Diversity-Oriented Synthesis of Biaryl-Containing Medium Rings Using a One Bead/One Stock Solution Platform

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Abstract: Diversity-oriented synthesis of structurally complex and diverse small molecules can be used as the first step in a process to explore cellular and organismal pathways. The success of this process is likely going to be dependent on advances in the synthesis of small molecules having natural product-like structures in an efficient and stereoselective manner. The development, scope, and mechanism of the oxidation of organocuprates was investigated and exploited in the atropdiastereoselective synthesis of biaryl-containing medium rings (9-, 10-, and 11-membered rings). The methodology was performed on high-capacity, large polystyrene beads by metalating aryl bromides with iPrBu₂MgLi, followed by transmetalating with CuCN₂LiBr and then oxidizing with 1,3-dinitrobenzene, and was used in a diversity-oriented synthesis of biaryl-containing medium rings (library total theoretical maximum 1412 members). The high capacity beads were arrayed into 384-well plates and, using a process optimized during the development of a one bead/one stock solution technology platform, converted into arrays of stock solutions, with each stock solution containing largely one compound. These stock solutions were used in numerous phenotypic and protein-binding assays. The process described outlines a pathway that we feel will contribute to a comprehensive and systematic chemical approach to exploring biology (chemical genetics).

Introduction

Small molecules can be used to understand biological pathways in a process analogous to genetics (chemical genetics). To enhance the generality of this approach, a method is required to discover small molecule partners (activators/inhibitors) for any protein target. A promising method begins by synthesizing structurally complex and diverse small molecules efficiently using diversity-oriented synthesis. Next, these small molecules are screened individually for their ability to either induce a desired cellular or organismal change (phenotypic screening) or bind to a protein (proteomic screening; the term proteomic is used to emphasize protein-binding screens that use many proteins in parallel). This paper illustrates the coupling of diversity-oriented synthesis to screening in a way that led to the discovery of several new probes of biological processes.

Nature provides guidelines for the characteristics of small molecules that are desirable for effective interactions with proteins, such as rigidity and stereochemical complexity. Natural products such as vancomycin and pterocaryanin C, which have an axially disymmetric biaryl moiety implanted within a ring, are particularly engaging due to their atropisomerism (Figure 1). Indeed, pterocaryanin C was especially stimulating on an axially disymmetric biaryl moiety implanted within a ring, which makes these rings relatively rigid conformationally. Medium rings are not only considered generally to be the most difficult ring sizes to synthesize, but they also require special attention since their formation en masse using split-pool synthesis presents special challenges. Thus, a general synthesis of medium rings would be useful, especially one that is operative on a polymer support and is high yielding and selective.

Any approach that aims to construct large collections of small molecules for individual use in biological assays needs to take

(1) Following the analogy with genetics, these two complementary screening approaches are known as forward and reverse chemical genetics, respectively.


(3) This difficulty is due to their associated torsional, transannular, and large-angle strain, which makes these rings relatively rigid conformationally. Illuminati, G.; Mandolini, L. Acc. Chem. Res. 1981, 14, 95–102. For a recent review of medium ring synthesis: Yet, L. Chem. Rev. 2000, 100, 2963–3007.

account of the format that will be required for the screen. When a diverse range of screens is desired, then a flexible and preferably automated approach will be essential. We have developed a procedure for synthesizing and arraying small molecules individually as stock solutions in quantities sufficient to permit hundreds of phenotypic and proteomic assays to be performed per compound—the one bead/one stock solution technology platform. The research described herein makes use of that platform.

We report the development, scope, and mechanism of general, efficient, and atropdiastereoselective reactions leading to compounds having biaryl-containing medium rings. The reactions were studied in solution and on a high capacity polymeric support, and the structural and conformational properties of the medium ring products were determined. Details of the strategies adopted in the library's design and specification, and its subsequent apportioning, which led to our being able to perform a range of biological assays on each of the resulting compounds, are also included.

