Asymmetric Catalysis in Diversity-Oriented Organic Synthesis: Enantioselective Synthesis of 4320 Encoded and Spatially Segregated Dihydropyrancarboxamides**

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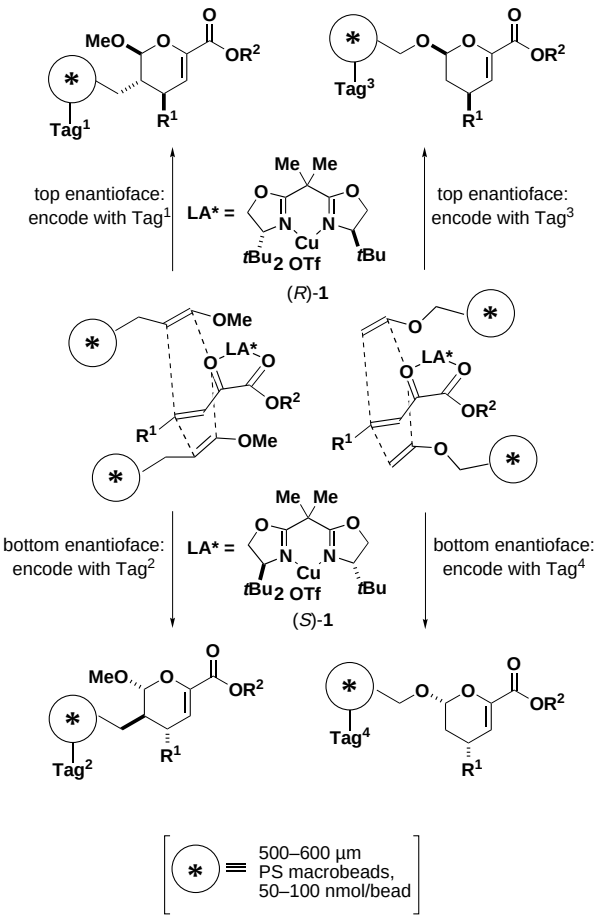
Small molecules have been used to explore many facets of biology for over a century. However, research in biology is not routinely performed using this approach, in the way that it is with biochemical, genetic, and increasingly, genomic approaches. Several problems limit the use of the former approach. Arguably, the primary one is the lack of routine access to structurally complex and diverse small molecules that can be used to modulate biological systems.[1] Diversity-oriented organic synthesis, especially when coupled with an economical and efficient technology platform, offers the means to change this situation, as it aims to synthesize complex and diverse small molecules efficiently.[2] Diversity-oriented synthesis is central to chemical genetics, which aims to explore biology with small molecules in a *systematic* way.[3]

Although enantioselective catalysis is often used in target-oriented synthesis, it is still relatively underexplored in diversity-oriented synthesis.[4, 5] We have been interested in reactions catalyzed by bis(oxazoline)metal Lewis acid complexes because of their high efficiency, selectivity, and broad substrate tolerance.[6] We chose to concentrate on inverse electron demand heterocycloadditions of vinyl ethers and β,γ-unsaturated ketoesters (Scheme 1).[7, 8] An account of related cycloadditions on solid support has been described;[9] however, the reported reactions were performed in the presence of achiral catalysts and with the heterodiene bound to the PS solid support through the ester. We initially investigated this mode of cycloaddition and found it to be highly selective when the enantiomerically pure catalysts (*S*)- or (*R*)-**1** were used.[5, 8] However, in the interest of effectively functionalizing the cycloadduct, we found an alternative mode using support-bound vinyl ethers, linked to a macrobead through either carbon or oxygen, to be more effective.



Scheme 1. Encoded, catalytic, asymmetric heterocycloaddition. The polystyrene macrobead serves as a microreactor, an important element of the "one-bead, one-stock-solution" technology platform.[10] LA* = the chiral lewis acid, PS = polystyrene, Tf = triflate = trifluoromethanesulfonyl.

Here we report our application of this asymmetric cycloaddition reaction to the synthesis of dihydropyrancarboxamides on high-capacity, 500–600 μm PS macrobeads, key elements in a one-bead, one-stock-solution technology platform.[6, 10] The diversity pathway explored resulted in the highly diastereo- and enantioselective synthesis of 4320 encoded[11] small molecules,[12] which were arrayed as 5 mM stock solutions from individual beads,[13] each containing predominantly a single dihydropyrancarboxamide. These stock solutions permit many phenotypic and proteomic assays to be performed.

