DNA-Encoded Chemistry: Drug Discovery From a Few Good Reactions

Patrick R. Fitzgerald[†], **Brian M. Paegel**[‡,¶]

[†]Skaggs Doctoral Program in the Chemical and Biological Sciences, The Scripps Research Institute

[‡]Departments of Pharmaceutical Sciences, Chemistry, & Biomedical Engineering, University of California, Irvine

[¶]Department of Chemistry, The Scripps Research Institute

Abstract

Click chemistry, proposed nearly 20 years ago, promised access to novel chemical space by empowering combinatorial library synthesis with a "few good reactions." These click reactions fulfilled key criteria (broad scope, quantitative yield, abundant starting material, mild reaction conditions, and high chemoselectivity), keeping the focus on molecules that would be easy to make, yet structurally diverse. This philosophy bears a striking resemblance to DNA-encoded library (DEL) technology, the now-dominant combinatorial chemistry paradigm. This review highlights the similarities between click and DEL reaction design and deployment in combinatorial library settings, providing a framework for the design of new DEL synthesis technologies to enable next-generation drug discovery.

Graphical Abstract
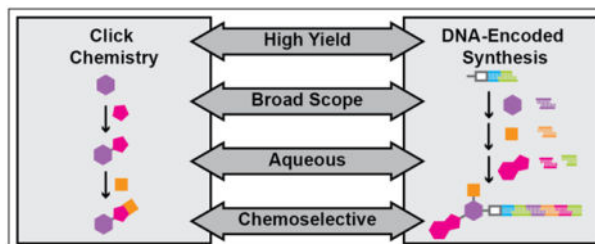
1 Introduction

Early-stage drug discovery in both pharmaceutical industry and academia has driven technology development efforts to revolutionize methods for generating and screening large collections of compounds for biological activity. What began as amassing compounds for robotic high-throughput screening (HTS) has evolved dramatically in recent years with the development of DNA-encoded library (DEL) technology.[1] DELs—combinatorial libraries of

bpaegel@uci.edu .

drug-like molecules, each barcoded with a DNA sequence that encodes the attached library member's structure—can be prepared at unprecedented scales of diversity and efficiently screened for ligands of a purified protein target. The many published examples of DEL screening hits becoming leads or even clinical candidates have in turn spurred intense interest in developing new chemistry to generate DELs of ever-increasing structural diversity that maximize the probability of successfully discovering additional leads. However, the constraints of DEL-compatible reaction development (e.g., in water, quantitative yield, dilute reactants, DNA-orthogonal) pose a great challenge to modern synthetic organic chemistry.

A surprising majority of the properties that define DNA-compatible chemistry coincide with those of click reactions. As initially described, the click philosophy is a pragmatist's approach to chemistry: achieve diverse chemical function from "a few good reactions."[2] Click reactions proceed with quantitative yield and with minimal side-product formation in aqueous or inoffensive solvent, are broad in substrate scope, and ideally require no chromatographic purification. These criteria are also highly advantageous for DEL preparation. For example, while 20 or more DEL-compatible reactions have been developed, a single DEL generally employs only 2–3 robust reactions to maximize library quality. Furthermore, DEL generation often entails parallel synthesis using hundreds (if not thousands) of substrates, thus demanding broad scope, and chromatography is impossible past the first coupling cycle. *The defining elements of click reactions in essence describe the ideal reaction for DEL.*

In this review we spotlight the remarkable relationship between click chemistry and DEL reaction development. In the first part of this review, we begin with a brief description of DEL technology and establish a framework for evaluating reaction development. We then apply these criteria to analyze six commonly practiced DEL reactions and three emerging reaction formats that potentially expand the scope of DEL synthesis. We restrict coverage to DNA-*encoded* synthesis (i.e., the DNA sequence encodes the synthetic history, it does not template or direct library synthesis) from 2008–2020, with some relevant background. DNA-templated[3,4] and dual pharmacophore[5] libraries are alternative encoded library approaches that have evolved significantly over the years and have delivered hits against numerous targets.[6–8] However, reaction development for these types of libraries diverges significantly since they use purified and validated DNA-small molecule heteroconjugates to yield well-defined combinations of building blocks. Our analysis of prospective strategies for expanding DEL synthesis, such as photocatalytic transformations and solid-phase reversible immobilization, is speculative as the state of the art is changing rapidly. However, we expect that this broader framework for reaction development will guide future efforts to achieve higher-quality and more structurally novel DELs, thereby delivering ever more successful screening outcomes in drug discovery.

2　Click Constraints Establish a Framework for DEL Design

2.1　DEL Fundamentals

Despite major technological advances in genome sequencing, structural biology, and computational drug design, drug discovery remains an empirical science. Screening large (100,000–5,000,000) compound collections by HTS is routine in the pharmaceutical

industry and a handful of academic centers. These collections vary in composition depending on the screening center, but often comprise compounds synthesized in house, natural product extracts, and compounds purchased through external vendors.[9,10] Advances in laboratory automation and analytical instrumentation have delivered the capacity to screen > 100,000 compounds per day, but HTS remains a costly endeavor that is limited by the chemical diversity inherent to collected compounds.

As HTS took root in industry, the rate of screening began to outstrip the rate at which chemical diversity could be generated, driving the development of more efficient chemical library synthesis methods. Combinatorial chemistry emerged as a prospective solution to the synthesis bottleneck. In contrast to HTS compound collections, which grow linearly with acquisition, combinatorial synthesis exponentially diversifies a target scaffold by split-and-pool strategies.[11,12] Combinatorial libraries could be screened against purified proteins or against whole cells,[13] but limited throughput of analytical methods available at the time for determining the hit structures after screening resulted in under-powered experiments.[14,15]

The advent of DEL technology in 2009 resurrected the field of combinatorial chemistry, and gave rise to a powerful new mode of designing and searching chemical space. Originating from a seminal thought experiment of Lerner and Brenner in 1992,[16] DNA-encoded synthesis matured as a technology through disclosures from Neri[17] and critically, Morgan,[1] who described DEL as it is widely practiced today (Figure 1). The power of DEL lies in linking compound identity with DNA-encoding tags. The DEL can be affinity panned as a highly complex mixture against the immobilized protein target of interest and the specifically bound fraction is amplified and deep sequenced[18] to reveal large collections of hit structures en masse. The statistical power of these experiments eclipses that of combinatorial chemistry from decades past by many orders of magnitude, simultaneously lending high confidence in hit authenticity and revealing detailed structure-activity relationship trends.[19] The analytical throughput advantages of DNA deep sequencing, however, are contingent on the library chemistry yielding solely the intended product while minimally compromising the fidelity of the encoding DNA.

2.2 Applying the Click Constraints to DEL

The quantitative yield, bioorthogonality, and other constraints associated with the ideal DEL reaction are highly evocative of the broader foundations of click reaction development. To the contemporary chemical biologist, click chemistry usually refers to the Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction for bioconjugation,[20–22] but click chemistry was originally a pragmatic, philosophical approach to defining a new mode of ligand discovery.[2] Given that the number of permutations of H, C, N, O, P, S, F, Cl, and Br atoms yielding a drug-like molecule is vast (10^{63})[23] compared to pharmaceutical industry compound collections (10^{6}), the click philosophy suggested looking for leads in the most strategically accessible regions of chemical space to expedite the process.[2,24]

The click ideals for ligand discovery simply restrict all searches to molecules that are easy to make.[2] As a corollary to this rule, the philosophy strongly advocates creating chemical diversity by using only the most efficient "click" reactions for coupling two building blocks rather than performing multiple reactions. Even a few reactions with broad scope can yield

libraries with sufficient diversity by capitalizing on readily available and deep building block sets. Aspects of click chemistry that resonate strongly with current practices in the field of DEL and underscore important commonalities between the reactions that define the two approaches include: 1) broad scope and high yield and 2) viable in water or benign solvent under mild conditions. Click chemistry has also evolved, becoming intertwined with bioorthogonal chemistry.[25] This final aspect of click chemistry also reflects an increasingly important consideration in DEL reaction development: 3) DNA-orthogonal reactivity. The following sections provide in-depth analysis of recent DEL reaction development efforts in the context of these click chemistry constraints.

2.2.1   Maximize Reaction Scope and Yield—High reaction yield is important to all chemists but yield requirements and reaction considerations vary with application, such as in process chemistry, total synthesis, medicinal chemistry, or combinatorial chemistry. Likewise, the importance of yield carries different concerns in click and DEL. The primary concern for yield in the context of click chemistry relates to expedient and efficient synthesis of individual molecules to populate large compound libraries. In contrast, DEL practitioners strive for maximum reaction yield during split-and-pool synthesis to ensure that all intended library members are prepared. As the composition of a full DEL cannot be analytically measured, nor each member individually purified, DEL generally adheres to the first principle of click, employing only high-yielding reactions.

Click reactions are by definition very high yielding. While this was not quantitatively defined, originally published click reactions proceeded with > 60% yields, with many reactions achieving > 95% yield.[2] These reactions produced C-heteroatom functionality through four main classes of reactions, including C-C multiple bond addition (epoxidation, aziridination, dihydroxylation, Michael addition), nucleophilic ring opening, cylcoaddition (Diels-Alder, Huisgen dipolar addition), and non-aldol carbonyl chemistry (formation of oxime ethers, hydrazones). Notably, the majority of these reactions are fusion reactions that can be conducted stoichiometrically, proceeding to completion without generating undesired side products. These few reactions can be combined in just 2–3 steps to generate new structures with interesting function (Scheme 1).

A high-yielding reaction with broad scope can be incredibly enabling for discovery science. The CuAAC reaction is perhaps the quintessential example. When it was discovered shortly after the formalization of click principles,[20,21] CuAAC offered significant regioselectivity and rate enhancement advantages over the uncatalyzed Huisgen condensation. CuAAC became a reaction of choice for large-scale library synthesis due to its high yield, orthogonal reactivity, broad scope, and mild conditions.[24] In fact, researchers at Lexicon pharmaceuticals used CuAAC and the first-generation click reactions to generate a 200k-member library of individually purified compounds (25–50 mg each).[24] Despite comprising few reactions, click chemistry has demonstrated the potential to create diverse libraries by sampling readily available building block sets[24,26–33] and continues to enable access to new chemical spaces by virtue of sustained reaction development, such as the recently disclosed Sulfur (VI) fluoride exchange (SuFEx) chemistry.[34–37]

Like click chemistry, DEL seeks to generate massive diversity expediently from large collections of building blocks. For example, the seminal disclosure of DEL technology[1] described two library plans for diversifying a central triazine scaffold with large building block pools (32–384 building blocks/cycle), resulting in impressive library diversity (7×10^6 and 8×10^8 members). However, subsequent computational analysis[38,39] has shown that these higher cycle number DELs contain large swathes of chemical space that deviate from typical drug-like molecules. Increasing the number of synthesis cycles generally increases molecular weight and all other relevant physicochemical property distributions (MW, logP, TPSA, H-bond donors/acceptors).[39,40] These considerations collectively prescribe constraining synthesis to 2–3 cycles using large diversity sets. For example, a 2-cycle DEL, which is likely the most densely populated with drug-like matter, will require two parallel synthesis steps of > 1,000 building blocks per coupling, underscoring the importance and scale of reaction scope in the state of the art.

To synthesize such a drug-like library with high chemical diversity, the requisite building blocks must be readily available and all members of these large building block sets must couple to the growing encoded compound in high yield to ensure interpretable screening data. Given the nature of split-and-pool synthesis, the presence and diversity of reaction byproducts increases exponentially with the number of additional synthetic steps, while the fraction of desired product corresponding to a given sequence decreases exponentially (Figure 2). As reaction yield is inevitably variable across building blocks, this means that among similarly potent library members, those with higher synthesis yield will be preferentially identified as hits during affinity selection. In fact, in computational screening simulations of 3-cycle libraries ($100 \times 100 \times 100$) that parameterized and modeled the outcome of variable synthesis yields (average 60%, standard deviation 20% per step), library member enrichment was more highly correlated with synthesis yield than with target association constant.[41,42] These simulations support the hypothesis that low yielding reactions decrease the signal to noise ratio in DEL, potentially leading to false SAR.

Further experimental analysis has substantiated the importance of incorporating high-yielding reactions. Sixteen DELs were synthesized and screened against a phosphodiesterase and a kinase.[43] The library that produced the most hit clusters in these experiments was generated by employing robust acylation/Fmoc deprotection conditions; library productivity did not correlate with numeric size. While this study was limited to Roche's DELs and only two protein targets, it is now routine practice across many groups to screen building block sets and exclude monomers that are problematic for DEL synthesis.[1,44–49] Notably, both of GSK's DEL-derived clinical candidates were the product of libraries prepared using robustly validated acylation or nucleophilic substitution of cyanuric chloride.[19,50–53] There is no agreed upon yield threshold for including a building block in a DEL synthesis, but standard practice seems to suggest that yield > 70% is suitable.[1,44–49]

Yield determination for large building block sets requires high-throughput, automated workflows that are sufficiently sensitive to analyze the rather unusual DNA-small molecule heteroconjugate products of DEL synthesis. DEL reactions are usually performed at the nanomole scale, and the product of building block coupling typically results in a mass shift of ~ 100–300 Da on DNA starting material > 5,000 Da. LCMS accompanied by UV

absorbance detection is the gold standard for DEL reaction yield determination. Reaction crude is separated and relative abundance of starting materials and products is quantitated by the DNA tag UV absorbance ($\lambda = 260$ nm), which is the dominant contributor to the molar extinction coefficient. The DNA tag also dominates chromatographic character, thus the same LC method usually provides adequate separation for all DNA-building block conjugates. LC method optimization (heating, hexafluoroisopropanol mobile phase, ion-pairing reagents, column particle size) has further enhanced separation efficiency and sensitivity while reducing analysis time.[1,44,54] MS analysis enables facile peak assignment and identification of side products; some also favor using MS abundance over UV absorbance for quantitation.[55] LCMS analysis cannot offer insight into reaction performance in a true combinatorial synthesis setting because DELs are too complex, but the workflow is routinely deployed for building block validation.

2.2.2 Employ Water- and Air-Compatible Reactions—Just as analytical characterization of DEL compounds is shaped by the dominant physical properties of DNA, optimal synthesis conditions must also accommodate DNA. DNA's hydrophilic nature and the lability of its glycosidic bonds impose the strictest constraints on DEL synthesis, limiting reaction conditions to aqueous solution with organic cosolvent, moderate temperatures (4–95 °C), and moderate pH (4–10). These coincide with click conditions, which prize simple reaction setup using a benign solvent (often water) and insensitivity to water/oxygen.[2,24] The similarity between click and DEL reaction development becomes even more apparent in the context of click-enabled bioorthogonal labeling chemistry (discussed later) where robust and selective reaction performance in aqueous conditions is necessary, for example, to preserve native protein structure or cell viability.

Using water as the solvent during click chemistry library generation is profoundly and counterintuitively enabling. Aqueous synthesis epitomizes ease of implementation and water's physical and chemical properties are favorable for characteristic reactions. While the low solubility of many organic compounds in water may seem like a liabilty, low compound solubility can be offset by the high free energy of organic compounds poorly solvated with water.[2] In fact, some reactions perform better in this fashion and some of the very best click reactions, such as 1,3-dipolar azide/alkyne cycloaddition, proceed best when reactants are "on water."[24] The nucleophilicity of water is also a potential liability, but again is offset by water's behavior specifically in the click context. Water's polar nature and tendency to form H-bonds allows it to facilitate rapid changes in H-bonding required for nucleophilic additions (epoxide/aziridine ring opening) that feature prominently in click. Moreover, water's high specific heat capacity (4.18 J·g^{-1}·K^{-1}) allows the solvent to double as a heatsink for highly exothermic click reactions. The low volatility and high surface tension of water also make it an ideal solvent for automated liquid handling, which facilitates large-scale parallel synthesis.

Water is similarly ideal and virtually required for solution-phase DEL chemistry because it is the only solvent in which the DNA polyanion is appreciably soluble. However, this solubility is still minimal (< 10 mM) compared to the concentrations of reactants found in traditional organic synthesis (0.1–1 M), which introduces kinetic reaction constraints. While click chemistry (as initially envisioned) may be more closely aligned with organic synthesis

conditions, click chemistry extended to bioorthogonal labeling is quite reminiscent of DEL. Like DEL, bioorthogonal labeling chemistry also faces the challenge of limiting reactant concentration in the form of minimal biological reactant (micromolar or lower concentration of antibody, native protein, metabolite, etc.). Bioorthogonal chemistry development often solves this challenge by designing reactions with large kinetic rate constants, a feature that is also desirable for DEL reactions.[25,56] Indeed, several routine bioorthogonal labeling reactions have been applied to DEL, including CuAAC,[47,48,57–63] SuFEx,[35] strain-promoted cycloaddition,[64] and inverse electron demand Diels-Alder reactions.[65] Furthermore, using high-throughput experimentation and reaction progress kinetic analysis protocols, it is now possible to design the highest yielding reactions within kinetic constraints by, for example, employing excess small molecule building block, different catalysts and/or ligands, higher temperature, solvent admixtures, or some complex combination of these parameters.[66] Even with these advances, DEL reaction development remains a challenging endeavor, especially considering the difficulties associated with designing aqueous reactions.

Water is an enabling solvent for many click reactions, but it is a challenging solvent for most of the medicinal chemistry transformations that feature prominently in DEL. Water is necessary to solubilize DNA, but organic cosolvent (DMA, ACN, MeOH) is also necessary to improve building block solubility, making reaction development an exercise in compromise. The suggested percentage of organic solvent that is compatible with on-DNA synthesis ranges from $<50\%$[67] to $<80\%$.[66] In our analysis of commonly employed DEL reactions, we observe that the majority of reactions are performed with $<50\%$ organic cosolvent (Tables 1–6) though certain on-DNA reactions tolerate higher percentages of organic solvent.[64,68–70] Balancing building block solubility with DNA solubility is a major challenge in DEL, so organic solvent percentage optimization is routine. Interestingly, as was observed for click reactions, DEL reactions can sometimes proceed even when building blocks are not fully soluble.[71] Methods for integrating solid-phase synthesis with DEL have emerged for circumventing building block solubility issues and for enabling water-sensitive reactions (discussed in detail later).

2.2.3   Prioritize High Chemoselectivity—In addition to having broad scope and high yield while proceeding in aqueous solvent, click reactions exhibit high chemoselectivity, a critical characteristic of DEL reactions. The specific considerations of reaction orthogonality are interesting to compare between these applications and even suggest new reactions for implementation in DEL. Click reactions used for parallel library synthesis leverage orthogonal reactivity to allow for sequential transformations without the need for protecting groups or purification. Click reactions used for bioconjugation demand conditions that are inert to biological nucleophiles, and in many cases the reaction must occur in the complex intracellular milieu. The most useful transformations for DEL often share these elements; highly chemoselective reactions allow for multiple sequential building block coupling reactions while minimally interfering with the DNA encoding tag.

Avoiding complicated protecting group strategies is a major motivating factor for employing click chemistry in library synthesis. This is achieved through orthogonal, high yielding chemistry. For example, in the library generation strategy we described earlier (Scheme 1), nucleophilic epoxide-ring opening produces a free hydroxyl group and an azide or hydrazine

group depending on the nucleophile. This reaction is followed by non-aldol carbonyl or cycloaddition reaction for the respective nucleophile. When performed in water, these reactions are inert to the -OH group that would otherwise require protection. Similarly, these reactions are also inert to most amine nucleophiles, allowing a broad range of building blocks.

Reactions in DEL share with click a need for chemoselectivity as both are library synthesis strategies involving highly diverse pools of building blocks that display different functional groups. As such, orthogonal reactivity in both building block sets and protecting group strategies (if used) is critical. For example, the Schreiber lab recently synthesized a stereochemically rich, 100,000-member library using orthogonal amidation, N-Boc deprotection, reductive amination, sulfonylation, and Suzuki coupling reactions.[44] In another recent example, researchers at Pfizer synthesized a library using amidation, reductive amination, sulfonylation, and carbamoylation coupling reactions while Azide, Fmoc-, and Boc-functionalities served as NH_2 protecting groups.[72]

Highly chemoselective reactions are also important in DEL for the critical reason that side reactions with the DNA tags can compromise library selection analysis. This is evocative of click applied to bioconjugation, such as activity-based protein profiling,[73–75] preparation of antibody drug conjugates,[76,77] and metabolic labeling in cells or animals.[78–82] Optimizing these reactions to perform under physiological conditions while avoiding reactivity with off targets, such as proteins or intracellular thiols, is a challenging endeavor. These concerns, as well as avoiding cellular toxicity, make the constraints of bioorthogonal reaction development stricter than those for DEL, but DNA still possesses structural features that can be problematic. The main structural liabilities present in DNA are the reactive heteroatoms of the nucleobases (exocyclic amines and purine N3, N7), the nucleophilic 3′-OH necessary for enzymatic ligation, the glycosidic bond, and the phosphodiester backbone.[83,84] Several reaction conditions, such as low pH and high temperature lead to DNA damage through depurination and concomitant phosphodiester strand scission, resulting in loss of encoded information. Radical species induce strand cleavage by oxidative abstraction of H from the deoxyribose-phosphate backbone and by introducing mutations by oxidation of guanine, the most easily oxidized nucleobase.[85] The potential pathways that introduce DNA damage are diverse and complex, which requires general methods for empirically determining the extent of DNA damage from a set of reaction conditions.

