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Evolution of a Strategy for Preparing Bioactive Small Molecules by Sequential Multicomponent Assembly Processes, Cyclizations, and Diversification[1]

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Abstract: A strategy for generating diverse collections of small molecules has been developed that features a multicomponent assembly process (MCAP) to efficiently construct a variety of intermediates possessing an aryl aminomethyl subunit. These key compounds are then transformed via selective ring-forming reactions into heterocyclic scaffolds, each of which possesses suitable functional handles for further derivatizations and palladium-catalyzed cross coupling reactions. The modular nature of this approach enables the facile construction of libraries of polycyclic compounds bearing a broad range of substituents and substitution patterns for biological evaluation. Screening of several compound libraries thus produced has revealed a large subset of compounds that exhibit a broad spectrum of medicinally-relevant activities.

Introduction: The wide range of human health disorders that lack effective agents for pharmaceutical intervention has engendered innovative approaches to creating molecular libraries for biological interrogation. These advances, coupled with the advent of high-throughput screening (HTS) technologies, has expedited the identification of novel chemical entities for development into drug leads or chemical probes to study biological systems.[1] For many years, screening decks used in HTS have been populated with compounds prepared through "combinatorial chemistry" methods, the origins of which can be traced back to Merrifield's pioneering work in which solid-phase synthesis was utilized to prepare polypeptides more efficiently and in higher purity than traditional coupling methods performed in solution.[2,3] Building upon Merrifield's work, the "split-mix" protocol[4] was introduced as a strategy to prepare large peptide libraries, after which the "one bead, one-peptide" concept[5] was reported as a means for rapidly identifying and sequencing bioactive peptides. This technology, which utilizes parallel synthesis and enables the convenient distribution of intermediates, was later extended to the synthesis of small molecule libraries, either as mixtures or as pure compounds, whereby matrices of reagent reservoirs are mixed and matched to produce the maximum number of unique chemical outputs.

The pharmaceutical industry adopted combinatorial chemistry and related methods as outlined generally in Figure 1 beginning in the 90's in hopes of expediting the drug discovery process and lead generation; however, larger screening libraries did not equate to an increase in the number of therapeutic leads as expected.[6] In retrospect, the disappointing output from the combinatorial chemistry era taught the scientific community that screening decks comprised of randomly generated molecules tend to suffer from low hit rates and poor

[1] Dedicated to Professor Richard J. K. Taylor on the occasion of his 65th birthday.

specificity,[7] a consequence stemming from non-optimal molecular parameters[8,9] and inadequate structural diversity. Accordingly, screening libraries evolved from relatively simple compound sets constructed from readily available building blocks, a so-called "strength in numbers" approach,[10] to judiciously designed assortments of drug-like collections that are characterized by relatively improved hit rates and lower attrition during subsequent development efforts.[11]

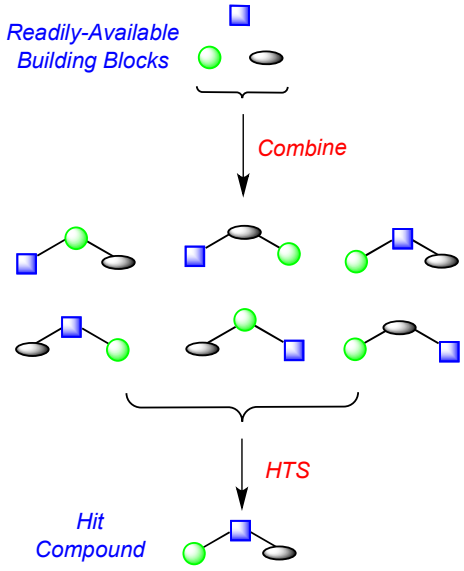
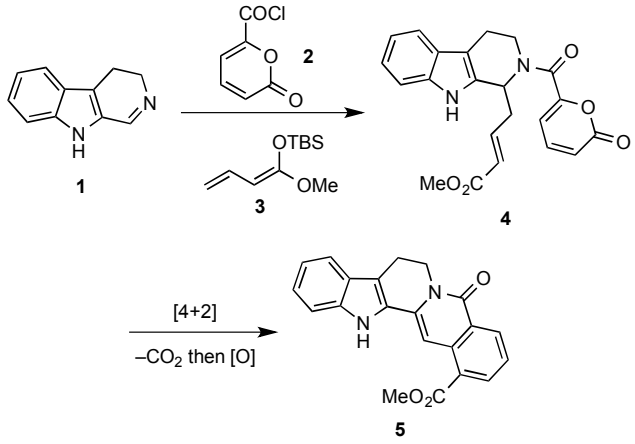


Figure 1 Typical combinatorial chemistry approach to hit identification.

Various strategies for creating novel collections of small molecules with potentially valuable biological properties have been reported,[12,13] with biology-oriented synthesis (BIOS)[14] and diversity-oriented synthesis (DOS)[15] emerging as two of the more successful ones. The BIOS approach, which was first introduced by Waldmann and co-workers in 2006, capitalizes on the biologically pre-validated cyclic, especially heterocyclic, scaffolds of natural and non-natural compounds. These frameworks then serve as starting points for structural and functional diversification to explore chemical space and to generate libraries of new substances for screening. In some respects BIOS thus represents an expansion of the related use of privileged scaffolds,[16] or molecular frameworks that bind to multiple proteins, an approach to small molecule discovery that was first described over 20 years ago. The goal of the DOS-based strategy is to create skeletal frameworks that broadly populate chemical space, especially less explored regions, with the objective of discovering new chemotypes that modulate biological pathways and probe the effects of specific protein targeting. Applications of this approach provide access to diverse, structurally- and stereochemically-complex sets of drug-like compounds from simple chemical building blocks.[17] When taken separately, the strategies of BIOS and DOS have enabled the discovery of several therapeutic lead compounds.[18,19]

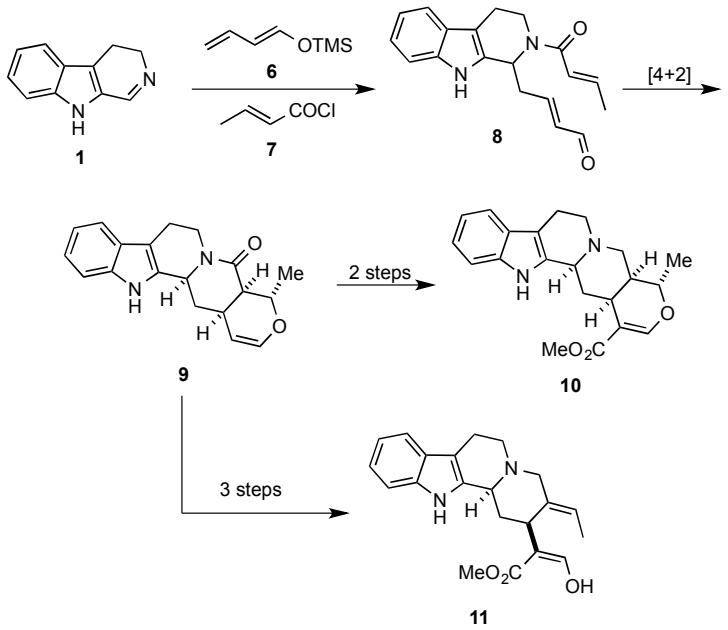
Multicomponent reactions (MCRs) enable the creation of multiple new bonds between a number of simple chemical inputs in a single step.[20] The potential to quickly access versatile intermediates, coupled with the efficiency and operational simplicity associated with such transformations, has inspired the development of a multitude of MCRs.[21] MCRs that are compatible with a broad range of functional groups are especially valuable because they present an opportunity for performing a variety of post-MCR transformations, such as cyclizations and refunctionalizations.[12,22] The sequencing of MCRs with subsequent ring-closing reactions is commonly referred to as the build/couple/pair strategy,[23] and its use has led to the generation of diverse heterocyclic scaffolds in short synthetic sequences.

Some years ago we became interested in developing improved strategies for discovering novel, bioactive compounds as a consequence of our discovery of a novel multicomponent reaction in which an imine, an acylating agent, and a π-nucleophile are combined in a vinylogous Mannich reaction to give intermediates that could be quickly elaborated into alkaloid natural products. For example, the key step in our synthesis of the pentacyclic indole alkaloid oxogambirtannine (**5**) involved a three-component assembly process in which the



Scheme 1 Total synthesis of oxogambirtannine **5**.

