a 2 x 2 DNA-encoded library was synthesised incorporating the optimised reductive amination conditions, with the goal of demonstrating library synthetic efficiency and conserved integrity of the DNA barcode. The two-cycle library consisted of an initial reductive amination step followed by a subsequent amide coupling where, prior to each chemical transformation, a unique DNA sequence was ligated onto the growing DNA strand (Scheme 3). In cycle one, the elongated DNA **HP2** (C_{11} alkyl amine) was split into two wells and subsequently coupled with two different aldehydes, benzaldehyde or 3,5-dimethylisoxazole-4-carbaldehyde, using the optimised conditions. The products were pooled and split into two wells where either 4-sulfamoylbenzoic acid or tetrahydrofuran-3-

carboxylic acid were subsequently amide coupled to the resulting secondary amines. In all cases, DNA ligation was confirmed to have proceeded efficiently by the appearance of a single band upon gel electrophoresis (Figure S126), with the two-cycle library prepared with an overall 70% yield. PCR amplification (40 cycles) of the pooled library with next-generation sequencing (NGS) elongation primers resulted in a major band of the expected 133 base pair length (Figure S127), suggesting efficient amplification of the DNA barcode following synthetic transformation. NGS of the amplified library confirmed the conserved integrity of the DNA barcode, with 79% of the ~10^6 reads corresponding to the expected sequences.



Scheme 3. Schematic representation of 2x2 library synthesis incorporating the developed on-DNA reductive amination. Conditions: i) phosphorylation (primer, complementary primer, first building block oligo), Ligation (phosphorylated DNA, headpiece oligo, first building block complementary oligo); ii) borate buffer (350 mM, pH = 10.8), 5% TPGS-750-M, aldehyde (400 mM), rt, 1.5 h, then $NaBH_4$ (440 mM), rt, 16 h; iii) phosphorylation (library oligo, second building block + complementary reverse primer oligo), Ligation (phosphorylated DNA, complementary second building block + reverse primer oligo); iv) carboxylic acid, HATU, 5% TPGS-750-M, 2,6-Lutidine, 45 °C, 24 h.

To quantify the amplifiable DNA remaining after the reductive amination, a sample of amplifiable DNA was subjected to the reaction conditions and amplified by quantitative PCR (qPCR). Equal amounts (0.1 pmol) of DNA, one subjected to the reaction conditions and the other untreated, were amplified by qPCR, with each repeated in triplicate. Almost equivalent amplification of DNA (98%, relative to untreated sample) was observed following subjection to reaction conditions (cycle threshold of 10.42 and 10.45 for control and post reaction DNA, respectively). Consequently, the integrity of the DNA barcode appears to be highly conserved under the optimised conditions, and thus the developed reductive amination is fully compatible with DEL synthesis, including the encoding and decoding steps.

Conclusion

The approach developed here provides a highly efficient method for the reductive amination of DNA-conjugated primary amines with a variety of aldehyde substrates promoted by micellar catalysis. This methodology exhibits a broad substrate scope and functional group compatibility, including the coupling of a range of benzylic, heteroaromatic, and aliphatic aldehydes with high efficiency. Transferability across a series of alternative primary amines has also been effectively demonstrated, alongside the development of a modified procedure that is applicable for the coupling of DNA-tagged secondary amines. This enables simple access to diverse secondary and tertiary amine scaffolds and resultant branched structures that are currently rare in the literature. Comparison to previous literature reported substrates revealed that this approach is superior to the existing methodologies, in particular offering a greater substrate scope, especially for the coupling of aldehyde substrates, with high control over single vs double alkylation, all of which have proved challenging previously. The application of micellar catalysis has proven to be critical to promoting greater reaction efficiency, as highlighted through lower conversions obtained under equivalent non-micellar conditions. Overall, the broad substrate scope, alongside the highlighted compatibility with DEL synthesis, underlines the value of this methodology for the development of increasingly diverse and effective DELs.

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Keywords: DNA-encoded libraries • reductive amination • micellar catalysis • reductive alkylation

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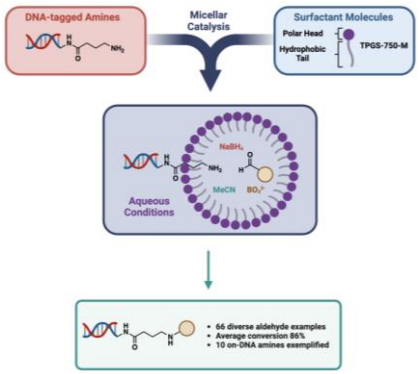

A two-step reductive amination reaction between DNA-conjugated amines and aromatic, aliphatic, or heterocyclic aldehydes, utilising the micelle-forming surfactant, TPGS-750-M, borate buffer, and $NaBH_4$. The methodology is applicable to a broad substrate scope, with regards to both on-DNA amine and aldehyde, and is fully compatible with DNA-amplification and sequencing; it is a new method for secondary and tertiary amine formation in DEL synthesis.

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