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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 i-PrBu$_2$MgLi, followed by transmetalating with CuCN·2LiBr 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).[1] 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.[2] 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).[3] 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).[4] Indeed, pterocaryanin C was especially stimulating on account of its biosynthesis via a stereoselective, C−C bond formation to provide the biaryl-containing medium ring.[4b] Medium rings are not only considered generally to be the most difficult ring sizes to synthesize,[5] but they also require special attention since their formation en masse using split-pool synthesis presents special challenges.[6] 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

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(1) This small molecule approach has the following features: (i) Effects of small molecules on cells and organisms are conditional; they are induced only following the addition of the small molecule. This allows the immediate effects of modulating protein function, rather than the steady-state effects resulting from mutations, to be observed. (ii) A dose-response can be used to gain more confidence of cause and effect and to determine the effects of only partial loss or gain of function. (iii) Protein function can be investigated in cases where a cell having a gene encoding a protein of interest deleted is not viable. For more information see: (a) Schreiber, S. L. *Bioorg. Med. Chem.* **1998**, *6*, 1127−1152. (b) Mitchison, T. J. *Chem. Biol.* **1994**, *1*, 3−6. (c) http://www-schreiber.chem.harvard.edu. (d) http://sbweb.med.harvard.edu/∼iccb/.

(2) (a) Schreiber, S. L. *Science* **2000**, *287*, 1964−1969. (b) Lee, D.; Sello, J. K.; Schreiber, S. L. *Org. Lett.* **2000**, *2*, 709−712. For leading references on split-pool synthesis see: (a) Furka, A.; Sebestyén, F.; Asgedom, M.; Dibó, G. *Int. J. Pept. Protein Res.* **1991**, *37*, 487−493. (b) Lam, K. S.; Salmon, S. E.; Hersh, E. M.; Hruby, V. J.; Kazmierski, W. M.; Knapp, R. J. *Nature* **1991**, *354*, 82−84. (c) Houghten, R. A.; Pinilla, C.; Blondelle, S. E.; Appel, J. R.; Dooley, C. T.; Cuervo, J. H. *Nature* **1991**, *354*, 84−86.

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

(4) (a) Nicolaou, K. C.; Boddy, C. N. C.; Brase, S.; Winssinger, N. *Angew. Chem., Int. Ed.* **1999**, *38*, 2097−2152. (b) Quideau, S.; Feldman, K. S. *Chem. Rev.* **1996**, *96*, 475−503.

(5) 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.

(6) Lee, D.; Sello, J. K.; Schreiber S. L. *J. Am. Chem. Soc.* **1999**, *121*, 10648−10649.

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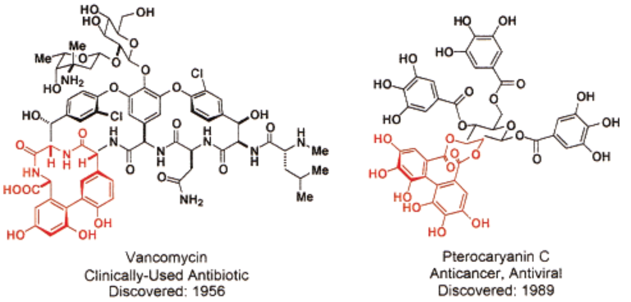
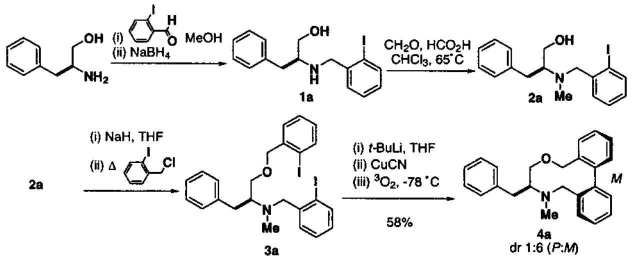


Figure 1. Both vancomycin and pterocaryanin C have axially disymmetric biaryl units contained within 12- and 10-membered rings, respectively (highlighted in red).

Scheme 1



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.[7] 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.[8] 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 pterocaryanin 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,[9] Suzuki and Miyaura,[10] and other methodologies,[11] were not sufficiently promising for a split-pool synthesis, which requires reactions to proceed in excellent yield and purity.[12]

Reaction Development. Biaryl synthesis by way of oxidation of organocopper complexes has been recognized for about a century, for example, in the Ullmann reaction.[13] Whitesides,[14] Kauffmann,[15] van Koten,[16] Ziegler,[17] Bertz,[18] and others[19] have used oxidants on aryl cuprates to give biaryls. More recently, Lipshutz and co-workers have expanded this work considerably in two ways: first, by using "kinetic" cuprates to cross-couple aryl units intermolecularly, generating unsymmetrical biaryls;[20] and second, by using a chiral tether to synthesize biaryls intramolecularly and atropdiastereoselectively.[21] The Lipshutz method was successfully extended to **3a**, a substrate bearing a new and readily accessible chiral tether (Scheme 1).[22] Acyclic substrates, such as **3a**, were synthesized readily in three steps: (a) amino alcohols were treated with an *ortho*-halobenzaldehyde, and the resultant imine was reduced with NaBH$_4$ to give **1a**,[23] (b) Eschweiler-Clarke *N*-methylation[24] or reductive alkylation using a borane−pyridine complex[25] yielded **2a**, and finally (c) **3a** was generated by *O*-alkylation with an *ortho*-halobenzyl halide via the sodium alkoxide.[26] Using the Lipshutz method, treatment of the cyclization precursor **3a** with *tert*-butyllithium, then with CuCN, and last with $^3O_2$, gave a 58% yield of biaryl

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[7] (a) Blackwell, H. E.; Pérez, L.; Stavenger, R. A.; Tallarico, J. A.; Eatough, E. C.; Foley, M. A.; Schreiber, S. L. *Chem. Biol.* **2001**, *8*, 1167−1182. (b) Clemons, P. A.; Koehler, A. N.; Wagner, B. K.; Sprigings, T. G.; Spring, D. R.; King, R. W.; Schreiber, S. L.; Foley, M. A. *Chem. Biol.* **2001**, *8*, 1182−1195.

[8] Spring, D. R.; Krishnan, S.; Schreiber, S. L. *J. Am. Chem. Soc.* **2000**, *122*, 5656−5657.

[9] Stille, J. K. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 508−524.

[10] (a) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457−2483. (b) Carbonnelle, A.-C.; Zhu, J. *Org. Lett.* **2000**, *2*, 3477−3480.