We first synthesized collections of vinyl ethers (Scheme 2A) and unsaturated ketoesters (Scheme 2B) as candidate partners for the cycloaddition reaction. Each of the vinyl ethers **BB1-A–N** was loaded onto pools of PS macrobeads via the silyl triflate **3** which is generated in situ (method shown in Scheme 3).[10] The support-bound vinyl ethers **4–6** were then treated with heterodienes (either **BB2-B** or **BB2-E**, R^3 = phenyl and 4-piperonyl, respectively; 3 equivalents) in THF in the presence of 20 mol% of the [(*t*BuBOX)Cu(OTf)$_2$] complex ((*S*)- or (*R*)-**1**) and 4 Å molecular sieves[14] to provide

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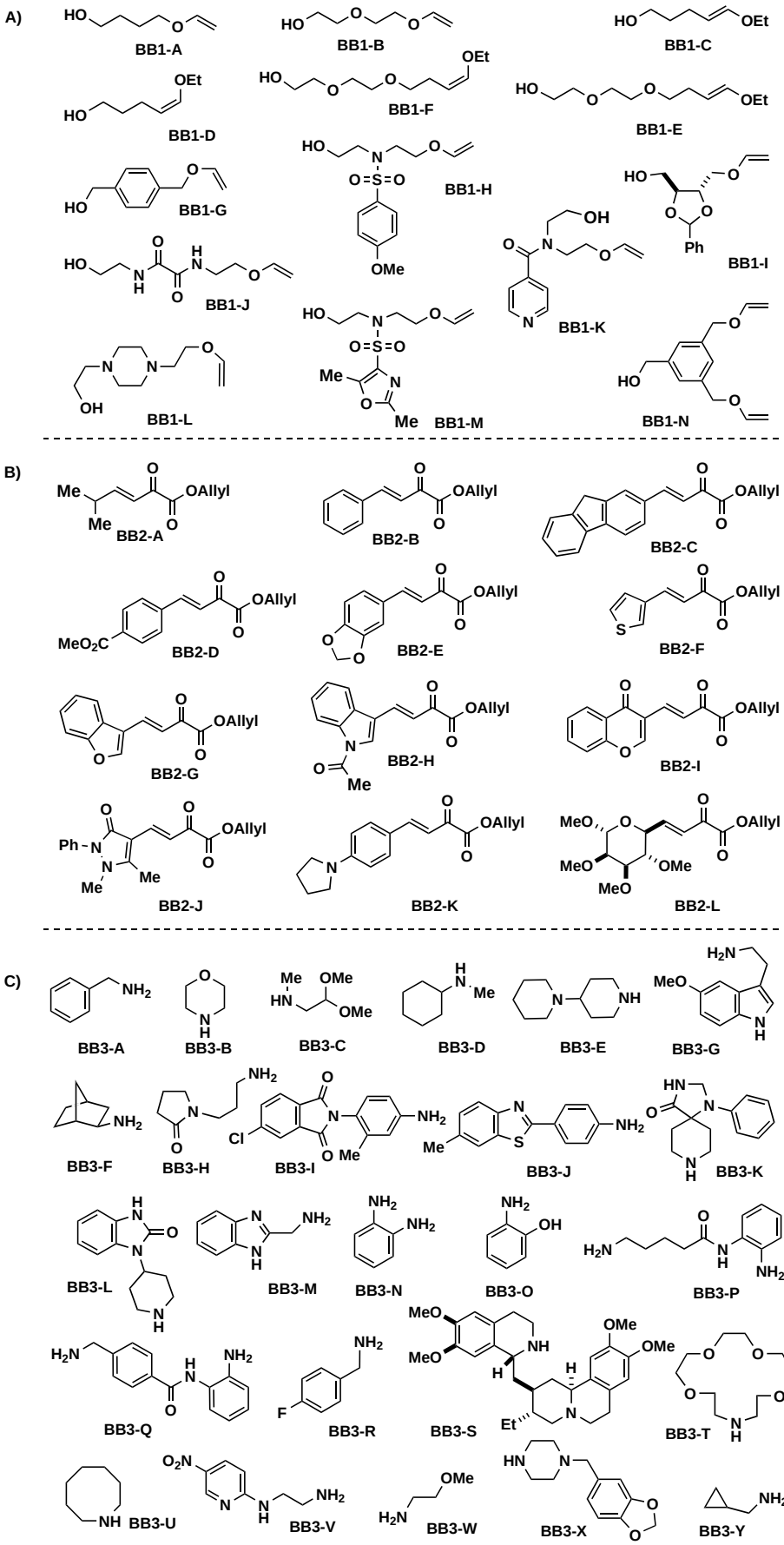

