J|A|C|S
ARTICLES
Published on Web 10/06/2004

A Synthesis Strategy Yielding Skeletally Diverse Small Molecules Combinatorially

Martin D. Burke,[1] Eric M. Berger, and Stuart L. Schreiber*

Contribution from the Broad Institute of Harvard and MIT, Department of Chemistry and Chemical Biology, Howard Hughes Medical Institute, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138

Received July 15, 2004; E-mail: stuart_schreiber@harvard.edu

Abstract: The efficient synthesis of small molecules having many molecular skeletons is an unsolved problem in diversity-oriented synthesis (DOS). We describe the development and application of a synthesis strategy that uses common reaction conditions to transform a collection of similar substrates into a collection of products having distinct molecular skeletons. The substrates have different appendages that pre-encode skeletal information, called σ-elements. This approach is analogous to the natural process of protein folding in which different primary sequences of amino acids are transformed into macromolecules having distinct three-dimensional structures under common folding conditions. Like σ-elements, the amino acid sequences pre-encode structural information. An advantage of using folding processes to generate skeletal diversity in DOS is that skeletal information can be pre-encoded into substrates in a combinatorial fashion, similar to the way protein structural information is pre-encoded combinatorially in polypeptide sequences, thus making it possible to generate skeletal diversity in an efficient manner. This efficiency was realized in the context of a fully encoded, split-pool synthesis of ~1260 compounds potentially representing all possible combinations of building block, stereochemical, and skeletal diversity elements.

Introduction

One aim of diversity-oriented synthesis (DOS) is to populate chemical space in defined ways with small molecules having diverse and complex structures.[2] In contrast to a one synthesis-one skeleton approach where many building blocks are appended to a common core structure, DOS aims to generate both stereochemical and skeletal diversity to create a diverse display of chemical information in three-dimensional space. It also aims to create products having attachment sites that facilitate the appending of substituents during a "follow-up" phase of research. Achieving these aims should facilitate the search for specific small-molecule modulators of each of the functions performed by the structurally diverse macromolecules that comprise living systems.[2,3]

In both combinatorial chemistry and DOS, building block diversity can be achieved in a highly efficient manner by harnessing the power of combinatorics (a multiplicative increase in the number of products with an additive increase in the number of building blocks). This efficiency can be exploited by using split-pool synthesis.

Generating skeletal diversity with this same efficiency has thus far been a formidable challenge;[2,4] it requires generating skeletal diversity combinatorially. We recently reported the development and application of a synthesis strategy for achieving this aim.[5] In this approach, a collection of substrates with different appendages that pre-encode skeletal information (named σ-elements) are transformed into a collection of products having distinct molecular skeletons using common reaction conditions. An important feature of this approach is that multiple σ-elements can be used to pre-encode skeletal diversity combinatorially and thereby provide efficient access to diverse displays of chemical information in three-dimensional space. Here, we describe in detail the development of this σ-element-based strategy for generating skeletal diversity combinatorially and its successful application in the context of a fully encoded split-pool synthesis.

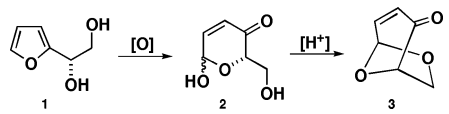


Figure 1. The Achmatowicz reaction.

The Achmatowicz Reaction

Our studies began with efforts to use the Achmatowicz reaction[6] in the context of DOS. As shown in Figure 1, this complexity-generating reaction transforms a relatively simple substrate **1** into a more complex product **3** having a [3.2.1]

(1) Adapted from the Ph.D. Thesis of Burke, M. D., Harvard University, 2003.
(2) (a) Schreiber, S. L. *Science* **2000**, *287*, 1964−1969. (b) Schreiber, S. L. *Chem. Eng. News* **2003**, *81*, 51−61. (c) Burke, M. D.; Schreiber, S. L. *Angew. Chem., Int. Ed.* **2004**, *43*, 46−58 and references therein.
(3) Schreiber, S. L. *Bioorg. Med. Chem.* **1998**, *6*, 1127−1152.
(4) (a) Weber, L. *Curr. Opin. Chem. Biol.* **2000**, *4*, 295−302 and references therein. (b) Ding, S.; Gray, N. S.; Wu, X.; Ding, Q.; Schultz, P. G. *J. Am. Chem. Soc.* **2002**, *124*, 1594−1596.
(5) Burke, M. D.; Berger, E. M.; Schreiber, S. L. *Science* **2003**, *302*, 613−618 and references therein.
(6) Achmatowicz, O., Jr.; Bukowski, P.; Szechner, B.; Zwierzchowska, Z.; Zamojski, A. *Tetrahedron* **1971**, *27*, 1973−1996.