Analytical characterization of DNA integrity during DEL reaction development is typically performed via LCMS, but these measurements cannot directly report whether the DNA remains amplifiable in PCR and intelligible by sequencing. However, analytical methods relying on tag ligation and qPCR now provide a reliable assessment of DNA damage.[54,86] In the first of these approaches, solid-phase DEL reactions are performed in the presence of DNA-functionalized "sensor beads," which are subsequently harvested post-synthesis for qPCR analysis to measure the quantity of amplifiable DNA.[86] Pfizer adapted this approach to the conventional on-DNA DEL synthesis format.[54] Their DNA compatibility assay begins by coupling building blocks to a DNA construct displaying both an appropriate site for coupling and an overhang for enzymatic DNA ligation (Figure 3). LCMS analysis provides reaction yield while enzymatic ligation of a qPCR Taqman probe sequence to the overhang,

and then analysis of the product by gel electrophoresis and qPCR provides quantitative assessment of the amplifiable DNA remaining. DNA-compatible reactions maximize the recovery of DNA measured by qPCR.

Using this approach, the Pfizer team demonstrated high yield and DNA compatibility of several common deprotection and coupling reactions. Notably, the conditions demonstrated for Boc-deprotection avoid trifluoroacetic acid in favor of thermal deprotection conditions (250 mM borate buffer, pH 9.5, 18 h, 90 °C).[54,67] Pfizer researchers subsequently implemented the validated amidation and Boc-deprotection reactions in a library setting as discussed above.[72] DNA-damage assays using qPCR are generalizable to other reactions as well, since both Pfizer and GSK have used this assay format to determine the compatibility of photoredox reactions.[69,87] Unfortunately, qPCR cannot directly identify the cause of DNA damage, but it nevertheless provides a rapid platform for hypothesis testing; qPCR assays are readily parallelized (96-well plates) for simultaneous and high-precision analysis of standards, controls, and sample replicates.

Although qPCR readily provides a holistic evaluation of post-synthesis DNA integrity, there are some liabilities. First, mutagenic damage is silent. Some reactions invoke known mutagens (e.g., hydroxylamine[44]) or induce mutagenesis while leaving the DNA intact (e.g., deamination). Incorporation of Sanger sequencing as an additional assay can detect whether mutagenesis has occured.[44,69] Appropriate encoding language design can also mitigate mutagenesis issues by increasing the genetic distance between sequences in the encoding sets.[62] Second, assigning an exact cutoff point for damage acceptability is difficult since systematic studies are still lacking. Nonetheless, these quantitative analyses assist planning library synthesis and aid in understanding screening outcomes. Although PCR can amplify single molecules from a library, the notoriously low yield of affinity selection means that 10^4–10^6 amplifiable copies of each library member are needed as input to detect signal after multiple rounds of selection.[88–90] As quantitative assessment of DNA damage by qPCR becomes more widely practiced, correlations between overall DNA fidelity and DEL quality are likely to emerge.

Regardless of the DNA damage threshold for describing useful DEL reactions, developing such chemistry will always be challenging. Reactions must not only be DNA compatible, but should also be high yielding for a broad scope of building blocks, enable sequential couplings, and proceed in aqueous conditions, evocative of click chemistry development. In fact, the strong overlap of reaction conditions between click and DEL suggests that click reactions would make excellent starting points for developing DEL-compatible reactions. As mentioned earlier, several biocompatible/click reactions have already been investigated in the DEL setting, including CuAAC,[47,48,57–60,62,63] SuFEx,[35] strain-promoted cycloaddition,[64] and inverse electron demand Diels-Alder reactions.[65] Additionally, several more common click reactions such as Diels-Alder[17] and epoxide ring opening[91] have also been implemented in DEL.

Enzymatic transformations are at the frontier of DEL chemistry development. Enzymatic reactions occur in water with limiting substrate concentrations and, by virtue of enzyme structure, are highly chemoselective (suggesting DNA compatibility). They are also typically

high yielding and feature large kinetic rate enhancements. Thus far, however, these advantages have only been explored in a proof-of-concept on-DNA synthesis.[92] The model reaction sequence began with carbamoylation between DNA-NH$_2$ and nine 2-ethylaminoglycosides, followed by modification using one of four enzymes (β-galactosyltransferase, sialyl transferase, trans sialidase, galactose oxidase). Scaling enzyme production for DEL synthesis and expensive cofactors may hinder widespread adoption of the approach. Narrow substrate scope could also render the approach incompatible with the relatively large and structurally diverse building block pools used in DEL. Nonetheless, this initial study sets the stage for future DEL synthesis using biocatalysis and, more broadly, demonstrates that novel linkages and structures are attainable in DEL using reactions that embody virtually all aspects of the click philosophy.

3 Reaction Constraints Applied to DEL Analysis

3.1 Practiced Reactions

We have thus far established a connection between click reaction constraints and DEL reaction development to inform and prioritize future DEL design. High-yielding reactions simplify hit deconvolution and reduce false negative rates. The physical properties of the DNA encoding tag strictly constrains reactions to aqueous conditions, which in turn limits the types of reactions that are possible on DNA. Finally, enforcing high chemoselectivity reduces the probability of damaging DNA, an emerging constraint of interest in the field as new DNA analysis methods emerge (qPCR, Sanger sequencing) and integrate with routine measurements of reaction yield (LCMS).

In the following section we apply the click criteria as a framework to overview the state of the art in DEL reactions and designs. Vipergen recently enumerated the complete set of reactions for DEL.[93] In contrast, we focus our discussion on six widely practiced reactions and their implementation in libraries. Generally, each section describes the reaction's advantages for library diversification, the evolution of reaction conditions to broaden scope and/or utility, and the DNA compatibility of the reaction.

3.1.1 Amide Bond Formation—Amide bond forming reactions feature prominently in DEL for their versatility and DNA compatibility. Amidation usually involves the condensation of an amine and a carboxylic acid, two of the largest commercially available building block pools[94] owing to its popularity in medicinal chemistry.[95] Further, amide chemistry permits ready diversification through heterobifunctional amino acids (another large pool of building blocks), which can be fashioned into both linear and macrocyclic products. Structurally diverse diamines and diacids that are amenable to decoration via amide chemistry provide additional strategies for accessing novel chemical space.[1] Finally, the routinely high yields and DNA compatibility of this reaction meet many of the click criteria.

Amine-terminated headpiece DNA is the most common starting material for DEL synthesis, [39] so amidation with functionalized carboxylic acid building blocks (Fmoc-amine, Boc-amine, aryl halide, etc.) is a nearly ubiquitous first step of library synthesis.[43,93,96] Many libraries have also relied on amide formation as critical diversification reaction.[53,72]

Activating agents are necessary reactants for converting carboxylic acids to active esters to promote condensation with amines. There is conflicting evidence for which activation conditions provide the highest average yields while minimizing DNA damage. Neri performed a thorough analysis of 8 different activating conditions for their ability to couple carboxylic acids to on DNA amines, finding that the combination of EDC/HOAt/DIPEA outperformed all others.[97] However, many DEL groups routinely use DMT-MM for modification of DNA-conjugated amines[49,72] or other coupling reagents.[54,98] Reaction conditions (organic cosolvent, buffer composition, pH, DNA concentration, and building block selection) varied widely between these studies, possibly explaining the diversity in optimal conditions.

While amidation of on-DNA amines tends to employ basic buffer conditions (pH 8–9.5) amidation of on-DNA carboxylic acids tends to employ acidic buffer conditions.[1,98,99] DMT-MM is the dominant coupling reagent for this reaction. Gillingham's recent comparison of reaction conditions for acylation with 126 amino acids determined that DMT-MM outperformed EDC/HOAt/DIPEA for coupling amino acids to on-DNA carboxylic acids.[47]

Building block validation is critical for chemistries like amidation that use deep, structurally diverse building block pools. To give a sense of scale, some commercial suppliers offer > 28,000 carboxylic acids alone. Many pharmaceutical companies also curate internal, proprietary building block collections.[100] As a consequence, broad and novel chemical functionalities (aromatic, cyclic, heterocyclic, aliphatic, bridged, etc.) can be added to a library using robust acylation reaction conditions. Running contrary to the widespread notion that amides are an intrinsic liability, both DEL compounds in late stage clinical trials feature amides.[19,50–53] The RIP1K candidate consists of a benzoxazipinone linked by an amide bond to a benzyl-isoxazole[53] and the parent DEL of the sEH candidate was synthesized by nucleophilic aromatic substitution of cyanuric chloride with amines/amino acids followed by acylation.[51]

The high yield and broad reaction scope of amidations have lead to the success of these and other library screens by tailoring amide bond forming reactions to aqueous reaction conditions. Outside of DEL, amide bond coupling reagent development has received significant attention within the chemical synthesis community,[101,102] but these chemistries are not usually designed to accommodate aqueous reaction conditions. DEL addresses the challenge of aqueous chemistry by most often employing EDC or DMT-MM as activating agents, which likely succeed in aqueous conditions since they are water-soluble cationic salts.

Critically, amide formation occurs under mild conditions and does not promote amidation of DNA's numerous nucleophilic sites. Amide formation does not require extreme pH, mutagenic reagents, metal catalysts, or high temperature. Moreover, qPCR analysis of recovered DNA has shown that amidation conditions minimally affect DNA amplifiability. In aqueous reaction conditions, Stress et al. found that acylation of dsDNA with acids or amino acids activated by DMT-MM leaves 80% amplifiable DNA remaining,[47] and Ratnayake et al. found that acylation of headpiece DNA using HATU/DIPEA leaves the

DNA completely unharmed.[54] Similarly, numerous DNA-compatible conditions now exist for removing common protecting groups associated with amidation, such as Fmoc, Boc, and azides.[54,72,86] Thus, between the array of coupling reagents and orthogonal nucleophile protection strategies available, amide formation represents the best-developed reaction class in DEL.

3.1.2   Reductive Amination/Alkylation—Reductive animation is another very popular reaction in DEL. It is one of the 10 most frequent transformations in traditional medicinal chemistry[95] and was one of the first reactions to be demonstrated for DNA-templated synthesis,[105] foretelling its utility in DEL. This reaction furnishes the C-N bonds found in many drugs[110] and is operationally simple, making it attractive for library preparation. Reductive amination reactions are generally high yielding and exhibit broad scope for both amine and aldehyde reaction partners (Table 2). While aqueous solvent is traditionally avoided for reductive amination,[111] reaction adaptation has allowed facile implementation in water at library scale. Finally, in the context of DEL, this reaction is reasonably tolerant of additional functional groups and reaction conditions are DNA compatible.

Reductive amination/alkylation generally occurs as a sequential one-pot reaction. In the first step, a primary or secondary amine reacts reversibly with a carbonyl, resulting in loss of water and concomitant imine or imminium ion formation. This species is subsequently reduced in the second step—frequently with borohydrides—rendering the reaction irreversible through formation of the desired C-N bond. Reagent selection is important because strong reducing agents reduce aldehydes and ketones, leading to unproductive alcohol synthesis. In more traditional organic synthesis, several reducing agents ($NaCNBH_3$, $NaBH_4$, $NaBH(OAc)_3$, etc.) are used in slight excess (2–3 eq),[111] but DEL has thus far almost exclusively used $NaCNBH_3$, and in large excess (Table 2). The reaction is quite attractive for DEL due to the sheer quantity of commercially available amines and aldehydes, which also tend to be the most economical.[94]

Reductive amination of on-DNA aldehydes has been a highly fruitful coupling mode for DEL synthesis. This direction of the reductive amination has been implemented in published work four times.[46,63,92,112] The largest amine sets included 2,259 and 2,341 amines,[63,112] but these publications did not describe building block validation or structural diversity. Of the published reductive amination reactions, GSK disclosed the most comprehensive scope for this transformation with an evaluation of 813 amines coupling to a model benzaldehyde DNA substrate.[46] Under the specified reaction conditions, 216 amines achieved yields > 70% and were subsequently included in the library. Generally, primary amines coupled more efficiently than secondary amines and aromatic amines coupled more efficiently than aliphatic amines. However, the majority of amines did not couple efficiently, again highlighting the importance of building block validation.

Reductive alkylation of on-DNA amines has similarly been a productive reaction for DEL synthesis, leveraging abundant commercial collections of amino acids and aldehydes. To avoid bis-alkylation during library synthesis, on-DNA amine reactants are often restricted to secondary amines, though conditions for mono-alkylation of primary amines have been described.[67] Conditions for reductive alkylation of the secondary amine of proline-

conjugated DNA were disclosed in the initial patent describing DEL filed by Praecis pharmaceuticals in 2004.[108] Since then, reductive alkylation of on-DNA amines in library synthesis has been published twice in recent reports from the Schreiber lab[44] and X-Chem pharmaceuticals/Arrakis therapeutics.[113] Schreiber's conditions achieved broad scope, coupling 72 out of 117 aldehydes with an azetidine-DNA substrate in > 70% yield (previous studies coupling 20 aldehydes per scaffold, showed little reactivity difference between azetidine- and pyrolidine-DNA). However 22 aldehydes were entirely unreactive, suggesting that certain classes of aldehydes are not suitable for this chemistry. In the other reported application, Litovchik et al. reacted 85 aldehydes, a similar sized building block set, with 1,024 secondary amine DNA conjugates, but building block validation data were not provided. Taken together, these studies substantiate the reaction of aldehydes with on-DNA secondary amines as useful for DEL synthesis.

Expanding reductive alkylation reaction scope to include addition of ketones would be highly beneficial for generating Csp^3 amine bonds. Scripps Research/Pfizer discovered aqueous phase conditions for reductive alkylation of on-DNA amines with ketones.[114] Nine of 14 ketones coupled with a primary amine at yields > 70%. Boric acid was key for this transformation, but the reaction also employed significantly higher concentrations of ketone and $NaCNBH_3$ (500 mM) than similar transformations with aldehyde building blocks (30–50 mM, see Table 2). Overall, reaction scope will require further study to confirm suitability for library synthesis.

In addition to the high yields achievable by reductive C-N bond formation in aqueous solution, these reactions are also orthogonal to other reactions, thereby facilitating more complex library design. Multifunctional building blocks can combine amidation reactions, reductive alkylation/amination, and Suzuki couplings. Recently, small collections of trifunctional building blocks have become commercially available for this purpose (Enamine), but so far, library synthesis with trifunctional building blocks has incorporated only custom building blocks. In all cases, order of reaction is important. Schreiber suggested that reductive alkylation should precede Suzuki coupling to avoid side reactions with the on-DNA amine, while GSK suggested that Suzuki coupling should precede reductive amination to avoid dehalogenation.[46] The relative orthogonality of reductive amination enables incorporation of other chemistries as well. For example, a recent library featured chemoenzymatic installation of aldehyde-labeled sugars, which were subsequently modified by reductive amination.[92]

Like amide formation, reductive C-N bond formation generally invokes reactivity that is orthogonal to DNA, and is another feature that makes the chemistry attractive for DEL. Quantitative analysis of the DNA compatibility of this reaction is limited to solid-phase DEL synthesis methods, but those studies indicated that there is no detectable effect on DNA amplifiability.[86] Even for untested reaction conditions, though, they are relatively mild, suggesting compatibility. For example, although the reaction usually proceeds at pH 5.5, this is not sufficiently acidic to promote depurination, which proceeds only sluggishy at pH > 5.0.[115] Similarly, one would not predict redox cross-reactivity of DNA with the commonly used borohydride reductants. Finally, the reaction occurs at mild temperature as well, with

most $< 60\ °C$. Altogether, reductive amination adheres to the suggested reaction criteria for DEL.

3.1.3 Suzuki-Miyaura Cross Coupling

—Since its discovery in 1981, the Suzuki-Miyaura cross coupling reaction has become one of the most popular reactions in medicinal chemistry[95] and it received a citation for the 2010 Nobel Prize in chemistry. Approximately 30 drugs contain biaryl C-C linkages,[118] suggesting that this reaction has high potential for generating suitable chemical matter. While designing Suzuki couplings that are compatible with DEL synthesis is particularly challenging, extensive reaction optimization has yielded improved scope and the reaction has been implemented in several libraries.

Among the now many DEL-compatible chemistries, the Suzuki cross coupling has been unique in its ability to deliver sufficiently robust C-C bond construction as to be useful in a library setting. Initially, Suzuki reactions were implemented in DEL using $Pd(PPh_3)_4$ as the catalyst and the scope was limited to the reaction of on-DNA aryl iodides (primarily) or pyrimidinyl bromides (far less explored) with excess boronates to yield the corresponding biaryl linkage (Table 3, entries 3,4,6).[45,46,63,119] Alternative Pd ligands explored recently have provided higher yields for aryl iodide building blocks[44] and some ligands catalyze cross coupling with challenging aryl chloride-DNA substrates,[46,120] and other coupling partners,[121,122] expanding the scope of Suzuki cross coupling in DEL.

The utility of the Suzuki reaction in library synthesis derives from its relatively broad substrate scope and its orthogonality with other common bond construction strategies. The first disclosed DEL[45] that used Suzuki coupling was a 3-cycle library (Table 3, entry 4) designed around 44 trifunctional building blocks (Fmoc-protected amine, aryl iodide, carboxylic acid). These trifunctional building blocks were used to amidate NH_2-DNA, then further elaborated in cycles two and three by Suzuki coupling with 265 boronic acids followed by amine capping reactions with 2976 electrophilic building blocks (carboxylic acids, sulfonyl chlorides, aldehydes, isocyanates, and heteroaryl chlorides). A screen for BCATm inhibitors using this 34.7-million-member Suzuki DEL yielded an optimized hit compound with $IC_{50} = 2\ \mu M$ in an enzyme activity assay. A second DEL from GSK used these Suzuki conditions in conjunction with trifunctional aldehydes and reductive amination[46] to yield a 3.5-million-member DEL of biaryls. X-Chem reported similar reaction conditions for DEL synthesis, coupling 222 bromoaryl carboxylates by Suzuki with 667 boronic acids, but yields for this transformation were not reported.[63]

Further studies of Suzuki reactions in on-DNA synthesis expanded the reaction scope to other aryl halide species for accessing other building block pools. Researchers at GSK discovered that pre-combining POPd with the Buchwald sSPhos precatalyst (1:2) enabled cross coupling to challenging pyridinyl and aryl chlorides.[123] To improve operational simplicity, Li and Huang reported that the commercially available precatalyst sSPhosPd G2 also catalyzed the aryl chloride cross coupling reaction.[120] In this work, 8 Buchwald precatalysts were tested, and only sSPhosPd G2 provided appreciable yield, possibly due to its water solubility derived from sulfation; the parent SPhosPd G2 compound lacking sulfation (but otherwise identical) did not catalyze the reaction. The optimized conditions using the sSPhosPd G2 catalyst were evaluated in a substrate scope study of 6 aryl/

heteroaryl chloride, one heteroaryl bromide, and one heteroaryl iodide headpiece coupled with 84 boronates. For all headpieces tested, > 60% of building blocks coupled in > 50% yield, but some boronates (e.g., sterically hindered, chlorinated, fluorinated) remained low yielding. Finally, a fluorosulfonate electrophile (accessible via phenols), enabled more permissive coupling at lower temperature under ligand-free conditions.[121]

Despite improvements in ligands and other reaction parameters, Suzuki coupling conditions are among the more demanding, and can be quite detrimental for DNA. Suzuki reactions are commonly alkaline and require both high temperature and metal catalyst. All of these are depurination hazards and, indeed, X-Chem observed that library recovery following Suzuki coupling was 3-fold lower than the other reactions used to generate this library.[63] Similarly, our laboratory observed that Suzuki coupling catalyzed by Pd(PPh$_3$)$_4$ yielded only 30% amplifiable DNA after the reaction.[86] The potential for Pd species to induce DNA damage is well appreciated: several unique library purification strategies have emerged from Pd-mediated reaction development, such as spin filtration,[45] centrifugation,[44] and addition of metal scavengers, including sodium sulfide[67] and sodium diethyldiothiocarbamate.[119] Additionally, ligation analysis[45] and Sanger sequencing[120] data have provided further characterization of the Suzuki reaction's DNA compatibility. Collectively, Suzuki-Miyaura cross coupling is highly advantageous for increasing druglikeness of DELs, but caution is necessary, as common reaction conditions compromise DNA fidelity.

3.1.4 Buchwald-Hartwig and Ullmann Cross Coupling—The rise of Suzuki coupling in DEL has driven strong parallel interest in C-N cross coupling reactions by way of Buchwald-Hartwig (Pd-catalyzed) or Ullmann type couplings (Cucatalyzed). The Buchwald and Ullmann reactions forge aryl C-N bonds, which occupy chemical space distinct from C-N bonds formed by reductive amination. Buchwald coupling is a particularly popular transformation in medicinal chemistry for its simplicity, the prevalence of C-N bonds in natural products, and the pharmacokinetic versatility of the secondary amine linkage. From a combinatorial chemistry perspective, C-N cross coupling reactions are more attractive than C-C Suzuki cross coupling reactions because amines are much more commercially abundant than aryl boronates and they are substrates for a wider array of reactions. However, the small reaction scale in DEL offers opportunities for exploring complementary C-N and C-C cross couplings, modularly increasing library diversity.[98,124]

Despite the appeal of C-N cross coupling for DEL diversification, discovering suitable reaction conditions has proven difficult. GSK's initial disclosure of C-N cross coupling for DNA-linked substrates included both Buchwald- and Ullmann-type reactions with large amine sets, but yields were generally low.[124] The Buchwald reaction, employing tBuXPhos precatalyst G1, was evaluated by coupling 6329 primary aromatic amines to a DNA-linked aryl iodide, but for 93% of building blocks, yield was < 50%, highlighting the challenge of achieving broad scope. The Cu(I)-catalyzed Ullmann reaction proved fruitful using primary aliphatic amines and amino acids, but yields were similarly low. Although these initial Buchwald and Ullmann conditions yielded narrow building block scope, both were used for library construction (screening yet to be disclosed), paving the way for future reaction development.