Scheme 2. A) Vinyl ether alcohol building blocks **BB1-A**–**N**. B) β,γ-Unsaturated ketoester building blocks **BB2-A**–**L**. C) Amine building blocks **BB3-A**–**Y**.

support-bound cycloadducts ***7**–***9**. Both enantiomers of the ligand were used in separate reactions to obtain a duplicate result and to detect potential matched/mismatched pairs when chiral starting materials were used. After washing and drying steps, each of the cycloadducts was cleaved from the silyl ether linker with hydrogen fluoride/pyridine (HF–py) and analyzed for purity with ^{1}H NMR spectroscopy and liquid chromatography/mass spectrometry (LC–MS).

These studies showed that support-bound vinyl ethers with amino or amido functionality led to low conversion, and the support-bound form of bis(vinyl ether) **BB1-N** underwent a single, rather than the desired double, cycloaddition, even when a stoichiometric amount of the copper complex was used. The chiral vinyl ether derived from threitol (support-bound form of **BB1-I**) reacted efficiently with only the *S* enantiomer of the catalyst, which suggests that double diastereoselection was taking place (see below). Upon treatment of the corresponding PS macrobeads with HF–py, the remaining vinyl ethers studied provided high purity cycloadducts **7**–**9** (Table 1). The ethenyl ethers **BB1-A**, **B**, **G**, **H** also yielded dihydropyrans **7** in high diastereo- and enantioselectivity.[15] Both configurations of substituted enol ethers **BB1-C**–**F** led to moderate to high diastereoselectivity of the tetrasubstituted dihydropyrans **8** and **9**. Although previous results[8a,c] had shown high diastereoselectivity with cyclic vinyl ethers, we found that *Z*-configured enol ethers (**BB1-D**, **F**) provided only moderate diastereoselection, whereas the *E* enol ethers (**BB1-C**, **E**) resulted in high levels of diastereoselection. The lower diastereoselectivity in the *Z* enol ether cycloadditions may arise from an endo–exo switch in the transition structure for cycloaddition.[16]

We next turned our attention to the substitution on the heterodiene partner. In most instances, treatment of the support-bound vinyl ether **BB1-H** with a hetereodiene under the previous conditions again

