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On the design of lead-like DNA-encoded chemical libraries

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ABSTRACT

DNA-encoded libraries (DELs) are becoming an established technology for finding ligands for protein targets. We have abstracted and analysed libraries from the literature to assess the synthesis strategy, selections of reactions and monomers and their propensity to reveal hits. DELs have led to hit compounds across a range of diverse protein classes. The range of reactions and monomers utilised has been relatively limited and the hits are often higher in molecular weight than might be considered ideal. Considerations for future library designs with reference to chemical diversity and lead-like properties are discussed.

1. Introduction

DNA-encoded chemical libraries (DELs), consisting of large numbers of organic compounds, covalently attached to a unique DNA sequence that can be used to decode their structure, offer great promise for the identification of small molecule ligands for proteins that can be used as chemical biological probes and start points for drug discovery.[1,2,3] DEL screening can be viewed as complementary to other techniques for finding hits, such as high throughput screening of lead-like libraries and fragment-based lead generation.[4] The advantages of DELs are manifold: because of their DNA-barcodes, libraries can be stored and screened as mixtures, greatly reducing the required resource and cost associated with processing traditional libraries; they are screened by affinity selection, which although restrictive in some aspects, has a number of advantages associated with measurement of direct binding. Perhaps the best publicised advantage of DELs, however, is their scale; libraries of potentially billions of compounds can be prepared using multicycle combinatorial approaches.[5] Hence, large numbers of compounds can be screened and the chances of finding high affinity hits against a protein target may be increased.

As with all hit finding methods, it is not possible to simply rely on scale to discover hits.[6] Whilst DELs allow access to numbers of compounds that far outstrip those accessible to traditional libraries, their scale remains insignificant compared to the theoretical size of chemical space.[7] Hence, the chemical composition of a DEL remains of critical importance and should be focused on biologically relevant space. Conceptually, a DEL may be designed for diversity or focused on a more defined region of chemical space. For a focused library, DELs are advantageous, in that deep sampling with a densely populated screening library can lead to potent, selective hits for a protein, should the area of biological activity be targeted by the DEL. Conversely, if it is not, they are not likely to yield hits, regardless of how large the libraries are.

In most applications, it is likely that chemical diversity in DELs will be desirable. Ideally, they should be focused on lead-like chemical space (low molecular weight and balanced lipophilicity)[8,9] such that hits are optimisable, accepting that the ability to screen large number of compounds increases the chances of finding hits for larger, more complex compounds.[10]

The chemical space populated by a DEL is entirely governed by the chemistry that is used in its construction: the reactions that are used to synthesise the library and the building blocks that are selected for each step. The need to carry-out DNA compatible chemistry[11] limits, to a degree, the choice of reactions that are employed, although the range of reactions that can be used is increasing.

The desire to produce large libraries might also dictate the chemical reactions that are used as large numbers of compounds can only be synthesised if large numbers of relevant monomers can be easily accessed, which tends to drive a focus on reactions that use acids or amines in the diversity step (43% of available building blocks from Enamine[12] are acids and amines, for example). Because most monomers might be considered monofunctional, the simplest library paradigms would involve sequential decorations of a central scaffold with selected monomer sets, leading to libraries with little or no scaffold diversity, in which the structural variation is derived from the monomers. Whilst this might not be a problem for individual libraries, if too many available DELs adhere to these concepts, the overall diversity of the space covered by the libraries could be limited. To investigate current practices in library design, we conducted an analysis of published DELs.

2. Dataset and analysis

A database of DELs in papers published up to the end of 2020 was compiled. Information on the maximum theoretical size of the library, the source, and any evidence of the library generating hits was recorded. Where possible, a description of the central

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scaffold of the library was derived and the type and number of monomers used in each step was collected. Instances for which this information could not be deduced from the publication were excluded. Many libraries were synthesised to exemplify methodology (in several cases just a single compound) rather than for screening, so those containing fewer than a theoretical maximum of 100 compounds were also excluded.

Whilst there are many variations on how DELs are constructed and screened, such as DNA-templated synthesis,[13,14] encoded self-assembled[15] and dynamic libraries,[16] the majority of published libraries were produced by stepwise combinatorial synthesis.[17] This is perhaps the simplest concept in DEL screening and provides the most direct route to validated hits in that the encoded hits can simply be synthesized without their DNA tag and screened in standard assays. For these reasons, they were the focus of our analysis.