**Results and Discussion**

A library of small molecules related to terpocarvinyl C was envisaged to be synthesized via a differentially acylated or alkylated 1,2-amino alcohol followed by intramolecular biaryl formation, where the atropdiastereoselectivity of the biaryl would be directed by the chiral amino alcohol. Unfortunately, all attempts at the medium ring-forming reactions, using Stille, Suzuki and Miyaura, and other methodologies, were not sufficiently promising for a split-pool synthesis, which requires reactions to proceed in excellent yield and purity.

**Reaction Development.** Biaryl synthesis by way of oxidation of organocopper complexes has been recognized for about a century, for example, in the Ullmann reaction. Whitesides, Kauffmann, van Koten, Ziegler, Bertz, and others have used oxidants on aryl cuprates to give biaryls. More recently, Lipshutz and co-workers have expanded this work considerably in two ways: by using “kinetic” cuprates to cross-couple aryl units intermolecularly, generating unsymmetrical biaryls, and second, by using a chiral tether to synthesize biaryls intramolecularly and atropdiastereoselectively. The Lipshutz method was successfully extended to 3a, a substrate bearing a new and readily accessible chiral tether (Scheme 1). Acyclic substrates, such as 3a, were synthesized readily in three steps: (a) amino alcohols were treated with an ortho-halobenzylaldehyde, and the resultant imine was reduced with NaBH₄ to give 1a, (b) Eschweiler-Clarke N-methylation or reductive alkylation using a borane—pyridine complex yielded 2a, and finally (c) 3a was generated by O-alkylation with an ortho-halobenzyl halide via the sodium alkoxide. Using the Lipshutz method, treatment of the cyclization precursor 3a with tert-butyllithium, then with CuCN, and last with O₂, gave a 58% yield of biaryl.


4a with a diastereomeric ratio (dr) of 1:6 (P:M) in favor of the thermodynamically more stable atropisomer. In attempts to improve the reaction’s suitability for split-pool synthesis, the yield and diastereoselectivity were found to depend on the choice of reaction solvent,27 temperature of oxidation,28 and the choice of oxidant.29 It is intriguing that the oxidant plays a crucial role in determining the degree and direction of atropdiastereoselectivity.

Optimized conditions entailed the use of 2-methyltetrahydrofuran (2-MeTHF) as the reaction solvent and 1,3-dinitrobenzene (1,3-DNB) as the oxidant at −40 °C. This improved procedure resulted reproducibly in the near-quantitative formation of the biaryl (88%, combined yield of biaryls after chromatography) with excellent atropdiastereoselectivity [20:1 (P:M)]. Dehalogenated starting materials are the only other byproducts (Figure 2) and originate from the presence of moisture.30 The reaction is all the more remarkable in that high dilution conditions were not necessary to give the biaryl. In fact, the acyclic substrate concentration has been as high as 0.15 M (1.4 g of 3r in 15 mL of 2-MeTHF), and oligomers were not detected. This eventuality permits the reaction to be performed on polymer supports since, as the reaction favors intrinsically an intramolecular pathway, it avoids the problem of site-site (intermolecular) interactions.31

The scope of the reaction was investigated by using the optimized conditions on a wide range of substrates (Table 1). The reaction proceeded efficiently with different aromatic rings (e.g., phenyls; electron-poor aryl fluorides, aryl chlorides, and benzothiophenes), different ring sizes (9-, 10-, and 11-membered rings), substrates with different substituents (alkyl, and benzothiophenes), different ring sizes (9-, 10-, and 11-membered rings), substrates with different substituents (alkyl, 86 NA NA