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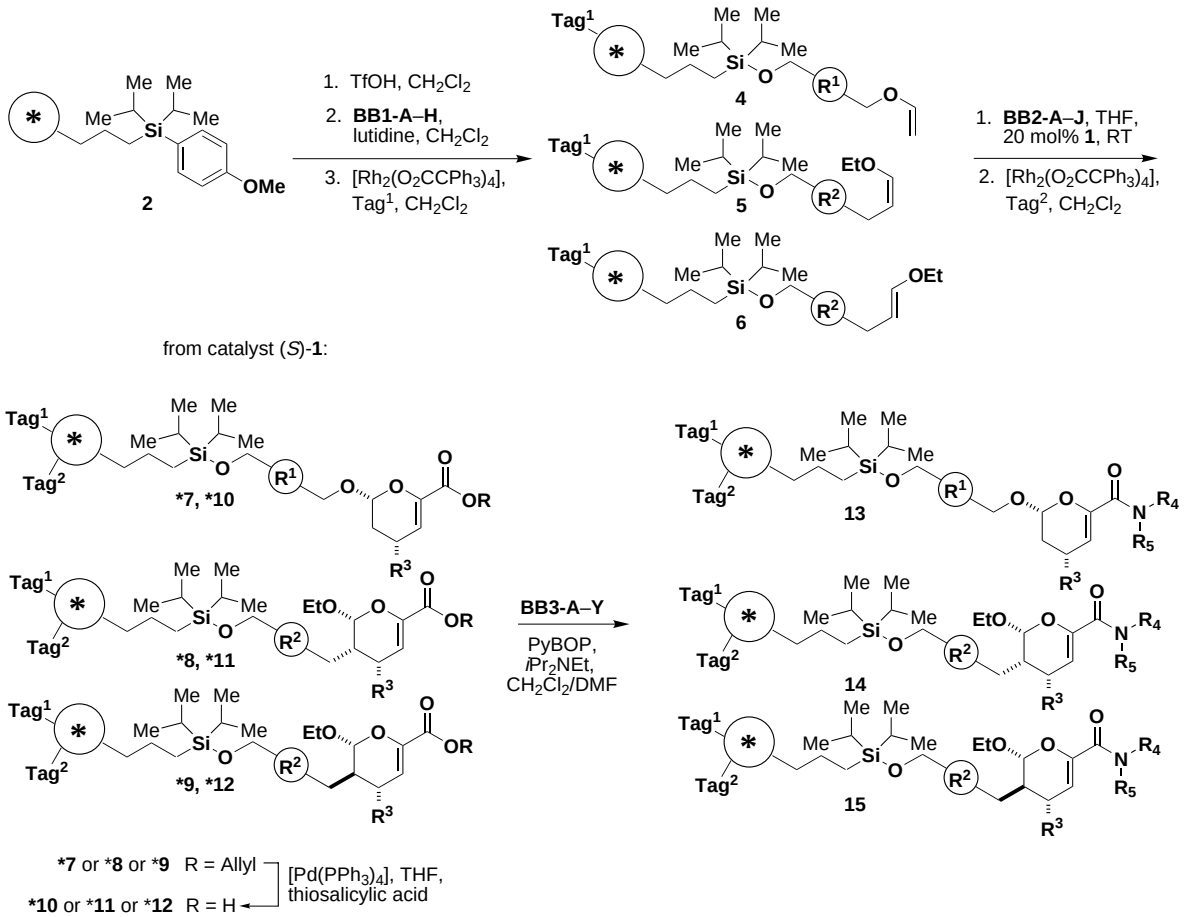
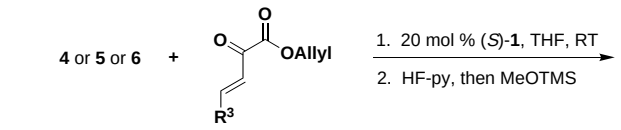
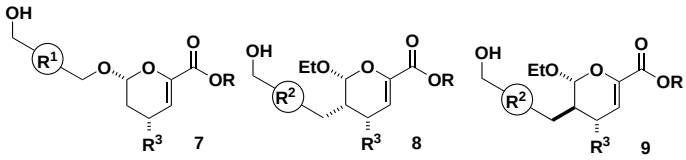


Scheme 3. The encoded split-pool synthesis of dihydropyrancarboxamides, with the (*S*)-**1** catalyst. The corresponding opposite enantiomers of compounds **7**–**15** are obtained when the (*R*)-**1** catalyst is used. Encircled R^1 and R^2 symbols represent elements found in building blocks **BB1-A**–**H** in Scheme 2. PyBOP = benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate, DMF = *N*,*N*-dimethylformamide, THF = tetrahydrofuran.

Table 1. Asymmetric cycloadditions of resin-bound vinyl ethers **BB1**.[a]





BB1-	BB2-	Product	Purity [%][b]	d.r.[c]	e.r.[d]
A	**E**	**7-A-E**	≥ 95	≥ 15:1	≥ 49:1
B	**E**	**7-B-E**	≥ 95	≥ 15:1	≥ 24:1
C	**B**	**9-C-B**	≥ 95	≥ 20:1	≥ 49:1
D	**B**	**8-D-B**	≥ 95	≥ 5:1	≥ 30:1
E	**B**	**9-E-B**	≥ 95	≥ 20:1	≥ 49:1
F	**B**	**8-D-B**	≥ 95	≥ 10:1	≥ 30:1
G	**E**	**7-G-E**	≥ 95	≥ 30:1	≥ 49:1
H	**E**	**7-H-E**	≥ 95	≥ 20:1	≥ 24:1

[a] Reactions were performed with 20 mol% of (*S*)-**1** or (*R*)-**1**; the results presented are an average of the two runs. [b] Estimated based on ^{1}H NMR analysis and HPLC–ESI MS. [c] Determined by ^{1}H NMR analysis and/or CSP HPLC or CSP SFC. [d] Determined by CSP HPLC or CSP SFC. HPLC–ESI MS = high-pressure liquid chromatography/electron spray ionization MS, CSP = chiral stationary phase, SFC = supercritical fluid chromatography, TMS = trimethylsilyl.

led to highly pure cycloadduct following HF–py cleavage from the PS macrobeads (Table 2), though again amine functionality (**BB2-K**) was incompatible. Similar to the case above with the threitol-derived vinyl ether, only the *S* enantiomer of the catalyst efficiently provided cycloadduct with the mannose-derived heterodiene **BB2-L**. Overall, ten heterodienes (**BB2-A**–**J**) resulted in somewhat variable, but uniformly high, diastereo- and enantioselectivities and high purities based on ^{1}H NMR spectroscopy and LC-MS analyses. These building blocks were chosen for subsequent incorporation into the library synthesis.