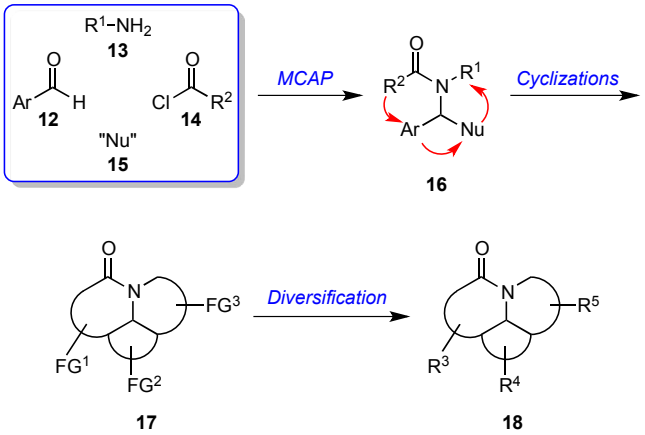
dihydro-β-carboline **1**, the acid chloride **2**, and the vinyl ketene acetal **3** were combined to give adduct **4** (Scheme 1). An intramolecular Diels-Alder reaction followed by spontaneous extrusion of CO_2 and subsequent oxidation completed the three step synthesis of oxogambirtannine (**5**).[24,25] The power of such three-component assembly processes is further illustrated by the reaction of the dihydro-β-carboline **1** with 1-trimethylsilyloxybutadiene (**6**) in the presence of crotonyl chloride (**7**) to deliver **8**. An intramolecular hetero Diels-Alder reaction transformed **8** into **9**, a key intermediate in extraordinarily concise syntheses of the heteroyohimboid alkaloid tetrahydroalstonine (**10**) and the corynantheoid alkaloid geissoschizine (**11**) (Scheme 2).[25,26]



Scheme 2 Total syntheses of tetrahydroalstonine (**10**) and geissoschizine (**11**).

The dramatic ease with which these three-component processes gave access to the complex heterocyclic frameworks found in biologically active alkaloids suggested that they might be easily adapted to develop a useful strategy for the preparation of diverse collections of small molecules. We envisioned that the strategic combination of certain aspects from BIOS and DOS would enable the assembly of such libraries via similar multicomponent assembly processes (MCAPs) (Scheme 4). MCAPs are differentiated from traditional MCRs and other domino or cascade processes[27] in that the reactants must be combined, at least in part, in a specific order rather than all at once to ensure selective chemical transformations.[12,28] In particular, we envisioned a MCAP in which aryl aldehydes **12** and primary amines **13** are first combined to give intermediate imines that then undergo reactions with acylating (or alkylating) agents **14** and nucleophiles **15** to give intermediate aryl aminomethyl derivatives **16**. It is noteworthy that aryl aminomethyl substructures in **16** are well-represented in natural products and many classes of medicinally-relevant therapeutics.[29] The four inputs in the MCAP may be programmed for further manipulation by the presence of orthogonal functional groups that can be paired in selective ring forming reactions to increase skeletal complexity and generate a series of target scaffolds **17**. For example, an aryl aldehyde might bear a variety of functional groups, including chloro, bromo, azido, and carboxyl. Carbon-carbon double and triple bonds might be easily incorporated into the amines and the acylating agents, and the nucleophiles could be functionalized organometallic reagents or a number of different π-nucleophiles. After the ring-forming reactions have been performed, the remaining functional groups on the heterocyclic scaffolds **17** may be used in cross-coupling and other functional group transformations to increase structural diversity and provide diverse compound collections **18** that maximally populate chemical space. The

modular nature and functional group compatibility associated with this approach insures that a broad range of substituted heterocyclic compounds will be accessible.



Scheme 3 Synthesis of diverse collections of small molecules via MCAPs.

Although the seeds for this novel entry to small compound collections had been sown during our efforts in alkaloid total synthesis, these concepts lay fallow until there was a motivation to develop the approach. The inspiration to access sets of structurally diverse, drug-like compounds for biological screening came with our involvement in the NIH Molecular Libraries Initiative (MLI). The goal of this important program was to discover new molecular probes and potential drug leads by screening collections of compounds within the Molecular Libraries Probe Production Center Network (MLPCN). In this report we highlight selected examples of synthetic approaches to a variety of scaffolds containing biologically pre-validated heterocyclic substructures, including substructures **19**-**28** (Figure 2), and we illustrate how these compounds may be transformed into novel derivatives that exhibit potentially useful bioactivities. Varying the nature of the functional groups present in the starting inputs for the MCAP is a critical design feature that enables the selective programming of available cyclization reactions in the post-MCAP transformations. Accordingly, this review is organized by the MCAP-type and the nature of the subsequent ring forming reactions.

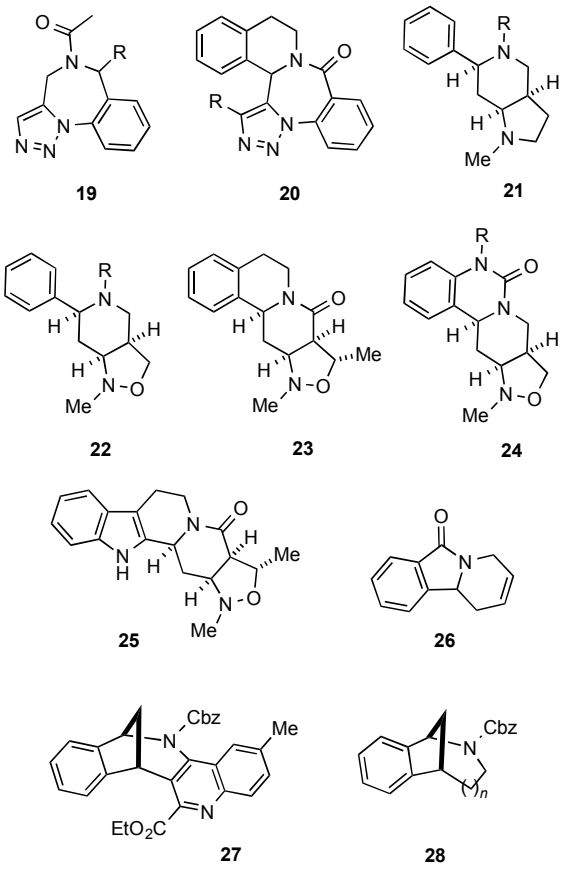


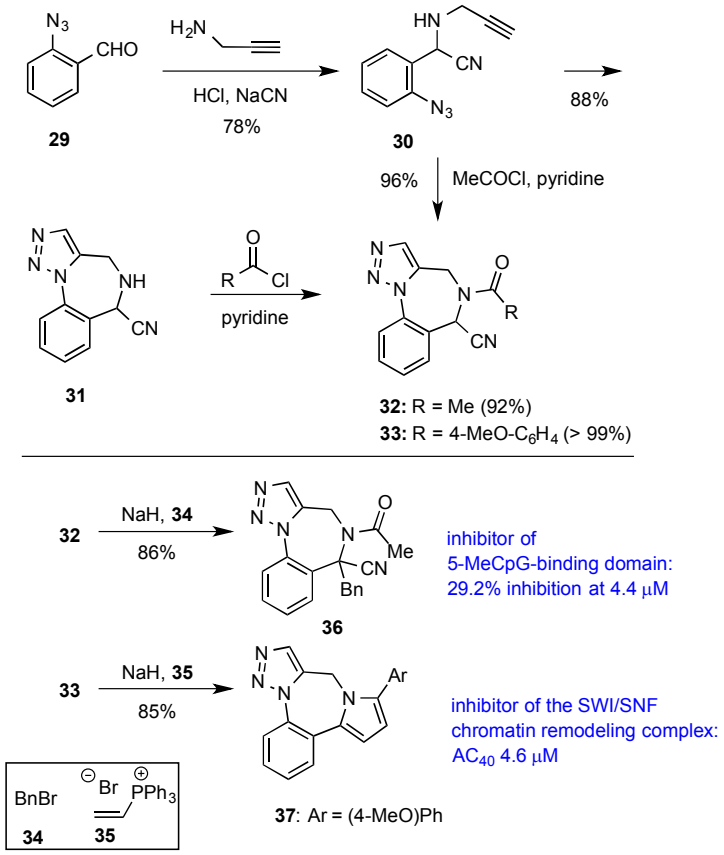
Figure 2 Tested scaffolds with confirmed biological activity.