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<th>Entry</th>
<th>Biaryl (d)</th>
<th>% Yield</th>
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- Kinetic atropisomer drawn; 3b-z were dibromides. Combined yield. P = S and M = R for a stereogenic axis. Unable to determine thermodynamic dr. No atropoisomerism observed at room temperature. Ratio refers to the configuration of the enantiomer drawn. Atropisomeric stereochemistry not assigned. Debromo-4x was also isolated (12%).
aryl), and substrates with different substituent stereochemistry, however, the reaction is constrained by the aryllithium intermediate on account of its basicity and nucleophilicity. Although the yield of the reaction remained high for the broad range of substrates, the atropdiastereoselectivity had a lower tolerance. As can be seen from the series 4a–e, the diastereomeric ratio was influenced dramatically by aryl substituents and ring size. Similarly, looking down the series 4j–1 and 4o–t, the nitrogen substituent and the amino alcohol substituent(s) affected acutely the atropdiastereoselectivity of the biaryl formation; sterically large substituents tended to reduce both the kinetic and the thermodynamic selectivity. At room temperature the heteroaromatic biaryl 4g–i did not display atropisomerism, presumably due to an insufficient restriction of rotation about the biaryl bond.

The atropisomer that was formed kinetically was not favored thermodynamically, allowing a thermal isomerization (10- and 11-membered rings only) to reverse the stereochemistry of the biaryl. This discovery permits ready access to both atropisomers in the case of 10- and 11-membered rings. The atropisomers were often separable by chromatography, so to determine the thermodynamic ratio for 10- and 11-membered ring products, each atropisomer was heated neat under argon at 120–150 °C for between 24 and 48 h. In the cases where the atropisomers were inseparable, the mixtures were heated until no change in the diastereomeric ratio was observed.

In the case of nine-membered ring products (4c, 4u, and 4w–z), the barrier to rotation about the biaryl bond was too high energetically such that even heating with a flame failed to interconvert atropisomers. In light of this finding, efforts were made to investigate whether the use of different oxidants in the ring-closing reaction would favor the opposite atropisomer kinetically. Although the investigation was unsuccessful, an intriguing result was obtained. Using a suspension of CuCl2 (10 equiv) in THF at −50 °C as the oxidant in the cyclization of 3z gave a 90% yield of 4z [1 : 14 (P : M)]. However, when a solution of CuCl2·2LiCl (10 equiv; THF; −50 °C) was used as the oxidant, biaryl 4z was not formed. Instead, only high molecular weight products were detected, indicative of extensive intramolecular coupling.

One of the many concerns involved in preparing a collection of small molecules for use in many biological screens over an extended period of time is the thermal stability of the compounds. All biaryl products (4a–z) were stable configurationally at room temperature indefinitely. Indeed, heating (P)–4a at 70 °C for 24 h resulted in little conversion to its atropisomer.

**Structural Analysis.** The biaryl configurations of the products were determined by X-ray crystallographic analysis in 18 cases, including 9-, 10-, and 11-membered ring sizes. In all but one of the remaining cases the stereochemistry of the biaryl atropisomers was identified reliably by the presence of nuclear Overhauser effect interactions between specific hydrogen atoms around the ring. With X-ray crystal structures of a variety of biaryl-containing medium rings in hand, structural analysis of these products was revealing (Figure 3).

Both 4g and 4h do not display atropisomerism at room temperature; however, the solid-state conformations of the biaryl axes are the same as the configuration of the thermodynamically more stable atropisomer (M) of biphenyl analogues, for example, (4j–4a). In the case of 10-membered ring products, the solid-state structure of the atropisomer favored kinetically had interring torsion angles (Φ) between 76.4° and 99.5° (median Φ = 82.0°; standard deviation = 10.5°), whereas the atropisomer...
using a zebrafish development assay,\textsuperscript{38} we discovered in the current study that an unusual observable effect on zebrafish development. As an example, different three-dimensional structures, it is hardly surprising to consider the resemblance of the mercury complex 8 does not display atropisomerism in solution at room temperature; however, it is interesting to note that the solid-state conformation that is adopted by the two aromatic rings along the biaryl axis is the same as the configuration of the atropisomer favored kinetically (P). This observation is depicted in the illustrations of 6 and 7 in Scheme 2 and assumes that the atropdiastereoselectivity could be explained by the one-electron oxidation and reductive elimination following the principle of least nuclear motion.\textsuperscript{45}

Along a similar vein, tetrahedral analogues of 7 were made by substituting the CuCN with either Me$_2$SiCl$_2$ or Ph$_2$SiCl$_2$ to form in 6 around the Cu$^+$ atom, which is bonded linearly; (b) a fast, energetically favorable single electron transfer occurs from 6 to form a 1,3-dinitrophenyl radical that is stable kinetically;\textsuperscript{40} and (c) diorganocuperc(II) complexes, such as 7, are unstable with respect to reductive elimination.