Further functionalization of the cycloadducts was then pursued. Conversion of the support-bound cycloadduct **7-H-E**, upon treatment with [$Pd(PPh_3)_4$] and thiosalicylic acid, into the corresponding acid **10-H-E** was achieved in high purity (Scheme 3). Treatment of the support-bound acid **10-H-E** with 20 equivalents of benzylamine, PyBOP, and diisopropylethylamine in CH_2Cl_2:DMF (3:1) led to the desired benzylamide. These conditions were applied to a diverse collection of amines with support-bound acid **10-H-E** to select 25 amines for use in the library synthesis (Scheme 2C).

These pathway development studies were necessary to select the reactions and building blocks for a library realization that would result in single-compound stock solutions from individual macrobeads. The library synthesis was

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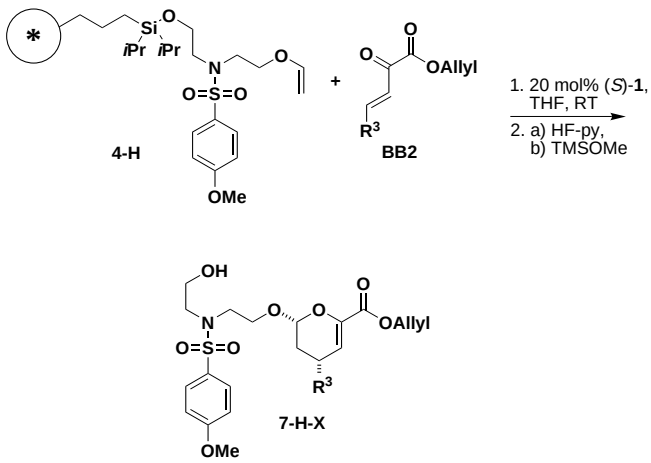
Table 2. Asymmetric cycloadditions of resin-bound vinyl ether **4-H** with various heterodienes.[a]



BB2-	Products	Purity [%][b]	d.r.[c]	e.r.[d]
A	**7-H-A**	≥ 95	≥ 16:1	≥ 16:1
B	**7-H-B**	≥ 95	≥ 20:1	≥ 24:1
C	**7-H-C**	≥ 95	≥ 9:1	≥ 24:1
D	**7-H-D**	≥ 95	≥ 9:1	≥ 9:1
E	**7-H-E**	≥ 95	≥ 20:1	≥ 24:1
F	**7-H-F**	≥ 95	≥ 25:1	≥ 24:1
G	**7-H-G**	≥ 95	≥ 9:1	≥ 49:1
H	**7-H-H**	≥ 95	≥ 15:1	≥ 24:1
I	**7-H-I**	≥ 95	≥ 12:1	≥ 49:1
J	**7-H-J**	≥ 95	≥ 9:1	≥ 49:1

[a] – [d] See footnotes for Table 1.

initiated with sufficient PS macrobeads (13000) to produce, on average, three beads containing each theoretical compound. The chosen vinyl ethers were attached to the supports and following the initial cycloaddition step, the two enantiomeric sets of cycloadducts were not pooled. (Each set includes cycloadduct attached to either the C1 oxygen or C2 carbon of the dihydropyran ring.) Instead, the two sets were carried through the remaining steps in parallel in order to provide an independent means (when coupled to mass spectrometry) to assess the ability of tags to infer the absolute configuration of library members (Scheme 1). The supports were not repooled following the amide coupling, thereby reducing the number of chemical encoding steps to which the macrobeads were subjected and simplifying the decoding of library members.[3e] In the end, 54 separate portions of macrobeads were produced (50 portions containing dihydropyrancarboxamides, 2 containing dihydropyrancarboxylic acids, and 2 containing dihydropyrancarboxylic esters, see the Supporting Information for details), each containing, theoretically, three copies of 80 compounds for a total of 4320 distinct, spatially-segregated, and stereochemically-defined dihydropyran derivatives.