[11] Ni^0 has been shown to be effective at medium ring formation; however, cyclization of **3a** (X = I) under literature conditions furnished only 18% of **4a** [2:3 (*P*:*M*)] and 18% of dehalogenated **3a** (i.e., X = H). Similarly, **3m** (X = I) yielded only 26% of **4m** [1:1 (*P*:*M*)] and 22% of dehalogenated **3m**.

[12] For recent reviews of biaryl synthesis: (a) Bringmann, G.; Menche, D. *Acc. Chem. Res.* **2001**, *34*, 615−624. (b) Lloyd-Williams, P.; Giralt, E. *Chem. Soc. Rev.* **2001**, *30*, 145−157. (c) Bringmann, G.; Breuning, M.; Tasler, S. *Synthesis* **1999**, 525−558. (d) Stanforth, S. P. *Tetrahedron* **1998**, *54*, 263−303.

[13] (a) Ullmann, F.; Bielecki, J. *Chem. Ber.* **1901**, *34*, 2174−2185. (b) Fanta, P. E. *Synthesis* **1974**, 9−21.

[14] (a) Whitesides, G. M.; San Filippo, J.; Casey, C. P.; Panek, E. J. *J. Am. Chem. Soc.* **1967**, *89*, 5302−5303. (b) Whitesides, G. M.; Fischer, W. F.; San Filippo, J.; Bashe, R. W.; House, H. O. *J. Am. Chem. Soc.* **1969**, *91*, 4871−4882. (c) Whitesides, G. M.; Stedronsky, E. R.; Casey, C. P.; San Filippo, J. *J. Am. Chem. Soc.* **1970**, *92*, 1426−1427.

[15] Kauffmann, T. *Angew. Chem., Int. Ed. Engl.* **1974**, *13*, 291−305.

[16] (a) van Koten, G.; Jastrzebski, J. T. B. H.; Noltes, J. G. *Chem. Commun.* **1977**, 203−204. (b) van Koten, G.; Jastrzebski, J. T. B. H.; Noltes, J. G. *J. Org. Chem.* **1977**, *42*, 2047−2053. (c) Janssen, M. D.; Corsten, M. A.; Spek, A. L.; Grove, D. M.; van Koten, G. *Organometallics* **1996**, *15*, 2810−2820.

[17] Ziegler, F. E.; Chliwner, I.; Fowler, K. W.; Kanfer, S. J.; Kuo, S. J.; Sinha, N. D. *J. Am. Chem. Soc.* **1980**, *102*, 790−798.

[18] Bertz, S. H.; Gibson, C. P. *J. Am. Chem. Soc.* **1986**, *108*, 8286−8288.

[19] Konduirov, N. V.; Fomin, D. A. *J. Russ. Phys. Chem. Soc.* **1915**, *47*, 190−198 (*CA* **1915**, *9*, 1473).

[20] (a) Lipshutz, B. H.; Siegmann, K.; Garcia, E. *J. Am. Chem. Soc.* **1991**, *113*, 8161−8162. (b) Lipshutz, B. H.; Siegmann, K.; Garcia, E. *Tetrahedron* **1992**, *48*, 2579−2588. (c) Lipshutz, B. H.; Siegmann, K.; Garcia, E.; Kayser, F. *J. Am. Chem. Soc.* **1993**, *115*, 9276−9282. (d) Lipshutz, B. H.; Kayser, F.; Siegmann, K. *Tetrahedron Lett.* **1993**, *34*, 6693−6696.

[21] (a) Lipshutz, B. H.; Kayser, F.; Liu, Z.-P. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1842−1844. (b) Lipshutz, B. H.; Kayser, F.; Maullin, N. *Tetrahedron Lett.* **1994**, *35*, 815−818. (c) Lipshutz, B. H.; Liu, Z.-P.; Kayser, F. *Tetrahedron Lett.* **1994**, *35*, 5567−5570.

[22] Use of the Lipshutz method: (a) Coleman, R. S.; Grant, E. B. *Tetrahedron Lett.* **1993**, *34*, 2225−2228. (b) Coleman, R. S.; Grant, E. B. *J. Am. Chem. Soc.* **1994**, *116*, 8795−8796. (c) Coleman, R. S.; Grant, E. B. *J. Am. Chem. Soc.* **1995**, *117*, 10889−10904. (d) Sugimura, T.; Yamada, H.; Inoue, S.; Tai, A. *Tetrahedron: Asymmetry* **1997**, *8*, 649−655. (e) Lin, G.-Q.; Zhong, M. *Tetrahedron: Asymmetry* **1997**, *8*, 1369−1372. (f) Andrus, M. B.; Asgari, D.; Sclafani, J. A. *J. Org. Chem.* **1997**, *62*, 9365−9368. (g) Kyasnoor, R. V.; Sargent, M. V. *Chem. Commun.* **1998**, 2713−2714. (h) Carbonnelle, A.-C.; Zamora, E. G.; Beugelmans, R.; Roussi, G. *Tetrahedron Lett.* **1998**, *39*, 4471−4472. (i) Kabir, S. M. H.; Iyoda, M. *Chem. Commun.* **2000**, 2329−2330.

[23] Saavedra, J. E. *J. Org. Chem.* **1985**, *50*, 2271−2273.

[24] Wikening, R. R.; Ratcliffe, R. W.; Doss, G. A.; Mosley, R. T.; Ball, R. G. *Tetrahedron* **1997**, *53*, 16923−16944.

[25] (a) Pelter, A.; Rosser, R. M.; Mills, S. *J. Chem. Soc., Perkin Trans. 1* **1984**, 717−720. (b) Moormann, A. E. *Synth. Commun.* **1993**, *23*, 789−795. (c) Bomann, M. D.; Guch, I. C.; DiMare, M. *J. Org. Chem.* **1995**, *60*, 5995−5996.

[26] (a) Stoochnoff, B. A.; Benoiton, N. L. *Tetrahedron Lett.* **1973**, *14*, 21−24. (b) Brown, C. A.; Barton, D.; Sivaram, S. *Synthesis* **1974**, 434−436.