The first major improvement to Buchwald coupling in DEL stemmed from the use of third-generation Buchwald catalysts. Incorporating these catalysts[125,126] in DEL[49,98,121] expanded amine and aryl halide scope. In the largest study, Eli Lilly found that 310/867 primary aromatic amines coupled in > 70% conversion with a model aryl bromide DNA conjugate. This compares favorably with GSK's previous conditions that required higher temperature, more base, and more reactive aryl iodide substrate to identify only 177/6,329 primary aromatic amines coupling in > 70% yield. Direct comparison of 6 reactions under both conditions uniformly proceeded in higher yield using Lilly's method. Lilly's conditions also expanded aryl halide scope to include several aryl bromide building blocks. In a two-step validation of 471 aryl bromide carboxylates, 225 acylated DNA in > 70% yield and 105 of these underwent further Buchwald coupling in > 70% yield. The utility of the developed conditions was demonstrated when Lilly synthesized a Buchwald DEL that was used in over 140 selection experiments.

More recent optimization of Buchwald for DEL improved reaction scope through use of a pyridine-enhanced precatalyst (PEPPSI) activated by ascorbate.[127] The optimized conditions required careful PEPPSI catalyst selection, high temperature (95 °C), and DMF cosolvent, but expanded both amine and aryl halide scope. While a small number of aryl chloride couplings suggested the potential suitability of these building blocks, the major focus of the work was identifying suitable aniline and secondary amine coupling partners. To this end, 197/328 and 123/292 aniline building blocks coupled to aryl bromide headpiece DNA and pyridinyl bromide headpiece DNA, respectively. Secondary amines remained challenging with 23/92 and 15/92 coupling in > 70% yield to the same aryl bromide and pyridinyl bromide headpieces respectively. The aryl halide scope investigation was more limited, but the newly developed reaction conditions were nevertheless applied to the synthesis of a 6×10^7 member library featuring 165 aryl halides in the second cycle followed by 386 anilines and 92 cyclic secondary amines in the third cycle.

The Ullmann coupling remains the most promising reaction for coupling aryl halides with aliphatic amines. Once again, ligand optimization was critical to enabling Ullmann-type couplings for DEL. Whereas commercial catalysts drove Buchwald coupling, Novartis tested 8 potential ligands, then synthesized 13 derivatives, to arrive at an optimal ligand.[68] Using this ligand and optimized conditions, 5/8 aliphatic amines coupled to aryl iodide headpiece in > 70% yield. In another study, 8 aryl iodide headpiece DNA substrates were reacted with 12 aliphatic amines. Both aryl iodides with ring-adjacent substituents and sterically hindered amines uniformly coupled poorly, but sterically-unhindered aryl iodides and amines generally coupled in > 70% yield. While the newly designed catalyst and optimized conditions have not yet been applied to library synthesis, they again highlighted the importance of catalyst selection.

Although Buchwald and Ullmann reaction conditions often resemble those of Suzuki couplings, C-N couplings are in several instances less damaging to DNA. For example, Ruff and Berst found that Ullmann coupling (Table 4, entry 2) leaves 65% amplifiable DNA,[68] while Ratnayake et al. found Buchwald (Table 4, entry 6) coupling left 73% amplifiable DNA by qPCR.[54] In the absence of quantitative data, the DNA compatibility of reactions employing PEPPSI precatalyst remains speculative. The higher temperature and increased

basicity in PEPPSI precatalyst conditions (95 °C) are likely to increase DNA damage, warranting further study. As in Suzuki couplings, Pd species in Buchwald couplings may be scavenged post reaction with thiol-containing compounds.[49] Cu may be scavenged with EDTA.[68]

Together with Suzuki, Buchwald and Ullmann reactions comprise a suite of highly valuable cross couplings that use commercially available and deep building block pools to access chemical matter of high druglikeness. Scope has rapidly and dramatically improved, both increasing diversification potential and allowing the DEL designer to pull from a collection of aryl halides to construct either C-N or C-C bonds. Finally, while DNA compatibility appears suitable for all of these metal-mediated couplings, arriving at these conditions has been a multivariate battle to enhance reaction kinetics through proper catalyst selection while minimizing reaction time. Metal scavenging has also played a crucial role, and one that has not been fully characterized to date. Further quantitative studies, particularly of Ullmann, will be helpful in establishing a click-like set of cross coupling conditions for the field.

3.1.5   Triazine Substitution—Nucleophilic displacement on the cyanuric chloride scaffold has yielded diverse published DELs. The seminal disclosure of DEL[1] showcased this scaffold and chemistry sequence, which uses economical and plentiful amines in 3 cycles of chemistry for readily accessing numerically large libraries (10^6–10^8). Libraries employing this chemistry have furnished several novel inhibitors,[1,128–131] including the lead for GSKs sEH inhibitor.[51] Although computational analysis identified potential liabilities,[38] the cyanuric chloride scaffold is still a logical starting material for creating branched libraries.

The simplicity of cyanuric chloride functionalization is a major aspect of its appeal for library synthesis. Cyanuric chloride is a trifunctional scaffold elaborated through symmetric and dynamic site reactivity[132] instead of protecting groups or orthogonal functionalities in other DEL scaffolds. Synthesis of the Praecis/GSK triazine "DEL A" entailed mild initial nucleophilic chloride substitution (1 h, 4 °C), a longer second substitution on the less-activated ring system (16 h, 4 °C), and an aggressive third substitution on the most deactivated ring system (6 h, 80 °C).[1]

The relatively large scope of nucleophiles that reacts with the cyanuric chloride scaffold is also a highly attractive feature of the chemistry. Competent nucleophile classes include amines, thiols, and alcohols,[132] but amines predominate for their utility in several different DEL chemistries. GSK identified 340/1000 amines that performed at least one step of substitution in > 70% yield. This reaction tolerated most classes of primary and secondary amines including aliphatic, aromatic, or cyclic amines. Substitution with phenolic acids, amino acids, or diamines introduces opportunities for a fourth cycle, increasing library size, but reducing library member druglikeness.[1,38] Incorporation of thiol nucleophiles or using the final aryl chloride in a Suzuki coupling poses alternative routes for exploiting the versatility of the cyanuric chloride scaffold while retaining druglike character.[133]

Despite the common synthesis of libraries focused around cyanuric chloride, quantitative understanding of the DNA compatibility of the associated S_NAr reactions remains lacking. For the first substitution, electrophilic cyanuric chloride reacts with amine-functionalized DNA to introduce the core library structure, but has potential for off target reactivity with nucleobase amines. The second and third nucleophilic substitutions appear mild, requiring low amine concentrations and temperatures < 80 °C. In comparison, Buchwald coupling with higher amine concentration, strong base, and metal catalyst leaves 73% amplifiable DNA.

The range of lead compounds discovered from triazine libraries is remarkable. Examples include metalloprotease inhibitors exhibiting high selectivity between highly homologous enzymes,[128,129] an LFA1-ICAM1 protein-protein interaction inhibitor,[130] and an OXA-48 β-lactamase inhibitor[131] to highlight a few target class firsts for DEL screening. Finally, the sEH inhibitor in late stage clinical trials exemplifies a few good reactions (amidation, cyanuric chloride substitution) enabling drug discovery through DEL.[51]

3.1.6   Copper-Catalyzed Azide Alkyne Cycloaddition—The quintessential click reaction, CuAAC, has served myriad purposes in encoded libraries. It was first shown in a DNA-templated reaction[57] and has since been deployed in DEL for introducing diversity elements,[47,48,58,61] macrocyclization,[59,60] coupling DNA to resin for solid-phase DEL synthesis,[62] and even chemical ligation of encoding tags in place of enzymatic ligation[63] (Table 5). CuACC is altogether convenient for DEL syntheses due to its facile reaction setup, broad scope, aqueous compatibility, and chemoselectivity, but commercial abundance of azides and alkynes is low relative to most other building block classes.

CuAAC in DELs employs conditions similar to those of bioconjugation. Most often, in situ reduction of Cu(II) with ascorbate furnishes the active Cu(I) catalyst, which tris-triazole ligands subsequently stabilize. The reaction benefits from sealing after gentle sparging with inert gas to protect the catalyst from decomposition by reaction with O_2. As is the case for bioconjugation,[22] an excess of coupling reagent and gentle heating (45–60 °C) enhance reaction kinetics. Ligand and solvent optimization generally maximizes reaction rate[134–136] while minimizing oxidative damage to off targets.[137] While the majority of DEL applications have used TBTA as the ligand, Neri recently explored a more water soluble TBTA derivative[138] and further ligand exploration may prove fruitful.

The chemoselectivity of CuAAC has prompted several innovative applications in DEL beyond just building block coupling. For example, CuAAC has been used for encoding tag ligation in place of enzymatic methods. X-Chem described library synthesis starting from ssDNA functionalized with a 5′-amine for diversification and a 3′- propargyl group for CuAAC ligation of encoding tags. Additional ssDNA oligonucleotides functionalized with a 3′-silane-protected propargyl group and a 5′- azide[63,139] enabled efficient chemical tag ligation. Klenow fragment could read through DNA triazole linkages, but efficiency was low. This strategy enabled split-and-pool library synthesis on ssDNA with encoding tag ligation by CuAAC to yield a 334-million-member DEL. Selection of this library identified a potent inhibitor of sEH ($IC_{50} = 2$ nM). As an additional example of CuAAC enabling

novel library design through orthogonality, it has been the reaction of choice for attaching DNA headpiece to resin for solid-phase DEL synthesis (see 3.2.2 below).[62]

Macrocyclization is another important application showcasing the versatility of the CuAAC in DEL design. GSK created a 6-mer macrocyclic peptide library with theoretical diversity of 2.3×10^{12} peptide backbones cyclized via CuAAC.[59] This application depended on the high yield and orthogonality of CuAAC. Selection patterns indicated that macrocylic library members were more potent ligands than linear counterparts obtained from an aliquot of the DEL that was not subjected to CuAAC. A similar strategy using CuAAC to cyclize scaffolds was also applied to DNA-recorded peptoid macrocycle libraries.[60] While acylation was the chosen reaction for macrocylization in a recent library from Stress et al., CuAAC was critical for attaching 21 non-peptidic macrocycle precursors to encoding tags.[47]

Given its roots as a library diversification strategy, CuAAC has appeared in several DEL syntheses. In one example, aliphatic and benzylic halides were converted in situ to azides by nucleophilic substitution with NaN_3, then coupled to propargyl glycine, pyrimidine, and benzodiazepine scaffolds.[58] Prior to library synthesis, building blocks were validated by coupling to a propargylamido-DNA conjugate, identifying 82/102 building blocks that coupled quantitatively. In alternative strategies, alkyne building blocks diversified azide-functionalized scaffolds. Stress et al. coupled 663 alkynes to azido homoalanine-functionalized library en route to a 1.4×10^6-member macrocycle library,[47] Li et al. coupled 136 alkynes to an azidolysine-functionalized scaffold during synthesis of a 3.5×10^7-member macrocycle library,[48] and Favalli found that 72/115 alkynes coupled to an azido iodiphenylalanine ssDNA conjugate in $> 75\%$ yield.[138]

Although CuAAC has seen limited application as a diversification strategy in DEL, synthetic methodology development is providing new opportunities for exploration. Perhaps the most significant impediment has been the relative paucity of commercially available azides and alkynes compared to, for example, amines or aldehydes. Novel azides are accessible from alkyl/aryl halide starting materials,[58] and drawing on a larger building block set, several approaches generate azides from primary amines.[140–142] However, on-demand building block generation is a fairly recent innovation in the field,[55] and deployment in library synthesis in not yet published.

CuAAC is generally high yielding and prized for its orthogonality, suggesting high compatibility with DNA, but the Cu(I) catalyst introduces known hazards to DNA. For example, Cu mediates oxidative DNA damage and radical chemistry.[97,143] Measurements of DNA damage from CuAAC vary with conditions. Skopic et al. ascribed a loss of 50% of amplifable DNA post CuAAC to oxidative damage.[58] In a separate study, CuAAC preserved 74% of amplifiable dsDNA, but only 10% of amplifiable ssDNA remained.[47] Avoiding oxidative DNA damage is a multivariate problem requiring optimization of Cu, reducing agent, ligand, and even solvent. Ligand optimization, particularly as it relates to solvent choice, is the likeliest starting point for further investigation.

3.2   Prospective DEL Reaction Schemes

Emergent synthesis technologies are both rapidly expanding the chemical space that DEL can access and enabling new screening modalities that have the potential to unlock previously intractable targets. For example, on-DNA photoredox coupling is delivering novel structural diversity via radical-mediated reactivity and solid-phase synthesis-inspired approaches are circumventing the aqueous reaction constraint of on-DNA synthesis while supporting activity-based DEL screening. The framework for evaluating the utility of these new technologies remains unchanged: rigorous analytical characterization of reaction yield and DNA compatibility are determinants of effective synthesis methodology development and building block availability continues to influence adoption prospects. Altogether, this section seeks to highlight the potential for new synthesis technologies to expand the scope of useful DEL reactions.

3.2.1   Covalent Attachment to Solid Support—Syntheses involving covalent binding of library members to solid supports has enabled both novel DEL reaction development and screening modalities. Drawing inspiration from conventional automated DNA synthesis, protected oligonucleotides attached to controlled porous glass (CPG) solid supports have been modified under reaction conditions likely to be incompatible with unprotected DNA to yield functionalized ssDNAs for initiating library synthesis. Libraries have also been prepared directly on polymeric solid supports using enzymatic encoding tag ligation reminiscent of conventional DELs, but circumventing the solubility limitations of DNA during chemical synthesis steps. Solid-phase DELs also introduce the unique ability to conduct activity-based screening by exploiting the polyvalent library member display on each bead.

Recent exploration of DEL synthesis initiated on CPG has suggested that solid-phase synthesis procedures can expand the scope of DNA-compatible chemistries. Building on the earliest work in DNA-encoded combinatorial chemistry,[103] Brunschweiger disclosed methodologies for accessing modified heterocycles. Initiating synthesis on CPG-linked DNA removes the aqueous reaction constraint and nucleobase protection increases the chemical robustness of DNA tags. Linkers composed of only pyrimidines such as hexathymidine ("hexT") or an alternating TC linker were most robust.[144,145] Exposure to 10% TFA or certain metal ions (Sc(III), Rh(II), Ru(II), Pd(0), and Pd(II)) still resulted in significant DNA damage detected by HPLC,[144,145] but on-CPG synthesis using protected oligonucleotides has nonetheless furnished previously inaccessible DNA conjugates.

On-CPG synthesis has introduced innovative approaches to library coding and access to several new chemistries on DNA. Brunschweiger's procedure begins with nucleobase-protected ssDNA bound to CPG at its 3′ terminus; the 5′ terminus displays an amine for compound synthesis. On-CPG reactions have included acid-catalyzed Pictet Spengler reaction to form β-carbolines, and gold-catalyzed pyrazoline and spirocycle formation.[144,146] Several multicomponent reactions, including Zn(II)-catalyzed aza Diels-Alder, Povarov, Biginelli, Castagnoli-Cushman, 1,3-dipolar azomethine ylide-alkene cycloaddition, and isocyanide reactions have also been developed.[61,144–148] After the initial on-CPG reaction, products are cleaved from solid support and splint-ligated to the encoding tag.

These reactions showcased the ability of on-CPG synthesis to enable otherwise difficult transformations that also explore Csp^3-rich chemical space.

An alternative DNA-encoded solid-phase synthesis (DESPS) approach has provided additional unique opportunities for reaction development and library screening. Drawing inspiration from the canonical one-bead-one-compound combinatorial synthesis strategy popularized by Lam,[12,149] solid-phase DELs are synthesized and screened entirely on beads[62,104] (Figure 4). Solid-phase technology development in DEL remained largely dormant until the 2015 publication of DESPS, which married the enzymatic encoding paradigm of DEL with the water-free and automation advantages of solid-phase synthesis.[62] Solid-phase DELs are constructed on a bifunctional linker that supports compound synthesis and substoichiometric DNA-encoding tag ligation. Like traditional DEL, split-and-pool synthesis delivers exponential diversification of chemical structure, while enzymatic ligation of encoding tags records each bead's synthetic history. Unlike conventional on-DNA DEL synthesis, DNA solubility considerations are irrelevant in DESPS, thus building block couplings are conducted in organic solvent.

High yield and DNA compatibility remain critical for successful solid-phase DEL reaction development. To evaluate chemical reactions for solid-phase DEL synthesis, our laboratory developed DNA-encoded reaction rehearsal. Solid-phase reactions of unknown yield or DNA compatibility are conducted on synthesis resin that has been mixed with magnetic sensor beads displaying a fully constructed DNA tag.[86] After the reaction, sensor beads are separated from synthesis beads. LCMS is used to determine the reaction yield from synthesis resin cleavage product and qPCR is used to measure the amount of amplifiable DNA remaining on the sensor beads. Several common coupling reactions, such as amidation, Suzuki cross coupling, and reductive amination were studied.[86] Along with azide reduction, protecting group removal (Fmoc, Mtt, and Alloc) proceeded to completion with acceptable DNA damage.[86] Several more specialized reactions, such as a proline-catalyzed aldol reaction,[150] Knoevanagel condensation,[151] and Mannich reaction[152] provide additional reactions for library generation. A variety of aldehyde reactions were also found to be both high yielding and highly DNA compatible.[153] While many DNA-compatible solid-phase reactions have been disclosed, only amidation,[109] nucleophilic displacement,[151,154] and Knoevenagel condensation[151] have been used to prepare solid-phase DELs.

The primary driver for developing solid-phase DEL technology has been achieving the ability to screen libraries in functional assays. As each DEL bead polyvalently displays one library member, it is possible to generate high local concentrations of an individual library member in the vicinity of the bead for screening. This is in stark contrast to conventional on-DNA DELs, which are inherently complex, inseparable mixtures. Synthesis of libraries on photocleavable linkers and microfluidic compartmentalization of beads allowed automated and miniaturized off-DNA screening of solid-phase DELs.[155,156] Using this technology, activity-based DEL screens identified inhibitors of the phosphodiesterase autotaxin,[109] while fluorescence polarization competition binding assays identified ligands of the receptor tyrosine kinase DDR1.[157] By separating the DEL member from the encoding tag, solid-phase DELs are poised to remove the barrier to investigating nucleic acid binding proteins[40] and enable direct interrogation of cellular signaling.

3.2.2 Solid-Phase Reversible Immobilization—Sustained interest in performing chemical modification of oligonucleotides in organic solvent has also motivated studies of solid-phase reversible immobilization to eliminate the DNA solubility constraint. These "pseudo solid-phase" strategies leverage the polyanionic phosphate backbone of DNA to immobilize it on cationic resin for suspension in organic solvent. Conceivably, this facilitates translation of organic synthesis conditions to DNA-encoded synthesis conditions. In addition, pseudo solid-phase chemistry offers ease of resin washing to allow rapid exchange of reagents and repeat couplings for increasing synthesis yield. Finally, these approaches expand the chemical space accessible to DEL by permitting transformations that do not tolerate water.

In 2004, Halpin and Harbury described amidation and an extensive suite of deprotection strategies for the automated chemical modification of DNA substrates immobilized on DEAE sepharose.[107,158] This approach enabled repeated cycles of synthesis and selection for two 10^6-member libraries.[106] In an investigation of several resin types, DEAE sepharose provided optimal DNA immobilization and proof-of-concept experiments demonstrated Fmoc-peptide synthesis on immobilized DNA.[107] Since then, pseudo solid-phase reactions on DNA using DEAE sepharose have been used for amide bond formation,[48,58,159,160] peptoid synthesis,[161] reductive amination,[116] and CuAAC.[58,61,138] Despite clear utility, whether DEAE-based synthesis provides yield improvements compared to reactions in solution remains unclear.

Subsequent development of pseudo solid-phase synthesis on DNA has focused on optimizing resin properties. The DEAE-functionalized sepharose core (Figure 5) displays numerous hydroxyl groups that are potentially cross-reactive, the resin retains sufficient water to render water-sensitive chemistries inaccessible, and the DEAE becomes deprotonated above ~ pH 8, abrogating DNA binding. Two parallel efforts sought to address this limitation with an essentially identical solution. Reversible adsorption to solid support (RASS)[114] and amphiphilic polymer-facilitated transformations under anhydrous conditions (APTAC)[162] both employed a resin with an organic core (PEG or PS) and quaternary amine functionality. These resins lack potentially cross-reactive nucleophiles, the organic resin core can be readily dehydrated, and quaternary amine DNA binding is pH independent.