To gain insight into the mechanism of the oxidative coupling of biaryl C–C bond and CuO.\textsuperscript{38} The success of this approach to medium ring synthesis is thought to be due to three factors: (a) a favorable chelate is favored thermodynamically had torsion angles between 65.4° and 75.0° (median Φ = 68.6°; standard deviation = 2.8°). When both atropisomers were characterized crystallographically [(M)- and (P)-4p; (M)- and (P)-4k], insights into why one atropisomer is more stable thermodynamically could then be gleaned by visual inspection and molecular modeling. Rather than one overriding factor, a cumulative combination of large-angle, torsional, and transannular strain appears to be responsible.

Considering that the atropisomeric small molecules have acutely different three-dimensional structures, it is hardly surprising to discover different biological activities for each. As an example, using a zebrafish development assay,\textsuperscript{38} we discovered in the current study that (M)-4p affects the cardiovascular system during zebrafish development, whereas (P)-4p had no effect (Figure 4).

**Figure 4.** The molecule shown on the left [(M)-4p] was found not only to cause a pericardial edema and a tube heart at a 5 μM concentration, but also to increase the ratio of atrium to ventricle contractions to 2:1, instead of the usual ratio of 1:1. The atrium is partially obscured by surface pigmentation. The P atropisomer, shown on the right [(P)-4p], had no unusual observable effects on zebrafish development.

**Scheme 2.** Postulated Mechanism for the Oxidative Coupling of Organocuprates

![Scheme 2](image)

