THE DISCOVERY OF ORGANIC REACTIONS VIA HIGH-THROUGHPUT SCREENING TECHNIQUES

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INTRODUCTION

In organic chemistry methodology, high-throughput screening is often used to identify suitable catalysts or to optimize conditions for a particular reaction. Recently, researchers have begun to use high-throughput screening methods to discover novel reactions as well.

Traditional synthetic methodology development starts with a certain transformation or product in mind, and uses a combination of chemical knowledge and directed screening to find conditions that give the desired product or level of selectivity. In contrast, a reaction discovery screen aims to find new reactivity, without any preference as to what the product may be. Reaction discovery screening strategies utilize a variety of starting materials and subject them to various reaction conditions. The resulting mixtures are analyzed for the production of a novel product of any kind. Any positive results from these screens can then be run using bench-top chemistry, and new reactions can be optimized using standard techniques. This review will cover high-throughput approaches for reaction discovery based on multi-well reactors or microfluidics technology, and those based on DNA-templated methods, and it will conclude with a comparison and critical analysis of these contrasting technologies.

CHEMICAL METHODS FOR REACTION DISCOVERY

Multi-Well Reaction Discovery Strategy

In 2007, the Porco group reported the development and use of a multi-well strategy for reaction discovery.[1] Using *o*-alkynylbenzaldehyde **A** in every reaction, they varied the reacting partner (**1**-**18**), metal catalyst, and reaction temperature (Figure 1). The substrates were mixed in dichloroethane in 96-well glass plates, and the plate was mixed in a shaker for 3 hours either at room temperature or at 60 °C. Each reaction was worked up by supported-liquid extraction and analyzed by LC/MS/ELSD. Evaporative light scattering detection (ELSD) is an analytic method that uses light scattering to detect the presence of non-volatile molecules.[2] Each well that showed >20% conversion to a major product based on the ELSD trace was reproduced on a larger scale (0.2-0.7 mmol), and the major product was isolated. For complex products, computational analysis of spectra was used to assist in structure determination. This involves the input of data from low-resolution mass spectra as well as from 1D and 2D NMR experiments into a program called ACD Structure Elucidator, which uses this data to generate possible structures. Of the total 396 reactions run, 21 resulted in known reactions, 15 yielded dimers, 10

resulted in products unisolable upon scale-up, and 5 gave new transformations.

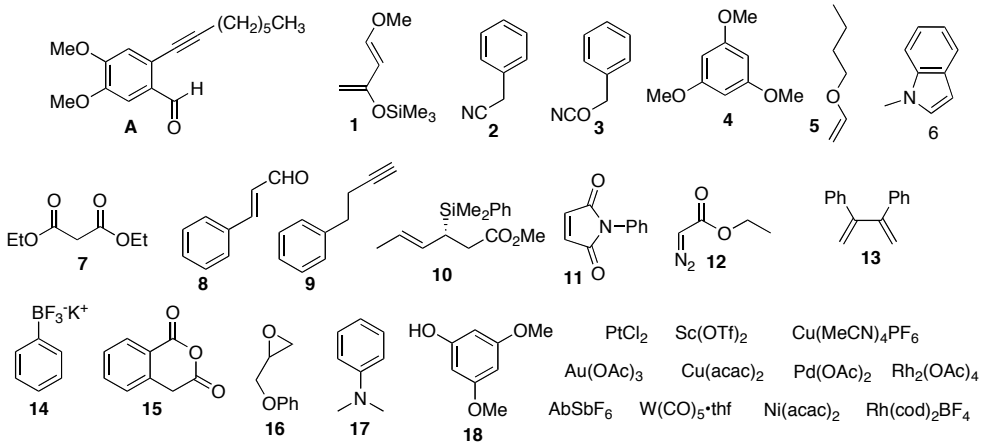

Figure 1. Substrates and catalysts used in multi-well screening

Novel dimerization of *o*-alkynyl benzaldehyde **A** was observed in many reactions, especially those employing $AgSbF_6$, $Au(OAc)_3$, $Rh(cod)_2BF_4$ and $Pd(OAc)_2$ (Figure 2a). The ring-opened dimer **21** was isolated in 65% yield upon treatment with AgOTf in acetonitrile. When the unsubstituted o-alkynyl benzaldehyde **20** was subjected to the same conditions, a different dimer (**22**) was isolated. The authors propose that both dimers are formed through a common benzopyrylium intermediate.