These resin improvements offered renewed incentive to develop pseudo solid-phase reactions that were difficult under aqueous conditions. RASS first enabled Ni-catalyzed $Csp^2 - Csp^3$ cross coupling of an aryl iodide-modified DNA with soluble redox-active esters (RAE), providing 82% yield in a model reaction.[114] Reactions with an additional 42 RAEs proceeded in > 60% yield, while the same reaction on DEAE resin or free in solution was unproductive. A Ni-catalyzed electrochemical amination of on-DNA aryl iodide was then investigated, yielding 74% conversion in a model reaction, while the corresponding reaction performed free in solution phase reaction lead to DNA loss, presumably due to electrode absorption. Yields for this reaction were roundly modest in a 21-substrate scope study (19 reactions < 55%), but this was the first electrochemical modification of DNA substrates. Subsequently, RASS afforded novel C–S and S–N bonds.[163] Aryl iodide-DNA and thiols/thiophenols were coupled using a water-sensitive Ni catalyst; 29 thiols reacted with variable C–S bond forming yield (19–83%). In a second demonstration, bromohexanamide-DNA

was reacted with thiols or sodium sulfinates; 12 sulfinate salts reacted in variable yields (29–83%) and 6 thiols/thiophenols all reacted in high yield (> 70%).

The APTAC method has similarly expanded the scope of DEL-compatible chemistry, but fewer reactions have been disclosed.[162] While Umpolong addition and tin amine protocol (SnAP) reactions[164] were demonstrated as potential APTAC reactions, these use rare building blocks and reaction scope is still unproven. APTAC was explored to enable photocatalytic decarboxylative cross coupling, which uses abundant carboxylic acids. Using a custom LED-illuminated 96-well photo reactor (λ = 470 nm), APTAC permitted Ni/Ir dual catalyst cross coupling between aryl iodide-DNA and 25 aliphatic carboxylic acids. This transformation provided high yields (> 70%) for 12 carboxylic acids.

Both RASS and APTAC warrant further investigation for introducing anhydrous reactions to library synthesis. These strategies require bead handling, which increases the complexity of library synthesis but provides access to new chemical space. Reactions such as decarboxylative cross coupling increase Csp^3 character and introduce stereochemistry, while C-S cross couplings form bonds that are not currently found in DEL. Abundant building blocks for these reactions increase their utility for library synthesis, but RASS and APTAC do not yet appear in published DELs. An analysis of resin binding capacity, however, supports scalability and similar pseudo solid-phase synthesis with DEAE resin has yielded several DELs.[48,106,160] In summary, solid-phase reversible immobilization seems generally promising, granting access to useful functionality through anhydrous conditions. Further studies are likely to confirm that these approaches exhibit the requisite high yield, broad scope, and DNA compatibility of other canonical DEL chemistry formats.

3.2.3 Photoredox Catalysis—Photoredox reactions have attracted significant attention in medicinal chemistry for their ability to generate novel C–C bonds, and they appear to be equally promising for DEL bond construction. Photoredox couplings between C-centered radicals and alkene groups (Giese coupling or defluorinative alkylation) can produce Csp^3 – Csp^3 linkages. Similarly, dual catalytic photoredox cross couplings between heteroaryl-halide-conjugated DNA and C-centered radicals can generate valuable Csp^2 – Csp^3 linkages. Photoredox reactions demonstrated thus far have great appeal for DEL synthesis as they proceed with fast kinetics despite dilute DNA concentrations, use abundantly available building block sets, and generate highly desirable functionality. Also, although radical-mediated, several studies suggest that these reactions do not significantly affect DNA recovery.

Photochemistry was one of the first reaction modes to receive attention for its inherent compatibility with DNA. Halpin and Harbury highlighted the use of the nitroveratryloxy-carbonyl (NVOC) group for mild and selective nucleophile protection in DNA-routed synthesis[107] and both o-nitroveratryl and o-nitrobenzyl linkers have seen heavy use in oligonucleotide synthesis for their ability to mediate mild and chemoselective DNA phosphodiester cleavage. Later, Liu's powerful reaction discovery platform uncovered an on-DNA photocatalytic azide reduction,[57] and GSK used NVOC deprotection in a triazine library,[128] setting the stage for implementation of photoredox chemistry in DEL. Drawing inspiration from the corresponding off-DNA reactions,[165,166] Pfizer disclosed a

photocatalytic Giese coupling[167] between acrylamide DNA and Boc-protected phenylalanine. After optimization, the desired 1,4 product was obtained in 89% yield. Boc-protected a-amino acids generally coupled in high yield (25/29 conversion > 65%), while other radical precursors were only preliminarily investigated. Further, the reaction tolerated several radical acceptors; 6/9 alkene-conjugated DNA species coupled with Boc-phenylalanine in > 65% conversion.

Photoredox catalysis-based reaction development for DEL has continued to be an intense area of investigation. Novel dual catalytic reaction cycles for decarboxylative arylation were high yielding and DEL compatible.[69,162,168] In the dual catalytic approach, photocatalyst (Ir or suitable organic dye) generates an a-carbon radical through decarboxylation, which inserts in Ni-activated aryl halides to yield the corresponding $Csp^3 - Csp^2$ linkages and regenerate the catalysts. Reaction conditions varied widely, employing 60% aqueous,[168] 20% aqueous with $MgCl_2$ to aid DNA solubility,[69] or nearly anhydrous conditions on solid support.[162] Reaction scope was similarly variable. Pfizer's conditions were best suited for coupling Boc-protected a-amino acids as 26/29 such substrates reacted in > 70% yield while 10/21 heteroaryl halide DNA conjugates coupled to N-Boc morpholine carboxylic acids in > 65% conversion.[168] Molander's conditions were simpler as they did not require exclusion of air, but yields were generally lower. Only 4 of 15 reactions between various Boc-protected a-amino acids and aryl/pyridinyl halides surpassed 60% yield.[69] Novartis's conditions were the most intensive, requiring both solid support and water/air exclusion, but provided access to aliphatic carboxylic acids (12/25 > 70% converison with aryl iodide substrate).[162]

Adaptation of cross electrophile coupling has further expanded the scope of photoredox chemistry.[70,169] Pfizer identified a novel bis(carboxamidine) ligand that provided 92% yield in a model reaction between aryl iodide DNA and N-Boc-3-bromopiperidine.[169] In a scope study of 26 aryl halides reacting with a DNA-linked aryl halide, primary and secondary alkyl bromides reacted favorably for many building blocks, but tertiary or sterically hindered alkyl bromides coupled poorly. In contrast, Molander developed cross electrophile coupling conditions that proceeded under standard atmosphere using 80% DMSO as the reaction solvent.[169] Optimal conditions included an Ir photocatalyst and a Ni catalyst, but used triethylamine as a mild reductant. The optimized reaction coupled 15 alkyl bromides with 4-bromobenzoic acid-tagged DNA (43–84% yield). Then, 15 heteroaryl bromides/iodides were tested against two alkyl bromides (34–74% yield).

Several additional photoredox reactions have been developed, increasing both reaction and building block scope. Molander demonstrated defluorinative alkylation using a range of radical precursors, including N-Boc/Fmoc-a-amino acids, alkyl 1,4-dihydropyridines (DHP), alkyl bis(catecholato)silicates,[69] and methyl trimethyl silyl amines.[70] Similarly, methyl trimethyl silyl amines[70] and alkyl DHP radical precursors[69] were coupled to aryl halide DNA. Finally, Pfizer recently showed photoredox [2+2] cycloaddition as another potential reaction for implementation in DEL.[169]

Altogether, photoredox couplings appear to be one of the most promising additions to the DEL repertoire. Certain photoredox conditions, including decarboxylative cross coupling,[162] cross electrophile coupling,[87] and [2+2] cycloaddition[169] minimally impacted DNA. DNA

recovery was 48%, 67%, and 60% post reaction, respectively. Of the available photoredox reactions, decarboxylative cross coupling reactions are particularly attractive given that they use versatile, stable, and abundant building block sets, introduce attractive library functionalities, and complement other cross couplings found in DEL. All photoredox couplings introduce unique challenges for optimizing catalytic cycles, designing high-throughput LEDs, and devising enclosures to exclude air. Both Pfizer and Novartis have already designed 96-well plate illuminators for this specific purpose,[162,168] hinting that the future of photoredox coupling in DEL may be bright.

4 Conclusions

DEL synthesis is a powerful new technology for drug discovery that depends critically on reaction development. In this review, we compared click reaction constraints with desirable features of DEL reactions, providing an outline for evaluating reaction utility. This comparison emphasized both the convenience of aqueous reaction setup and the importance of building block validation and quantitative post-synthesis DNA recovery assessment. Analysis of six of the most popular reactions in modern DEL as well as emerging synthesis technologies demonstrated the utility of the click-inspired framework.

The success of DEL technology is ultimately measured by the quality of lead molecules that result from screening. Employing reactions that maximize library quality is critical for providing reliable data to discover molecules that can be readily optimized to druglike compounds. Additionally, exploring new bond construction is advantageous for pioneering new chemical space, particularly for the purposes of investigating increasingly difficult targets. Despite the challenges associated with designing robust reactions for DEL synthesis, several established reactions have realized dramatic performance improvements in DEL and novel reaction development efforts have greatly expanded in recent years. It will be exciting to see how future library design influences the next generation of DEL-derived clinical candidates and how these new synthesis technologies enable other efforts to circumvent the canonical limits of druggability.

Acknowledgement

Biographies

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Patrick R Fitzgerald earned his B.S. degree in chemistry from the University of California, Davis in 2016. After a year developing GCMS and LCMS assays at Quest Diagnostics (San Juan Capistrano, CA), he undertook doctoral studies with Paegel at Scripps Research (Jupiter, FL). In 2019, he moved with Paegel to the University of California, Irvine, where he continues his research developing advanced DNA-encoded library screening technology with an emphasis on antibacterial discovery and characterization.

Brian M Paegel holds the rank of Professor at the University of California, Irvine, with appointments in the Departments of Pharmaceutical Sciences, Chemistry, and Biomedical Engineering. He earned a B.S. degree in chemistry from Duke University and a Ph.D. in chemistry from UC Berkeley under the direction of Richard Mathies, whose laboratory pioneered high-throughput DNA sequencing technology for the Human Genome Project. He undertook postdoctoral studies with Gerald Joyce (then at Scripps Research and now at Salk Institute, La Jolla, CA) applying laboratory automation and instrumentation engineering to problems in molecular evolution and the chemical origins of life. In 2009 he started his independent faculty career in the Department of Chemistry at Scripps Research (Jupiter, FL) where he developed miniaturized and automated technology for drug discovery, including solid-phase DNA-encoded library synthesis and microfluidic activity-based DEL screening. He was recruited back to the UC system in 2019 to help build the new School of Pharmacy and Pharmaceutical Sciences at Irvine. His lab is developing cellular measurement strategies for DEL and using activity-based DEL to pursue classically undruggable targets. Paegel has received a Ruth L Kirschstein NRSA, a Pathway to Independence Award, the NIH Director's New Innovator Award, and the NSF CAREER Award for his work in reaction miniaturization and chemical evolution.

References

(1). Clark MA; Acharya RA; Arico-Muendel CC; Belyanskaya SL; Benjamin DR; Carlson NR; Centrella PA; Chiu CH; Creaser SP; Cuozzo JW et al. Design, Synthesis and Selection of DNA-Encoded Small-Molecule Libraries. Nat. Chem. Biol 2009, 5, 647–654. [PubMed: 19648931]

(2). Kolb HC; Finn MG; Sharpless KB Click Chemistry: Diverse Chemical Function from a Few Good Reactions. Angew. Chemie - Int. Ed 2001, 40, 2004–2021.

(3). Gartner ZJ; Tse BN; Grubina R; Doyon JB; Snyder TM; Liu DR DNA-Templated Organic Synthesis and Selection of a Library of Macrocycles. Science 2004, 305, 1601–1605. [PubMed: 15319493]

(4). Hansen MH; Blakskjaer P; Petersen LK; Hansen TH; Hoøjfeldt JW; Gothelf KV; Hansen NJV A Yoctoliter-Scale DNA Reactor for Small-Molecule Evolution. J. Am. Chem. Soc 2009, 131, 1322–1327. [PubMed: 19123795]

(5). Melkko S; Scheuermann J; Dumelin CE; Neri D Encoded Self-Assembling Chemical Libraries. Nat. Biotechnol 2004, 22, 568–574. [PubMed: 15097996]

(6). Petersen LK; Blakskjær P; Chaikuad A; Christensen AB; Dietvorst J; Holmkvist J; Knapp S; Koínek M; Larsen LK; Pedersen AE et al. Novel p38α MAP Kinase Inhibitors Identified from yoctoReactor DNA-Encoded Small Molecule Library. MedChemComm 2016, 7, 1332–1339.

(7). Zhou Y; Li C; Peng J; Xie L; Meng L; Li Q; Zhang J; Li XD; Li X; Huang X et al. DNA-Encoded Dynamic Chemical Library and Its Applications in Ligand Discovery. J. Am. Chem. Soc 2018, 140, 15859–15867. [PubMed: 30412395]

(8). Neri D; Lerner RA DNA-Encoded Chemical Libraries: A Selection System Based on Endowing Organic Compounds with Amplifiable Information. Annu. Rev. Biochem 2018, 87, 479–502. [PubMed: 29328784]

(9). Pereira DA; Williams JA Origin and Evolution of High Throughput Screening. Br. J. Pharmacol 2007, 152, 53–61. [PubMed: 17603542]

(10). Volochnyuk DM; Ryabukhin SV; Moroz YS; Savych O; Chuprina A; Horvath D; Zabolotna Y; Varnek A; Judd DB Evolution of Commercially Available Compounds for HTS. Drug Discov. Today 2019, 24, 390–402. [PubMed: 30399443]

(11). Furka A; Sebestyén F; Asgedom M; Dibó G General Method for Rapid Synthesis of Multicomponent Peptide Mixtures. Int. J. Pept. Protein Res 1991, 37, 487–93. [PubMed: 1917305]

(12). Lam KS; Salmon SE; Hersh EM; Hruby VJ; Kazmierski WM; Knapp RJ A New Type of Synthetic Peptide Library for Identifying Ligand-Binding Activity. Nature 1991, 354, 82–84. [PubMed: 1944576]

(13). Silen JL; Lu AT; Solas DW; Gore MA; Maclean D; Shah NH; Coffin JM; Bhinderwala NS; Wang Y; Tsutsui KT et al. Screening for Novel Antimicrobials from Encoded Combinatorial Libraries by Using a Two-Dimensional Agar Format. Antimicrob. Agents Chemother 1998, 42, 1447–1453. [PubMed: 9624492]

(14). Kennedy JP; Williams L; Bridges TM; Daniels RN; Weaver D; Lindsley CW Application of Combinatorial Chemistry Science on Modern Drug Discovery. J. Comb. Chem 2008, 10, 345–354. [PubMed: 18220367]

(15). Kodadek T The Rise, Fall and Reinvention of Combinatorial Chemistry. Chem. Commun 2011, 47, 9757–9763.

(16). Brenner S; Lerner RA Encoded combinatorial chemistry. Proc. Natl. Acad. Sci 1992, 89, 5381–5383. [PubMed: 1608946]

(17). Buller F; Zhang Y; Scheuermann J; Schäfer J; Bühlmann P; Neri D Discovery of TNF Inhibitors from a DNA-Encoded Chemical Library Based on Diels-Alder Cycloaddition. Chem. Biol 2009, 16, 1075–1086. [PubMed: 19875081]

(18). Mannocci L; Zhang Y; Scheuermann J; Leimbacher M; De Bellis G; Rizzi E; Dumelin C; Melkko S; Neri D High-Throughput Sequencing Allows the Identification of Binding Molecules Isolated from DNA-Encoded Chemical Libraries. Proc. Natl. Acad. Sci 2008, 105, 17670–17675. [PubMed: 19001273]

(19). Satz AL What Do You Get from DNA-Encoded Libraries? ACS Med. Chem. Lett 2018, 9, 408–410. [PubMed: 29795750]

(20). Rostovtsev VV; Green LG; Fokin VV; Sharpless KB A stepwise huisgen cycloaddition process: Copper(I)-catalyzed regioselective "ligation" of azides and terminal alkynes. Angew. Chemie - Int. Ed 2002, 41, 2596–2599.

(21). Tornøe CW; Christensen C; Meldal M Peptidotriazoles on Solid Phase: [1,2,3]-Triazoles by Regiospecific Copper(I)-Catalyzed 1,3-Dipolar Cycloadditions of Terminal Alkynes to Azides. J. Org. Chem 2002, 67, 3057–3064. [PubMed: 11975567]

(22). Presolski SI; Hong VP; Finn M CopperCatalyzed AzideAlkyne Click Chemistry for Bioconjugation. Curr. Protoc. Chem. Biol 2011, 3, 153–162. [PubMed: 22844652]

(23). Bohacek RS; McMartin C; Guida WC The Art and Practice of Structure-based Drug Design: a Molecular Modeling Perspective. Med. Res. Rev 1996, 16, 3–50. [PubMed: 8788213]

(24). Kolb HC; Sharpless KB The Growing Impact of Click Chemistry on Drug Discovery. Drug Discov. Today 2003, 8, 1128–1137. [PubMed: 14678739]

(25). Lang K; Chin JW Bioorthogonal Reactions for Labeling Proteins. ACS Chem. Biol 2014, 9, 16–20. [PubMed: 24432752]

(26). Meldal M; Tomøe CW Cu-Catalyzed Azide - Alkyne Cycloaddition. Chem. Rev 2008, 108, 2952–3015. [PubMed: 18698735]

(27). Tron GC; Pirali T; Billington RA; Canonico PL; Sorba G; Genazzani AA Click Chemistry Reactions in Medicinal Chemistry: Applications of the 1,3-Dipolar Cycloaddition Between Azides and Alkynes. Med. Res. Rev 2008, 28, 278–308. [PubMed: 17763363]

(28). Hein CD; Liu XM; Wang D Click Chemistry, a Powerful Tool for Pharmaceutical Sciences. Pharm. Res 2008, 25, 2216–2230. [PubMed: 18509602]

(29). Moorhouse AD; Moses JE Click Chemistry and Medicinal Chemistry: A case of "Cyclo-Addiction". ChemMedChem 2008, 3, 715–723. [PubMed: 18214878]

(30). Hou J; Liu X; Shen J; Zhao G; Wang PG The Impact of Click Chemistry in Medicinal Chemistry. Expert Opin. Drug Discov 2012, 7, 489–501. [PubMed: 22607210]

(31). Jiang X; Hao X; Jing L; Wu G; Kang D; Liu X; Zhan P Recent Applications of Click Chemistry in Drug Discovery. Expert Opin. Drug Discov 2019, 14, 779–789. [PubMed: 31094231]

(32). Thirumurugan P; Matosiuk D; Jozwiak K Click Chemistry for Drug Development and Diverse Chemical-Biology Applications. Chem. Rev 2013, 113, 4905–4979. [PubMed: 23531040]

(33). Wang X; Huang B; Liu X; Zhan P Discovery of Bioactive Molecules From CuAAC Click-Chemistry-Based Combinatorial Libraries. Drug Discov. Today 2016, 21, 118–132. [PubMed: 26315392]

(34). Dong J; Krasnova L; Finn MG; Barry Sharpless K Sulfur(VI) Fluoride Exchange (SuFEx): Another Good Reaction for Click Chemistry. Angew. Chemie - Int. Ed 2014, 53, 9430–9448.

(35). Liu Z; Li J; Li S; Li G; Sharpless KB; Wu P SuFEx Click Chemistry Enabled Late-Stage Drug Functionalization. J. Am. Chem. Soc 2018, 140, 2919–2925. [PubMed: 29451783]

(36). Zheng Q; Woehl JL; Kitamura S; Santos-Martins D; Smedley CJ; Li G; Forli S; Moses JE; Wolan DW; Barry Sharpless K SuFEx-Enabled, Agnostic Discovery of Covalent Inhibitors of Human Neutrophil Elastase. Proc. Natl. Acad. Sci. U. S. A 2019, 116, 18808–18814. [PubMed: 31484779]

(37). Wan X; Yang T; Cuesta A; Pang X; Balius TE; Irwin JJ; Shoichet BK; Taunton J Discovery of Lysine-Targeted eIF4E Inhibitors through Covalent Docking. J. Am. Chem. Soc 2020, 142, 4960–4964. [PubMed: 32105459]

(38). Zhu H; Flanagan ME; Stanton RV Designing DNA Encoded Libraries of Diverse Products in a Focused Property Space. J. Chem. Inf. Model 2019, 59, 4645–4653. [PubMed: 31689098]

(39). Franzini RM; Randolph C Chemical Space of DNA-Encoded Libraries: Miniperspective. J. Med. Chem 2016, 59, 6629–6644. [PubMed: 26914744]

(40). Goodnow RA; Davie CP Annu. Rep. Med. Chem, 1st ed.; Elsevier Inc., 2017; Vol. 50; pp 1–15.

(41). Satz AL DNA Encoded Library Selections and Insights Provided by Computational Simulations. ACS Chem. Biol 2015, 10, 2237–2245. [PubMed: 26176408]

(42). Satz AL Simulated Screens of DNA Encoded Libraries: The Potential Influence of Chemical Synthesis Fidelity on Interpretation of Structure-Activity Relationships. ACS Comb. Sci 2016, 18, 415–424. [PubMed: 27116029]

(43). Eidam O; Satz AL Analysis of the Productivity of DNA Encoded Libraries. MedChemComm 2016, 7, 1323–1331.