\begin{align*}
\text{ organocuprate} & \rightarrow \text{oxidant} \rightarrow \text{biaryl} \\
\text{CuCN with HgBr$_2$ in the cyclization reaction furnishes the 1,3-dinitrophenyl radical that is stable kinetically; but the Cu$^+$ atom is bonded linearly; the M–C$_\text{Ar}$ bond length of Hg$^{\text{II}}$ (Hg$^{\text{II}}$–C$_\text{Ar}$ = 2.07 Å) is the same as that of Cu$^+$ (Cu$^{\text{II}}$–C$_\text{Ar}$ = 1.92 Å). The mercury complex 8 does not display atropisomerism in solution at room temperature; however, it is interesting to note that the solid-state conformation that is adopted by the two aromatic rings along the biaryl axis is the same as the configuration of the atropisomer favored kinetically (P). This observation is depicted in the illustrations of 6 and 7 in Scheme 2 and assumes that this is the ground-state conformation, that is, P rather than M. If this assumption is correct, then the atropdiastereoselectivity could be explained by the one-electron oxidation and reductive elimination following the principle of least nuclear motion. Among a similar vein, tetrahedral analogues of 7 were made by substituting the CuCN with either Me$_2$SiCl$_2$ or Ph$_2$SiCl$_2$ to form in 6 around the Cu$^+$ atom, which is bonded linearly; the M–C$_\text{Ar}$ bond length of Hg$^{\text{II}}$ (Hg$^{\text{II}}$–C$_\text{Ar}$ = 2.07 Å) is the same as that of Cu$^+$ (Cu$^{\text{II}}$–C$_\text{Ar}$ = 1.92 Å). The mercury complex 8 does not display atropisomerism in solution at room temperature; however, it is interesting to note that the solid-state conformation that is adopted by the two aromatic rings along the biaryl axis is the same as the configuration of the atropisomer favored kinetically (P). This observation is depicted in the illustrations of 6 and 7 in Scheme 2 and assumes that this is the ground-state conformation, that is, P rather than M. If this assumption is correct, then the atropdiastereoselectivity could be explained by the one-electron oxidation and reductive elimination following the principle of least nuclear motion. Among a similar vein, tetrahedral analogues of 7 were made by substituting the CuCN with either Me$_2$SiCl$_2$ or Ph$_2$SiCl$_2$ to form in 6 around the Cu$^+$ atom, which is bonded linearly; the M–C$_\text{Ar}$ bond length of Hg$^{\text{II}}$ (Hg$^{\text{II}}$–C$_\text{Ar}$ = 2.07 Å) is the same as that of Cu$^+$ (Cu$^{\text{II}}$–C$_\text{Ar}$ = 1.92 Å). The mercury complex 8 does not display atropisomerism in solution at room temperature; however, it is interesting to note that the solid-state conformation that is adopted by the two aromatic rings along the biaryl axis is the same as the configuration of the atropisomer favored kinetically (P). This observation is depicted in the illustrations of 6 and 7 in Scheme 2 and assumes that this is the ground-state conformation, that is, P rather than M. If this assumption is correct, then the atropdiastereoselectivity could be explained by the one-electron oxidation and reductive elimination following the principle of least nuclear motion. Among a similar vein, tetrahedral analogues of 7 were made by substituting the CuCN with either Me$_2$SiCl$_2$ or Ph$_2$SiCl$_2$ to form in 6 around the Cu$^+$ atom, which is bonded linearly; the M–C$_\text{Ar}$ bond length of Hg$^{\text{II}}$ (Hg$^{\text{II}}$–C$_\text{Ar}$ = 2.07 Å) is the same as that of Cu$^+$ (Cu$^{\text{II}}$–C$_\text{Ar}$ = 1.92 Å). The mercury complex 8 does not display atropisomerism in solution at room temperature; however, it is interesting to note that the solid-state conformation that is adopted by the two aromatic rings along the biaryl axis is the same as the configuration of the atropisomer favored kinetically (P). This observation is depicted in the illustrations of 6 and 7 in Scheme 2 and assumes that this is the ground-state conformation, that is, P rather than M. If this assumption is correct, then the atropdiastereoselectivity could be explained by the one-electron oxidation and reductive elimination following the principle of least nuclear motion. Among a similar vein, tetrahedral analogues of 7 were made by substituting the CuCN with either Me$_2$SiCl$_2$ or Ph$_2$SiCl$_2$ to form in 6 around the Cu$^+$ atom, which is bonded linearly; the M–C$_\text{Ar}$ bond length of Hg$^{\text{II}}$ (Hg$^{\text{II}}$–C$_\text{Ar}$ = 2.07 Å) is the same as that of Cu$^+$ (Cu$^{\text{II}}$–C$_\text{Ar}$ = 1.92 Å). The mercury complex 8 does not display atropisomerism in solution at room temperature; however, it is interesting to note that the solid-state conformation that is adopted by the two aromatic rings along the biaryl axis is the same as the configuration of the atropisomer favored kinetically (P). This observation is depicted in the illustrations of 6 and 7 in Scheme 2 and assumes that this is the ground-state conformation, that is, P rather than M. If this assumption is correct, then the atropdiastereoselectivity could be explained by the one-electron oxidation and reductive elimination following the principle of least nuclear motion.
The macrobeads are functionalized with a diisopropylalkylsilyl group, ideal for alcohol attachment. The most attractive way to attach the amino alcohol building blocks to a polymer support was via reductive amination onto a polymer-supported aldehyde. Unfortunately, because of the poor atropdiastereoselectivity observed in the cyclization of N-benzyl substrates \(4j\) and \(4k\) (Table 1), the wide range of commercially available hydroxybenzaldehydes has not yet been exploitable. Instead, the alkyl aldehyde 11 was used, generated by silyl ether formation (via the silyl triflate) with Z-octen-5-ol, followed by treatment with \(\text{O}_3\) and then \(\text{Me}_2\text{S}\) (Scheme 4).51

The reductive amination was conducted by soaking the macrobeads in a solution containing an excess of amino alcohol to form the oxazolidine, which was reduced by the addition of \(\text{NaBH}_4\) to furnish the polymer-supported oxazolidines (Table 1), the wide range of commercially available hydroxybenzaldehydes not yet been exploitable.