New products resulted from reactions with diethyl malonate **7** in the presence of $Au(OAc)_3$, $AgSbF_6$, $PtCl_2$, and $Rh(cod)_2BF_4$ and were determined to be isochromene **23** and an isochromene-derived ring-opened product **24** (Figure 2b). Reaction optimization revealed that 10 mol% $Au(OAc)_3$ with 10 minutes of microwave irradiation at 110 °C gave 62% of isochromene **23** and 20% of the ring-opened product **24** (no conditions were found where the formation of **24** was completely suppressed). When acetonitrile was used as the solvent, the same conditions gave 90% of the ring-opened product **24**.

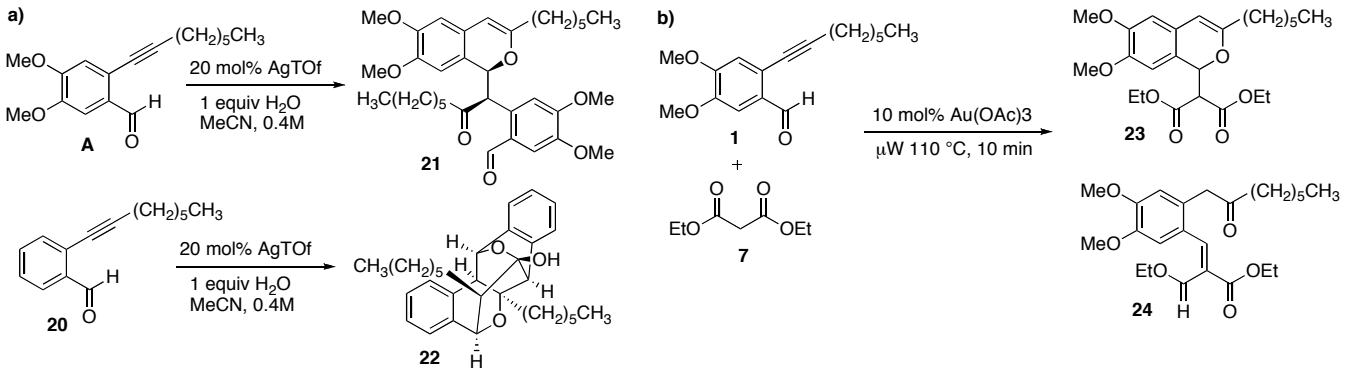

Figure 2. a) Novel dimerizations of **A**. b) Novel reactions with diethyl malonate **8**.

Investigation into the scope of this isochromene synthesis resulted in the unexpected formation of a different, more complex bicyclic ketal (**26**) product when cyclohexadione was employed (Figure

3a). Moderate yields could be obtained with β-hydroxy lactones such as hydroxy pyrone and hydroxy coumarin. This reaction produces the core structure of natural product cyclolycoserone (**27**, Figure 3d).[3]

A similar Friedel-Crafts addition pathway was credited with forming the product produced by 3,5-dimethoxyphenol (**18**) and $PtCl_2$ (Figure 3b). Optimization and use of microwave irradiation gave a 58% yield of tetracylic ketal **29**. The proposed mechanism proceeds through a benzopyrylium intermediate, which, after Friedel-Crafts addition, yields a hydrogen bond-stabilized isochromene intermediate. A 6-exo-trig cyclization gives the observed bicyclic ketal **29**. This structural motif is found in the natural product anthrabenzoxocinone (Figure 3c), which is a very potent ligand for the liver X receptor and has antibacterial activity against Gram-positive bacteria.[4]