(44). Gerry CJ; Wawer MJ; Clemons PA; Schreiber SL DNA Barcoding a Complete Matrix of Stereoisomeric Small Molecules. J. Am. Chem. Soc 2019, 141, 10225–10235. [PubMed: 31184885]

(45). Deng H; Zhou J; Sundersingh FS; Summerfield J; Somers D; Messer JA; Satz AL; Ancellin N; Arico-Muendel CC; Bedard KL et al. Discovery, SAR, and X-ray Binding Mode Study of BCATm Inhibitors from a Novel DNA-Encoded Library. ACS Med. Chem. Lett 2015, 6, 919–924. [PubMed: 26288694]

(46). Ding Y; Franklin GJ; DeLorey JL; Centrella PA; Mataruse S; Clark MA; Skinner SR; Belyanskaya S Design and Synthesis of Biaryl DNA-Encoded Libraries. ACS Comb. Sci 2016, 18, 625–629. [PubMed: 27571034]

(47). Stress CJ; Sauter B; Schneider LA; Sharpe T; Gillingham D A DNAEncoded Chemical Library Incorporating Elements of Natural Macrocycles. Angew. Chemie Int. Ed 2019, 58, 9570–9574.

(48). Li Y; de Luca R; Cazzamalli S; Pretto F; Bajic D; Scheuermann J; Neri D Versatile Protein Recognition by the Encoded Display of Multiple Chemical Elements on a Constant Macrocyclic Scaffold. Nature Chem 2018, 10, 1–8.

(49). De Pedro Beato E; Priego J; Gironda-Martínez A; González F; Benavides J; Blas J; Martín-Ortega MD; Toledo MÁ; Ezquerra J; Torrado A Mild and Efficient Palladium-Mediated C-N Cross-Coupling Reaction between DNA-Conjugated Aryl Bromides and Aromatic Amines. ACS Comb. Sci 2019, 21, 69–74. [PubMed: 30615417]

(50). Lazaar AL; Yang L; Boardley RL; Goyal NS; Robertson J; Baldwin SJ; Newby DE; Wilkinson IB; Tal-Singer R; Mayer RJ et al. Pharmacokinetics, Pharmacodynamics and Adverse Event Profile of GSK2256294, a Novel Soluble Epoxide Hydrolase Inhibitor. Br. J. Clin. Pharmacol 2016, 81, 971–979. [PubMed: 26620151]

(51). Belyanskaya SL; Ding Y; Callahan JF; Lazaar AL; Israel DI Discovering Drugs with DNA-Encoded Library Technology: From Concept to Clinic with an Inhibitor of Soluble Epoxide Hydrolase. ChemBioChem 2017, 18, 837–842. [PubMed: 28281333]

(52). Harris PA; King BW; Bandyopadhyay D; Berger SB; Campobasso N; Capriotti CA; Cox JA; Dare L; Dong X; Finger JN et al. DNA-Encoded Library Screening Identifies Benzo[b]

[1,4]oxazepin-4-ones as Highly Potent and Monoselective Receptor Interacting Protein 1 Kinase Inhibitors. J. Med. Chem 2016, 59, 2163–2178. [PubMed: 26854747]

(53). Harris PA; Berger SB; Jeong JU; Nagilla R; Bandyopadhyay D; Campobasso N; Capriotti CA; Cox JA; Dare L; Dong X et al. Discovery of a First-in-Class Receptor Interacting Protein 1 (RIP1) Kinase Specific Clinical Candidate (GSK2982772) for the Treatment of Inflammatory Diseases. J. Med. Chem 2017, 60, 1247–1261. [PubMed: 28151659]

(54). Ratnayake AS; Flanagan ME; Foley TL; Smith JD; Johnson JG; Bellenger J; Montgomery JI; Paegel BM A Solution Phase Platform to Characterize Chemical Reaction Compatibility with DNA-Encoded Chemical Library Synthesis. ACS Comb. Sci 2019, 21, 650–655. [PubMed: 31425646]

(55). Zambaldo C; Geigle SN; Satz AL High-Throughput Solid-Phase Building Block Synthesis for DNA-Encoded Libraries. Org. Lett 2019, 21, 9353–9357. [PubMed: 31747291]

(56). Oliveira BL; Guo Z; Bernardes GJ Inverse Electron Demand Diels-Alder Reactions in Chemical Biology. Chem. Soc. Rev 2017, 46, 4895–4950. [PubMed: 28660957]

(57). Chen Y; Kamlet AS; Steinman JB; Liu DR A Biomolecule-Compatible Visible-Light-Induced Azide Reduction from a DNA-Encoded Reaction-Discovery System. Nature Chem 2011, 3, 146–153. [PubMed: 21258388]

(58). Škopić MK; Bugain O; Jung K; Onstein S; Brandherm S; Kalliokoski T; Brunschweiger A Design and Synthesis of DNA-encoded Libraries Based on a Benzodiazepine and a Pyrazolopyrimidine Scaffold. MedChemComm 2016, 7, 1957–1965.

(59). Zhu Z; Shaginian A; Grady LC; O'Keeffe T; Shi XE; Davie CP; Simpson GL; Messer JA; Evindar G; Bream RN et al. Design and Application of a DNA-Encoded Macrocyclic Peptide Library. ACS Chem. Biol 2018, 13, 53–59. [PubMed: 29185700]

(60). Shin MH; Lee KJ; Lim HS DNA-Encoded Combinatorial Library of Macrocyclic Peptoids. Bioconjugate Chem 2019, 30, 2931–2938.

(61). Kunig VBK; Potowski M; Akbarzadeh M; Klika Škopić M; Santos Smith D;Arendt L; Dormuth I; Adihou H; Andlovic B; Karatas H et al. TEAD-YAP Interaction Inhibitors and MDM2 Binders from DNA-Encoded Indole-Focused Ugi Peptidomimetics. Angew. Chemie Int. Ed 2020, 1–6.

(62). MacConnell AB; McEnaney PJ; Cavett VJ; Paegel BM DNA-Encoded Solid-Phase Synthesis: Encoding Language Design and Complex Oligomer Library Synthesis. ACS Comb. Sci 2015, 17, 518–534. [PubMed: 26290177]

(63). Litovchick A; Dumelin CE; Habeshian S; Gikunju D; Guié M.-a.; Centrella P; Zhang Y; Sigel EA; Cuozzo JW; Keefe AD et al. Encoded Library Synthesis Using Chemical Ligation and the Discovery of sEH Inhibitors from a 334-Million Member Library. Sci. Reports 2015, 5, 1–8.

(64). Westphal MV; Hudson L; Mason JW; Pradeilles JA; Zécri FJ; Briner K; Schreiber SL Water-Compatible Cycloadditions of Oligonucleotide-Conjugated Strained Allenes for DNA-Encoded Library Synthesis. J. Am. Chem. Soc 2020, 142, 7776–7782. [PubMed: 32267148]

(65). Li H; Sun Z; Wu W; Wang X; Zhang M; Lu X; Zhong W; Dai D Inverse-Electron-Demand Diels-Alder Reactions for the Synthesis of Pyridazines on DNA. Org. Lett 2018, 20, 7186–7191. [PubMed: 30365326]

(66). Wang J; Lundberg H; Asai S; Martín-Acosta P; Chen JS; Brown S; Far-rell W; Dushin RG; O'Donnell CJ; Ratnayake AS et al. Kinetically Guided Radical-Based Synthesis of $C(sp^3)$–$C(sp^3)$ linkages on DNA. Proc. Natl. Acad. Sci 2018, 115, E6404–E6410. [PubMed: 29946037]

(67). Satz AL; Cai J; Chen Y; Goodnow R; Gruber F; Kowalczyk A; Petersen A; Naderi-Oboodi G; Orzechowski L; Strebel Q DNA Compatible Multistep Synthesis and Applications to DNA Encoded Libraries. Bioconjugate Chem 2015, 26, 1623–1632.

(68). Ruff Y; Berst F Efficient Copper-Catalyzed Amination of DNA-Conjugated Aryl Iodides Under Mild Aqueous Conditions. MedChemComm 2018, 9, 1188–1193. [PubMed: 30109007]

(69). Phelan JP; Lang SB; Sim J; Berritt S; Peat AJ; Billings K; Fan L; Molander GA Open-Air Alkylation Reactions in Photoredox-Catalyzed DNA-Encoded Library Synthesis. J. Am. Chem. Soc 2019, 141, 3723–3732. [PubMed: 30753065]

(70). Badir SO; Sim J; Billings K; Csakai A; Zhang X; Dong W; Molander GA Multifunctional Building Blocks Compatible with Photoredox-Mediated Alkylation for DNA-Encoded Library Synthesis. Org. Lett 2020, 22, 1046–1051. [PubMed: 31940210]

(71). Luk K-C; Satz AL A Handb. DNA-Encoded Chem; John Wiley & Sons, Inc.: Hoboken, NJ, 2014; pp 67–98.

(72). Kung P-P; Bingham P; Burke BJ; Chen Q; Cheng X; Deng Y-L; Dou D; Feng J; Gallego GM; Gehring MR et al. Characterization of Specific N-α-Acetyltransferase 50 (Naa50) Inhibitors Identified Using a DNA Encoded Library. ACS Med. Chem. Lett 2020, 11, 1175–1184. [PubMed: 32550998]

(73). Speers AE; Adam GC; Cravatt BF Activity-Based Protein Profiling in vivo Using a Copper(I)-Catalyzed Azide-Alkyne [3 + 2] Cycloaddition. J. Am. Chem. Soc 2003, 125, 4686–4687. [PubMed: 12696868]

(74). Weerapana E; Wang C; Simon GM; Richter F; Khare S; Dillon MB; Bachovchin DA; Mowen K; Baker D; Cravatt BF Quantitative Reactivity Profiling Predicts Functional Cysteines in Proteomes. Nature 2010, 468, 790–797. [PubMed: 21085121]

(75). Hacker SM; Backus KM; Lazear MR; Forli S; Correia BE; Cravatt BF Global Profiling of Lysine Reactivity and Ligandability in the Human Proteome. Nature Chem 2017, 9, 1181–1190. [PubMed: 29168484]

(76). Oller-Salvia B; Kym G; Chin JW Rapid and Efficient Generation of Stable AntibodyDrug Conjugates via an Encoded Cyclopropene and an Inverse-Electron-Demand DielsAlder Reaction. Angew. Chemie - Int. Ed 2018, 57, 2831–2834.

(77). Peplow M Click Chemistry Targets Antibody-drug Conjugates for the Clinic. Nat. Biotechnol 2019, 37, 835–837. [PubMed: 31375794]

(78). Wang Q; Chan TR; Hilgraf R; Fokin VV; Sharpless KB; Finn MG Bioconjugation by Copper(I)-Catalyzed Azide-Alkyne [3 + 2] Cycloaddition. J. Am. Chem. Soc 2003, 125, 3192–3193. [PubMed: 12630856]

(79). Laughlin ST; Baskin JM; Amacher SL; Bertozzi CR In Vivo Imaging of Membrane-Associated Glycans in Developing Zebrafish. Science 2008, 320, 664–667. [PubMed: 18451302]

(80). Rossin R; Verkerk PR; Van Den Bosch SM; Vulders RC; Verel I; Lub J; Robillard MS In vivo Chemistry for Pretargeted Tumor Imaging in Live Mice. Angew. Chemie - Int. Ed 2010, 49, 3375–3378.

(81). Uttamapinant C; Tangpeerachaikul A; Grecian S; Clarke S; Singh U; Slade P; Gee KR; Ting AY Fast, Cell-Compatible Click Chemistry with Copper-Chelating Azides for Biomolecular Labeling. Angew. Chemie - Int. Ed 2012, 51, 5852–5856.

(82). Rashidian M; Keliher EJ; Dougan M; Juras PK; Cavallari M; Wojtkiewicz GR; Jacobsen JT; Edens JG; Tas JM; Victora G et al. Use of 18f-2-Fluorodeoxyglucose to Label Antibody Fragments for Immuno-positron Emission Tomography of Pancreatic Cancer. ACS Cent. Sci 2015, 1, 142–147. [PubMed: 26955657]

(83). Gates KS Rev. React. Intermed. Chem; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2006; pp 333–378.

(84). Götte K; Chines S; Brunschweiger A Reaction Development for DNA-encoded Library Technology: From Evolution to Revolution? Tetrahedron Lett 2020, 61, 1–8.

(85). Burrows CJ; Muller JG Oxidative Nucleobase Modifications Leading to Strand Scission. Chem. Rev 1998, 98, 1109–1151. [PubMed: 11848927]

(86). Malone ML; Paegel BM What is a "DNA-Compatible" Reaction? ACS Comb. Sci 2016, 18, 182–187. [PubMed: 26971959]

(87). Kolmel DK; Ratnayake AS; Flanagan ME; Tsai MH; Duan C; Song C Photocatalytic [2 + 2] Cycloaddition in DNA-Encoded Chemistry. Org. Lett 2020, 22, 2908–2913. [PubMed: 32239950]

(88). Sannino A; Gabriele E; Bigatti M; Mulatto S; Piazzi J; Scheuermann J; Neri D; Donckele EJ; Samain F Quantitative Assessment of Affinity Selection Performance by Using DNA-Encoded Chemical Libraries. ChemBioChem 2019, 20, 955–962. [PubMed: 30549160]

(89). Satz AL; Hochstrasser R; Petersen AC Analysis of Current DNA Encoded Library Screening Data Indicates Higher False Negative Rates for Numerically Larger Libraries. ACS Comb. Sci 2017, 19, 234–238. [PubMed: 28287689]

(90). McCarthy KA; Franklin GJ; Lancia DR; Olbrot M; Pardo E; O'Connell JC; Kollmann CS The Impact of Variable Selection Coverage on Detection of Ligands from a DNA-Encoded Library Screen. SLAS Discov 2020, 25, 515–522. [PubMed: 32107961]

(91). Fan L; Davie CP Zirconium(IV)-Catalyzed Ring Opening of on-DNA Epoxides in Water. ChemBioChem 2017, 18, 843–847. [PubMed: 27905174]

(92). Thomas B; Lu X; Birmingham WR; Huang K; Both P; Reyes Martinez JE; Young RJ; Davie CP; Flitsch SL Application of Biocatalysis to on-DNA Carbohydrate Library Synthesis. ChemBioChem 2017, 18, 858–863. [PubMed: 28127867]

(93). Madsen D; Azevedo C; Micco I; Petersen LK; Hansen NJV Prog. Med. Chem, 1st ed.; Elsevier B.V., 2020; Vol. 59; pp 181–249. [PubMed: 32362328]

(94). Kalliokoski T Price-Focused Analysis of Commercially Available Building Blocks for Combinatorial Library Synthesis. ACS Comb. Sci 2015, 17, 600–607. [PubMed: 26371511]

(95). Brown DG; Boström J Analysis of Past and Present Synthetic Methodologies on Medicinal Chemistry: Where Have All the New Reactions Gone? J. Med. Chem 2016, 59, 4443–4458. [PubMed: 26571338]

(96). Richter H; Satz AL; Bedoucha M; Buettelmann B; Petersen AC; Harmeier A; Hermosilla R; Hochstrasser R; Burger D; Gsell B et al. DNA-Encoded Library-Derived DDR1 Inhibitor Prevents Fibrosis and Renal Function Loss in a Genetic Mouse Model of Alport Syndrome. ACS Chem. Biol 2019, 14, 37–49. [PubMed: 30452219]

(97). Li Y; Gabriele E; Samain F; Favalli N; Sladojevich F; Scheuermann J; Neri D Optimized Reaction Conditions for Amide Bond Formation in DNA-Encoded Combinatorial Libraries. ACS Comb. Sci 2016, 18, 438–443. [PubMed: 27314981]

(98). Du H-C; Simmons N; Faver JC; Yu Z; Palaniappan M; Riehle K; Matzuk MM A Mild, DNA-Compatible Nitro Reduction Using B 2 (OH) 4. Org. Lett 2019, 21, 2194–2199. [PubMed: 30860855]

(99). Li JY; Miklossy G; Modukuri RK; Bohren KM; Yu Z; Palaniappan M; Faver JC; Riehle K; Matzuk MM; Simmons N Palladium-Catalyzed Hydroxy-carbonylation of (Hetero)aryl Halides for DNA-Encoded Chemical Library Synthesis. Bioconjugate Chem 2019, 30, 2209–2215.

(100). Helal CJ; Bundesmann M; Hammond S; Holmstrom M; Klug-Mcleod J; Lefker BA; McLeod D; Subramanyam C; Zakaryants O; Sakata S Quick Building Blocks (QBB): An Innovative and Efficient Business Model to Speed Medicinal Chemistry Analog Synthesis. ACS Med. Chem. Lett 2019, 10, 1104–1109. [PubMed: 31413793]

(101). Valeur E; Bradley M Amide Bond Formation: Beyond the Myth of Coupling Reagents. Chem. Soc. Rev 2009, 38, 606–631. [PubMed: 19169468]

(102). El-Faham A; Albericio F Peptide Coupling Reagents, More Than a Letter Soup. Chem. Rev 2011, 111, 6557–6602. [PubMed: 21866984]

(103). Nielsen J; Brenner S; Janda KD Synthetic Methods for the Implementation of Encoded Combinatorial Chemistry. J. Am. Chem. Soc 1993, 115, 9812–9813.

(104). Needels MC; Jones DG; Tate EH; Heinkel GL; Kochersperger LM; Dower WJ; Barrett RW; Gallop MA Generation and Screening of an Oligonucleotide-Encoded Synthetic Peptide Library. Proc. Natl. Acad. Sci. U. S. A 1993, 90, 10700–10704. [PubMed: 7504279]

(105). Gartner ZJ; Kanan MW; Liu DR Expanding the Reaction Scope of DNA-Templated Synthesis. Angew. Chemie - Int. Ed 2002, 41, 1796–1800.

(106). Halpin DR; Harbury PB DNA Display II. Genetic Manipulation of Combinatorial Chemistry Libraries for Small-Molecule Evolution. PLoS Biol 2004, 2, 1022–1030.

(107). Halpin DR; Lee JA; Wrenn SJ; Harbury PB DNA Display III. Solid-phase Organic Synthesis on Unprotected Dna. PLoS Biol 2004, 2, 1031–1038.

(108). Morgan B; Hale S; Arico-Muendel CC; Clark M; Wagner R; Kavarana MJ; Creaser SP; Franklin GJ; Centrella PA; Israel DI et al. Methods for Synthesis of Encoded Libraries 2005.

(109). Cochrane W; Malone ML; Dang VQ; Cavett VJ; Satz AL; Paegel BM Activity-Based DNA-Encoded Library Screening. ACS Comb. Sci 2019, 21, 425–435. [PubMed: 30884226]

(110). Afanasyev OI; Kuchuk E; Usanov DL; Chusov D Reductive Amination in the Synthesis of Pharmaceuticals. Chem. Rev 2019, 119, 11857–11911. [PubMed: 31633341]

(111). Podyacheva E; Afanasyev OI; Tsygankov AA; Makarova M; Chusov D Hitchhiker's Guide to Reductive Amination. Synth 2019, 51, 2667–2677.

(112). Cuozzo JW; Centrella PA; Gikunju D; Habeshian S; Hupp CD; Keefe AD; Sigel EA; Soutter HH; Thomson HA; Zhang Y et al. Discovery of a Potent BTK Inhibitor with a Novel Binding Mode by Using Parallel Selections with a DNA-Encoded Chemical Library. ChemBioChem 2017, 18, 864–871. [PubMed: 28056160]

(113). Litovchick A; Tian X; Monteiro MI; Kennedy KM; Guié MA; Centrella P; Zhang Y; Clark MA; Keefe AD Novel Nucleic Acid Binding Small Molecules Discovered Using DNA-encoded Chemistry. Molecules 2019, 24, 1–14.

(114). Flood DT; Asai S; Zhang X; Wang J; Yoon L; Adams ZC; Dillingham BC; Sanchez BB; Vantourout JC; Flanagan ME et al. Expanding Reactivity in DNA-Encoded Library Synthesis via Reversible Binding of DNA to an Inert Quaternary Ammonium Support. J. Am. Chem. Soc 2019, 141, 9998–10006. [PubMed: 31136164]

(115). An R; Jia Y; Wan B; Zhang Y; Dong P; Li J; Liang X Non-Enzymatic Depurination of Nucleic Acids: Factors and Mechanisms. PLoS One 2014, 9, 1–7.

(116). Franzini RM; Samain F; Abd Elrahman M; Mikutis G; Nauer A; Zimmermann M; Scheuermann J; Hall J; Neri D Systematic Evaluation and Optimization of Modification Reactions of Oligonucleotides With Amines and Carboxylic Acids for the Synthesis of DNA-Encoded Chemical Libraries. Bioconjugate Chem 2014, 25, 1453–1461.

(117). Yang H; Medeiros PF; Raha K; Elkins P; Lind KE; Lehr R; Adams ND; Burgess JL; Schmidt SJ; Knight SD et al. Discovery of a Potent Class of PI3Kα Inhibitors with Unique Binding Mode via Encoded Library Technology (ELT). ACS Med. Chem. Lett 2015, 6, 531–536. [PubMed: 26005528]

(118). Wishart DS DrugBank: a Comprehensive Resource for in silico Drug Discovery and Exploration. Nucleic Acids Res 2006, 34, D668–D672. [PubMed: 16381955]

(119). Ding Y; Clark MA Robust Suzuki-Miyaura Cross-Coupling on DNA-Linked Substrates. ACS Comb. Sci 2015, 17, 1–4. [PubMed: 25459065]

(120). Li JY; Huang H Development of DNA-Compatible Suzuki-Miyaura Reaction in Aqueous Media. Bioconjugate Chem 2018, 29, 3841–3846.