1. MCAP followed by Dipolar Cycloadditions

Dipolar cycloadditions have long been employed to construct heterocyclic subunits that comprise both natural products and non-natural pharmaceutical agents.[30] Dipolar cycloadditions have the capacity to form multiple bonds and stereocenters to generate products having greater complexity than their precursors, a feature that contributes to the high synthetic utility of the transformation. Because of the broad variety of 5-membered heterocycles present in biologically-important compounds, we sought to pair dipolar cycloadditions with MCAPs as a means to access polycyclic products bearing such structural subunits. Some representative examples of the use of this strategy to prepare novel collections of bioactive compounds are outlined herein.

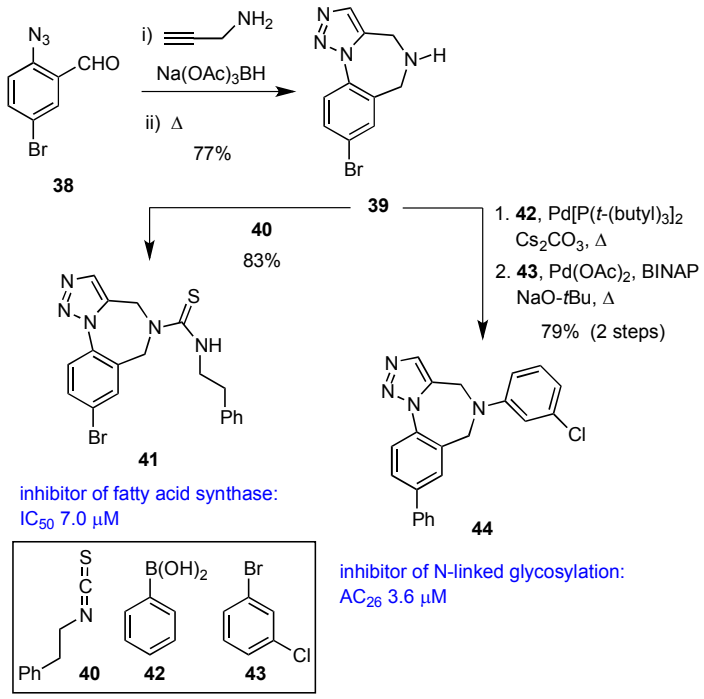
***a. Azide-Alkyne Dipolar Cycloadditions*.** In our early work[31,32] we applied the Huisgen [3+2]-dipolar cycloaddition[33] to the synthesis of 1,2,3-triazolo-1,4-benzodiazepines,[34] and building upon these findings, we developed an MCAP/cyclization sequence to prepare several series of novel members of this structural class. In one version of this approach, cyanide ion was employed as the nucleophilic partner in a MCAP involving propargylamine and a 2-azidobenzaldehyde, such as **29,** to produce the α-aminonitrile **30** (Scheme 4),[35,36] which underwent a Huisgen dipolar cycloaddition when heated in toluene to afford the 1,4-benzodiazepine **31**. It is noteworthy that *N*-acetylation of the acyclic α-aminonitrile **30** facilitated spontaneous [3+2]-dipolar

cycloaddition to give the tricyclic amide **32** in a single step. Alternately, the free amine in **31** could be derivatized with a variety of *N*-functionalizing agents to provide a range of analogs, including amide **33**. The acidic nature of the proton alpha to the cyano group provided other possibilities for generating derivatives. For example, the anion of **32** was alkylated with benzyl bromide (**34**) to afford the benzodiazepine **36**, and the anion of **33** was also allowed to react with vinyltriphenylphosphonium bromide (**35**, Schweizer's reagent) to produce the pyrrole-fused benzodiazepine **37** through an addition/intramolecular Wittig reaction/elimination sequence.[37]



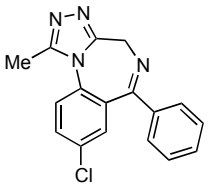
Scheme 4 Synthesis of triazole-fused benzodiazepines **36** and **37**.

In a simple variant of this approach, the brominated scaffold **39** was obtained through a reductive amination of bromoaldehyde **38** and subsequent thermally-induced dipolar cycloaddition (Scheme 5). The aryl bromide and the secondary amine in **39** were both obvious embarkation points from which analogs for biological evaluation could be obtained by cross-coupling and *N*-derivatization reactions, respectively. Treatment of amine **39** with electrophiles, such as phenethyl isothiocyanate **40**, gave quick access to a range of benzodiazepine analogs, including thiourea **41**. Alternately, palladium catalyzed arylations were attractive reactions for installing aryl groups on the nitrogen atom and the aryl bromide of **39** to afford biaryl derivatives as typified by **44**.



Scheme 5 Benzodiazepines **41** and **44**.

The 1,4-benzodiazepine ring system is present in a large number of pharmaceuticals, of which alprazolam (**45**) is a notable example as it is reported to be the most prescribed psychotropic drug in the Unites States (Figure 3).[38] 1,4-Benzodiazepines are potent binders of GPCRs and ligand-gated ion channels[39] and are recognized as prominent frameworks in medicinal chemistry.[40] Significantly, the triazolobenzodiazepines generated with the synthetic transformations described in schemes **4** and **5** represent novel members of the benzodiazepine class, the screening of which has led to the discovery of a number of compounds having diverse biological activities. For example, the α-amidonitrile **36** (Scheme 4) was identified as an inhibitor of 5-meCpG-binding domain protein 2 (MBD2)-DBD binding to methylated oligonucleotide.[41,42] Hits identified in this screen have potential therapeutic applications in oncology and may be utilized as inhibitors of epigenetic gene silencing mediated by MBD2, thus facilitating reactivation of silenced genes in cancer cells leading to restored gene function. The pyrrole-fused benzodiazepine **37** (Scheme 4) was found to be an inhibitor of the SWI/SNF chromatin remodeling (SCR) complex.[43] Because of their essential role in pluripotency, human tumor suppression, and cellular senescence,[44] small molecule SCR complex inhibitors might be utilized as chemical tools to study stem cells, chromatin remodeling, and cancer.



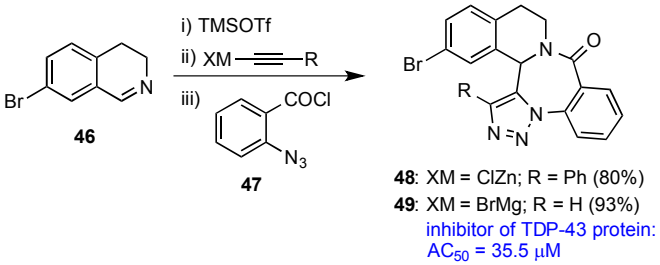
alprazolam (**45**)

Figure 3 Triazolobenzodiazepine **45.**

Screening efforts also revealed that benzodiazepine **44** (Scheme 5) possessed inhibitory activity towards *N*-linked glycosylation (NLG), a protein modification process that is critical to the stability and proper folding of transmembrane proteins.[45,46] Receptor tyrosine kinases are a subclass of *N*-glycosylated enzymes that are implicated in neoplastic development and resistance to chemotherapies. The drug tunicamycin reduces protein tyrosine kinases via NLG, yet its use as a therapeutic agent is limited due to hepatotoxicity.[47] Hits identified in this screen may aid the development of novel, non-toxic NLG inhibitors as potential chemotherapeutic agents that improve outcomes in radiation therapy.

HTS screening revealed the thiourea **41** (Scheme 5) to be an inhibitor of fatty acid synthase (FAS),[48] an enzyme that is overexpressed in a variety of cancer cell types and is essential for the growth of solid tumors.[49] As there are no known inhibitors of the thioesterase domain of FAS, efforts are underway to discover novel small molecules with inhibitory activity towards this subunit of the enzyme. Novel FAS inhibitors will serve as tools to probe the therapeutic potential of FAS modulation.