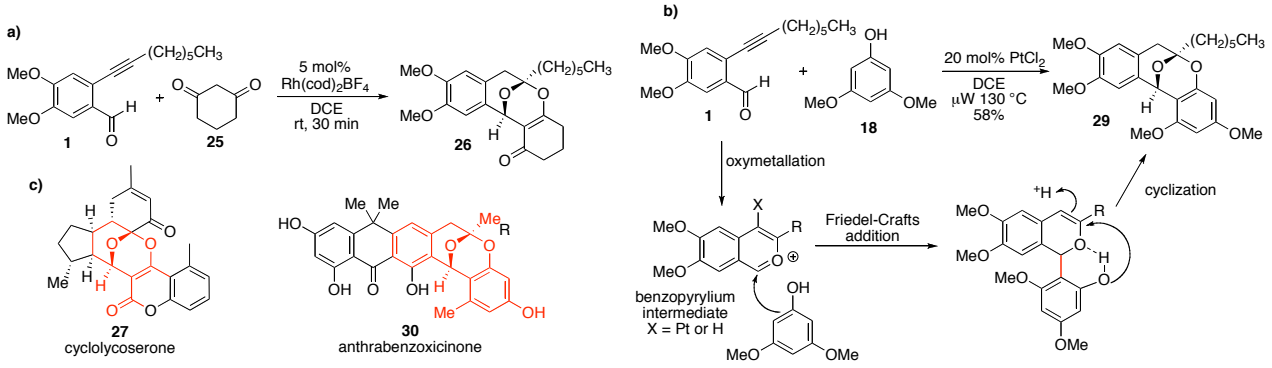

Figure 3. a) New reaction with cyclohexadione. b) Novel reaction with dimethoxyphenol and proposed mechanism d) Natural products with bicyclic ketal motifs, highlighted - core produced by new reaction

Using a multi-well approach, the Porco group demonstrated that a reaction discovery screen can successfully identify new reactions and can inspire further investigations into new methodology.

Application of Microfluidics for Reaction Discovery

A collaboration between the Porco and Jensen groups yielded an automated microfluidic method for reaction discovery screening.[5] This automated system allowed screening of over 1000 reactions in a small-scale format, and easily handled air- and water-sensitive reagents outside of a glove box.

In this reaction screening series, one of two multifunctional bicyclo[3.2.1]octanoid substrates (**31**, **32)** was employed in each reaction. These were mixed with 55 different reacting partners in the presence of either DBU or TBD under five different conditions. Each reaction mixture was "pulsed" into a stream of solvent and run through a series of zones, consisting of mixing, reaction, and quenching zones, and then analyzed by UPLC/MS/ELSD. Any reaction with an ELSD profile showing >20% conversion to a major product was scaled up on the bench, and the reaction products were characterized.

All of the primary amines tested resulted in a retro-Dieckmann-type ring-opening, creating a variety of functionalized cycloheptenones, such as **33** (Figure 4a). Additions of this type with oxygen

nucleophiles were previously reported,[6] but this is the first example of an amine addition. Reaction with an aryl magnesium bromide resulted in exclusive addition to the enone carbonyl over the bridgehead carbonyl to give a single diastereomer **35** (Figure 4b). Upon further investigation, most Grignard reagents resulted in the same addition, but allyl magnesium bromide was the only Grignard reagent that underwent multiple additions. Addition of the allyl group to both carbonyls gave intermediate **36,** which, upon heating, underwent cyclization to yield polycyclic ketal **37**. In general, the Grignard reagent addition displayed excellent chemoselectivity and gave high yields and high diastereoselectivity.

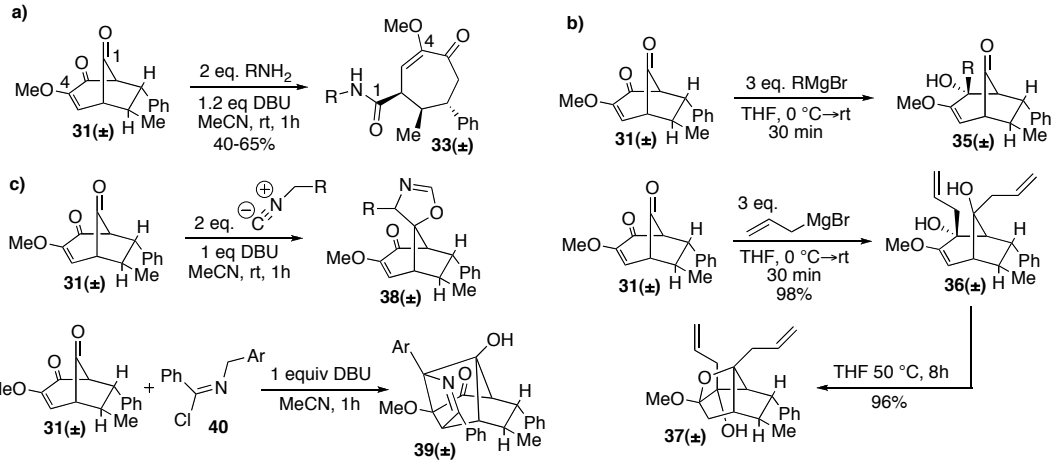

Figure 4. a) Retro-Dieckmann-type ring openings b) Grignard additions c) Cycloadditions of isonitriles