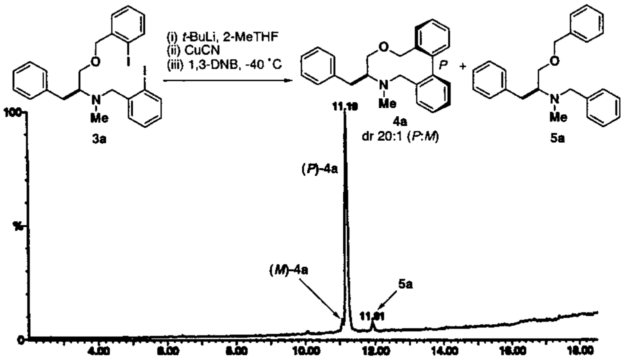


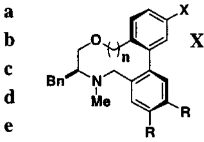
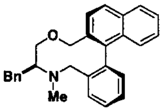
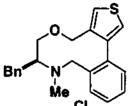
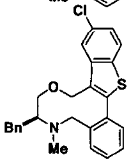
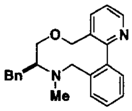
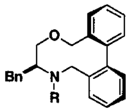
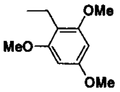
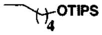
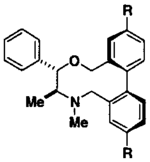
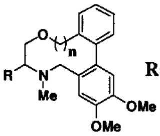
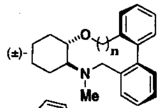
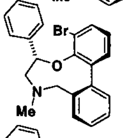
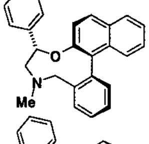
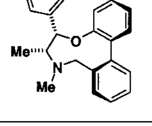
Figure 2. Optimized conditions for the atropdiastereoselective synthesis of the biaryl-containing medium ring **4a**. The LCMS total ion count (TIC) trace for the crude reaction mixture depicts the efficiency and selectivity of the cyclization. The signal-to-noise ratio decreased along the solvent gradient from H_2O (0.1% HCO_2H) to MeCN (0.1% HCO_2H) on the reverse-phase column and accounts for the baseline angle.

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, naphthylenes, and pyridines; electron-rich aryl ethers, thiophenes, and benzothiophenes), different ring sizes (9-, 10-, and 11-membered rings), substrates with different substituents (alkyl,

Table 1. Kinetic and Thermodynamic Ratios

Entry	Biaryl (4)[a]				% Yield[b]	Kinetic dr (*P*:*M*)[c]	Thermodynamic dr (*P*:*M*)
a		X= H	R=H	n=1	88	20:1	1:6
b		F	H	1	84	13:1	1:10
c		H	H	0	97	3:1	UD[d]
d		H	H	2	85	1.3:1	1:4
e		H	OMe	1	92	16:1	1:11
f					92	1.5:1	1:2
g					86	NA[e]	NA[e]
h					73	NA[e]	NA[e]
i					70	NA[e]	NA[e]
j		R= Bn			94	1.1:1	1:7
k					88	2.2:1	1:3
l					83	7:1	1:7
m		R= H			88	17:1	1:1.5
n		Cl			94	6:1	1:2
o		R= (*R*)-Ph		n= 1	84	1:23	2:1
p		(*S*)-*t*-Bu		1	83	4:1	1:2
q		(*S*)-CH_2Cy		1	88	22:1	1:11
r		(*S*)-*i*-Bu		1	97	35:1	1:10
s		(*R*)-Et		1	91	1:25	9:1
t		(*R*)-Me		1	94	1:32	6:1
u		(*S*)-*i*-Bu		0	93	2:1	UD[d]
v				n=1	77	11:1[f]	1:3[f]
w				0	96	>50:1[g]	UD[d]
x					81[h]	1:>50	UD[d]
y					73	1.5:1	UD[d]
z					68	1:10	UD[d]

[a] Kinetic atropisomer drawn; **3b**−**z** were dibromides. [b] Combined yield. [c] *P* ≡ *S* and *M* ≡ *R* for a stereogenic axis. [d] Unable to determine thermodynamic dr. [e] No atropisomerism observed at room temperature. [f] Ratio refers to the configuration of the enantiomer drawn. [g] Atropisomeric stereochemistry not assigned. [h] Debromo-**4x** was also isolated (12%).

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(27) Reaction solvent: 2-MeTHF [dr = 9:1 (*P*:*M*)]; Et_2O [dr = 6:1]; PhMe [dr = 6:1]; THF [dr = 5:1]; DME [dr = 1:1]. Conditions: **3a** to **4a**; oxidant, 1,3-DNB; temperature of oxidation, 0 °C.

(28) Temperature of oxidation: −78 °C [dr = 2:1 (*P*:*M*)] unclean reaction; −40 °C [dr = 5:1]; −20 °C [dr = 5:1]; 0 °C [dr = 5:1]; 25 °C [dr = 4:1]. Conditions: **3a** to **4a**; oxidant, 1,3-DNB; reaction solvent, THF. Temperature of oxidation: −100 °C [dr = 2:1 (*P*:*M*)]; −78 °C [dr = 1:6]; 0 °C [dr = 1:1] unclean reaction. Conditions: **3a** to **4a**; oxidant, $^3O_2$; reaction solvent, THF.

(29) Oxidant: $PhNO_2$ [dr = 5:1 (*P*:*M*)]; 1,2-DNB [6:1]; 1,3-DNB [20:1]; 1,4-DNB [14:1]; 2,4-dinitrotoluene (2,4-DNT) [dr = 3:1]; 3,4-DNT [dr = 1:1]. Conditions: **3a** to **4a**; temperature of oxidation, −40 °C; reaction solvent, 2-MeTHF.

(30) Other products, such as phenols (presumably from ArLi + oxidant) and *tert*-butyl adducts (from the use of more than 4.0 equiv of *t*-BuLi), can be avoided if exact reagent quantities are used. The crude reaction mixture also contains the excess 1,3-DNB and 3,3′-dinitroazoxybenzene (from oxidized 1,3-DNB).

(31) (a) Blackwell, H. E.; Clemons, P. A.; Schreiber, S. L. *Org. Lett.* **2001**, *3*, 1185−1188. (b) Hodge, P. *Chem. Soc. Rev.* **1997**, *26*, 417−424.

aryl), and substrates with different substituent stereochemistry;[32] 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**−**l** 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 biaryls **4g**−**i** did not display atropisomerism,[33] presumably due to an insufficient restriction of rotation about the biaryl bond.[34]

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.[35] 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 $CuCl_2$ (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*)];[36] however, when a solution of $CuCl_2 \cdot 2LiCl$ (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 intermolecular coupling.[37]

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).