The reductive amination was conducted by soaking the macrobeads in a solution containing an excess of amino alcohol to form the oxazolidine, which was reduced by the addition of \(\text{NaBH}_4\) to furnish 12 (>90% pure by HPLC and NMR).52 The reductive alkylation of the polymer-supported secondary amine was achieved (>95% pure by HPLC and NMR) in high conversion to give 13 by the use of 2-bromobenzylaldehyde and borane−pyridine complex as a reductant.53 O-Alkylation of 13 proved to be a difficult reaction to accomplish successfully on the macrobeads.54 After screening many permutations of bases (e.g., \(\text{NaH/15-crown-5, KH/18-crown-6, BEMP, CsOH, r-BuOK, AgO}_2\)) and solvents, the most successful combination proved to be formation of the polymer-supported potassium alkoxydide with potassium bis(trimethylsilyl)amide (KHMDs) in THF,57 followed by treatment with 2-bromobenzyl bromide. This

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(50) The compounds are attached to the bead by way of a silyl ether. Release of the compound from the bead is achieved by treatment with HF-pyridine; then excess reagent is removed by the addition of TMSOMe followed by concentration under reduced pressure.
(51) Yields on the polymer support were calculated by performing the reactions with 100 mg of macrobeads. The products were released from the polymer support, and the purified mass was compared with the mass of starting material released from 100 mg of unreacted beads.
(52) This reaction illustrates two concerns when transforming a solution-phase reaction on to large polystyrene beads. First, the reaction rate decreases, requiring increased reaction times. Second, the choice of solvent is critical to the success of the reaction.
(54) This may be due to unfavorable charge−charge interactions required when the reactants are held in close proximity to each other on the polymer support.
alkylation procedure resulted in an 80% conversion of the starting material to 0-benzylated product 14. Nevertheless, since the reaction was apparently without side-products, it could be repeated to drive the reaction to completion.

With the polymer-supported cyclization precursor 14 in hand, the copper-mediated cyclization was investigated. It was surprising initially to discover that the lithium–halogen exchange, which had preceded on polymer supports, would not proceed on the macrobeads. Eventually, the exchange was found to occur cleanly at room temperature after 24 h in benzene using an excess of t-BuLi. Using this dilithium intermediate, transmetalation was achieved in a cooled solution of CuCN-2LiBr in 2-MeTHF, and oxidation of the cuprate was accomplished by the addition of 1,3-DNB to give 15. The biaryl-containing medium ring compound 15 could be released from the macrobead at this stage to give 16 in 55% yield [dr = 6:1 (P:M); >80% pure by HPLC and NMR]. Alternatively, the macrobeads (15) could be heated prior to compound release to reverse the atropodiastereomeric ratio [dr = 1:7 (P:M); yield 65%]. Although it was gratifying to have accomplished the medium ring synthesis on the macrobeads, it was evident that to use this reaction in a split-pool synthesis, a less aggressive procedure for the metal–halogen exchange was required.

Not only would the present procedure not be compatible with many of the building blocks that might be considered, but also it would rule out the use of the Still method60 of encoding that would have indistinguishable molecular ions. Alternatively metalation procedures were attempted, specifically application of zinc,61 copper,62 or magnesium63 ate complexes (e.g., Me₄ZnLi₂, Me₂Zn(CN)Li₂, t-Bu₂ZnLi₆, Me₂Cu(CN)Li₃, Me₂Cu(CN)Li₃, i-PrBu₂MgLi, t-Bu₂MgLi)⁶₅ and preferably with the more available aryl bromides rather than iodides. It was rewarding to discover that Me₂ZnLi₂, i-PrBu₂MgLi, and t-Bu₂-

(57) The macrobeads were washed after treatment with base, since KHMDMS reacts with benzyl bromides to form stilbene. For example, the addition of 2-bromobenzyl bromide (1 equiv) to a THF solution of CuCN afforded a 92% solution of KHMDMS at room temperature affords an instantaneous and quantitative formation of (E)-1-(1,2-ethenediyl)b(is[bromobenzene].