Two of the three isonitriles tested in the screen gave [3+2] cycloaddition products with the bridgehead ketone, yielding spirooxazolines **38** with no diastereoselectivity (Figure 4c). Unfortunately, these products were very prone to hydrolysis, and one was too unstable on silica to be isolated. Since the isonitrile without an α-acidic proton did not react, the authors hypothesized that the formation of an isonitrile ylide was a key step in the cycloaddition. When an isonitrile ylide (**40**) was utilized, it underwent stepwise condensation to form the complex polycylic imine **39**.

The automated microfluidic platform allowed the screening and analysis of over a thousand reactions using sensitive reagents outside of an inert atmosphere. Five different reactions were found that yielded unique and highly functionalized scaffolds, demonstrating the potential of this approach to identify reactions that produce complex structures.

DNA-LINKED METHODS FOR REACTION DISCOVERY

Hybridization-Dependent Strategy for Reaction Discovery

Over the past decade, the Liu group has developed two DNA-based methods for discovering bond-forming reactions. The first relies on hybridization of complementary strands of DNA to bring specific pairs of substrates together and to identify which pairings resulted in a bond-formation (Figure

5).[7] Each substrate in pool B was attached to an oligonucleotide containing a unique identifying region.

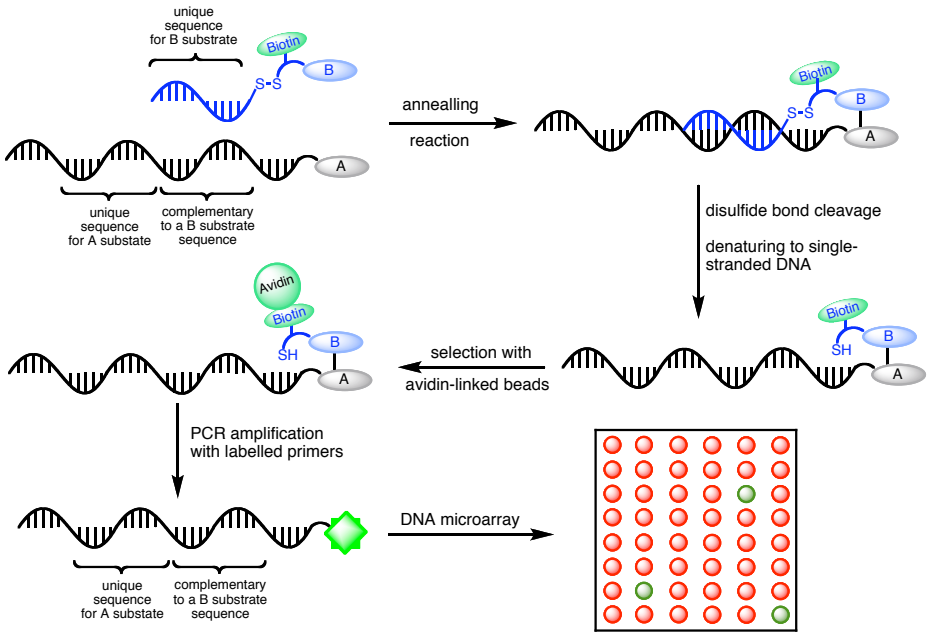

Figure 5. DNA-templated bond-forming reaction selection

A spacer between this oligonucleotide and substrate B contained a disulfide linkage and an appended biotin. Each substrate from pool A was attached to an oligonucleotide containing a unique identifying region and a region complementary to a unique sequence for one of the pool B substrates. Thus, a different oligonucleotide must be prepared and attached to each pool A substrate for every possible combination of A and B substrates.

All of the DNA-linked substrates from pools A and B were mixed in aqueous buffer in one pot and exposed to various reaction conditions. The conditions were chosen such that the complementary oligonucleotides were able to anneal and bring the substrates close together in a predetermined manner. After a certain reaction time, the disulfide bonds were cleaved and the DNA strands separated, leaving a single oligonucleotide attached to both the product and the appended biotin only in those cases where an A and a B component were joined together. Streptavidin beads were used to isolate these oligonucleotides, and after PCR amplification with fluorophore-labeled primers, the mixture was incubated with a DNA microarray.