A total of 62 libraries were included in the dataset, of which 28 were peptidic.[2,18-72] There was a significant increase in the publication of DELs since 2015, with 45 of the libraries published since then (Fig. 1). 30 of the libraries came from academia with ETH and Baylor being the most prominent, GSK and X-Chem were the most prominent companies. The libraries varied dramatically in size to a maximum of 10^{12} for a peptidic and 10^{9} for a non-peptidic library (Fig. 2). The average library size was $10^{6.7}$, varying significantly between industry and academia ($10^{7.9}$ vs. $10^{5.4}$). This may be a result of reduced capacity for building block sets in academia due to cost and handling capability or could reflect different motivations in each setting.

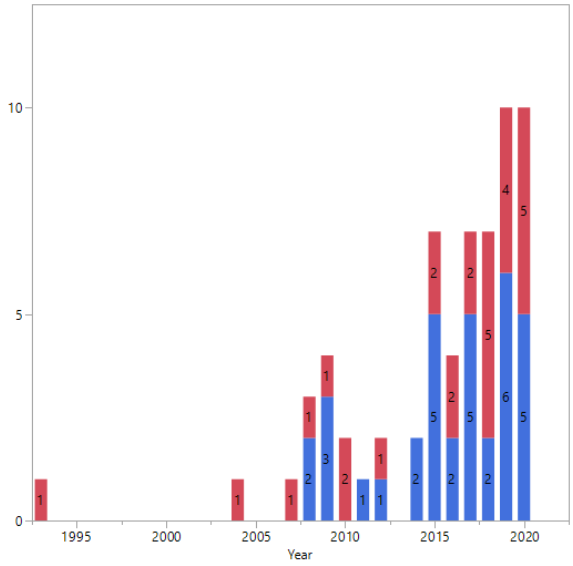


Figure 1. DELs published up until December 2020. Peptidic libraries are shown in red, non-peptidic libraries are shown in blue.