(121). Xu H; Ma F; Wang N; Hou W; Xiong H; Lu F; Li J; Wang S; Ma P; Yang G et al. DNA-Encoded Libraries: Aryl Fluorosulfonates as Versatile Electrophiles Enabling Facile On-DNA Suzuki, Sonogashira, and Buchwald Reactions. Adv. Sci 2019, 1901551, 1–6.

(122). Qu Y; Liu S; Wen H; Xu Y; An Y; Li K; Ni M; Shen Y; Shi X; Su W et al. Palladium-Mediated Suzuki-Miyaura Cross-Coupling Reaction of Potassium Boc-Protected Aminomethyltrifluoroborate with DNA-Conjugated Aryl Bromides for DNA-Encoded Chemical Library Synthesis. Biochem. Biophys. Res. Commun 2020, 1–6.

(123). Ding Y; DeLorey JL; Clark MA Novel Catalyst System for Suzuki-Miyaura Coupling of Challenging DNA-Linked Aryl Chlorides. Bioconjugate Chem 2016, 27, 2597–2600.

(124). Lu X; Roberts SE; Franklin GJ; Davie CP On-DNA Pd and Cu Promoted C-N Cross-Coupling Reactions. MedChemComm 2017, 8, 1614–1617. [PubMed: 30108872]

(125). Bruno NC; Tudge MT; Buchwald SL Design and Preparation of New Palladium Precatalysts for C-C and C-N Cross-Coupling Reactions. Chem. Sci 2013, 4, 916–920. [PubMed: 23667737]

(126). Bruneau A; Roche M; Alami M; Messaoudi S 2-Aminobiphenyl Palladacycles: the "Most Powerful" Precatalysts in C-C and C-Heteroatom Cross-Couplings. ACS Catal 2015, 5, 1386–1396.

(127). Chen YC; Faver JC; Ku AF; Miklossy G; Riehle K; Bohren KM; Ucisik MN; Matzuk MM; Yu Z; Simmons N C-N Coupling of DNA-Conjugated (Hetero)aryl Bromides and Chlorides for DNA-Encoded Chemical Library Synthesis. Bioconjugate Chem 2020, 31, 770–780.

(128). Deng H; O'Keefe H; Davie CP; Lind KE; Acharya RA; Franklin GJ; Larkin J; Matico R; Neeb M; Thompson MM et al. Discovery of Highly Potent and Selective Small Molecule ADAMTS-5 Inhibitors that Inhibit Human Cartilage Degradation via Encoded Library Technology (ELT). J. Med. Chem 2012, 55, 7061–7079. [PubMed: 22891645]

(129). Ding Y; O'Keefe H; DeLorey JL; Israel DI; Messer JA; Chiu CH; Skinner SR; Matico RE; Murray-Thompson MF; Li F et al. Discovery of Potent and Selective Inhibitors for ADAMTS-4

through DNA-Encoded Library Technology (ELT). ACS Med. Chem. Lett 2015, 6, 888–893. [PubMed: 26288689]

(130). Kollmann CS; Bai X; Tsai CH; Yang H; Lind KE; Skinner SR; Zhu Z; Israel DI; Cuozzo JW; Morgan BA et al. Application of Encoded Library Technology (ELT) to a Protein-Protein Interaction Target: Discovery of a Potent Class of Integrin Lymphocyte Function-Associated Antigen 1 (LFA-1) Antagonists. Bioorganic Med. Chem 2014, 22, 2353–2365.

(131). Taylor DM; Anglin J; Park S; Ucisik MN; Faver JC; Simmons N; Jin Z; Palaniappan M; Nyshadham P; Li F et al. Identifying Oxacillinase-48 Carbapenemase Inhibitors Using DNA-Encoded Chemical Libraries. ACS Infect. Dis 2020, 6, 1214–1227. [PubMed: 32182432]

(132). Banerjee R; Brown DR; Weerapana E Recent Developments in the Synthesis of Bioactive 2,4,6-Trisubstituted 1,3,5-Triazines. Synlett 2013, 24, 1599–1605.

(133). Saleh M; Abbott S; Perron V; Lauzon C; Penney C; Zacharie B Synthesis and Antimicrobial Activity of 2-Fluorophenyl-4,6-Disubstituted [1,3,5]Triazines. Bioorganic Med. Chem. Lett 2010, 20, 945–949.

(134). Rodionov VO; Presolski SI; Díaz DD; Fokin VV; Finn MG Ligand-Accelerated Cu-Catalyzed Azide-Alkyne Cycloaddition: A Mechanistic Report. J. Am. Chem. Soc 2007, 129, 12705–12712. [PubMed: 17914817]

(135). Presolski SI; Hong V; Cho SH; Finn MG Tailored Ligand Acceleration of the Cu-Catalyzed Azide-Alkyne Cycloaddition Reaction: Practical and Mechanistic Implications. J. Am. Chem. Soc 2010, 132, 14570–14576. [PubMed: 20863116]

(136). Besanceney-Webler C; Jiang H; Zheng T; Feng L; Soriano Del Amo D; Wang W; Klivansky LM; Marlow FL; Liu Y; Wu P Increasing the Efficacy of Bioorthogonal Click Reactions for Bioconjugation: A Comparative Study. Angew. Chemie - Int. Ed 2011, 50, 8051–8056.

(137). Hong V; Presolski SI; Ma C; Finn MG Analysis and Optimization of Copper-Catalyzed Azide-Alkyne Cycloaddition for Bioconjugation. Angew. Chem 2009, 121, 10063–10067.

(138). Favalli N; Bassi G; Zanetti T; Scheuermann J; Neri D Screening of Three Transition Metal-Mediated Reactions Compatible with DNA-Encoded Chemical Libraries. Helv. Chim. Acta 2019, 102, 1–11.

(139). Keefe AD; Clark MA; Hupp CD; Litovchick A; Zhang Y Chemical Ligation Methods for the Tagging of Dna-encoded Chemical Libraries. Curr. Opin. Chem. Biol 2015, 26, 80–88. [PubMed: 25756406]

(140). Fischer N; Goddard-Borger ED; Greiner R; Klapötke TM; Skelton BW; Stierstorfer J Sensitivities of Some Imidazole-1-Sulfonyl Azide Salts. J. Org. Chem 2012, 77, 1760–1764. [PubMed: 22283437]

(141). Gironda-Martínez A; Neri D; Samain F; Donckele EJ DNA-Compatible Diazo-Transfer Reaction in Aqueous Media Suitable for DNA-Encoded Chemical Library Synthesis. Org. Lett 2019, 21, 9555–9558. [PubMed: 31747292]

(142). Meng G; Guo T; Ma T; Zhang J; Shen Y; Sharpless KB; Dong J Modular Click Chemistry Libraries for Functional Screens Using a Diazotizing Reagent. Nature 2019, 574, 86–89. [PubMed: 31578481]

(143). Abel GR; Calabrese ZA; Ayco J; Hein JE; Ye T Measuring and Suppressing the Oxidative Damage to DNA During Cu(I)-Catalyzed Azide-Alkyne Cycloaddition. Bioconjugate Chem 2016, 27, 698–704.

(144). Škopić MK; Salamon H; Bugain O; Jung K; Gohla A; Doetsch LJ; Santos DD; Bhat A; Wagner B; Brunschweiger A Acid- and Au(I)-Mediated Synthesis of Hexathymidine-DNA-Heterocycle Chimeras, an Efficient Entry to DNA-Encoded Libraries Inspired by Drug Structures. Chem. Sci 2017, 8, 3356–3361. [PubMed: 28507705]

(145). Potowski M; Losch F; Wünnemann E; Dahmen JK; Chines S; Brunschweiger A Screening of Metal Ions and Organocatalysts on Solid Support-Coupled DNA Oligonucleotides Guides Design of DNA-Encoded Reactions. Chem. Sci 2019, 10, 10481–10492. [PubMed: 32055372]

(146). Klika Škopić M; Willems S; Wagner B; Schieven J; Krause N; Brunschweiger A Exploration of a Au(I)-Mediated Three-Component Reaction for the Synthesis of DNA-Tagged Highly Substituted Spiroheterocycles. Org. Biomol. Chem 2017, 15, 8648–8654. [PubMed: 28981134]

(147). Potowski M; Kunig VB; Losch F; Brunschweiger A Synthesis of DNA-coupled Isoquinolones and Pyrrolidines by Solid Phase Ytterbium- and Silver-Mediated Imine Chemistry. MedChemComm 2019, 10, 1082–1093. [PubMed: 31391880]

(148). Kunig VBK; Ehrt C; Dömling A; Brunschweiger A Isocyanide Multicomponent Reactions on Solid-Phase-Coupled DNA Oligonucleotides for Encoded Library Synthesis. Org. Lett 2019, 21, 7238–7243. [PubMed: 31464126]

(149). Lam KS; Lebl M; Krchák V The "One-Bead-One-Compound" Combinatorial Library Method. Chem. Rev 1997, 97, 411–448. [PubMed: 11848877]

(150). Shu K; Kodadek T Solid-Phase Synthesis of β-Hydroxy Ketones Via DNA-Compatible Organocatalytic Aldol Reactions. ACS Comb. Sci 2018, 20, 277–281. [PubMed: 29578681]

(151). Pels K; Dickson P; An H; Kodadek T DNA-Compatible Solid-Phase Combinatorial Synthesis of β-Cyanoacrylamides and Related Electrophiles. ACS Comb. Sci 2018, 20, 61–69. [PubMed: 29298042]

(152). Tran-Hoang N; Kodadek T Solid-Phase Synthesis of β-Amino Ketones Via DNA-Compatible Organocatalytic Mannich Reactions. ACS Comb. Sci 2018, 20, 55–60. [PubMed: 29316387]

(153). Paciaroni NG; Ndungu JM; Kodadek T Solid-Phase Synthesis of DNA-Encoded Libraries: Via an "Aldehyde Explosion" Strategy. Chem. Commun 2020, 56, 4656–4659.

(154). Mendes KR; Malone ML; Ndungu JM; Suponitsky-Kroyter I; Cavett VJ; McEnaney PJ; MacConnell AB; Doran TD; Ronacher K; Stanley K et al. High-throughput Identification of DNA-encoded IgG Ligands that Distinguish Active and Latent Mycobacterium Tuberculosis Infections. ACS Chem. Biol 2017, 12, 234–243. [PubMed: 27957856]

(155). Price AK; MacConnell AB; Paegel BM HνSABR: Photochemical Dose-Response Bead Screening in Droplets. Anal. Chem 2016, 88, 2904–2911. [PubMed: 26815064]

(156). MacConnell AB; Price AK; Paegel BM An Integrated Microfluidic Processor for DNA-Encoded Combinatorial Library Functional Screening. ACS Comb. Sci 2017, 19, 181–192. [PubMed: 28199790]

(157). Hackler AL; FitzGerald FG; Dang VQ; Satz AL; Paegel BM Off-DNA DNA-Encoded Library Affinity Screening. ACS Comb. Sci 2020, 22, 25–34. [PubMed: 31829554]

(158). Halpin DR; Harbury PB DNA Display I. Sequence-Encoded Routing of DNA populations. PLoS Biol 2004, 2, 1015–1021.

(159). Leimbacher M; Zhang Y; Mannocci L; Stravs M; Geppert T; Scheuermann J; Schneider G; Neri D Discovery of Small-Molecule Interleukin-2 Inhibitors from a DNA-Encoded Chemical Library. Chem. - A Eur. J 2012, 18, 7729–7737.

(160). Krusemark CJ; Tilmans NP; Brown PO; Harbury PB Directed Chemical Evolution With an Outsized Genetic Code. PLoS One 2016, 11, 1–16.

(161). Wrenn SJ; Weisinger RM; Halpin DR; Harbury PB Synthetic Ligands Discovered by in Vitro Selection. J. Am. Chem. Soc 2007, 129, 13137–13143. [PubMed: 17918937]

(162). Ruff Y; Martinez R; Pellé X; Nimsgern P; Fille P; Ratnikov M; Berst F An Amphiphilic Polymer-Supported Strategy Enables Chemical Transformations under Anhydrous Conditions for DNA-Encoded Library Synthesis. ACS Comb. Sci 2020, 22, 120–128. [PubMed: 32040908]

(163). Flood DT; Zhang X; Fu X; Zhao Z; Asai S; Sanchez BB; Sturgell EJ; Vantourout JC; Richardson P; Flanagan ME et al. RASS-Enabled S/P–C and S–N Bond Formation for DEL Synthesis. Angew. Chemie - Int. Ed 2020, 59, 7377–7383.

(164). Vo CVT; Luescher MU; Bode JW SnAP Reagents for the One-Step Synthesis of Medium-Ring Saturated N-Heterocycles from Aldehydes. Nature Chem 2014, 6, 310–314. [PubMed: 24651197]

(165). Chu L; Ohta C; Zuo Z; MacMillan DW Carboxylic Acids as a Traceless Activation Group for Conjugate Additions: A Three-Step Synthesis of (±)-Pregabalin. J. Am. Chem. Soc 2014, 136, 10886–10889. [PubMed: 25032785]

(166). Bloom S; Liu C; Kölmel DK; Qiao JX; Zhang Y; Poss MA; Ewing WR; Macmillan DW Decarboxylative Alkylation for Site-selective Bioconjugation of Native Proteins via Oxidation Potentials. Nature Chem 2018, 10, 205–211. [PubMed: 29359756]

(167). Kölmel DK; Loach RP; Knauber T; Flanagan ME Employing Photoredox Catalysis for DNA-Encoded Chemistry: Decarboxylative Alkylation of α-Amino Acids. ChemMedChem 2018, 13, 2159–2165. [PubMed: 30063289]

(168). Kölmel DK; Meng J; Tsai MH; Que J; Loach RP; Knauber T; Wan J; Flanagan ME On-DNA Decarboxylative Arylation: Merging Photoredox with Nickel Catalysis in Water. ACS Comb. Sci 2019, 21, 588–597. [PubMed: 31283168]

(169). Kölmel DK; Ratnayake AS; Flanagan ME Photoredox Cross-Electrophile Coupling in DNA-Encoded Chemistry. Biochem. Biophys. Res. Commun 2020,
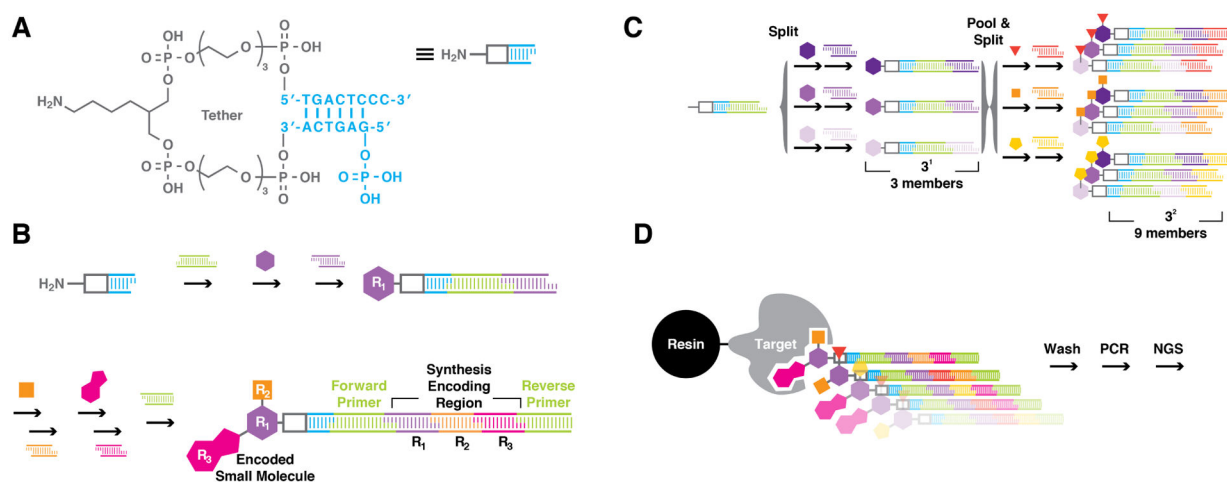
Figure 1.

DNA-encoded library synthesis and screening. (A) The linchpin "headpiece" DNA molecule is a covalently tethered (gray) dsDNA heteroduplex (cyan) displaying a primary amine for chemical synthesis, and a $5'$–CC–$3'$ dinucleotide overhang and $5'$ phosphate for enzymatic cohesive end ligation. (B) Encoded synthesis proceeds in interleaved steps of enzymatic ligation and building block coupling. Synthesis begins with ligation of a $5'$-phosphorylated dsDNA (green) to the headpiece. Coupling of the position 1 building block (R_1, purple hexagon) and enzymatic encoding (purple dsDNA) follow. Synthesis continues with analogous coupling and encoding steps for the position 2 building block (R_2, orange square) and position 3 building block (R_3, magenta bicycle), and a final dsDNA ligation (green). Green sequences flanking the synthesis encoding region are constant PCR primer binding sites. The product is a small molecule attached to a DNA whose sequence encodes the synthesis history of the small molecule. (C) Split-and-pool diversification entails parallel position 1 building block coupling and encoding (purple hues) to yield 3 different example DEL members. A second encoded split-and-pool synthesis step (orange hues) yields $3^2 = 9$ different DEL members. (D) Protein target (gray) immobilized to resin captures all molecules in the library that are ligands. Washing removes unbound species, leaving the bound fraction encoding DNA sequences for PCR amplification, sequencing, and structure decoding.
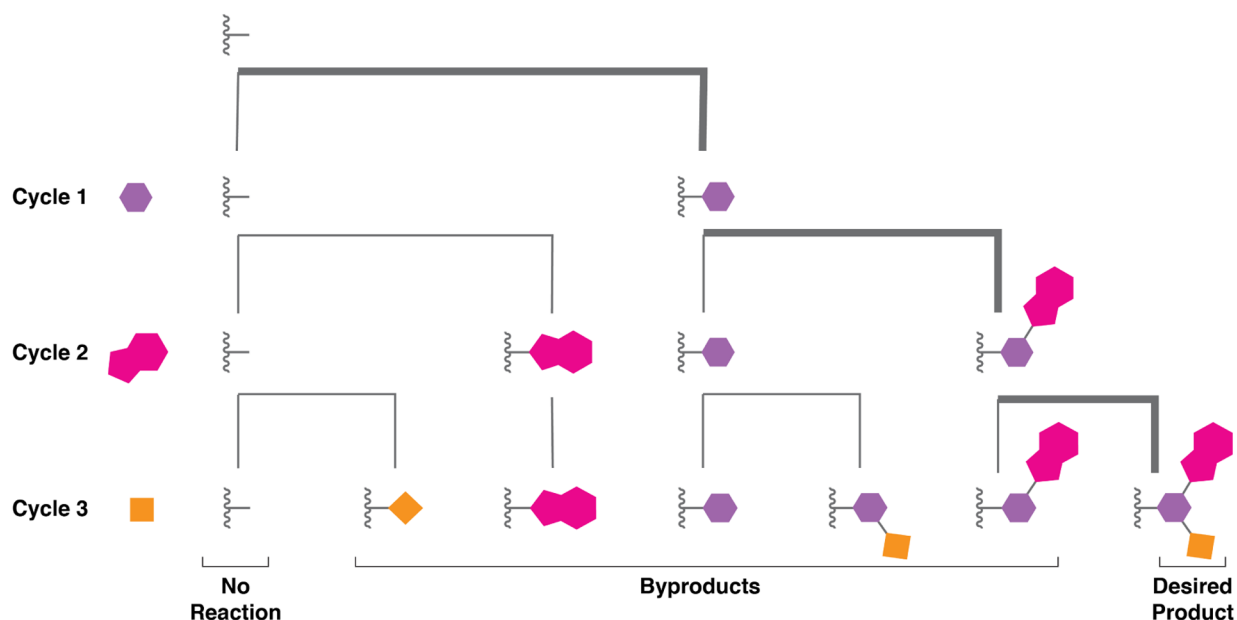
Figure 2.
DNA-encoded library synthesis decision tree. Combinatorial synthesis yields exponentially increasing diversity of products. The decision tree maps all possible molecular outcomes of a 3-cycle DEL synthesis (excluding unanticipated side reactions). The attachment point to DNA is shown with a wavy line. The DEL synthesis begins with coupling of a trifunctional hub (purple hexagon) and ends with 2 capping groups (magenta, orange). Three successful building block couplings lead to formation of the desired product 3-cycle DEL member(bold decision branches). Incomplete building block coupling leads to one of five different truncate byproducts or, in the case of no coupling, an unmodified DNA results. The scheme is representative of the triazine DEL, which are the product of two sequential nucleophilic aromatic substitution of a cyanuric chloride hub using amine building blocks. The desired product, byproducts and no reaction all share the same encoding sequence. As a consequence, schemes with fewer steps employing only high-yielding chemistry universally lead to higher fidelity library outputs by maximizing the number of encoding tags that display the desired product. Adapted from Ref. 42. Copyright 2016 American Chemical Society.
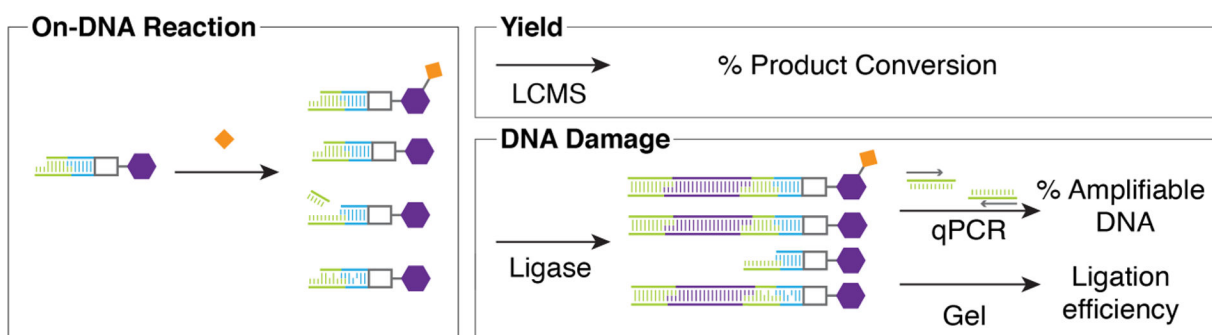
Figure 3.