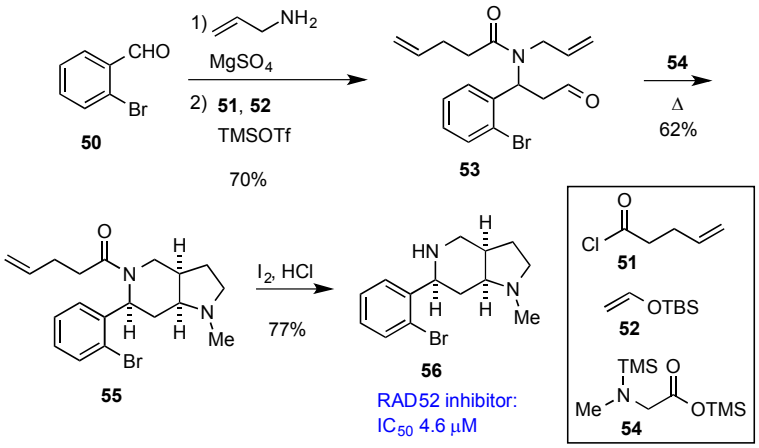
An azide/alkyne cycloaddition also served as a key step for preparing other bioactive scaffolds. For example, employing metal acetylides as nucleophilic partners in MCAPs affords intermediates that can be quickly elaborated into polycyclic ring systems through an azide/alkyne cycloaddition. We found that activation of the starting imine **46** with the Lewis acid TMSOTf prior to the addition of zinc or magnesium acetylides produced intermediate *N*-silyl amines that underwent acylation with *o*-azidobenzoyl chloride (**47**) (Scheme 6).[50] The amides thus produced underwent a [3+2]-dipolar cycloaddition to deliver tetrahydroisoquinoline-fused triazolo-benzodiazepines **48** and **49.**



Scheme 6 One-pot formation of pentacycles **48** and **49**.

Biological screening of **49** (Scheme 6) within the MLPCN revealed that it was an inhibitor of the transactive response DNA-binding protein-43 (TDP-43).[51] Mutations in the gene that encodes for TDP-43, the TAR DNA binding protein gene (TARDBP), are responsible for the aggregation of TDP-43 within nerve cells, a phenomenon that has become a marker for several neurodegenerative disorders including ALS and frontotemporal lobar degeneration (FTLD).[52] Compounds that modulate TDP-43 should facilitate further understanding of the complex mechanisms underlying these diseases.

b. Azomethine Ylide Dipolar Cycloadditions. In exploratory work, we found that π-nucleophiles including vinyl ethers may be employed as nucleophilic partners in reactions with activated imines that are formed as intermediates in a number of MCAPs.[31,32] The adducts thus obtained bear an aldehyde function that may be condensed with selected amines to generate 1,3-dipoles that then cyclize via dipolar cycloadditions with proximal carbon-carbon double or triple bonds to generate novel heterocyclic systems. For example, when the imine derived from 2-bromobenzaldehyde (**50**) and allylamine were treated with pentenoyl chloride (**51**) and *tert*-butyldimethyl(vinyloxy)silane (**52**) in the presence of TMSOTf, aldehyde **53** was produced (Scheme 7).[53] Heating aldehyde **53** with *bis*(trimethylsilyl)sarcosine (**54**) generated an intermediate azomethine ylide that cyclized upon heating to furnish pyrrolidine **55**. Removal of the pentenamide group was achieved under the action of iodine and aqueous HCl to provide the secondary amine **56**.[54] A diverse collection of compounds were generated through palladium catalyzed cross-coupling reactions with the aryl bromide, as well as nitrogen atom acylation and alkylation reactions (*vide infra*).

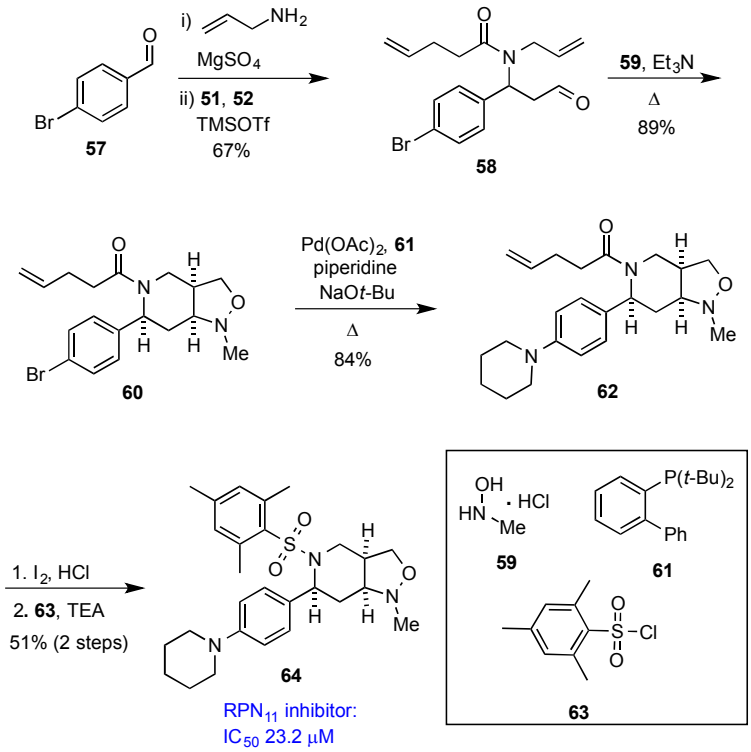


Scheme 7 Synthesis of pyrrolidine-fused piperidines.

Screening by the MLPCN revealed that the fused *N*-methylpyrrolidine **56** (Scheme 7) is a RAD52 inhibitor with an IC_{50} of 4.6 μM.[55] Mutations in breast cancer type 1 and 2 susceptibility protein (BRCA1 and BRCA2) genes are well documented indicators for increased risk in hereditary breast and ovarian cancer.[56] BRCA2 overlaps in functionality with RAD52, an evolutionarily conserved protein that assists with the repair of DNA double-strand breaks and interstrand cross-links. The recent discovery that RAD52 inhibition leads to lethality in BRCA2

deficient cells but not in healthy cells suggests that a "synthetic lethal" approach may be harnessed to treat cancers arising from BRCA2 mutations.[57] Since there are no known specific inhibitors of RAD52, a screening assay was developed to identify small molecule inhibitors of RAD52 to study the inhibitory effect on human cells and ultimately, discover lead compounds that may be developed into novel therapeutics for breast cancer.[57]

c. Nitrone Dipolar Cycloadditions. Differentially-substituted bromobenzaldehydes could be employed at the outset of the synthetic sequence to facilitate the construction of compound libraries with variation of the regiochemistry of the aromatic substituent. For example, the use of 4-bromobenzaldehyde (**57**, Scheme 8) in the MCAP gave the aldehyde **58** in analogous fashion to **53** (Scheme 7). Treatment of aldehyde **58** with *N*-methylhydroxylamine hydrochloride (**59**) under basic conditions formed an intermediate nitrone that underwent facile dipolar cycloaddition to create novel fused isoxazolidine **60**.[53] The protected nitrogen atom and the aryl bromide moieties resident in **60** were then exploited to access focused libraries for biological assessment. Exemplary of such conversions is the Buchwald-Hartwig cross-coupling of **60** with piperidine in the presence of $Pd(OAc)_2$ and JohnPhos® (**61**) to give the aniline **62**, which was *N*-deprotected and treated with mesitylenesulfonyl chloride (**63**) to provide sulfonamide **64**.

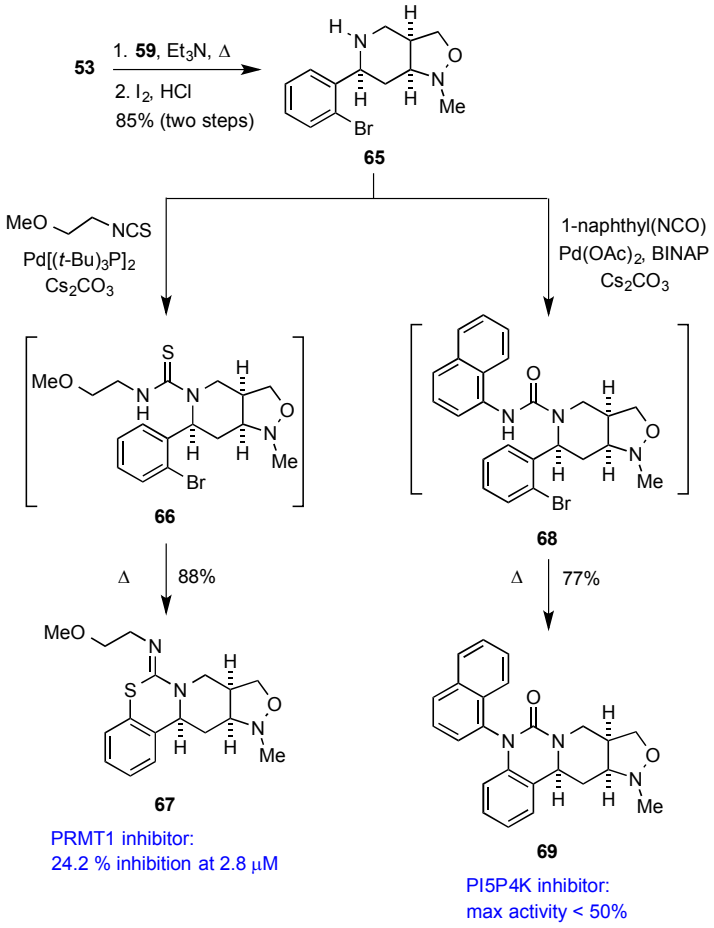


Scheme 8 Synthesis of isoxazolidine-fused piperidines.