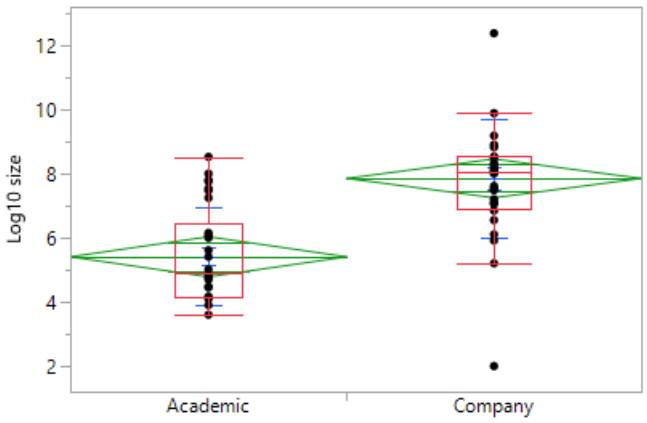


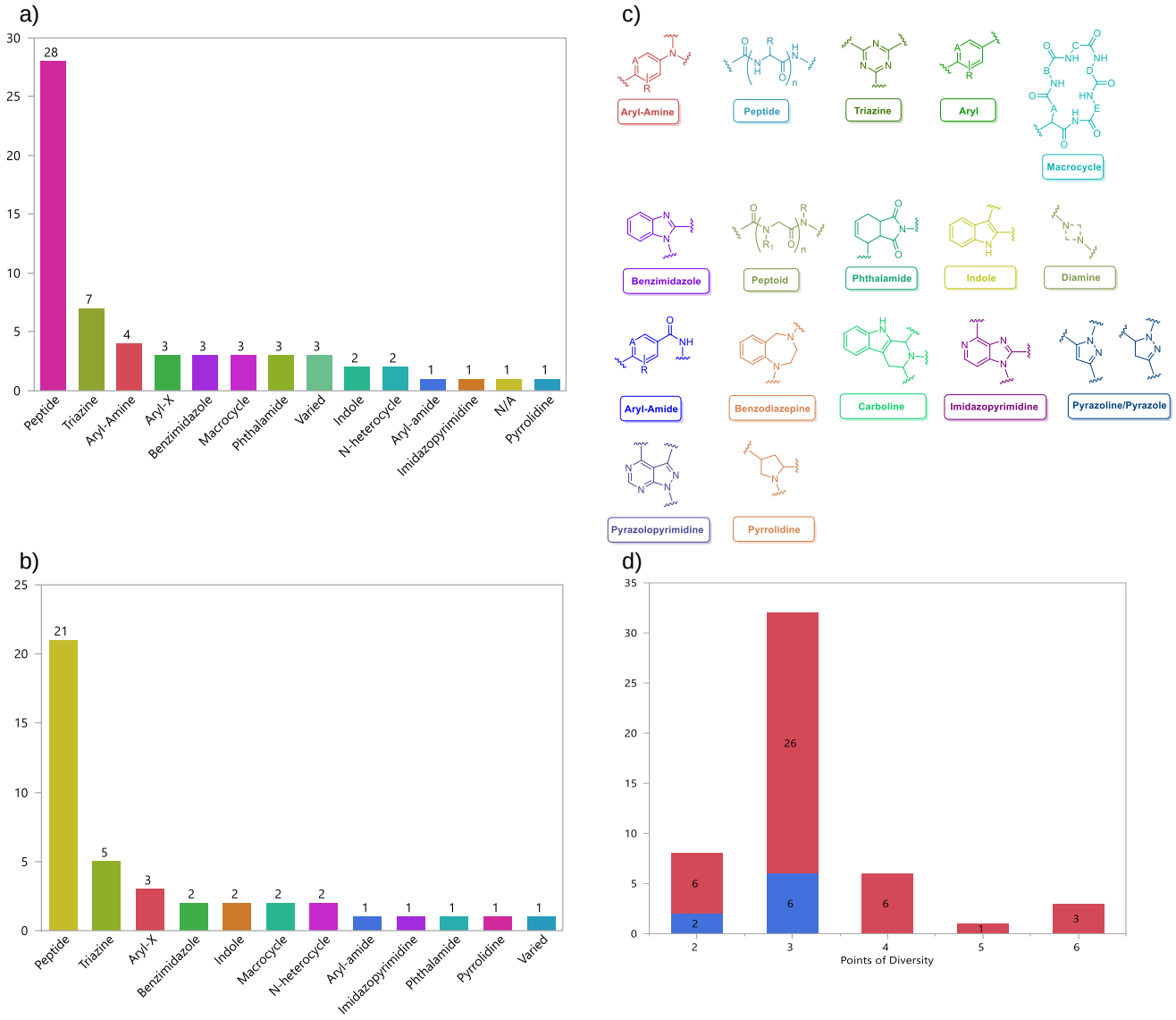
Figure 2. Distributions of library size for the academic and industrial libraries (academia mean $\log_{10}$ size = 5.4, std dev. 1.5, n=30, company mean $\log_{10}$ size = 7.9, std dev = 1.9, n=32).

Although some of the disclosed libraries are proof of concept and screening results have not been disclosed, from the libraries surveyed, hits for 55 different proteins across a range of target classes were described. The libraries that revealed hits are more likely to be published than those that do not and the results of many screening campaigns will inevitably remain undisclosed, hence it is difficult to draw firm conclusions from the hit rate. Nevertheless, this highlights a significant number of successful screens, indicating that DELs are rapidly becoming established as a robust method of hit discovery.

Producing high quality libraries requires careful design of library synthesis sequences and robust reactions to ensure the library is chemically diverse, consists of compounds with desirable physicochemical properties and is of high fidelity. This means that robust chemistry must be employed that delivers good conversions across a wide substrate scope. Chemical diversity within a DEL is a function of three components: the scaffold used in the headpiece, the monomers employed in each library step and the library synthesis scheme. Combinatorial synthesis protocols are often based on a central functionalized scaffold that is derivatised by appending collections of monomers in either a linear or branched manner. This means that in many cases the central scaffold is common to all members of the library, limiting the chemical diversity. Such a library could be considered focused rather than diverse. A focused library is not necessarily disadvantageous. If the structures are focused on chemotypes that bind the target protein, the large numbers of compounds mean that the DEL could lead to highly optimised compounds directly from the screen. Conversely, if the common element is not tolerated by the target protein, the library will not reveal hits, regardless of its size.