DNA compatibility assay. Comprehensive qPCR and LCMS characterization of DEL reactions is critical for preparing high-quality libraries. A candidate reaction is evaluated by coupling one or several model building blocks (orange diamond) in an on-DNA reaction with an elaborated DNA headpiece. The elaborated headpiece displays the appropriate reaction site (purple hexagon) for the candidate reaction and a partial DNA encoding tag. The resulting reaction mixture is analyzed by LCMS to obtain the on-DNA % conversion. The mixture is also enzymatically ligated (T4 DNA ligase) to dsDNA oligonucleotide modules that install binding sites for a Taqman exonuclease probe (purple) and closing primer (green). The ligase reaction is analyzed for ligation yield by gel electrophoresis and for the molecules of amplifiable DNA remaining by qPCR.The qPCR analysis is also conducted on DNA that has experienced control conditions (e.g., incubation in buffer) and the result used to calculate % amplifiable DNA remaining after exposure to conditions of chemical synthesis. Adapted from Ref. 54. Copyright 2019 American Chemical Society.
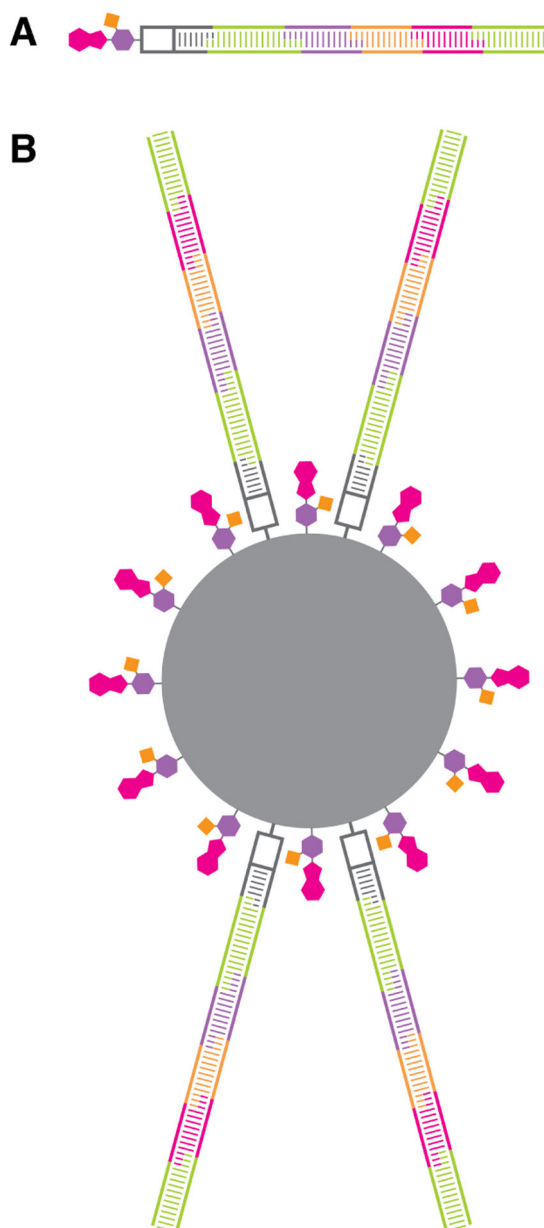
Figure 4.
Solid-phase versus conventional on-DNA DELs. (A) A conventional 3-cycle DEL member contains a DNA-encoding tag that is stoichiometrically conjugated to the encoded small molecule. (B) A solid-phase 3-cycle DEL member comprises a bead that polyvalently displays the DNA-encoding tag and the encoded small molecule. DNA functionalization is substoichiometric to small molecule loading. Solid-phase DELs are prepared using analogous encoded split-and-pool combinatorial synthesis.
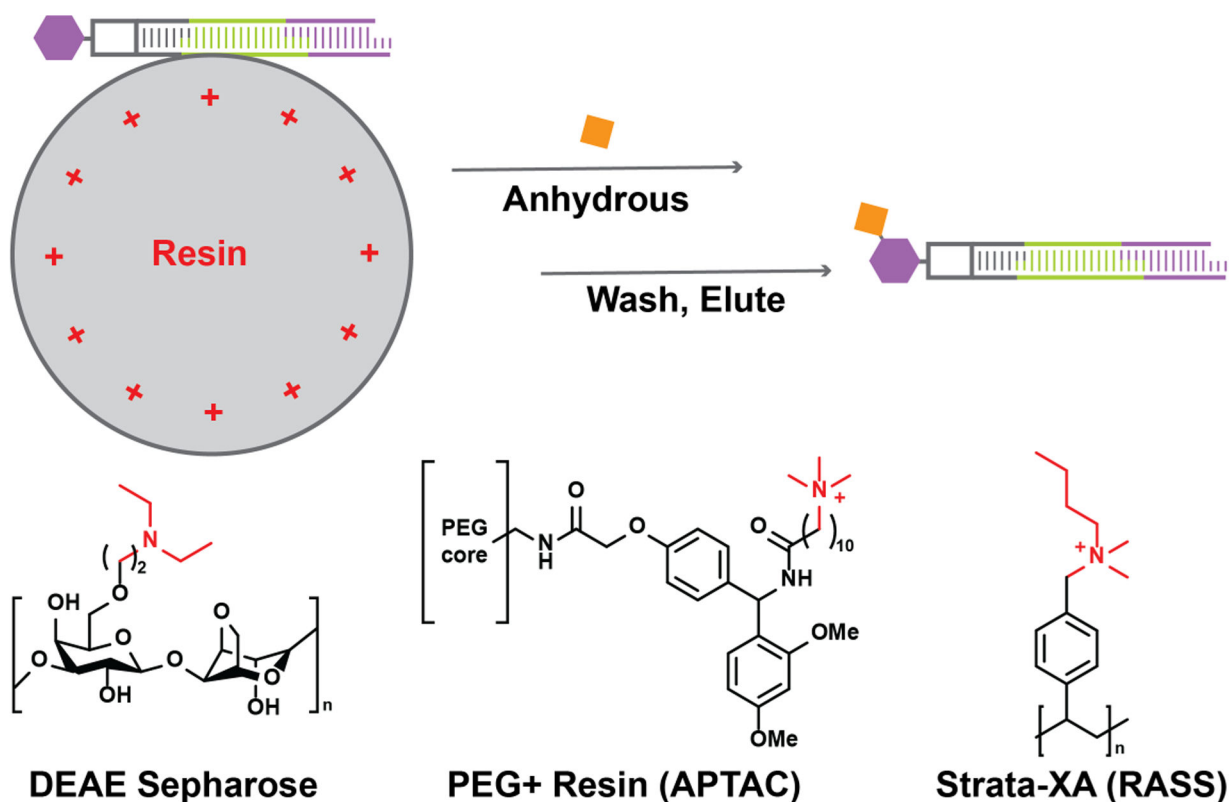
Figure 5.
Solid-phase reversible immobilization-enabled DEL synthesis. The cationic bead surface immobilizes DNA conjugates through ionic interactions with the DNA phosphodiester backbone. Resin is combined with DNA in aqueous media, washed with water-miscible solvent, and then washed with dry solvent to provide near-anhydrous conditions for DNA modification. Anion exchange resins used in this process include DEAE sepharose, a modified ChemMatrix resin (PEG+), and Strata-XA.
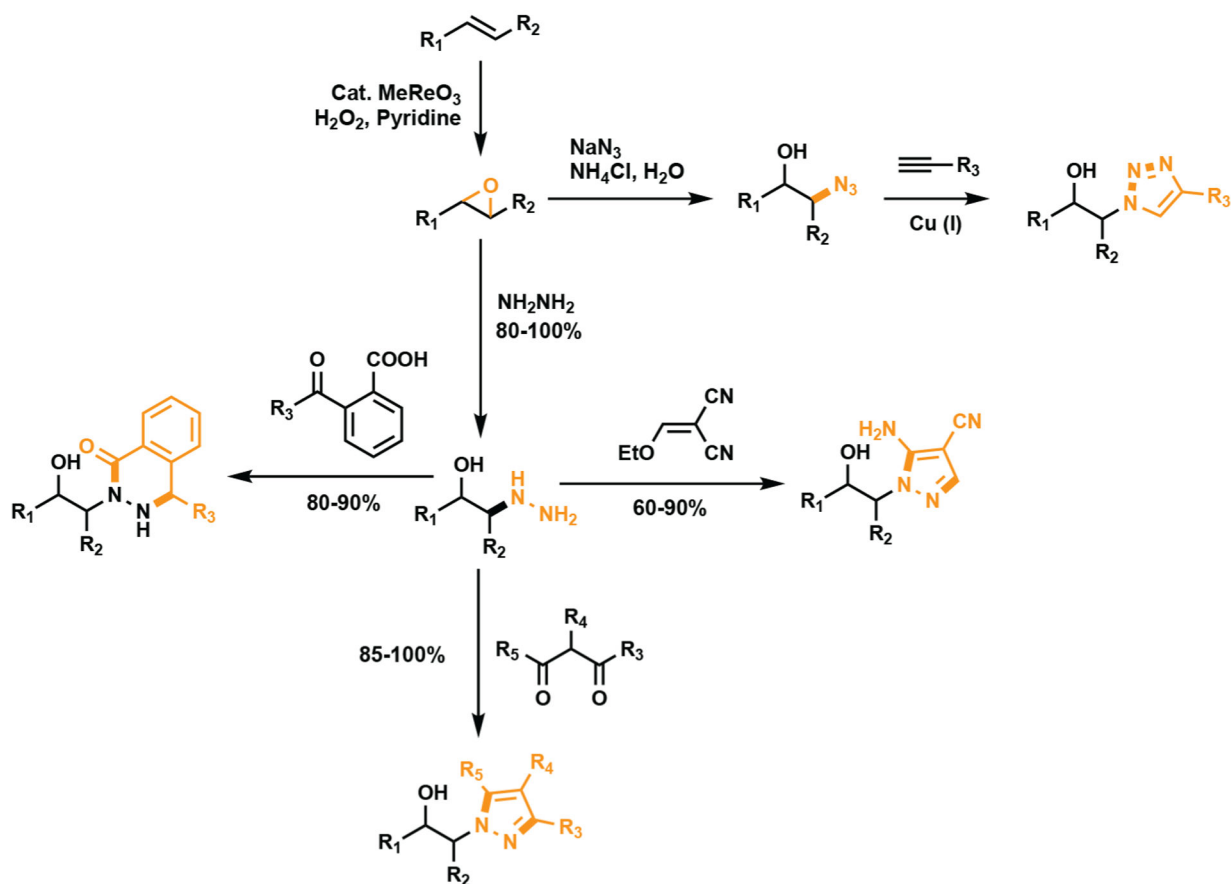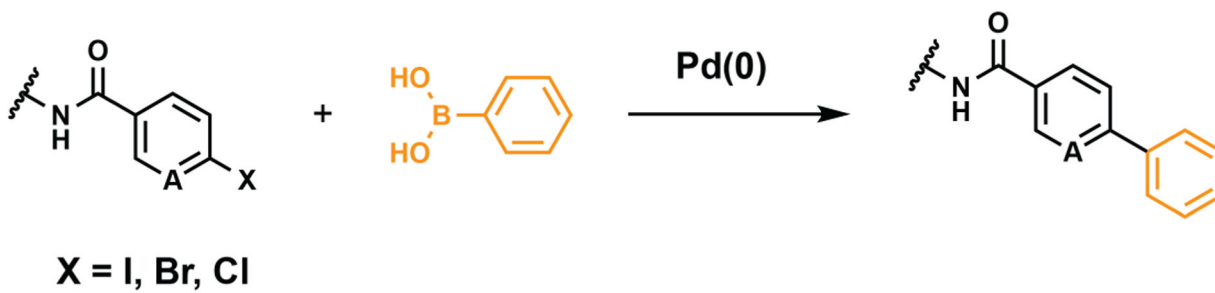
Scheme 1.

Structural diversification using a few good reactions. Adapted with permission from Ref. 2.

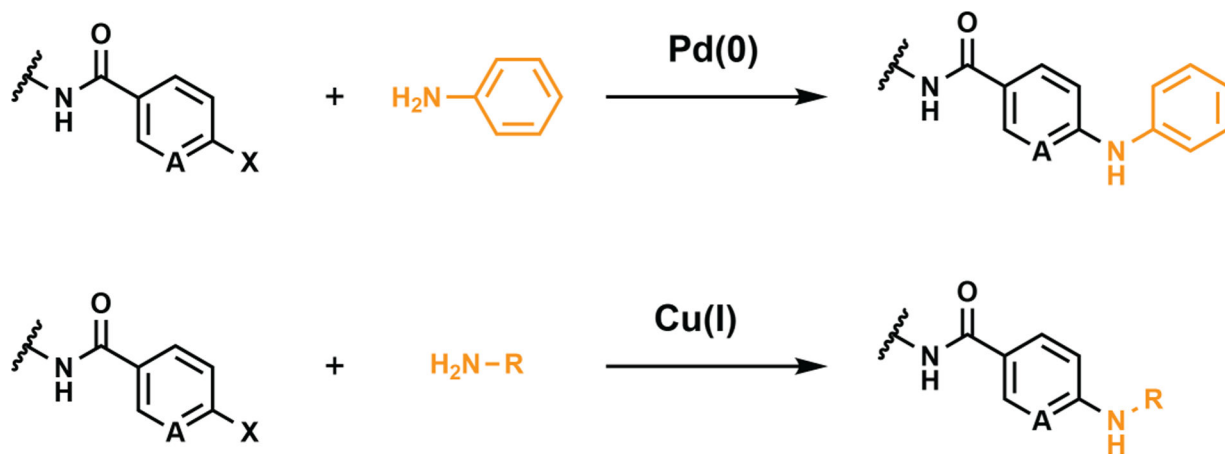Copyright 2001 Angewandte Chemie International Edition.
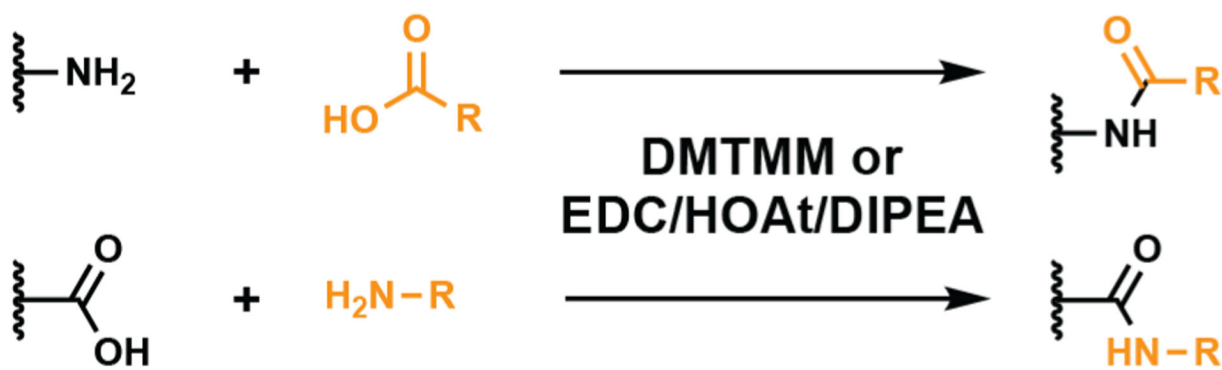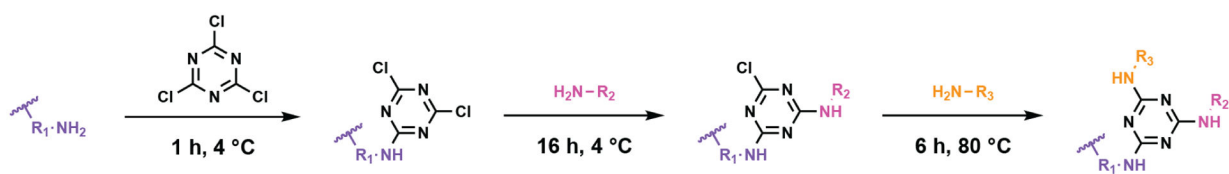
Scheme 2.
Amide bond formation.
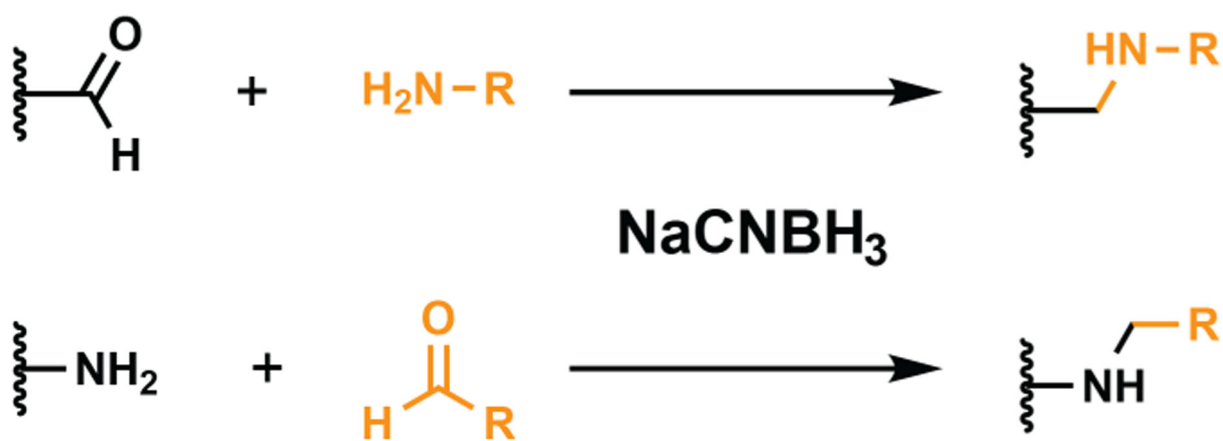
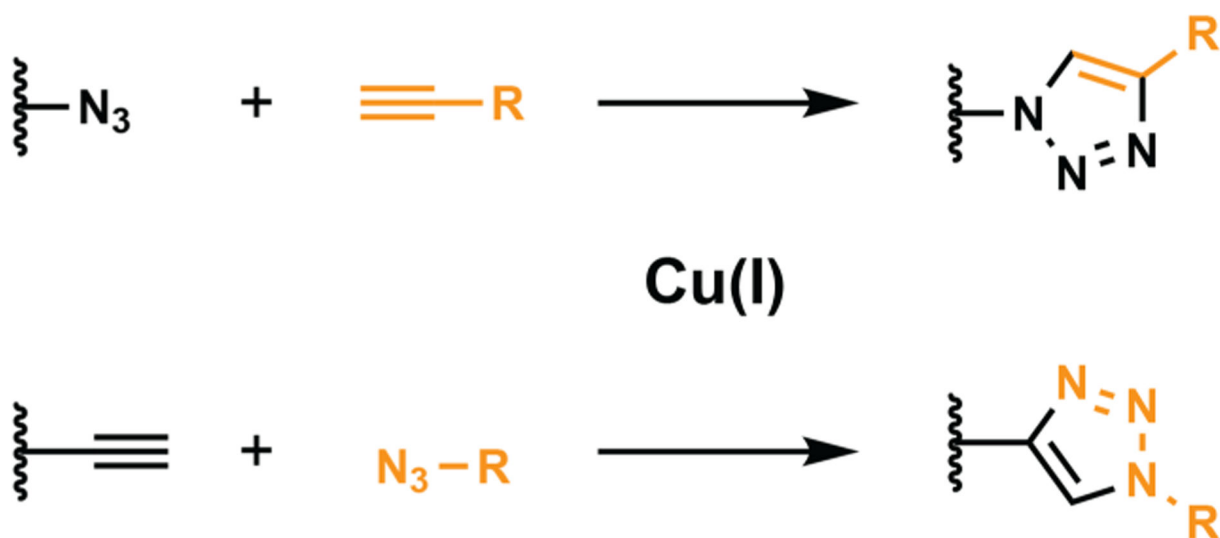**Scheme 3.**
Reductive amination/alkylation.