The DNA microarray used in this study was prepared such that each spot corresponded to a specific oligonucleotide containing both the A substrate coding region and the B substrate complementary region. Spots which showed a strong signal from the post-selection fluorophore suggested a bond-forming reaction. The substrate pairings identified from this microarray were then repeated both in DNA-templated form and in a DNA-free format.

A screen utilizing palladium(II) uncovered a palladium-mediated alkyne/alkene coupling. To test this reaction without DNA but with the same "tethering", a linear substrate with an alkyne and an alkene at opposing ends was prepared (Figure 6, **40**). Optimized conditions using 5 mol% Pd(II) with 1 equivalent of copper(II) chloride in 9:1 THF:water gave a 91% yield in just 4 hours. In a subsequent publication, the Liu group reported an intermolecular version of this reaction, coupling ynamides to terminal alkenes (Figure 6b).[8]

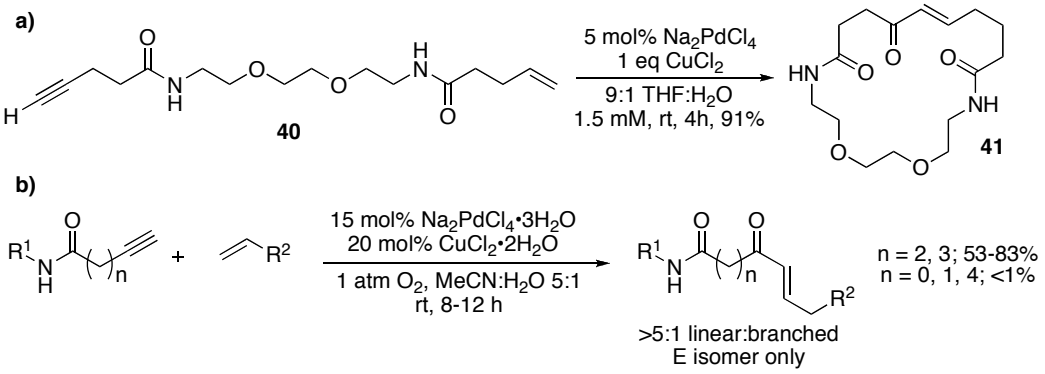

Figure 6. a) Palladium-catalyzed macrocyclization b) intermolecular enyne coupling

Hybridization-Independent Strategy for Reaction Discovery

As the requirement for DNA hybridization limits the conditions under which reactions can be carried out, the Liu group developed a hybridization-independent version of this strategy that is compatible with organic solvents and elevated temperatures (Figure 7).[9] In this system, one substrate from each pool was appended to a single-stranded oligonucleotide containing a coding region for each substrate. The pool A substrate was attached through a linker to a modified adenine. As before, the pool B substrate was attached to the DNA though a biotin-containing disulfide linker, which is cleaved after the reaction. The same sequence of isolation with avidin beads, PCR amplification, and incubation with a DNA microarray gave a fluorescence read-out as did the original method. Again, only in those cases where an A and a B component have been joined together is a signal observed.

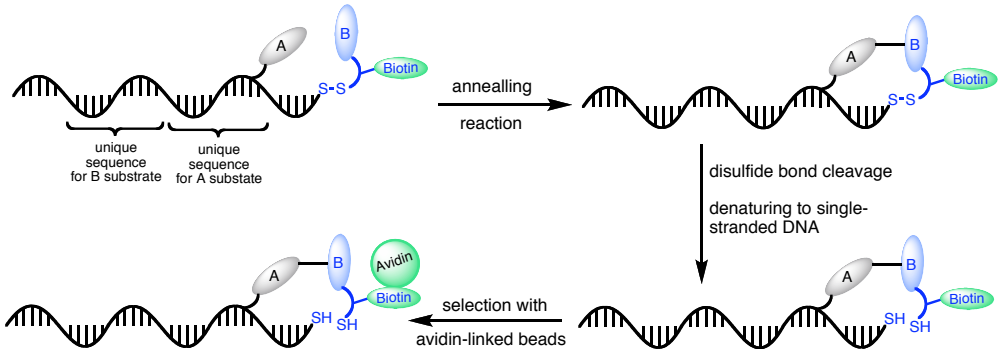

Figure 7. Hybridization-independent bond-forming reaction selection

A screen with gold(III) revealed a gold-catalyzed indole-styrene coupling (Figure 8). The only previous example of this reaction was a platinum-catalyzed reaction that resulted in an inseparable mixture of isomers (Figure 8b).[10] Optimization of the non-DNA-linked coupling gave high yields of the