Many of the libraries consisted of sub-libraries, which were synthesised using differing reaction sequences on a central building block and then combined once synthesis was complete. For structural analysis, these sub-libraries were considered separately, resulting in 105 separate library synthesis protocols.

Figure 3. Common scaffolds for the 105 libraries. a) Overall frequency distribution; b) Distribution within the subset returning hits; c) Substructures; d) Frequencies of points of diversity in the scaffolds, red portion of the bars show the subset that delivered reported hits.



Each library was analysed to determine a common scaffold that was central to all library members (Fig. 3). Triazine templates have been widely exploited in the DEL field.[2] The combination of the efficiency of sequential S_NAr displacement reactions of triazine cores coupled with the wide availability of amine monomers allows large libraries to be constructed. Accordingly. triazines represented the most common non-peptidic template (9 libraries). The arylamine template was the third most common library scaffold, contributing 4 separate libraries. These arose from 3 larger libraries with differing chemical derivatisation steps.[20,27,42] 3 of the libraries were based on a simple aryl scaffold, 3 were macrocycles and most of the remainder were based on derivatisation of various heterocyclic cores.

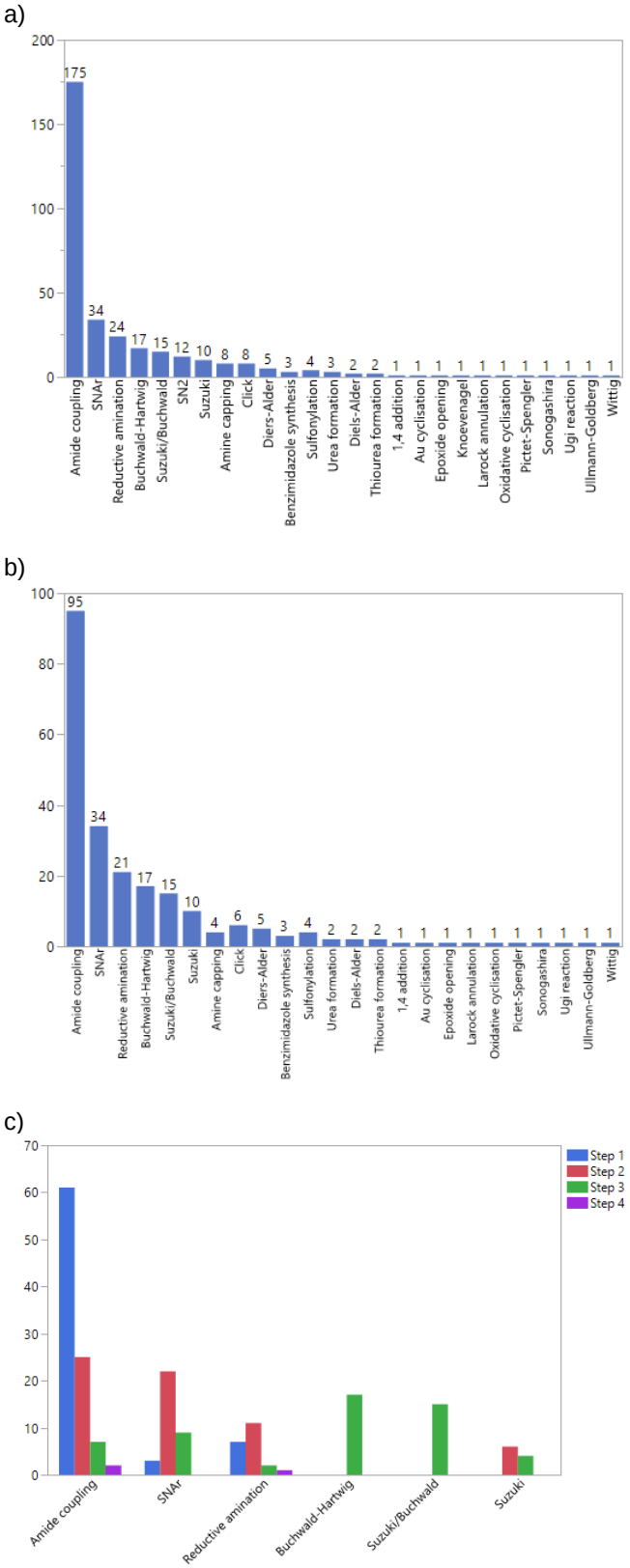
Hits were reported across a variety of different scaffolds, with the peptide libraries most commonly reporting hits (Fig. 3b). Of course, other libraries may have also given hits that have not been reported, but the distribution of scaffolds within this subset relative to the overall dataset do not indicate the presence of privileged structures and perhaps indicate that the choice of central scaffold is not an essential component of a successful DEL.