(59) It is believed that this problem is due to the difficulty in the b-ButLi penetrating the bead, since the reaction proceeded normally if small (diameter 15 μm) polystyrene beads were used or if the large beads were crushed before the reaction.


(64) Some of the other reagents attempted included Litriphalochene, Rieke Cu (Rieke, R. D. Aldrichimica Acta 2000, 33, 52–60), Rieke Cu (Rieke, R. D.; Dawson, B. T.; Stack, D. E.; Stinn, D. E. Synth. Commun. 1990, 20, 2711–2721), and cyclopentylMgBr (Boudier, A.; Bronm, L. O.; Lotz, M.; Kirschel, P. Angew. Chem., Int. Ed. 2000, 39, 4414–4435). Rieke Cu (4.0 equiv) was successful in solution; for example, 3N = (Br) gave 50% 4v [9.5.1 (P:M)] using 1,3-DNB as the oxidant. However, the procedure was not transferable to polymer-supported substrates.
Figure 6 illustrates all of the building blocks that were selected for use in the split-pool synthesis. To gauge the similarity of atropdiastereoselectivity when the cyclization is performed in solution versus on a polymer support, several cyclization precursors were prepared where the C-5 alcohol was either protected by a triisopropylsilyl (TIPS) group or attached to large polystyrene beads. Analysis of the cyclization reaction products is presented in Table 2. Clearly, a similar solution- and gel-phase result was observed each time. This result indicates that the previous solution-phase results, displayed in Table 1, should be relevant to library members prepared on a polymer support.

During the biaryl-forming reaction, the only side-product that was always observed, albeit in less than 5% yield, was dehalogenated starting material, that is, where the bromines in 14 were substituted by hydrogens. The side-product’s appearance was due, presumably, to the presence of moisture during the reaction. Because it proved difficult practically to eliminate these products completely, and to enhance the diversity of library members, the dehalogenated products were synthesized deliberately by quenching the reaction with MeOH after metalation with i-PrBu2MgLi. Again, for the sake of library diversity, the cyclization precursors were included for biological screening (12—14). Computing all the combinations of building blocks and products revealed that the theoretical maximum number of compounds from the split-pool library was 1412. In total, over 10 000 beads were used in the split-pool library synthesis. This not only ensured that there would be a high statistical coverage of the library members, but also, more than adequately, it allowed for any bead loss and breakage.

The library was synthesized over 2 weeks and comprised 42 individual steps from the polymer-supported aldehyde (Scheme 4). To ensure the purity of the library, five or more macrobeads from each step were analyzed by LCMS before the macrobeads were pooled. The finished library consisted of three pools of macrobears (kinetic and thermodynamic and 17) for both the (S) and (R) series. Ten macrobears chosen randomly from each pool (total of 60 beads) were analyzed by HPLC and LCMS; 37 samples (62%) were over 70% pure, whereas 11 samples (18%) were less than 50% pure, and 1 sample showed no compound was present. Although not perfect, the library has enormous potential for use in biological screens. The macrobears from each pool that looked in best shape physically were arrayed into 384-well plates, one macrobead per well. Each plate was treated with HF-pyridine and then TMSOMe following an optimized, automated operation of compound release (Figure 7). This was followed by another automated procedure to divide each compound into “daughter” 384-well plates. Most of the compound was divided into plates of varying concentration in DMSO to be used in phenotypic assays; 20% was apportioned for small molecule printing, which is used to make the small molecule microarrays used in protein-binding assays, and 10% was sidelined for LCMS analysis.

Note that “amino alcohol” (S)-17i was derivatized as an aryl ether; thus, it did not react in the O-alkylation step and furnishes ultimately a nine-membered ring product. Also, 1,3-amino alcohol (S)-17j yields ultimately an 11-membered ring product.
Members of the library were next screened in many phenotypic assays, including zebrafish Danio rerio and plant Arabidopsis developmental assays and cell-based assays, and in protein-binding assays using small molecule microarrays. The microarrays were constructed by covalently attaching each alcohol-containing small molecule from the library onto a single chlorinated glass slide, by spotting 1 nL of the DMF stock solution using a quill pin robot. Proteins of interest, labeled with fluorescent tags, were used to probe the glass slide to identify small molecule partners.