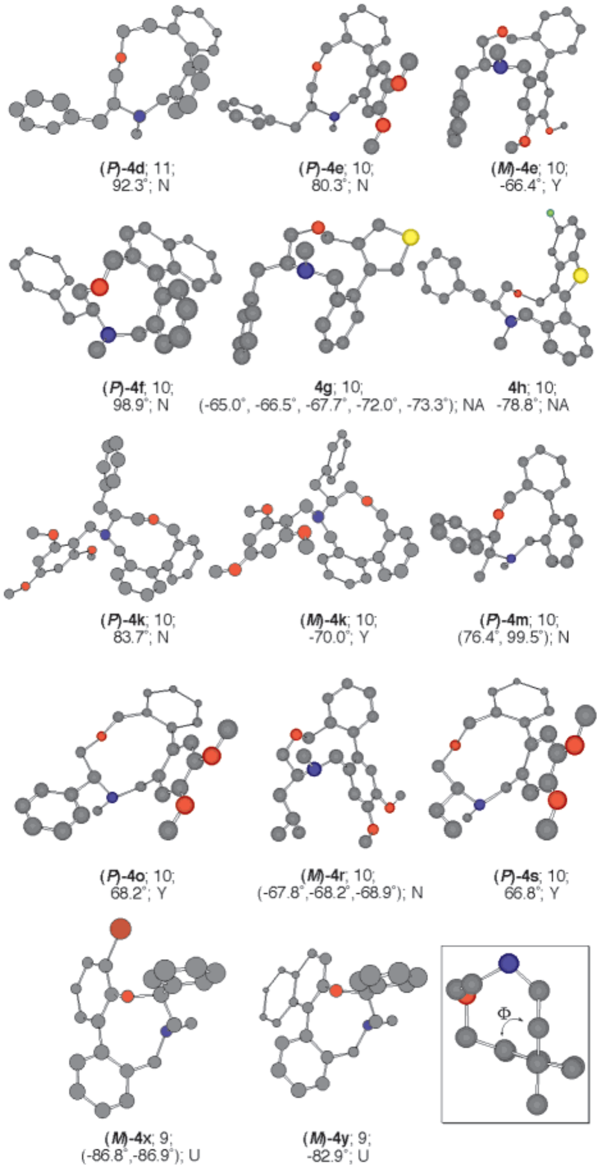

Figure 3. X-ray crystal structures of selected biaryl-containing medium ring compounds. Below each structure is listed the compound identifier, ring size, angle Φ, and whether the atropdiastereomer is the thermodynamically more stable atropisomer. Multiple torsion angles (Φ) observed in different crystallographic polymorphs are depicted within parentheses. Key: *M* = minus; *P* = positive; Y = yes; N = no; NA = not applicable; U = unknown; Φ = biaryl torsion angle within the medium ring (see inset).

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, ***(M)*-4a**. In the case of 10-membered ring products, the solid-state structure of the atropisomer favored kinetically had inter-ring torsion angles (Φ) between 76.4° and 99.5° (median Φ = 82.0°; standard deviation = 10.5°), whereas the atropisomer

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[32] All the cyclization precursors shown contain *bisortho*-iodo/bromoaromatic rings. Attempts to cyclize different substrates to give the biaryl implanted within a medium ring in an *ortho,meta*-relationship (e.g., within the 12-membered ring of vancomycin) failed.

[33] Atropisomerism has been defined arbitrarily as existing where the isomers can be isolated and have a half-life, $t_{1/2}$, of at least 1 000 s. Oki, M. *Top. Stereochem.* **1983**, *14*, 1−81.

[34] Note that when the *ortho*-N is replaced by C−H (compare **4i** with **4a**) the increase to the energy barrier of rotation about the biaryl bond is enough to observe atropisomerism at room temperature.

[35] Higher temperatures were required to determine the thermodynamic diastereomeric ratio of **4f**.

[36] A suspension of $FeCl_3$ (10 equiv) in THF improved the atropdiastereoselectivity [1:>50 (*P*:*M*)].

[37] This result was confirmed with other nine-membered ring substratres. Also, a suspension of $CrCl_3$ (10 equiv) in THF yielded high molecular weight products only.

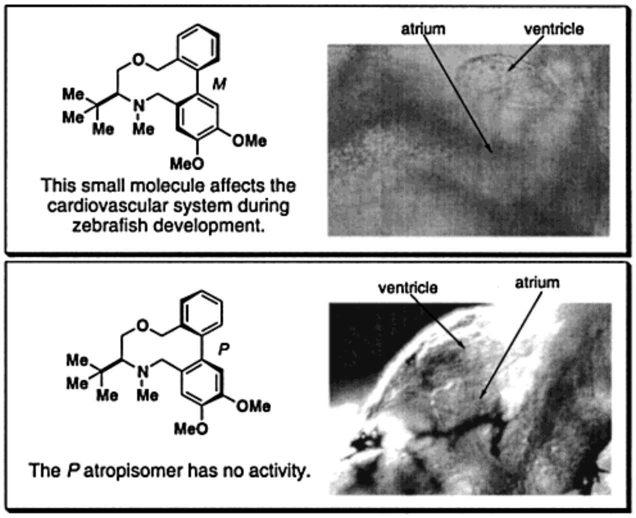
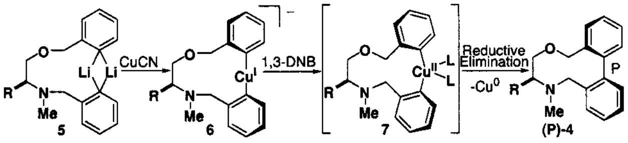


Figure 4. The molecule shown on the left [**(*M*)-4p**] was found not only to cause a pericardial edema and a tube heart at a 5 μM concentration, but also to increase the ratio of atrium to ventricle contractions to 2:1, instead of the usual ratio of 1:1. The atrium is partially obscured by surface pigmentation. 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



favored thermodynamically had torsion angles between 65.4° and 75.0° (median $\Phi = 68.6°$; standard deviation = 2.8°). When both atropisomers were characterized crystallographically [**(*M*)-** and **(*P*)-4e**; **(*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,[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).

Reaction Mechanism. The mechanism of this remarkably efficient ring-closing reaction is believed to involve the following: (a) lithium–halogen exchange to give the dilithium intermediate **5** (Scheme 2), (b) transmetalation resulting in the formation of the 11-membered copper(I) intermediate **6**, where the Cu^I atom is bonded linearly, and (c) this intermediate (**6**) is oxidized upon addition of 1,3-dinitrobenzene to furnish the square planar or tetrahedral copper(II) intermediate **7**, which itself eliminates reductively to form the biaryl C–C bond and Cu^0.[39] The success of this approach to medium ring synthesis is thought to be due to three factors: (a) a favorable chelate is formed in **6** around the Cu^I 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;[40] and (c) diorganocopper(II) complexes, such as **7**, are unstable with respect to reductive elimination.