Most frequently, the libraries were based on 3 points of diversity both overall and for the subset with reported hits (Fig. 3d). Libraries with greater than 3 diversity points generally arose from multiple cycles of linear diversification of a single vector, rather than additional diversification points attached directly to the core scaffold. Interestingly, hits were reported for 75% of the libraries with 2 points of diversity, which was similar to those with 3 points (81%), suggesting that less elaborate libraries are as valuable in hit finding as larger more complex ones, as has been proposed for hit finding in general.[73,74] It is possible that the large numbers of compounds accessed by DELs mean that more complex structures can be identified more readily than from traditional screening libraries, and that the numbers involved can overcome the increased probability of pharmacophoric mismatches that are inherent in more complex structures. The observation of good hit rates with 2-point diversity libraries indicates that simpler DELs are also of value and are likely to lead to simpler, more lead-like hits (see below).

The synthesis protocols used amide coupling as by far the most common reaction both overall (34% of all reactions) and for the non-peptidic libraries (31%) (Fig. 4 and Scheme 1). The next most frequent reaction was the S_NAr, followed by reductive amination, palladium mediated coupling (Suzuki or Buchwald, sometimes used in parallel) and S_N2 reactions. Several other reactions were

used less frequently, sometimes with only a single report. Interestingly, there were distinct differences among the most common reactions regarding where in the synthesis sequence they occurred, with amide couplings often employed in the first step and palladium couplings used at the end.

Figure 4. Chemical reactions used in the libraries. a) Overall frequency; b) Frequency in non-peptidic libraries; c) Occurrence of the most common reaction types by position in the synthesis (non-peptidic).

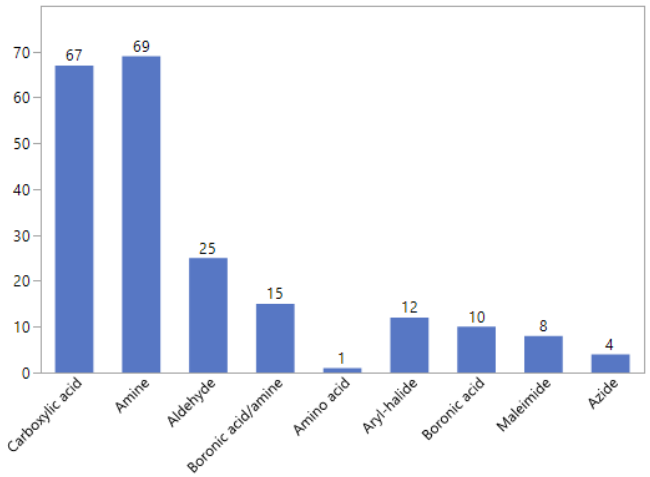


This analysis reveals a somewhat limited repertoire of reactions that are used in the construction of DELs, which may, in part, be driven by the limits of DNA-compatible reactions. However, it is qualitatively similar to the distribution of reactions used in traditional medicinal chemistry.[75,76] This is not necessarily a disadvantage, these reactions types are frequently used for good reasons and generally lead to drug-like structures. However, overreliance on a small number of reactions will severely limit the chemical space that is accessed both by constraining the structural elements formed in the reactions and by requiring the same monomer sets for each library (see below). The observation that amide couplings are often used early in the synthesis and palladium couplings are used later represents a further restriction on chemical diversity. This may be due to the amide coupling being the most established method to attach the first monomer to the headpiece and may also be related to the lack of availability or compatibility of polyfunctional monomers for other reaction types, such as palladium mediated couplings.