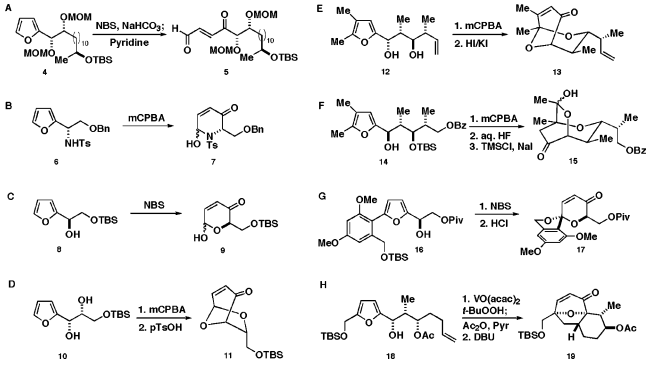


Figure 2. Achmatowicz reaction and related transformations in the context of TOS. References: (A) Kobayashi, Y.[7] (B) Zhou, W.-S.[8] (C) O'Doherty, G. A.[9] (D) Ogasawara, K.[10] (E) Martin, S. F.[11] (F) Deshong, P.[12] (G) O'Doherty, G. A.[13] (H) Wender, P. A.[14]

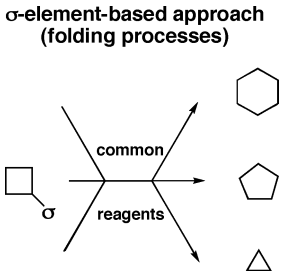


Figure 3. The σ-element-based approach for generating skeletal diversity. Substrates having different appendages that pre-encode skeletal information, named σ-elements, are transformed into products having distinct molecular skeletons using a common set of reaction conditions.

bicyclic ketal skeleton with potential for diversification via diastereoselective functionalization of the resultant enone. It also offers the potential for skeletal diversification, as hinted by the intermediacy of a monocycle on route to the bridged ketal. Stereochemical diversification was envisioned as arising from, among others, the application of stereocontrolled reactions leading to the diol-containing side chain.

Since the pioneering studies by Achmatowicz in 1971, skeletal transformations based on furan oxidation have found widespread use in the context of target-oriented synthesis (TOS). Some selected examples are shown in Figure 2.

These reactions, involving transformations of relatively simple and similar substrates into more complex and diverse products using similar reaction conditions, are reminiscent of the process of protein folding. Similar to the way the three-dimensional structures of proteins are pre-encoded by distinct primary amino acid sequences,[15] the skeletal information for each small molecule product is pre-encoded in the corresponding substrate, not the applied reaction conditions. This conceptual analogy led to a strategy for generating skeletal diversity in DOS, that is, pre-encoding skeletal information into relatively simple and similar substrates, and transforming them under single reaction conditions into more complex and diverse products having different molecular skeletons (see Figure 3).[16]

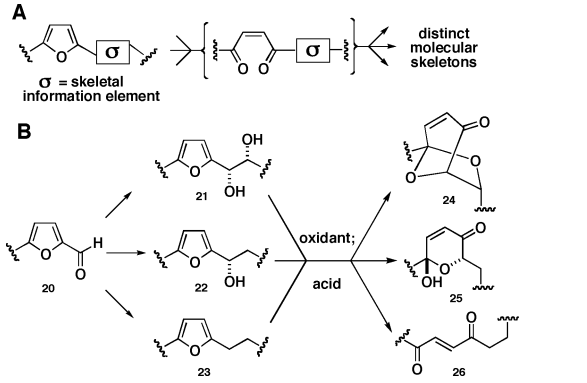


Figure 4. Synthetic plan for a skeletal diversity-generating process.