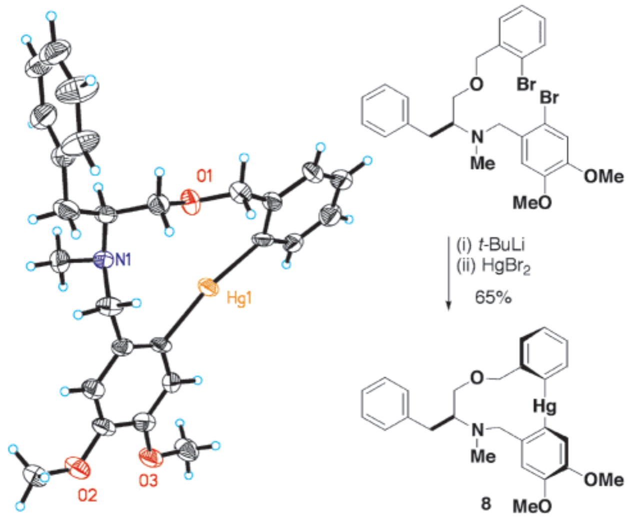


Figure 5. Synthesis and X-ray crystal structure of the organomercurial **8**, presumably analogous structurally to the reaction intermediate **6**.

To gain an insight into the foundation of the atropdiastereoselectivity, stable analogues of the intermediates **6** and **7** were sought.[41] The linear bonding mode of copper(I), illustrated in **6**, is adopted by mercury(II) complexes. The substitution of CuCN with $HgBr_2$ in the cyclization reaction furnishes the crystalline **8** in 65% yield,[42] after recrystallization from EtOH (Figure 5).[43] Confidence in the resemblance of the mercury analogue **8** to the speculated copper intermediate **6** is enhanced, because the $M-C_{(Ar)}$ bond length of Hg^{II} ($Hg^{II}-C_{Ar} = 2.07$ Å) is similar to that of Cu^I ($Cu^I-C_{Ar} = 1.92$ Å).[44] 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.[45]

Along a similar vein, tetrahedral analogues of **7** were made by substituting the CuCN with either Me_2SiCl_2 or Ph_2SiCl_2 to

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(38) Peterson, R. T.; Link, B. A.; Dowling, J. E.; Schreiber, S. L. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 12965–12969.

(39) It has been speculated that the organocuprate may be oxidized to a copper(III) intermediate. While this cannot be ruled out conclusively, it is considered unlikely, especially since a slight excess of $CuCl_2$ can be used alternatively as the oxidant to give the biaryl in good yield.

(40) The dinitrobenzene radical species persist until the reaction is quenched with acid, at which point they disproportionate to form 3,3′-dinitroazoxybenzene and 1,3-dinitrobenzene.

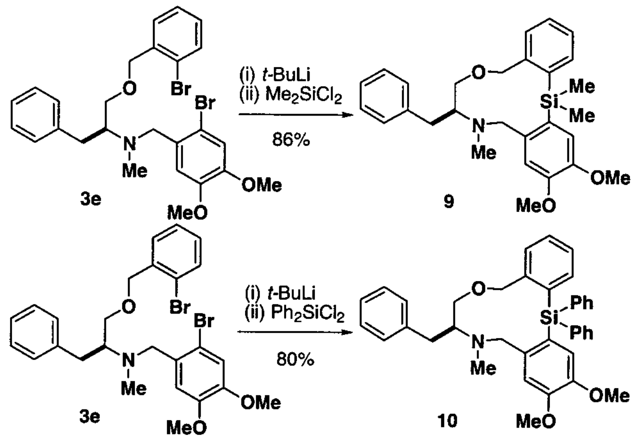
(41) Also, attempts were made to crystallize the dilithium intermediate **5** via standard Schlenk techniques in the hope of obtaining evidence for the proposed structure illustrated in Scheme 2 (cf. Beno, M. A.; Hope, H.; Olmstead, M. M.; Power, P. P. *Organometallics* **1985**, *4*, 2117–2121); however, these efforts only resulted in the crystallization of moisture- and temperature-sensitive $[LiBr{\cdot}Et_2O]_4$ (see Supporting Information), which has been characterized previously: Neumann, F.; Hampel, F.; Schleyer, P. V. R. *Inorg. Chem.* **1995**, *34*, 6553–6555.

(42) Bähr, G.; Küpper, F. W. *Chem. Ber.* **1967**, *100*, 3992–3995.

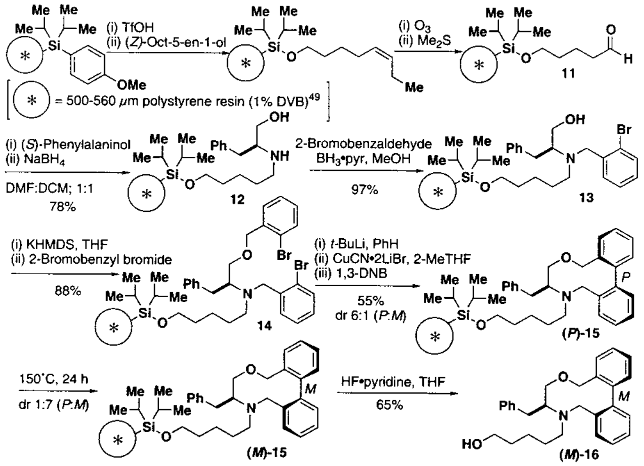
(43) The mercury complex **8** is stable to air, moisture, and room temperature. A 10-membered ring analogue of **8** was made with **3y** in 81% yield after chromatography. Although the product was not crystalline, it was characterized spectroscopically; this showed an increased conformational rigidity as compared with **8**, but atropisomerism was not observed.

(44) Leoni, P.; Pasquali, M.; Ghilardi, C. A. *J. Chem. Soc., Chem. Commun.* **1983**, 240–241.

Scheme 3



Scheme 4



give **9** and **10**, respectively (Scheme 3); however, these silacycles failed to crystallize, and atropisomerism was not observed at room temperature. Nevertheless, we find these structures fascinating from the perspective of their unknown potential as modulators of biological pathways. The reactions that lead to metal insertion and ring formation are also of interest from the perspective of planning diversity-oriented reaction pathways. Together with the biaryl-forming reactions, they constitute a branch point in the pathway, where the addition of distinct reagents transforms the acyclic bis(bromoarenes) into products having distinct scaffolds. Such reactions are of prime significance in efforts to synthesize complex and diverse compounds efficiently, a goal of diversity-oriented synthesis.