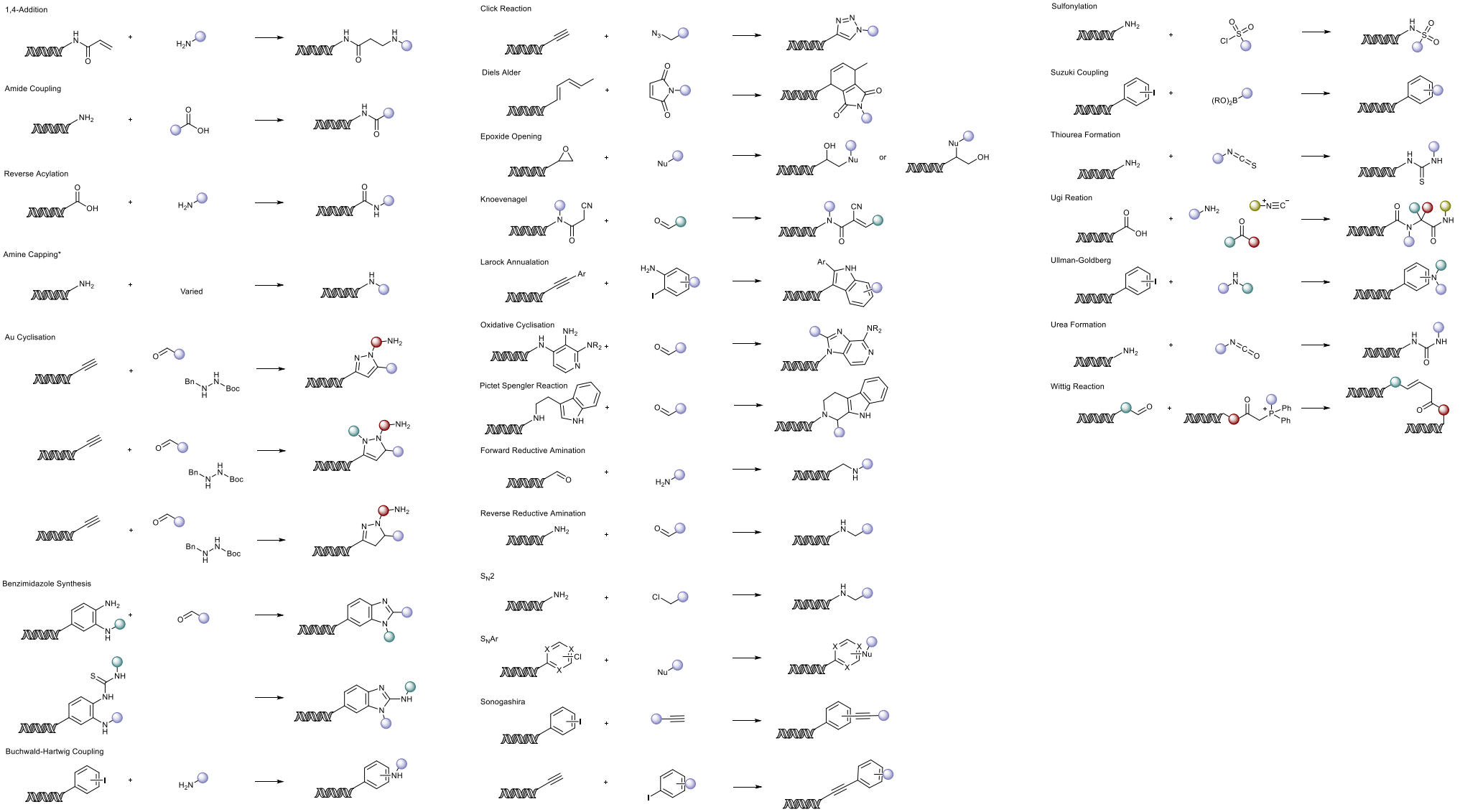
The monomers employed in library construction were dominated by acids and amides, which together made up 44% of the entries with aldehydes, boronic acids and aryl halides being the next most prevalent (Fig. 5). This distribution obviously parallels the reactions that have been employed in library synthesis but is also consistent with the availability of reagents. For example, acids and amines represent about half the available monomers in the Enamine catalogue (55k amines and 33k acids).[12]