Investigators within the MLPCN identified isoxazolidine **64** as a regulatory particle non-ATPase11 (RPN_{11}) inhibitor with an IC_{50} of 23.2 μM (Scheme 8). RPN_{11} belongs to a class of de-ubiquitinating enzymes, and it is essential to the protein-degrading activity of the proteasome.[58] RPN_{11} catalyzes the cleavage of isopeptide bonds

by employing a zinc-metalloprotease active site. Small molecule inhibitors of RPN_{11} metalloprotease are currently under investigation as novel antitumor agents, which putatively elicit their activity through inhibiting degradation of proteasome substrates resulting in stymied tumor growth.

When isoxazolidine- and pyrrolidine scaffolds contained a 2-C_{Ar} bromine atom, a second cyclization could be utilized to provide access to fused tetracyclic ring systems. This transformation is highlighted by the sequence in Scheme 9, in which the secondary amine **65**, prepared in 2 steps from aldehyde **53** (Scheme 7), was treated with 2-methoxyethyl isothiocyanate to give an intermediate thiourea **66**.[53] Upon heating in the presence of Pd(*t*-$Bu_3P)_2$, S-C_{Ar} bond formation ensued to give the 2-imino-1,3-benzothiazinane (**67**). In a related transformation, a mixture of 1-naphthylisocyanate, $Pd(OAc)_2$, BINAP, and amine **65** was stirred at room temperature until formation of the binaphthyl urea **68** was complete, whereupon the mixture was simply heated to deliver the dihydroquinazolin-2-one **69** via an intramolecular *N*-arylation.



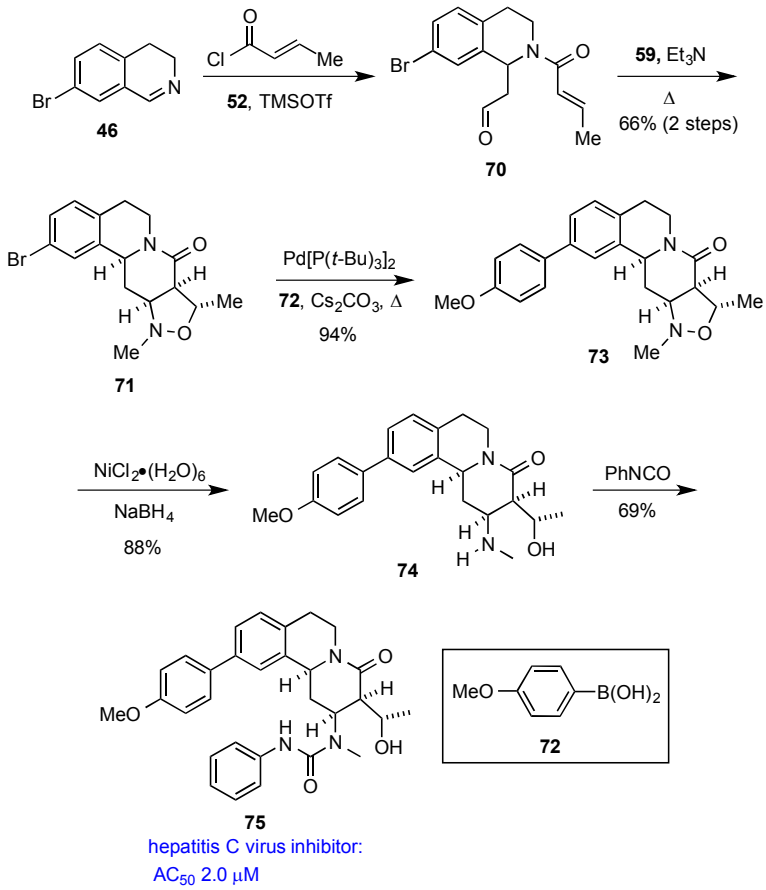
Scheme 9 Synthesis of bioactive urea derivatives **67** and **69**.

Dihydroquinazolin-2-one (**67**) was identified as an inhibitor of protein arginine methyltransferase 1 (PRMT1) (24.2% at 2.8 μM) in a HTS assay.[59] PRMT1 is responsible for over 85% of cellular arginine methylation, and improper PRMT1 function has been linked to a range of inflammatory disorders, including asthma and certain

types of cancers.[60] The identification of novel PRMT1 inhibitors will help evaluate the potential medicinal value that could be harnessed through small molecule modulation of this enzyme.[61]

An MLPCN HTS for small molecule inhibitors of the phosphatidylinositol 4-phosphate 5-kinases (PI4P5K) tentatively identified 2-imino-1,3-benzothiazinane (**69**) as an active compound.[62] The PI4P5Ks regulate AKT signaling and tumor cell growth,[63] and recent findings have illuminated PI4P5K's potential involvement in oncogenesis, suggesting that modulating its activity may be an effective therapeutic approach to treating cancers.

A nitrone dipolar cycloaddition also served as a key step to access novel tetrahydroisoquinolines as is illustrated by the preparation of **75** (Scheme 10).[50,64] A three-component MCAP involving imine **46**, crotonyl chloride, and **52** provided the aldehyde **70**. This MCAP was optimally performed using sub-stoichiometric quantities of TMSOTf, and the crude aldehyde **70** thus obtained was treated with **59** to furnish a nitrone that cyclized upon heating to deliver isoxazolidine **71** as a single diastereomer. The presence of the bromine atom on scaffold **71** enabled a variety of palladium-catalyzed cross-coupling reactions, as exemplified by a Suzuki reaction with 4-methoxyphenylboronic acid (**72**) to give the biaryl **73**. When isoxazolidine **73** was exposed to nickel boride, *N-O* bond cleavage ensued to give the amino alcohol **74**. The secondary amine in **74** was then modified to prepare a variety of analogs for screening, such as the urea **75**.



Scheme 10. Synthesis of urea **75**.

The prominence of the hexahydropyridoisoquinoline motif (**76**) in molecules with potent biological activities, ranging from opioid antagonism[65] (**77**) to α_2-adrenoceptor antagonism[66] (**78**), has made compound libraries embodying this subunit an attractive screening platform for identifying hits that may be advanced to drug leads (Figure 4). HTS of a set of hexahydropyridoisoquinoline analogs identified urea **75** as a 2.0 μM inhibitor of hepatitis C virus (HCV),[67,68] for which the standard line of treatment utilizes pegylated interferon and ribavirin, which has significant adverse side effects and is curative in only 40–75% of patients.[69] The hexahydropyridoisoquinoline ring system may thus serve as a template for the discovery of novel small molecule inhibitors of HCV that have more favorable side-effect profiles.

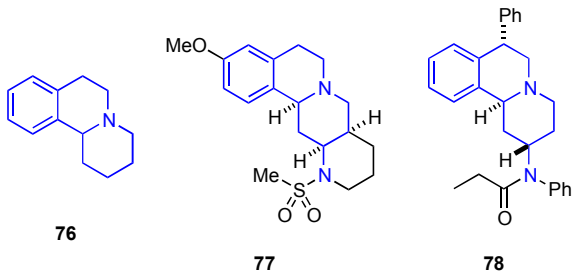
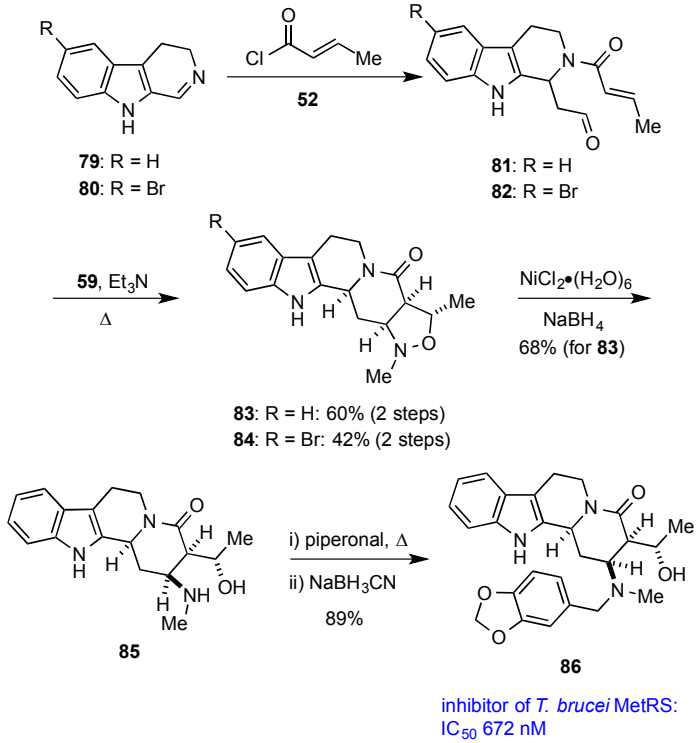