Polymer-Supported Synthesis. The small polystyrene beads (e.g., diameter of 50 μm; capacity of 0.1 nmol per bead) that have been used in traditional applications of solid-phase synthesis, as a purification technique, provide a quantity of compound insufficient for multiple assays. In the current study, solid-phase supports are used to take advantage of the power of split-pool synthesis, which results in a predominantly single compound on each individual bead.

We have shown that by using larger “macrobeads” (e.g., diameter of 500 μm; capacity of 100 nmol per bead), we are able to obtain enough compound to perform many assays.[7] We have also been able to adapt an encoding method to these macrobeads to determine a macrobead’s chemical history; one key feature of this method is that decoding is accomplished by using a tiny fraction of the final stock solution[46] (e.g., using GC tags).[47] In addition, by analyzing the compound after it has been released from the bead (e.g., using liquid chromatograph/mass spectrometry [LCMS]), we have been able to determine directly the structure of a compound associated with a given macrobead.[48]

The macrobeads are functionalized with a diisopropylalkylsilyl group,[49] ideal for alcohol attachment.[50] 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 O_3 and then Me_2S (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 $NaBH_4$ 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-bromobenzaldehyde 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., NaH/15-crown-5, KH/18-crown-6, BEMP,[55] CsOH,[56] *t*-BuOK, Ag_2O) and solvents, the most successful combination proved to be formation of the polymer-supported potassium alkoxide with potassium bis(trimethylsilyl)amide (KHMDS) in THF,[57] followed by treatment with 2-bromobenzyl bromide. This

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[45] It is likely that, even at the low temperatures used in the reaction, the cuprate **6** will entertain rotation about the biaryl axis; however, the ground-state conformation will be most populated. If the hypothesis that the later mechanistic steps follow the principle of least nuclear motion is correct, then the atropisomer stereochemistry and selectivity are ascertained by the relative conformational population (*P* vs *M*) of the cuprate **6**. Following this conjecture through, however, does not immediately provide an explanation for the atropdiastereoselectivity of other oxidants. For reviews of the principle of least nuclear motion, see: (a) Hine, J. *Adv. Phys. Org. Chem.* **1977**, *15*, 1−61. (b) Sinnott, M. L. *Adv. Phys. Org. Chem.* **1988**, *24*, 113−204.

[46] (a) Stavenger, R. A.; Schreiber, S. L. *Angew. Chem., Int. Ed.* **2001**, *40*, 3417−3421. (b) Blackwell, H. E.; Pérez, L.; Schreiber, S. L. *Angew. Chem., Int. Ed.* **2001**, *40*, 3421−3425.

[47] Barnes, C.; Balasubramanian, S. *Curr. Opin. Chem. Biol.* **2000**, *4*, 346−350.

[48] Sternson, S. M.; Louca, J. B.; Wong, J. C.; Schreiber, S. L. *J. Am. Chem. Soc.* **2001**, *123*, 1740−1747.

[49] Tallarico, J. A.; Depew, K. M.; Pelish, H. E.; Westwood, N. J.; Lindsley, C. W.; Shair, M. D.; Schreiber, S. L.; Foley, M. A. *J. Comb. Chem.* **2001**, *3*, 312−318.

[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.

[53] Khan, N. M.; Arumugam, V.; Balasubramanian, S. *Tetrahedron Lett.* **1996**, *37*, 4819−4822.

[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.

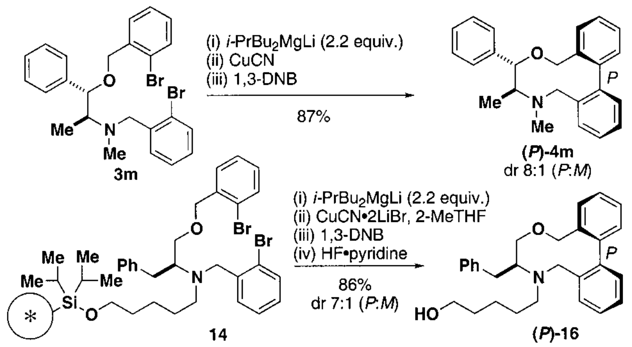
[55] O’Donnell, M. J.; Zhou, C.; Scott, W. L. *J. Am. Chem. Soc.* **1996**, *118*, 6070−6071.

[56] Dueno, E. E.; Chu, F.; Kim, S. I.; Jung, K. W. *Tetrahedron Lett.* **1999**, *40*, 1843−1846.

alkylation procedure resulted in an 80% conversion of the starting material to *O*-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 precedent on polymer supports,[58] would not proceed on the macrobeads.[59] Eventually, the exchange was found to occur cleanly at room temperature over 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 atropdiastereomeric 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.

Scheme 5



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 method[60] of encoding that had previously been adapted to the macrobeads.[7a] Alternative metalation procedures were attempted, specifically application of zinc,[61] copper,[62] or magnesium[63] ate complexes (e.g., Me_4ZnLi_2, $Me_3Zn(CN)Li_2$, *t*-Bu_3ZnLi,[64] $Me_2Cu(CN)Li_2$, $Me_3Cu(CN)Li_3$, *i*-$PrBu_2MgLi$, *t*-Bu_3MgLi)[65] and preferably with the more available aryl bromides rather than iodides. It was rewarding to discover that Me_4ZnLi_2, *i*-$PrBu_2MgLi$, and *t*-Bu_3MgLi all work very well with arylbromides in solution;[66] however, on the polymer-supported cyclization precursor **14**, *i*-$PrBu_2MgLi$ gave consistently the best results (Scheme 5).[67] At this stage it was interesting to look at the yield of compound (**16**) available per macrobead. HPLC was used to analyze the amount of compound released from 20 beads. The mean average was found to be a yield of 76 nmol/macrobead, which ranged between 53 and 106 nmol/macrobead with a standard deviation of 16 nmol/macrobead.