Reported hit compounds from the surveyed libraries were extracted and their physicochemical properties were analysed (Fig. 6). The hits were generally of higher molecular weight than would be ideal for lead-like start points (median 572 Da, 76% above 500 Da. The average clogP values were closer to the ideal range (median 2.8) although 26% had values above 5. Hits also tended to populate ranges of hydrogen bond donors / acceptors and polar surface area that was higher than ideal. This distribution is consistent with previous reports on the properties of DEL hits and with the expected distribution of properties within the DELs, which is a consequence of the library synthetic strategies and the selection of large numbers of building blocks, the diversity of which necessitates selection of some compounds of higher molecular weight.[10,77]

Figure 5. Most common monomers used in non-peptidic DELs.



Scheme 1. Schematics for chemical reactions used in the synthesis of the libraries.



3. Discussion

The observations presented highlight the recent rapid expansion of interest in DEL research in both industry and academia. Recent years have seen a significant increase in the number of DELs being published. Many DELs that have been constructed do not have their details published but the subset that was selected allows for meaningful insights into the chemistry used in their construction and are likely to be representative of the wider set of libraries.

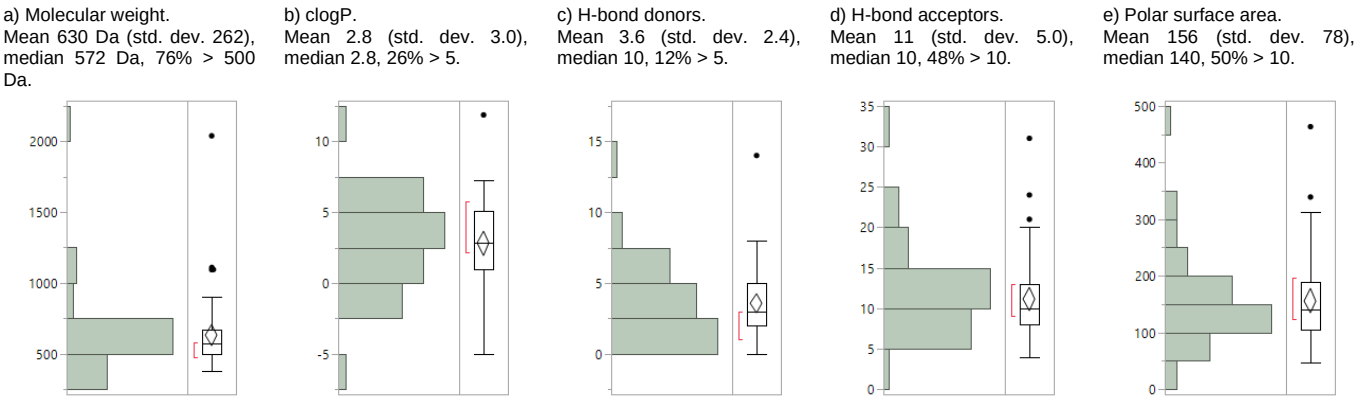
Reports of identified hits across a wide distribution of the libraries give confidence that DELs provide a robust technology for hit finding. The increase in reports of DEL synthesis and library hits over recent years we would expect this will continue in future as more organisations use the technology and success stories increase. Hence, DELs will become a primary strategy for hit finding. The observation of similar hit rates for smaller, 2-dimensional relative to those of the larger 3-cycle DELs suggest that the attraction of DELs extends beyond the ability to synthesise large numbers of compounds. Smaller, less complex DELs may be increasingly important in future, in particular for organisations lacking access to traditional high throughput screening capability.

The situation described here suggests that existing DELs have been based to a large extent on a limited number of reactions and reagents, most prominently amide coupling chemistry and acid and amine monomers. This may well be desirable: amides are prominent and desirable functionality in drug molecules, being advantageous for physical properties and pharmacophoric interactions and the large array of diverse amine and acid monomers enables very large and highly diverse libraries to be built. However, if the central tenet of a library design is the display of such reagents on a central scaffold, relying on the monomer sets to provide the diversity, there is a limit to the chemical space that can be accessed within that library.