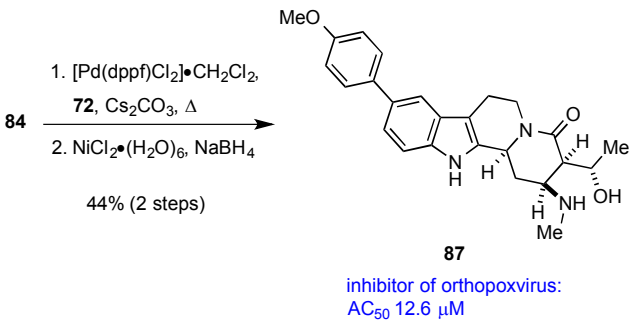
Figure 4. Biologically active hexahydropyridoisoquinolines.

In an extension of our work with dihydroisoquinolines (*e.g.,* Scheme 10), we examined the feasibility of using functionalized dihydro-β-carbolines as starting materials for MCAPs that would give intermediates suitable for subsequent elaboration via nitrone dipolar cycloadditions. The MCAPs using **79** and **80** were conducted in a fashion to give the aldehydes **81** and **82**, but in this case the use of TMSOTf was not required (Scheme 11).[70,71] When **81** and **82** were heated with **59**, stereoselective [3+2]-dipolar cycloadditions ensued to deliver isoxazolidines **83** and **84**. Cleavage of the *N,O*-bonds in **83** and **84** was induced with nickel boride to produce amino alcohols such as **85** that could be subsequently *N*-alkylated, as exemplified by the preparation of **86** via reductive amination with piperonal.



Scheme 11. Synthesis of *N*-methylbenzylamine **86**.

The presence of a bromine atom on the indole nucleus provided the opportunity to utilize cross-coupling reactions to further diversify the scaffolds. For example, the Suzuki cross-coupling reaction between bromoindole **84** and boronic acid **72** proceeded smoothly, and subsequent reductive cleavage of the *N,O*-bond gave the biaryl amino alcohol **87** (Scheme 12).



Scheme 12. Synthesis of *N*-methylamino alcohol **87**.

Owing to the well-known, potent and diverse biological activities of members of the *Yohimbine* and *Corynanthe* class of alkaloids,[72] it was not surprising that screening of collections of analogs of **10** and **11** (Scheme 2) exhibited potentially useful biological properties. For example, *N*-methylbenzylamine **86** (Scheme 11)[73] is a potent inhibitor of *Trypanosoma brucei* methionyl tRNA synthetase (*T. brucei* MetRS), an enzyme critical to the etiological agent of human African trypanosomiasis (HAT; sleeping sickness).[74,75] There is an unmet need for effective therapeutic agents for African trypanosomiasis, because currently available drugs possess high toxicity, lack good oral bioavailability, or require difficult dosing regimens.[76] As small molecule inhibition of MetRS is regarded as a viable treatment approach for this neglected tropical disease,[75] *N*-methylbenzylamine **86** may be an attractive starting point for the development of improved drugs that combat HAT.

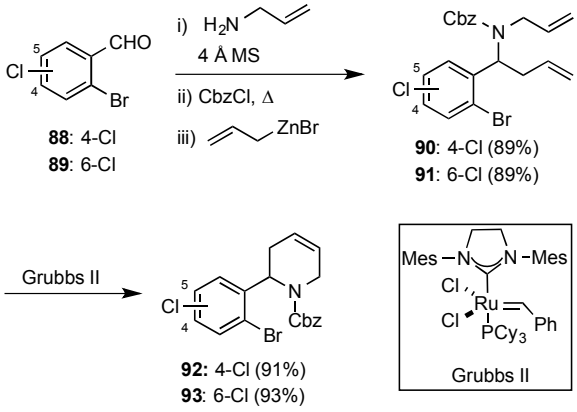
MLPCN screening identified *N*-methylamino alcohol **87** (Scheme 12) as an orthopoxvirus inhibitor with an IC_{50} of 12.6 μM.[77] The eradication of smallpox and subsequent discontinuation of routine vaccinations has resulted in populations with attenuated immunity towards orthopoxviruses, of which monkeypox and smallpox are members.[78] This, compounded with the lack of a single FDA-approved drug to treat infected individuals, has spurred efforts to identify orthopoxvirus inhibitors.[79] As such, novel small molecule viral inhibitors such as **87** may help guide the development of much needed therapeutic agents for monkeypox.

2. MCAP Followed by Ring-Closing Metathesis (RCM) and Other Ring-Forming Reactions

The piperidine and homopiperidine ring systems figure prominently into pharmaceuticals and biologically active natural products.[80,81] Constructing scaffolds embodying these motifs thus seemed to be an attractive approach for accessing molecular libraries for biological evaluation. We found that 2-aryl substituted derivatives of these azacycles could quickly be prepared through a MCAP / RCM reaction sequence when the MCAP was conducted with inputs containing unsaturated functionality.[82] In this variation of the MCAP, the amine and nucleophile reagents possess carbon-carbon double bonds that render products that can be cyclized by a RCM reaction to form tetrahydropyridines and tetrahydroazepines. Significantly, functionality resident in the metathesis products enable a range of subsequent ring forming reactions that provide structurally diverse,

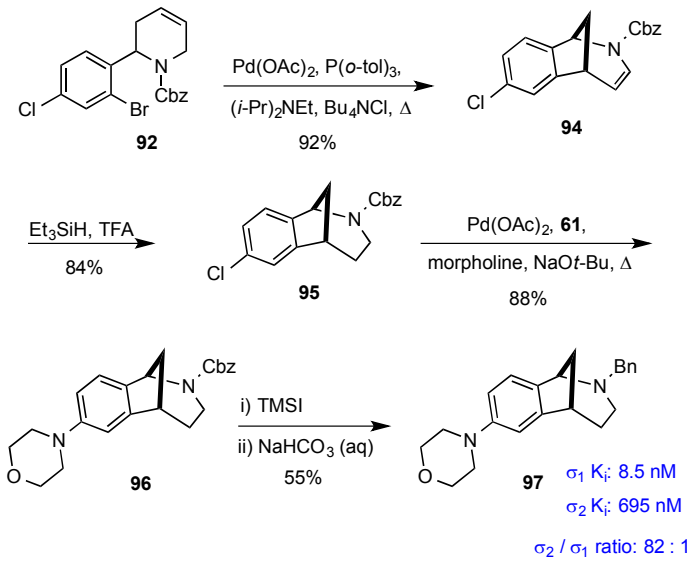
polycyclic frameworks. Representative examples of the application of these concepts that led to the discovery of collections of novel bioactive compounds are outlined herein.

a. Elaboration of RCM Products via Heck Cyclizations. In early accounts, we showed that tetrahydropyridines prepared by RCM of dienes formed by selected MCAPs could be cyclized by Heck reactions to give bridged polycyclic systems.[31,32] A straightforward modification of this methodology using dihalo aldehydes **88** and **89**[83] as inputs together with allylamine, benzyl chloroformate, and allylzinc bromide afforded the dienes **90** and **91**, respectively (Scheme 13).[84,85] Cyclizations of these compounds via RCM to give the corresponding tetrahydropyridines **92** and **93** were induced using Grubbs II catalyst.