With a series of suitable reactions available, consideration was given to their use in a split-pool synthesis. Unfortunately, this modified procedure was still not orthogonal to polychlorinated aromatics used for GC encoding of macrobeads;[68] in fact it was observed that a pentachloro-aromatic ring is metalated before a monobromo-aromatic ring. This nonorthogonality dictated that each library member, released from an individual bead in the one bead/one stock solution format,[7] should be identified by direct analysis of the material, for example, by LCMS. Fortunately, electrospray mass spectrometry was extremely sensitive in detecting these tertiary amine products. However, consideration of the building blocks must be made to avoid making library members with the same empirical formula, which would have indistinguishable molecular ions. Fragmentation of the molecular ion,[48] next to the heteroatoms, could be used to differentiate the two halves of the molecule; nevertheless, building block constitutional isomers, such as leucinol and isoleucinol, still should be avoided. In the case of atropisomers, these will, by definition, have the same empirical formula; hence, macrobeads that have undergone a thermal equilibration would not be mixed with beads that have not been heat-treated. In this way, by not pooling beads at the last step, the library would be encoded positionally. Similarly, enantiomers cannot be differentiated by mass spectrometry; thus, the library was carried out in parallel with (*S*)- and (*R*)-amino alcohols.

Building blocks for the library synthesis were selected individually on the basis of sufficient success (>80% purity by HPLC and NMR after release from the polymer support) with a chosen substrate in the reaction where they were introduced.[69]

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(57) The macrobeads were washed after treatment with base, since KHMDS reacts with benzyl bromides to form stilbenes. For example, the addition of 2-bromobenzyl bromide (1 equiv) to a THF solution of KHMDS at room temperature affords an instantaneous and quantitative formation of (*E*)-1,1′-(1,2-ethenediyl)bis(2-bromobenzene).

(58) (a) Farrall, M. J.; Fréchet, J. M. J. *J. Org. Chem.* **1976**, *41*, 3877−3882. (b) Darling, G. D.; Fréchet, J. M. J. *J. Org. Chem.* **1986**, *51*, 2270−2276. (c) Itsuno, S.; Darling, G. D.; Lu, P. Z.; Fréchet, J. M. J. *Polym. Mater. Sci. Eng.* **1987**, *57*, 570−574. (d) Tempest, P. A.; Armstrong, R. W. *J. Am. Chem. Soc.* **1997**, *119*, 7607−7608.

(59) It is believed that this problem is due to the difficulty in the *t*-BuLi penetrating through the bead, since the reaction proceeded normally if small (diameter 75 μm) polystyrene beads were used or if the large beads were crushed before the reaction.

(60) (a) Ohlmeyer, M. J. H.; Swanson, R. N.; Dillard, L. W.; Reader, J. C.; Asouline, G.; Kobayashi, R.; Wigler, M.; Still, W. C. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10922−10926. (b) Nestler, H. P.; Bartlett, P. A.; Still, W. C. *J. Org. Chem.* **1994**, *59*, 4723−4724.

(61) (a) Kondo, Y.; Takazawa, N.; Yamazaki, C.; Sakamoto, T. *J. Org. Chem.* **1994**, *59*, 4717−4718. (b) Uchiyama, M.; Kameda, M.; Mishima, O.; Yokoyama, N.; Koike, M.; Kondo, Y.; Sakamoto, T. *J. Am. Chem. Soc.* **1998**, *120*, 4934−4946. (c) Kondo, Y.; Komine, T.; Fujinami, M.; Uchiyama, M.; Sakamoto, T. *J. Comb. Chem.* **1999**, *1*, 123−126.

(62) Kondo, Y.; Matsudaira, T.; Sato, J.; Murata, N.; Sakamoto, T. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 736−738.

(63) (a) Kitagawa, K.; Inoue, A.; Shinokubo, H.; Oshima, K. *Angew. Chem., Int. Ed.* **2000**, *39*, 2481−2483. (b) Inoue, A.; Kitagawa, K.; Shinokubo, H.; Oshima, K. *J. Org. Chem.* **2001**, *66*, 4333−4339.

(64) This reagent succeeds in exchange with aryl iodides; however, treatment with CuCN and then 1,3-DNB results in a 21% isolated yield of the biaryl [10:1 (*P*:*M*)] and a 76% yield of the dehalogenated starting material.

(65) Some of the other reagents attempted included Li/naphthalene, 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.; Bromm, L. O.; Lotz, M.; Knochel, P. *Angew. Chem., Int. Ed.* **2000**, *39*, 4414−4435). Rieke Cu (4.0 equiv) was successful in solution; for example, **3v** (X = 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.

(66) Me_4ZnLi_3 (2.2 equiv); **3m** to **4m**; yield: 84%; dr 13:1 (*P*:*M*). *t*-Bu_3MgLi (2.2 equiv); **3m** to **4m**; yield: 90%; dr 12:1 (*P*:*M*).

(67) It is notable that metalation with *i*-$PrBu_2MgLi$ is more functional-group tolerant than that with *t*-BuLi. This advance has allowed the reaction to be performed successfully in solution in the presence of esters, and no doubt allows the cyclization reaction to be compatible with a much wider range of functionalities.

(68) Blackwell, H. E.; Pérez, L.; Schreiber, S. L. *Angew. Chem., Int. Ed.* **2001**, *40*, 3421−3425.

(69) All the reactions were performed on a relatively large scale, 100 mg of macrobeads, followed by release from the polymer support to ascertain purity and yield.

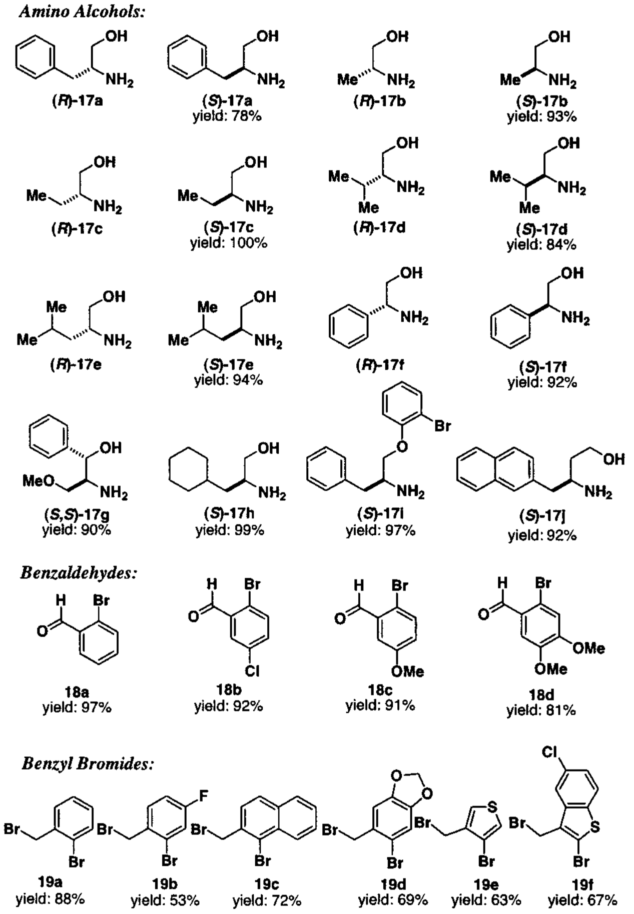


Figure 6. Building blocks for the biaryl-containing medium ring library.