To fully exploit DEL technology, development of a wider range of compatible chemistries will be highly desirable. Recent advances in synthetic methodology suggest that this situation will improve further in the near future.[78-84] In addition to the need for chemistry compatible with DNA, an additional constraint for an ideal reaction for DEL construction is the ability to incorporate a monomer class that is populated with a large number of diverse, readily available compounds. Further diversity could be introduced using synthetic schemes that do not rely on a single scaffold.

It was apparent that the reaction classes used in synthesis sequences differ according to their position in the synthesis, most notably with amide coupling employed in the early steps with metal mediated couplings at the final stage. This may be due to the reliability of the amide coupling for attaching monomers to the DNA headpiece but may also be due to the availability of reagents that permit this sequence, aryl halide containing acids, for example and the compatibility of the intermediates without the need for protecting groups. Different areas of chemical space could be accessed if the

Figure 6. Physical property distributions of representative hits from the DEL screens (n = 50). a) Molecular weight; b) clogP; c) Number of H-bond donors; d) Number of H-bond acceptors; e) Polar surface area.



common orders of synthetic operations were reversed, providing the required monomers were available in sufficient quantity, such as diverse boronic acids with masked amine functionality. The scope to alter the sequence order would also be expanded by expanded protecting group methodology, which has perhaps not received sufficient attention thus far.

The drive to produce large libraries has led to the incorporation of large monomers into libraries, resulting in DELs that have what might traditionally be perceived as sub-optimal physicochemical properties (high molecular weight and lipophilicity, for example). The analysis here suggests that this is often the case, with a number of hits having high molecular weight in particular. However, it is apparent from these examples that more lead-like hits can be found from DELs, which may be more desirable in some cases and highlights the value of less complex DELs with fewer points of diversity and more lead-like structures.

It has been argued that including larger monomers also has advantages,[77] since the structural elements within the monomers may be the important chemotype. Such start points do not necessarily preclude the optimisation towards traditional drug-like chemical space but require a different optimisation strategy to that adopted for lead-like hits or fragments, i.e. reduction and simplification, rather than expansion). However, it has been observed that many optimisations of DEL hits thus far have not reduced molecular weight, perhaps indicating that this is not a facile process.[10]

Higher molecular weight screening libraries might also be of value given the need to access less tractable protein targets that necessitate a compromise of drug-like properties, such as protein-protein interactions. The understanding of strategies to discover drug candidates in "Beyond rule of 5 space" is increasing with notable recent successes.[85-87] In this case, higher molecular weight start points may be advantageous and DELs could offer a very attractive means of identifying them since screening libraries focused on larger, more complex structures likely require larger numbers of compounds to maintain an acceptable hit rate.

Nevertheless, DELs centred on traditional lead-like space would also be desirable, and probably preferred in most cases. Synthesis of large libraries that achieve this focus is difficult as the combination of a central scaffold with diverse sets of monomers inevitably leads to inflated physicochemical properties, especially in libraries with more than two diversity cycles. It may not always be necessary to use large numbers of monomers, the advantages of DELs extend beyond simply the ability to make and screen lots of compounds, still, new libraries strategies for the incorporation of multiple monomers on minimal scaffolds would lead to large, more lead-like libraries. Computational tools to facilitate monomer selection that allow consideration of physicochemistry will also be of great value.[88,89]

4. Conclusion

The analysis presented here is consistent with the growing importance of DELs in hit finding, and that their establishment as a core component of drug discovery processes. The increased number of reports of successful DEL screens show that they are a robust technology for finding hits. The surveyed libraries are only based on those that are published, and we acknowledge that many remain undisclosed, nevertheless, the size of the compiled dataset is sufficiently large for meaningful conclusions to be drawn.

The survey of existing strategies for DEL construction and the properties of the resultant hits shows that existing synthesis protocols can lead to libraries that are productive in finding hits, which will be valuable to those new to the field. Our analysis of the current status of published DELs will hopefully be influential in guiding where future developments may be focused to further improve the scope of this exciting technology with regard to chemical diversity and physical properties.

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