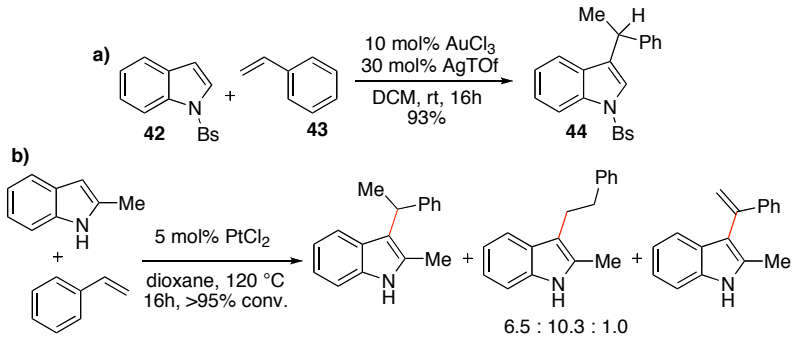

Figure 8. a) Au-catalyzed alkene hydroarylation b) Pt-calayzed alkene hydroarylation

Markovnikov product (90-91%) with either 10 mol% $AuCl_3$ and 30 mol% AgOTf, or with 5 mol% TfOH alone. The alkene scope was found to extend to substituted styrenes and 2-methyl-2-pentene, but not to olefins such as 1-pentene and cyclohexene.

The Liu group recently reported the development and validation of a reactivity-dependent PCR selection method for reaction discovery that eliminates the need for selection of bond-forming pairings with streptavidin beads.[12] Since this method relies on hybridization for selection, it is not as applicable for the discovery of organic reactions and thus will not be discussed further.

EVALUATION OF APPROACHES, AND CONCLUSION

Critical Evaluation of Approaches

One measure by which to assess these various reaction discovery methods is the scale on which the screen reactions are run. Undoubtedly, the DNA-templated methods require the smallest amounts of substrate. This is due to the use of PCR, which allows the reaction to be run on femtomoles of material, but the unique sequences corresponding to successful reactions to be amplified up to a level detectable by microarray. The microfluidic and multi-well methods run each reaction on the 1 and 5 micromole scale, respectively, which is still less than most directed reaction screens which use 50-100 micromoles.

Additionally, unique substrate restrictions apply to each screening method. The multi-well approach has not been shown to be amenable to highly air- or water-sensitive reagents. In the microfluidic approach, solubility in the chosen solvent is required, and metals may not always be tolerated. The DNA-templated method requires a functional group handle with which to link the substrate, and this method cannot be used with substrates that readily react with DNA.

The synthetic and instrumental overhead required to conduct a reaction discovery screen will

also help determine how readily it can be adopted by other laboratories. While the multi-well and microfluidic methods can be carried out with commercial materials, a suitable tandem separation/analysis instrument is required to analyze the outcome of each reaction. Although microreactors are available commercially, a customized system may be required to achieve the same level of automation and/or specialized handling described here. The necessity of covalently linking substrates to a separately prepared DNA oligonucleotide adds significant synthetic overhead for each substrate, although small amounts can be used thousands of times in DNA-linked screens before re-synthesis is necessary. Preparation of a custom DNA microarray slide is also required for this method.

Conclusion

While most methodology development and reaction screening concentrates on obtaining a specific outcome, high-throughput screening for reaction discovery has a much broader goal: to identify new reactions. Approaching reaction discovery without bias towards the products allows for a unique exploration of chemical reactivity and the discovery of diverse sets of reactions. While directed efforts towards specific reactions have a higher probability of success, reaction discovery screens have the potential to give unexpected and unusual outcomes, which offsets their lower success rates.

Multiple methods for high-throughput reaction discovery have been reported and each has demonstrated its ability to discover unique and robust reactions. Even from the small screens conducted in these initial publications, a wide variety of unique reactions were discovered. These initial discoveries have provided starting points for further investigations into the scope and limitations of each methodology. Very likely, the best is yet to come.

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