Scheme 4.
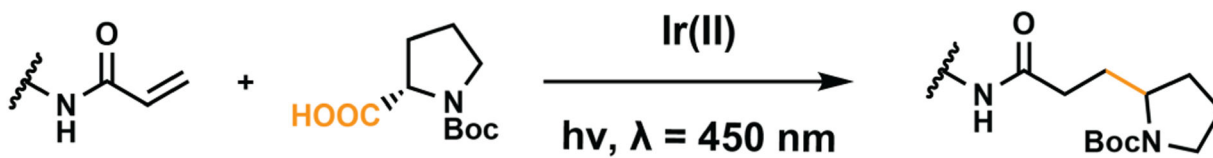Suzuki-Miyaura cross coupling.

Scheme 5.
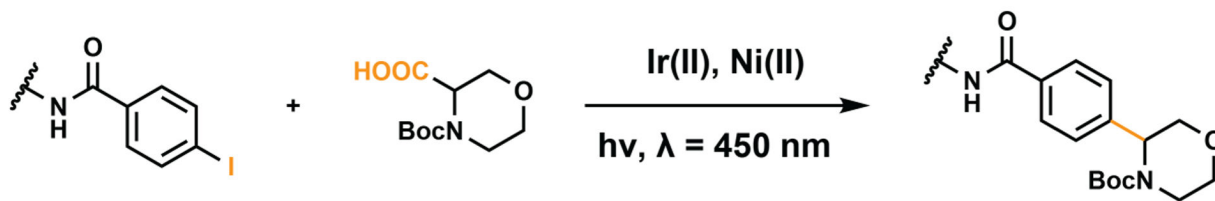Buchwald and Ullmann cross coupling.

Scheme 6.
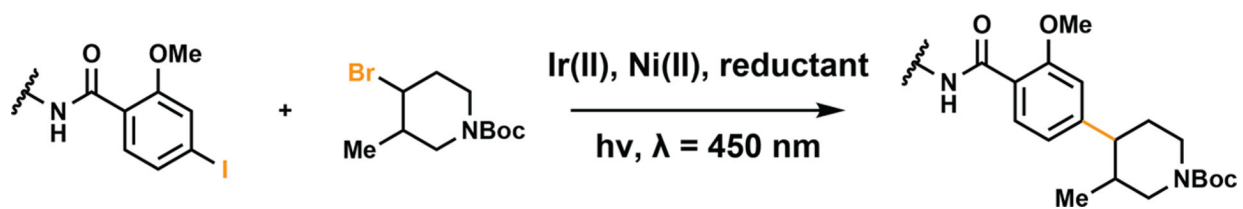Nucleophilic substitution.

Scheme 7.
CuAAC.

Scheme 8.
Photoredox Giese reaction.

Scheme 9.
Decarboxylative cross coupling.

Scheme 10.
Cross electrophile coupling.

Table 1.

Amide Formation Conditions

Activating agent	Conditions	Scope Investigated	Library	Year
PyBOP/DIPEA[a]	-	AAs L,F,G,K,Y, P	5	1994 [103]
HBTU/HOBt/ DIPEA	0.1M AA/HBTU/ HOBT ; 0.3 M DIPEA	(L) AAs R, Q, K, V, and T; (D) V	8.2×10^5 (5 cycle)	1994 [104]
DMT-MM or EDC/ Sulfo-NHS (sNHS)	Templated (60 nM NH_2-DNA, 120 nM COOH-DNA, 50 mM DMT-MM in 0.1 M MOPS buffer pH 7.0, 1 M NaCl, 16 h, 25 °C	2 NH_2-DNA, 4 COOH-DNA	-	2002 [105]
EDC/HOAt	DEAE immobilization, 50 mM Fmoc-AA/EDC, 5 mM HOAt, MeOH, 30 min, RT	33 AAs	10^6 (6 cycle, DNA-routed)[106]	2004 [107]
DMT-MM or HATU/ DIPEA	0.7 mM NH_2-DNA, 28 mM DMT-MM/Fmoc-AA	96 AAs (yield not reported)	8.4×10^7 tetrapeptides	2005 [108]
EDC/sulfo-NHS[a]	50 μM NH_2-DNA, 4 mM Fmoc-AA/EDC, 10 mM sNHS, 70% DMSO, 30% 80 mM TEA pH 9	20 AAs and 200 RCOOH	4×10^3 (2 cycle)	2008 [18]
DMT-MM[a]	0.7 mM NH_2-DNA, 27 mM Fmoc-AA, 27 mM DMT-MM, 18% DMF, 82% 130 mM borate pH 9.5, 18 h, 4 °C	> 192/400 Fmoc-AAs (yield > 70%)	DEL A: 7×10^6 (3 cycle), DEL B: 8×10^8 (4 cycle)	2009 [1]
	0.44 mM $COOH_2$-DNA, 22 mM amine, 44 mM DMT-MM, 111 mM phosphate pH 5.5, 44 mM HCl, 94% H_2O, 6% MeCN, 72 h, RT, 50 additional equiv. DMTMM added at 48 h and 60 h	383 amines		
9 tested, EDC/HOAt/ DIPEA recommended[a]	4 μM NH_2-DNA, 9 mM EDC, 2 mM HOAt, 9 mM DIPEA, 56% MOPS pH 8, 44% DMSO, 16 h, RT	543 RCOOH tested for DMT-MM and EDC/HOAt/DIPEA	-	2016 [97]
DIC/HOAt[b]	Resin-bound DNA, resin-bound 1° amine: 40 mM Fmoc-AA, 40 mM HOAt, 57 mM DIC, DMF 1 h, 37 °C; resin-bound 2° amine: 80 mM Fmoc AA/Oxyma/TMP, 100 mM DIC, 3 h, 37 °C	1 Fmoc-AA	6×10^4 (2 cycle)[109]	2016 [86]
HATU/DIPEA[a,b]	71 mM Boc-AA/DIPEA/HATU, 43% DMA, 57% 250 mM borate pH 9.5, 2 h, RT	Boc-Phe-OH	-	2019 [54]
DMT-MM[a,b]	60 μM NH_2-DNA, 60 mM AA/DMT-MM, 120 mM N-methyl morpholine, 58% DMSO 42% 50 mM MOPS pH 8.2, 24 h, RT	126 AAs in library	1.4×10^6 macrocycles	2019 [47]
DEPBT/DIPEA[a]	0.24 mM NH_2-DNA, 24 mM COOH/DEPBT/ DIPEA, 100 mM borate pH 9.5, 63% H_2O, 37% MeCN, 2h, RT	57 N-Boc AAs, 23 nitro benzoic acids	7.5×10^7	2019 [98]
DMT-MM (reverse acylation)	0.2 mM COOH-DNA, 20 mM amine/DMTMM, 100 mM MES buffer pH 5.8, 65% H_2O, 35% MeCN, ON,RT	183 N-Boc Diamines, 23 nitro anilines		
DMT-MM	-	Library included 57 N_3-AAs (Fmoc/Boc) and 1846 RCOOH (used in two positions)	6.6×10^8 (3 cycle)	2020 [72]

[a]Analysis of DNA conjugates by HPLC(A260) and LCMS.

[b]Quantitative analysis of DNA compatibility by qPCR.

Table 2.

Reductive Amination Conditions

Reducing Agent	Conditions	Scope Investigated	Library	Year
NaCNBH$_3$	60 nM NH$_2$-DNA, 60 nM CHO-DNA, 3 mM NaCNBH$_3$, 0.1 M MES pH 6.0, 1.5 h, 25 °C	1 NH$_2$-DNA, 1 CHO-DNA or one glyoxal-DNA	-	2002 [105]
NaCNBH$_3$	0.76 mM proline-DNA, 30 mM RCHO/NaCNBH$_3$, 75% 150 mM Phosphate pH 5.5, 25% DMF, 2 h, 80 °C or 1 mM CHO-DNA,30 mM RNH$_2$/NaCNBH$_3$, 2 h, 80 °C	Proline-DNA and CHO-DNA	-	2005 [108]
NaCNBH$_3$[a]	50 μM CHO-DNA, 500 mM amine/NaCNBH$_3$, 300 mM MOPS, pH 7.4, 16 h, 37 °C	12 amines	-	2014 [116]
NaCNBH$_3$	0.63 mM CHO-DNA, 50 mM Amine/ NaCNBH$_3$, 62.5% 500 mM phosphate pH 5.5, 12.5% H$_2$O/MeCN/DMF, 16 h, 60 °C	2,259 amines (no validation)	3.3×10^8	2015 [63]
NaCNBH$_3$	0.5 mM CHO-DNA, 50 mM amine/NaCNBH$_3$, 50% 250 mM phosphate pH 5.5, 25% DMA/ACN, 16 h, RT	218/831 amines (yield > 70%)	3.5×10^6	2015, [117] 2016 [46]
NaCNBH$_3$[a]	0.63 mM NH$_2$-DNA, 50 mM R-CHO/NaCNBH$_3$, 62.5%, 25% DMF, 12.5% DMA 250 mM phosphate pH 5.5, 16 h, 60 °C	636 R-CHO in library, > 50% yield in validation with 1° and 2° amine-DNA substrates	3.4×10^7	2015 [45]
NaCNBH$_3$[b]	Resin-bound 2° amine, 1) 0.5 M R-CHO, 1% AcOH in DMF, 10 min, RT; 2) 0.5 M NaCNBH$_3$, 1% AcOH in MeOH, 1 h, RT	4-iodobenzaldehyde	-	2016,[86]
NaCNBH$_3$[c]	33 μM carbohydrate/CHO-DNA, 333 mM amine/ NaCNBH$_3$, 16 h, 37 °C	1 carbohydrate/CHO-DNA with 19 RNH$_2$, 7 carbohydrate/CHO-DNA with benzylamine	-	2017 [92]
NaCNBH$_3$[a]	0.19 mM 2° amine-DNA, 37.5 mM RCHO/NaCNBH$_3$, 25% NaHCO$_3$, 37.5% 1 M phosphate pH 4.2/DMF, 8 h, 37 °C	4 NR$_2$H-DNA with 20 aldehydes, 1 NR$_2$H-DNA with 118 R-CHO (72/118 > 70% yield)	1.07×10^6	2019 [44]
NaCNBH$_3$[a]	500 mM ketone, 1 M NaCNBH$_3$, 400 mM B(OH)$_3$, 80% NMP, 20% H$_2$O, 20 h, 60 °C	17 ketones	-	2019 [114]
NaCNBH$_3$	100 eq RCHO, 100 eq NaCNBH$_3$, O/N, 60 °C	1347 aldehydes	1.9×10^8	2020 [72]

[a]Analysis of DNA conjugates by HPLC(A260) and LCMS.

[b]Quantitative analysis of DNA compatibility by qPCR.

[c]Analysis of DNA conjugates by MALDI-TOF.

Table 3.

Suzuki-Miyaura Cross Coupling Conditions

Catalyst	Conditions	Scope Investigated	Library	Year
Pd(PPh₃)₄[a]	1 mM ArI-DNA, 20 mM Ar-B(OR)₂[b], 1 mM Pd(PPh₃)₄, 40 mM Na₂CO₃, 90 min, 80 °C	15 R-B(OH)₂, 8 boronic esters, 2 ArI-DNA, 1 ArBr-DNA, 3 PyBr-DNA	-	2015 [119]
Pd(PPh₃)₄[a]	0.73 mM ArI-DNA, 30 mM Ar-B(OR)₂[b], 60 mM Na₂CO₃, 0.4 mM Pd(PPh₃)₄, 5 h, 80 °C,	44 ArI (> 70% yield in 2 step valdiation) and 265 Ar-B(OR)₂ (> 50% yield in validation)	3.4×10^7	2015 [45]
Pd(PPh₃)₄[a]	0.5 mM ArI-DNA, 50 mM Cs₂CO₃, 40 mM Ar-B(OR)₂[b], 0.6 mM Pd(PPh₃)₄, 75% aqueous, 25% DMA, 3 h, 80 °C; 15 mM NaS₂CN(C₂H₅)₂ Pd scavenger	222 ArBr, 667 Ar-B(OR)₂	3.3×10^8	2015 [63]
Pd(PPh₃)₄[c]	Resin-bound ArI, 344 mM Ar-B(OH)₂, 690 mM DIPEA, 0.13 mM Pd(PPh₃)₄, NMP, 7 h, 70° C	4-(iPr)PhB(OH)₂	-	2016 [86]
POPd/sSPHOS (Pd₂(dba)₃, POPd1, POPd2)[a]	0.83 mM (het)ArCl-DNA, 33 mM KOH, 17 mM Ar-B(OR)₂[b], 1.7 mM sSPHOS, 0.83 mM POPd, 3 h, 80 °C	3 (het)ArCl-DNA with 8 Ar-B(OR)₂ each	-	2016 [123]
sSPhos-Pd-G2[a]	0.42 mM ArX-DNA, 170 mM CsOH, 42 mM Ar-B(OR)₂[b], 0.84 mM sSPhos-Pd-G2, 81% H₂O, 11% dioxane, 8% DMA, 15 min, 80 °C,	8 (het)ArX-DNA with 84 Ar-B(OR)₂ each	-	2018 [120]
Pd(OAc)₂[a]	0.19 mM ArSO₂F-DNA, 77 mM Ar-B(OH)₂, 192 mM TEA, 3.8 mM Pd(OAc)₂, 60% H₂O, 40% dioxane, 2 h, 25 °C; NaS₂CN(C₂H₅)₂ Pd scavenger	33 Ar-B(OH)₂ with ArSO₂F-DNA; 8 (het)ArX-DNA with PhB(OH)₂	-	2019 [121]
Pd(dppf)Cl₂[a]	0.13 mM ArI-DNA, 25 mM Ar-B(OH)₂, 0.15 mM Pd(dppf)Cl₂, 20/51/13/16 100 mM carbonate pH 8.2/1 M phosphate pH 9.2/EtOH/MeCN, 1 h, 90 °C	241 Ar-B(OR)₂ (yield 117/241 > 85%)	10^6	2019 [44]

[a] Analysis of DNA conjugates by HPLC(A260) and LCMS.

[b] (OR)₂ = (OH)₂ or pinacol.

[c] Quantitative analysis of DNA compatibility by qPCR.

Table 4.

Buchwald-Hartwig and Ullmann C-N Cross Coupling Conditions

Catalyst	Conditions	Scope Investigated	Library	Year
tBuXPhos Pd G1[a]	0.4 mM ArI-DNA, 400 mM ArNH$_2$, 400 mM CsOH, 0.8 mM tBuxPhos Pd G1, 48% Aq, 52% DMA, 3 h, 100 °C	1033/6329 1° ArNH$_2$ > 50% yield	1.7×10^8	2017 [124]
CuSO$_4$, ascorbate, (proline)[a]	0.38 mM ArI-DNA, 280 mM 2° amine, 11 mM CuSO$_4$/proline/KOH, 14 mM ascorbate, 2 h, 100 °C	96/557 AAs (yield > 50%); 300/2776 aliphatic 1° RNH$_2$ (> 50% yield)	3×10^7	
Cu(OAC)$_2$, ascorbate, 2-((2,6-dimethoxyphenyl)amino)-2-oxoacetic acid[a]	62.5 μM ArI-DNA, 500 mM amine, 25 mM Cu(OAC)$_2$, 50 mM ascorbate, 200 mM ligand, 500 mM K$_3$PO$_4$, 1:3 DMSO:H$_2$O, 3 h, 40 °C	8 ArI-DNA with 12 2° amines	-	2018 [68]
tBuBrettPhos Pd G3[a]	0.31 mM ArSO$_2$F-DNA, 62.5 mM ArNH$_2$, 312.5 mM TEA, 3.1 mM tBuBrettPhos Pd G3	1 ArSO$_2$F-DNA with 24 ArNH$_2$ and 8 ArSO$_2$F-DNA with aniline	-	2019 [121]
BrettPhos Pd G3[a]	0.25 mM ArX-DNA, 50 mM aniline, 1.25 mM BrettPhos Pd G3, 125 mM CsOH, 50/50 H$_2$O/1-methoxy-2-propanol, 20 min, 80 °C; Cysteine Pd scavenger	222 anilines (yield not provided)	7.5×10^7	2019 [98]
tBuXPhos Pd G3[a]	0.74 mM ArX-DNA, 112 mM ArNH$_2$, 112 mM NaOH, 11 mM tBuXPhos Pd G3, 2 h, 60 °C	867 ArNH$_2$ with one ArI-DNA and one ArBr-DNA. 471 ArBr-DNA with 3-fluoro aniline.	4×10^7	2019 [49]
tBuXPhos Pd G3[a,b]	0.8 mM ArI-DNA, 63 mM amine, 230 mM NaOH, 1 mM tBuXPhos d G3, 1 h, 80 °C	-	-	2019 [54]
Pd-PEPPSI-*i*Pent[Cl], ascorbate[a]	0.25 mM ArX-DNA, 250 mM CsOH, 5 mM ascorbate, 0.5 mM Pd-PEPPSI-*i*Pent[Cl], 15 min, 95 °C; Cysteine Pd scavenger.	ArBr-DNA with 328 anilines and 92 2° amines; PyBr-DNA with 292 anilines and 92 2° amines	6.2×10^7	2020 [127]

[a] Analysis of DNA conjugates by HPLC(A260) and LCMS.

[b] Quantitative analysis of DNA compatibility by qPCR.

Table 5.

CuAAC Conditions

Catalyst	Conditions	Scope Investigated	Library	Year
CuCl	2 μM alkyne DNA, 100 mM RN$_3$, 10 mM CuCl, 9:1 MeCN:H$_2$O	one alkyne-DNA, 2 R-N$_3$	-	2011 [57]
TBTA, CuSO$_4$, ascorbate	0.5 mM alkyne-DNA, 0.5 mM N$_3$-DNA, 1 mM CuSO$_4$, 2 mM ascorbate, 0.5 mM TBTA, O/N, RT	3′-propargyl-DNA and 5′-N$_3$-DNA	3.3 × 10^8	2015 [63]
TBTA, CuSO$_4$, ascorbate	Solid-phase: 40 mg alkyne-resin (17.2 μmol resin, 4 mM sites), 16 μM N$_3$-DNA, 4.7 mM CuSO$_4$, 23 mM ascorbic acid, 8 μM TBTA, 50% DMSO, 30 mM TEAA, pH 7.5, 0.04% Tween 20, 4 h, 40 °	resin-bound alkyne, N$_3$-DNA	-	2015 [62]
TBTA, CuBr[a]	0.35 mM N$_3$-DNA, 2.9 mM CuBr, 11.4 mM TBTA, 42.9 mM alkyne (generated in situ from R-CHO), 16 h, RT	N$_3$-DNA with 4 alkynes (in situ generated and commercially)	-	2015 [67]
TBTA, CuSO$_4$, ascorbate[a]	Pseudo solid-phase: DEAE sepharose, 50 pmol alkyne-DNA (400 nM on resin), 200 μM RN$_3$ (generated in situ), 10 μM TBTA, 10 μM ascorbate, 0.5 μM CuSO$_4$, O/N, 45 °C; EDTA Cu scavenger.	104 RN$_3$	3.4 × 10^4	2016 [58]
CuSO$_4$, ascorbate	Intramolecular reaction: 1 mM azide-/alkyne-DNA, 3.8 mM CuSO$_4$, 3.8 mM ascorbate, 30 min, 60° C	Propargylglycine with azido acetic acid linked by variable hexapeptide	2.4 × 10^{12}	2018 [59]
TBTA, CuSO$_4$, ascorbate[a]	Pseudo solid-phase synthesis: DEAE, 0.7 nmol N$_3$-macrocyde-DNA, 20 mM alkyne, 10 mM TBTA, 2.5 mM CuSO$_4$, 10 mM ascorbate, 4 h, 25 °C	136 alkynes to constant macrocyclic scaffold	3.5 × 10^7	2018 [120]
THPTA, CuSO$_4$, ascorbate	0.13 mM propyne DNA, 12 mM N$_3$-natural product, 10 mM THPTA, 10 mM CuSO$_4$, 20 mM ascorbate, 1:1 DMSO:H$_2$O, O/N, RT	110 natural products (N$_3$-labeled by photoreactive diazarene linker)	110	2019 [121]
TBTA, CuSO$_4$, ascorbate[a,b]	Scaffold attachment: 0.21 mM alkyne DNA, 0.42 mM N$_3$-macrocylce precursor, 1.1 mM ascorbate, 1 mM CuSO$_4$, 1.1 mM TBTA, 425mM TEA buffer pH 7.2, 9:1 H$_2$O:DMSO, 20 min, RT	21 N$_3$-macrocylce precursors, 663 alkyne diversifying elements	1.4 × 10^6	2019 [47]
TBTA derivative, CuSO$_4$, ascorbate[a]	63 μM N$_3$-iodophenylalanine oligonu-cleotide, 2.5 mM alkyne, 6.3 μM Cu(OAc)$_2$, 26 μM ligand, 630 μM sodium ascorbate, 530 μM K$_2$CO$_3$, 94% aq, 6% DMSO, 3 h, 35 °C	73/116 alkynes (yield > 75%)	-	2019 [138]
THPTA, CuSO$_4$, ascorbate	1.6 μM alkyne-DNA, 400 μM RN$_3$ (generated in situ), 200 μM THPTA, 200 μM CuSO$_4$, 320 μM ascorbate, 96% H$_2$O, 4% DMF, 1 h, 40 °C	pool of 78 alkyne-DNA with 104 RN$_3$	8.1 × 10^3	2020 [61]

[a] Analysis of DNA conjugates by HPLC(A260) and LCMS.

[b] Quantitative analysis of DNA compatibility by qPCR.