As a graphic illustration of the potential of this chemical genetic approach, the results from two developmental screens are described below. Observational (phenotypic) screening after random genetic mutagenesis has revealed much information about the development of organisms. Small molecules can modulate gene products to give observable phenotypes; however, they can be used with temporal control and have other advantages, as we have reported elsewhere.

Arabidopsis thaliana seeds (thale cress, ecotype Lansberg erecta [Ler]) were germinated and grown on DMSO (1%) and small molecule-containing (10 μM) agar in 96-well plates at 25 °C under continuous white light. The seedlings were inspected visually under a dissecting light microscope from 1 to 7 days post-germination. Seedlings grown on DMSO-containing agar alone developed normally, with a hypocotyl (seedling stem), two cotyledons, and primary root apparent by 2 days of growth. Plants treated with (P)-4k were found to exhibit stunted development that eventually led to noticeable pigment loss by day 4 (potential inhibition of chlorophyll and/or carotenoid biosynthesis) and death by day 7 (Figure 8).

Vertebrate Danio rerio (zebrafish) embryos develop ex utero and are largely transparent, allowing visual inspection of most anatomical systems during development. Synchronized and fertilized zebrafish embryos were collected and arrayed in 96-well plates (three eggs per well) containing embryo buffer. Library members were added to the embryo buffer from the DMSO stock solutions, one compound per well. The embryos were incubated at 28.5 °C and inspected visually using a dissecting light microscope for developmental defects 1, 2, and 3 days post-fertilization (dpf). Compound 13ab was identified to interfere reproducibly with zebrafish development at a concentration of 100 nM. By the second day post-fertilization, all fish were delayed developmentally; they exhibited lower than normal pigmentation, weak hearts, abnormal brains, and misshapen jaws. Remarkably, in side-by-side experiments performed at a concentration of either 500 or 50 nM, zebrafish developed normally. Compounds 13aa, 13ac, and 13ad do not reproduce this phenotype, suggesting that 13ab may be modulating a particular gene product specifically.

Although the targets of (P)-4k and 13ab have not been pursued yet, they may be discovered by a variety of techniques, such as affinity chromatography followed by microsequencing of the target protein. Identification of the targets of the developmental modulators should provide insight into developmental processes at the molecular level and contribute to the understanding of protein function.

Conclusions

In conclusion, methodology involving the synthesis and oxidation of bis-aryl organocuprates, producing biaryls contained within a medium ring, was developed into an efficient, general, and atom-economical selective ring-closing reaction. The reaction pathway was modified for use on high-capacity macrobeads, key elements of a one bead/one stock solution platform, and used to synthesize a collection of small molecules (1412 theoretical total). Mass spectrometry was demonstrated as an effective way to identify biologically active compounds. Phenotypic and protein-binding assays were performed on members of the library, and results from zebrafish and plant develop-

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(77) Zebrafish developed normally at 5 μM, 2.5 μM, 500 nM, and 50 nM concentrations; however, developmental defects were observed reproducibly at 10 μM and 100 nM concentrations (repeated five times). The developmentally delayed zebrafish die 3 dpf if they continue to be exposed to embryo buffer containing 13ab; however, they can be rescued if transferred to 13ab-free embryo buffer and allowed to progress.

(78) These and other experiments indicate that the small molecules from the library are cell-permeable and capable of interacting directly with intracellular protein targets.

mental assays were described. The ability to use complexity-generating reactions on high capacity beads suitable for a one bead/one stock solution platform and the subsequent use of library members in phenotypic and protein-binding screens demonstrate key elements of the systematic (chemical genetic) approach to exploring biology.

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Supporting Information Available: Complete experimental procedure, characterization data, and library LCMS traces (PDF). An X-ray crystallographic file (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.