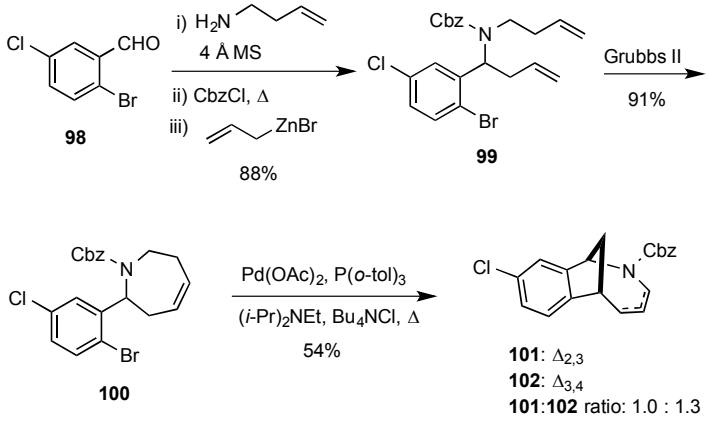
Scheme 13. Preparation of tetrahydropyridines **92** and **93**.

The presence of the aryl bromide moiety in **92** enabled an intramolecular Heck reaction in the presence of Bu_4NCl[86] to fabricate the bridged azabicyclic enecarbamate **94**. Processing **94** by an ionic reduction with triethylsilane and trifluoroacetic acid[87] completed a short entry to the norbenzomorphan **95**, which is endowed with two sites for chemical diversification (Scheme 14). For example, Buchwald-Hartwig cross-coupling reactions using the aryl chloride **95** led to a number of anilines as exemplified by the preparation of **96**. The benzylamine **97** could be accessed directly by simply treating the benzyl carbamate **96** with TMSI followed by workup with aqueous sodium bicarbonate.[88]



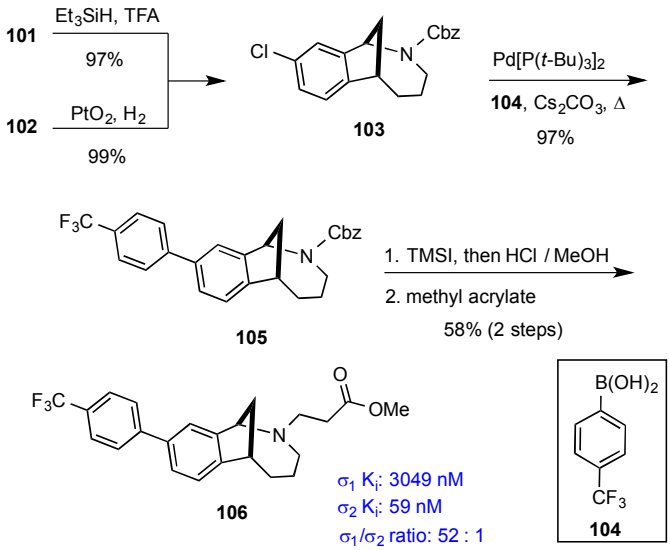
Scheme 14. Novel entry to norbenzomorphans.

A simple modification of the sequence depicted in Scheme 13 allowed for a facile entry to the homonorbenzomorphan scaffold. In the event, the MCAP involving benzaldehyde **98**[89] and homoallylamine gave the diene **99**, which underwent a RCM reaction followed by an intramolecular Heck cyclization to provide a mixture (1.0:1.3) of isomeric unsaturated homonorbenzomorphans **101** and **102** (Scheme 15).[90]



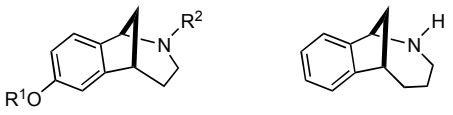
Scheme 15 Preparation of homonorbenzomorphans.

Obtention of the olefin isomers represented nothing more than an inconvenience, because **101** and **102** were readily separated via chromatography and reduced under ionic and catalytic hydrogenation conditions, respectively, to yield the homonorbenzomorphan scaffold **103** (Scheme 16). A Suzuki cross-coupling reaction between 4-(trifluoromethyl)benzeneboronic acid (**104**) and aryl chloride **103** proceeded in high yield to deliver the biaryl **105**. TMSI-mediated removal of the carbamate and reaction of the secondary amine thus produced with methyl acrylate gave the β-amino carboxylate **106**.



Scheme 16 Synthesis of β-amino carboxylate **106**.

The norbenzomorphan (**107** and **108**) and homonorbenzomorphan (**109**) scaffolds comprise investigational compounds with medicinally-relevant biological activities (Figure 5). Both amines **107** and **109** elicit antinociceptive effects in murine pain models, with the former demonstrating activity comparable to codeine.[91] Moreover, the carbamate **108** was found to be a potent acetylcholinesterase (AChE) inhibitor, leading to significant ACh elevation in mouse forebrain.[92] The documented biological activities associated with these azabicycles, coupled with their lack of overt toxicity in animal models, suggest that these scaffolds may be harnessed for the discovery and development of novel medicinal agents.



107: R^1 = Me, R^2 = Me
108: R^1 = $CO(NH)C_6H_{13}$, R^2 = Me

109

Figure 5 (Homo)norbenzomorphans with biological activity.

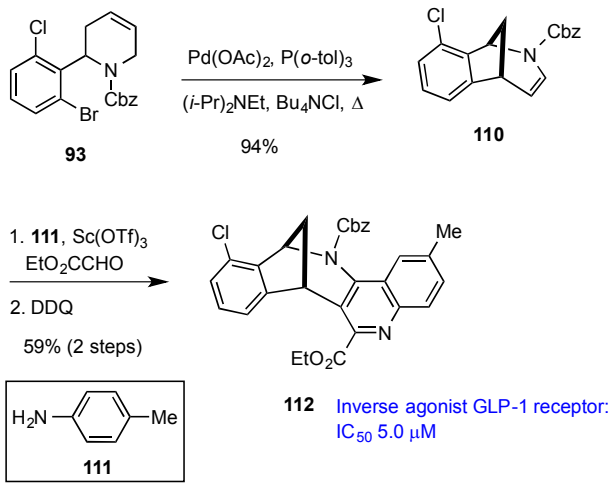
Comprehensive receptor screening at the Psychoactive Drug Screening Program (PDSP)[93] revealed that norbenzomorphan **97** (Scheme 14) is a potent sigma 1 receptor binding ligand with high selectivity over the sigma 2 receptor (σ_1 K_i = 8.5 nM, σ_2 K_i = 695 nM). A handful of sigma 1 receptor ligands (agonists) exhibit *in vitro* neuroprotection towards amyloid-β peptide[94] and anti-amnesic effects in rodents infused with amyloid-β peptide.[95] Indeed, sigma 1 receptor modulators are commanding increasing interest as potential therapeutics for neuropathic pain, substance abuse and amyotrophic lateral sclerosis (ALS).[96] Continued development of

selective sigma 1 ligands may also help advance understanding of the basic cellular mechanisms relevant to a range of chronic diseases that currently lack adequate pharmacological treatment.[97]

Complementary to the sigma 1 receptor binding properties of norbenzomorphan **97**, receptor screening at the PDSP identified homonorbenzomorphan **106** as a potent sigma 2 receptor ligand with high selectivity over the sigma 1 receptor (σ_2 K_i = 59 nM, σ_1 K_i = 3049 nM, Scheme 16). Putatively identified as the progesterone receptor membrane component 1 (PGRMC1),[98] the sigma 2 receptor is highly overexpressed in proliferating tumor cells. The sigma 2 receptor is thus regarded as a cancer cell biomarker,[99] and ligands that bind to it are being explored as molecular probes for imaging solid tumors. Sigma 2 receptor agonists also induce apoptosis in a number of human cancer cell lines, and they potentiate the activity of other anticancer drugs, including gemcitabine and paclitaxel, when used in combination therapy.[97,100] Ongoing research in this area suggests that sigma 2 receptor modulators may soon play an important role in cancer therapy.