Table 2. Kinetic Diastereomeric Ratios and Yields with a Soluble Substrate or with a Substrate on an Insoluble Support

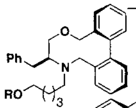
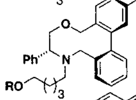
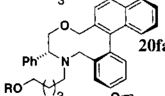
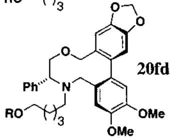
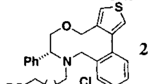
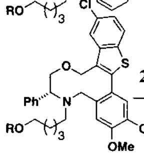
	Solution Phase (R=TIPS)			Gel Phase (R=H)			
	Entry	% Yield	Kinetic dr (*P*:*M*)	Entry	% Yield	Kinetic dr (*P*:*M*)	Thermodynamic dr (*P*:*M*)
	**20aaa**	83	7:1	**16aaa**	86	7:1	1:7
	**20fab**	88	1:5	**16fab**	72	1:5	2:1
	**20fac**	74	1:1	**16fac**	87	1:1.5	UD
	**20fdd**	85	1:10	**16fdd**	74	1:8	2:1
	**20fae**	93	NA	**16fae**	74	NA	NA
	**20fdf**	58	NA	**16fdf**	90	NA	NA

Figure 6 illustrates all of the building blocks that were selected for use in the split-pool synthesis.[70]

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 **17**, 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 (**17**) were synthesized deliberately by quenching the reaction with MeOH after metalation with *i*-$PrBu_2MgLi$. 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.[71] 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 **11** (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 macrobeads (kinetic and thermodynamic **15** and **17**)[72] for both the (*S*) and (*R*) series. Ten macrobeads 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.[73] Although not perfect, the library has enormous potential for use in biological screens. The macrobeads from each pool that looked in best shape physically were arrayed into 384-well plates, one macrobead per well.[74] Each plate was treated with HF•pyridine and then TMSOMe following an optimized, automated operation of compound release (Figure 7).[7b] 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.

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(70) 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.

(71) The totals of (*S*) = 854 and (*R*) = 558 include both atropisomers with benzyl bromide building blocks **a**−**d**.

(72) The macrobeads of the cyclization precursors were distributed equally between the three other pools; since they all contain bromine they will be distinguishable instantly, once released from the macrobead, by virtue of the isotope pattern observed in the mass spectrum.

(73) See Supporting Information.

(74) Columns 23 and 24 were left empty allowing for their use as internal controls in assays.

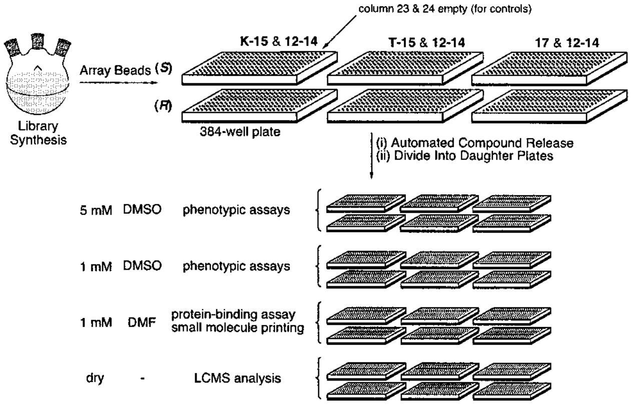

Figure 7. Postsynthesis formatting of the split-pool library.

Members of the library were next screened in many phenotypic assays, including zebrafish *Danio rerio*[38] and plant *Arabidopsis*[75] 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.[76] 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.[38]

Arabidopsis thaliana seeds (thale cress, ecotype Lansberg erecta [L*er*]) 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). **(*M*)-4k** was only very weakly active in this assay.

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.[77] Compounds **13aa**, **13ac**, and **13ad** do not reproduce this phenotype, suggesting that **13ab** may be modulating a particular gene product specifically.[78]

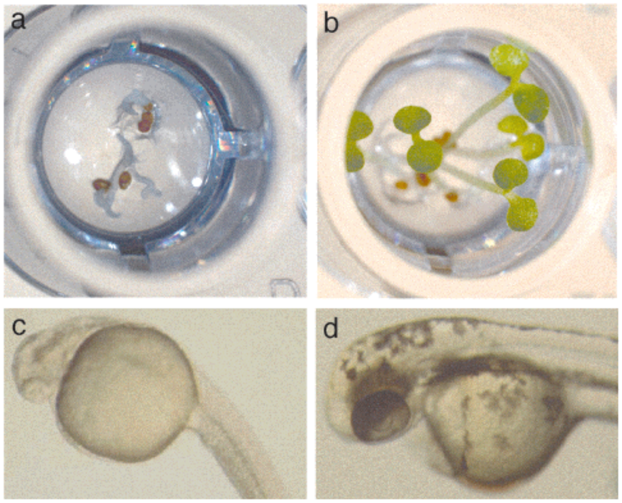

Figure 8. Plant (a and b) and zebrafish (c and d; 2 dpf) developmental assays. (a) Seven day old *Arabidopsis* seedlings germinated on agar containing 1% DMSO and **(*P*)-4k** (10 μM). (b) Seven day old control seedlings germinated on agar containing 1% DMSO. (c) Synchronized zebrafish embryos were treated with **13ab** 100 nM. Zebrafish are delayed developmentally; they exhibit lower than normal pigmentation, weak hearts, abnormal brains, and misshapen jaws. (d) Synchronized embryos were treated with **13ab** 5 μM; zebrafish look indistinguishable to untreated controls.

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.[79] 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 atropdiastereoselective 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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(75) Grozinger, C. M.; Chao, E. D.; Blackwell, H. E.; Moazed, D.; Schreiber, S. L. *J. Biol. Chem.* **2001**, *276*, 38837−38843.

(76) Hergenrother, P. J.; Depew, K. M.; Schreiber, S. L. *J. Am. Chem. Soc.* **2000**, *122*, 7849−7850.

(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.

(79) King, R. W. *Chem. Biol.* **1999**, *6*, R327−R333.

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.

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