In order to analyze the purity of members of the library, two macrobeads from each of the above 54 pools were removed, arrayed, and treated with HF-py, and fractions of the eluted products (10 μL of 5 mM stock solutions) were assayed by LC-MS.[12, 17] In summary, 78 samples (72%) were ≥ 95% pure, 93 samples (86%) were ≥ 90% pure, 104 samples (96%) were ≥ 75% pure, and the remaining 4 samples were of roughly 50% purity. Direct structure determination by MS was successful in 83 of the 108 cases,[18] and indirect structure inference by decoding of the chloroaromatic diazoketone tags[11b, 12] was successful in all cases. Full details of this procedure are described in the following Communication.[12]

Although this library synthesis succeeded in using stereochemistry as a diversity element and extended the asymmetric heterocycloaddition reaction to solid phase,[19] only one of two potential diastereomers (for the unsubstituted vinyl ethers) was accessed. Catalyst systems with truly complete external control over enantio- and diastereoselectivity are required to realize fully the potential of stereoselective catalysis in diversity-oriented organic synthesis. The generation of spatially-segregated stock solutions from individual macrobeads guarantees that the compounds are amenable to both phenotypic and protein-binding assays, and their common primary hydroxy group ensures that every compound can be robotically arrayed onto a glass microscope slide for protein-binding assays.[20] Indeed, small molecule microarrays of the dihydropyrancarboxamides have already been manufactured and screened, which led to the discovery of a small molecule that binds to a protein of interest.[21, 22]

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[1] There are examples of simple, achiral modulators of biological systems, notably "drug-like" molecules, although in these cases the smaller size and complexity of the species have more to do with delivery and pharmacokinetic parameters than with affinity and selectivity for a protein target. It is our hypothesis that structurally complex and diverse collections of "natural product like", rather than "drug-like", molecules will be better suited as biological probes and this report is part of a collective effort to address this very hypothesis.

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[13] A fully automated procedure for deriving and arraying stock solutions from the dihydropyrancarboxamide-containing macrobeads described herein has been developed and will be reported elsewhere; ref. [10c].

[14] After surveying several loading/ligand/metal/solvent combinations, 20 mol% of **1** in THF was found to provide the best combination of kinetics and selectivity.

[15] The configurations of the cycloadducts were assigned by analogy. See refs. [8a, c].

[16] We have ruled out isomerization of the alkenyl ether and epimerization of the acetal center as reasons for the lower selectivity.

[17] The details of this procedure and the relationship between the 108 cleaved compounds and their associated electrophoretic tags are reported in ref. [12].

[18] For 25 of the 108 samples, the molecular ion observed upon ionization corresponded to a fragment of the compound.

[19] To the best of our knowledge, this is the first report of the use of a substoichiometric amount of chiral controller to perform a carbon – carbon bond forming reaction on solid phase.

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[22] Supporting Information available: Full experimental details and spectral data for building block and library syntheses.

Decoding Products of Diversity Pathways from Stock Solutions Derived from Single Polymeric Macrobeads**

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Efficient phenotypic and proteomic screening of small molecules derived from diversity-oriented organic syntheses[1] requires a library realization platform[2] that 1) produces a sufficient quantity of compound per bead to perform many hundreds of assays[3] and 2) supports reliable compound structure identification. To facilitate the latter, solid-phase library-encoding strategies[4] have been developed that allow the identity of the compounds to be inferred postsynthesis directly from individual beads.[5] We recently adapted the chemical-encoding strategy introduced by Still and co-workers[6] to a high-capacity (1.4 mequiv $g^{-1} \approx 100$ nmol/bead), 500 – 600 μm polystyrene (PS) solid support (Scheme 1),[3] a key element of a "one-bead, one-stock-solution" technology platform.[7] We have now discovered that the stock solutions of compounds cleaved from individual beads contain sufficient tags to allow the structures of their corresponding small molecules to be inferred reliably. Two methods used for the decoding of the library of 4320 dihydropyrancarboxamides reported in the preceding Communication are described.[8]

The encoding method features structurally related chloroaromatic diazoketone "tags",[6b] which are introduced through an acylcarbene insertion into the phenyl rings of PS catalyzed by $[Rh_2(O_2CCPh_3)_4]$ (**1**) to yield cycloheptatrienes (Scheme 1).[9] To decode a library compound, the tags are cleaved oxidatively from the solid support with ceric ammonium nitrate (CAN) to yield free alcohols,[10] which are then silylated (with *N*,*O*-bis-(trimethylsilyl)acetimide, BSA) and injected directly onto a gas chromatograph equipped with electron-capture detection (GC/ECD) for analysis (each tag trimethylsilyl ether has a unique GC retention time). For low-loaded solid support (≈100 pmol/bead) it has been postulated that the carbene inserts predominantly into the support due to

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