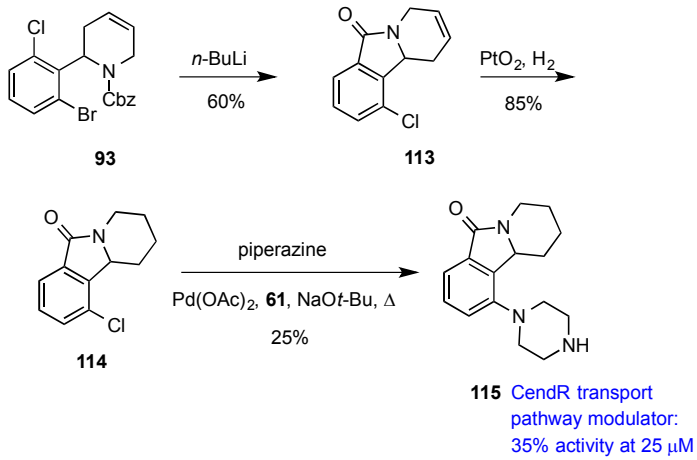
b. Elaboration of RCM Products via Sequential Heck and Povarov Cyclizations. The electron rich olefin in the enecarbamate **110**, which was formed via Heck cyclization of **93**, could be exploited as a dienophile in an imino Diels-Alder reaction (Povarov cyclization). For example, when exposed to *p*-toluidine (**111**), ethyl glyoxylate and scandium triflate, enecarbamate **110** underwent a Povarov reaction to give a mixture of cycloadducts (1.2:1.0), which converged to a single quinoline-fused norbenzomorphan **112** upon oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (Scheme 17).[84] The aryl chloride and protected nitrogen in **112** provide opportunity for subsequent derivatization reactions.

Screening within the MLPCN identified **112** as a 5 μM inverse agonist of the glucagon-like peptide 1 receptor (GLP-1R),[101] a member of the B1 class of GPCRs that are activated by peptide hormones.[102] GLP-1R is a GPCR with many physiologically-significant roles, one of which involves the synthesis and regulation of insulin. Since only a few small molecules with weak B1 GPCR agonist activity are known, a novel screening module was developed to establish proof-of-principle for the approach through the identification of small molecule GLP-1R inverse agonists.[103] It is anticipated that novel GLP-1R inverse agonists may be advanced to drugs that treat diabetes and neurodegenerative disease.



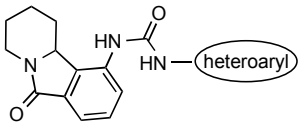
Scheme 17 Quinoline-fused norbenzomorphan **112**.

c. Elaboration of RCM Products via Parham Cyclization. The Parham cyclization involves ring-formation by reaction between an aryllithium that is generated via lithium-halogen exchange and a pendant electrophilic center.[104] It thus occurred to us that the *N*-acyl tetrahydropyridine **93** might undergo the Parham cyclization to produce isoindolinones, so we explored the feasibility of applying this reaction to the synthesis of a collection of novel isoindolinones. Indeed, we discovered that **93** underwent selective halogen-metal exchange upon treatment with *n*-BuLi, and spontaneous cyclization ensued to form isoindolinone **113** (Scheme 18).[84] Reduction of **113** with Adam's catalyst (PtO_2) delivered the reduced scaffold **114** with no observed loss of halogen. Both biaryl and aniline derivatives were prepared through cross-coupling reactions with aryl chloride **114**. In one instance, a Buchwald-Hartwig cross-coupling reaction between isoindolinone **114** and piperazine generated the aniline derivative **115**, which contains a secondary nitrogen atom that could be functionalized to prepare a set of analogs.



Scheme 18. Isoindolinone **115** via the Parham cyclization.

Significantly, isoindolinone **115** bears structural similarity to known cyclin-dependent kinase (CDK)/glycogen synthase kinase (GSK) 3 inhibitors, collectively termed valmerins (*i.e.,* tetrahydropyrido-[1,2-a]isoindolone (heteroaryl)ureas (Figure 6).[105] Many members from this class inhibit CDK5 and GSK3 with an IC_{50} < 100 nM. More importantly, isoindolinone **116** not only exhibited sub-micromolar activity towards various human cancer cell lines, but also inhibited tumor growth in HCT-116 mouse xenograft models without overt toxicity.[105] It is envisioned that isoindolinone **114** (Scheme 18) could serve as a late stage intermediate for the synthesis of valmerin-type molecules.



valmerins

116: heteroaryl = 3-(5-phenylisoxazol-3-yl)

Figure 6 Valmerin kinase inhibitor **116**.

An MLPCN screening center revealed that isoindolinone **115** (Scheme 18) is a modulator of the "C-end Rule" (CendR) transport pathway (35% activity at 25 μM).[106] A class of synthetic CendR peptides sharing a common amino acid sequence with vascular endothelial growth factor (VEGF) A-165 has recently been identified that can induce cell internalization and transport cargo deep into extravascular tissue via binding to the pleiotropic cell surface receptor neuropilian–1 (NRP–1).[107] Small molecule CendR agonists may thus represent potential tools for introducing compounds into tissues, whereas antagonists could limit tissue penetration of toxins and inflammatory agents that use the CendR pathway.

In addition to the specific case studies presented in previous sections of this account, we have applied the fundamental principles summarized herein to synthesize a broad spectrum of other novel small molecules having natural product- and drug-like scaffolds. For example, a number of polyheterocyclic compounds,

including **117**-**131**, have been prepared that have not yet been evaluated in biological assays (Figure 7). It seems likely that testing of these and other collections of small molecules will unveil novel entities possessing medicinally-useful biological properties.

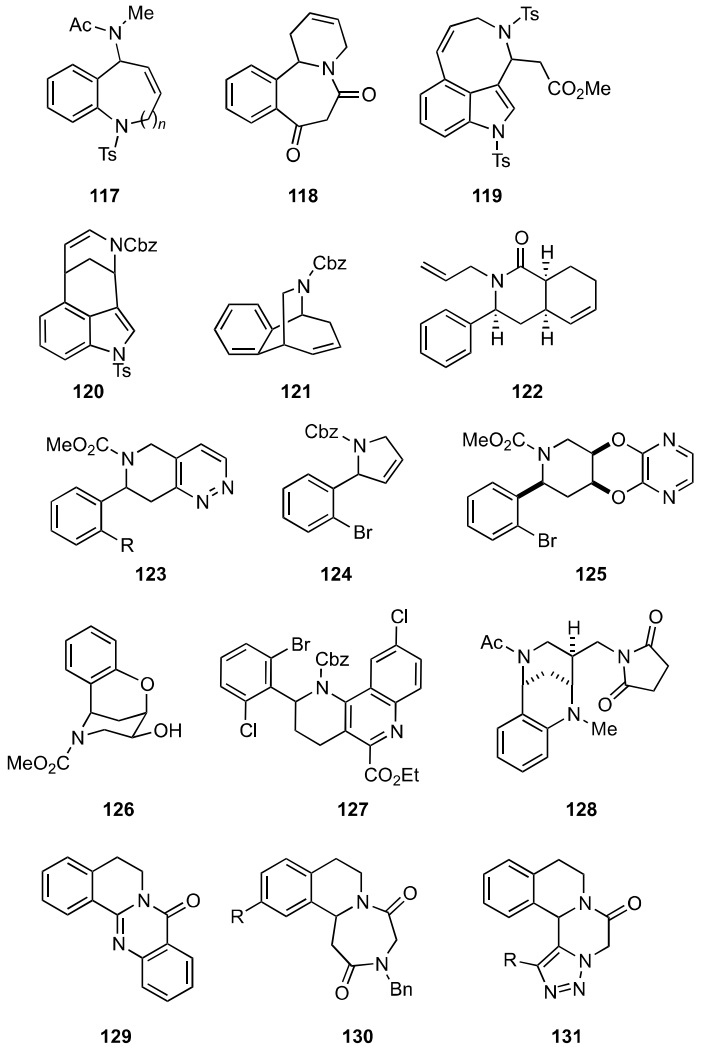


Figure 7 Novel scaffolds awaiting biological assessment.

Summary and Conclusion.

The foregoing survey reveals that the modular nature of the MCAP outlined in Scheme 3 enables rapid entry to a multitude of versatile intermediates that can be advanced via numerous ring-closing transformations to generate a variety of heterocyclic ring scaffolds. Functionality embedded in the starting inputs, or unmasked at a later stage, allows the subsequent preparation of collections of analogs bearing diverse substituents within each scaffold class. Screening these collections of small molecules has already led to the identification of compounds that exhibit a broad range of biological activities. Some of these will likely serve as tools to probe biological function and mechanism, whereas others may be further developed as therapeutic agents for treating neurodegenerative and infectious diseases and a range of human cancers. In this context, it is noteworthy that

application of guidelines set forth by Lipinski[8] and Veber[9] reveals that a majority of the compounds we have created possess physicochemical parameters that are associated with favorable oral bioavailability. Accordingly, some of these compounds are attractive candidates for analog design and advancement from hit to drug lead and beyond.[108] Indeed, a number of compounds derived from hits that were generated by implementing the strategy outlined herein are currently undergoing preclinical development. These promising findings augur well for future applications of this approach to access sets of drug-like small molecules with activities against diverse biological targets.

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