Planning DOS Pathways

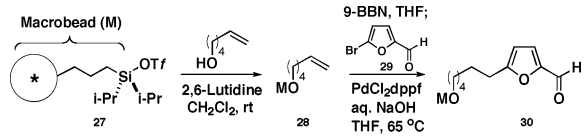
In TOS, synthesis pathways are devised most effectively using retrosynthetic analysis, a planning strategy in which a target structure is systematically deconstructed by formally performing chemical reactions in the reverse-synthetic direction.[17] In this way, syntheses in TOS are planned in the direction of products → reactants. Because DOS aims to generate a collection of compounds with maximized diversity (rather than a single target structure), retrosynthetic methods cannot be applied effectively in this context. Forward-synthetic analysis aims to facilitate the planning of efficient synthesis pathways that maximize molecular complexity and diversity.[2]

Synthesis pathways in DOS are planned in the forward-synthetic direction, that is, from reactants → products. The basic subunit of forward-synthetic planning is the process, defined as the transformation of a collection of substrates into a collection of products. Furthermore, transformations of relatively similar substrates into collections of more diverse products are referred to as diversity-generating processes, and DOS pathways are developed by identifying series of such processes having products = substrates relationships. In this context, the σ-element-based strategy proposed above represents a potentially general type of skeletal diversity-generating process, which we have named a folding process in recognition of its conceptual analogue.

Key to this approach is the transformation of substrates having different σ-elements into products having different skeletons using common reaction conditions (split-pool synthesis renders ad-hoc optimization for each substrate impractical). This type of forward-synthetic planning can begin with the identification of a core structure or functional group with the capacity for transformation into a more reactive intermediate using, ideally, a set of mild and selective reaction conditions. As shown in Figure 4A, the furan ring is one example of this type of latent intermediate: it can be transformed into a more reactive *cis*-enedione using mild oxidative reaction conditions. The planning of a folding process proceeds by conceptually appending

(7) Kobayashi, Y.; Nakano, M.; Kumar, G. B.; Kishihara, K. *J. Org. Chem.* **1998**, *63*, 7505−7515.
(8) Yang, C.-F.; Xu, Y.-M.; Liao, L.-X.; Zhou, W.-S. *Tetrahedron Lett.* **1998**, *39*, 9227−9228.
(9) Harris, J. M.; O'Doherty, G. A. *Tetrahedron Lett.* **2000**, *41*, 183−187.
(10) Takeuchi, M.; Taniguchi, T.; Ogasawara, K. *Synthesis* **1999**, *2*, 341−354.
(11) Martin, S. F.; Gluchowski, C.; Campbell, C. L.; Chapman, R. C. *J. Org. Chem.* **1984**, *49*, 2512−2513.
(12) Deshong, P.; Ramesh, S.; Perez, J. J. *J. Org. Chem.* **1983**, *48*, 2117−2118.
(13) Balachari, D.; O'Doherty, G. A. *Org. Lett.* **2000**, *2*, 4033−4036.
(14) Wender, P. A.; Rice, K. D.; Schnute, M. E. *J. Am. Chem. Soc.* **1997**, *119*, 7897−7898.
(15) Anfinsen, C. B. *Science* **1973**, *181*, 223−230.
(16) Burke, M. D.; Schreiber, S. L. *Harvard-MIT Division of Health Sciences and Technology Forum, The Speech Chain, Book of Abstracts*; Cambridge, MA, March, 2000; p 5.
(17) (a) Corey, E. J.; Cheng, X.-M. *The Logic of Chemical Synthesis*; John Wiley and Sons: New York, 1995. (b) Corey, E. J. *Angew. Chem., Int. Ed. Engl. (Nobel Lecture)* **1991**, *30*, 455−465.

Scheme 1 [a]



[a] Reagents and conditions: 5-hexen-1-ol, 2,6-lutidine, CH_2Cl_2, room temperature, 12 h; 9-BBN, THF, room temperature, 5 h; 5-bromofuraldehyde (**29**), $PdCl_2$dppf, NaOH, THF:H_2O 5:1, 65 °C, 20 h; loading = 0.679 mequiv/g, purity >85% (LCMS).

substituents (σ-elements) with complementary chemical reactivity to the reactive intermediate. Common conditions can then be used to liberate the latent intermediate and to allow the trapping of this reactive intermediate with the appended σ-elements, leading to the formation of distinct molecular skeletons.

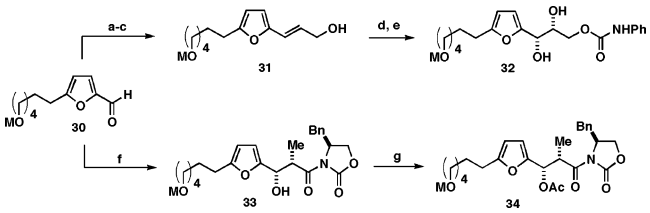
This logic was used to develop the synthetic plan shown in Figure 4B. First, the common furaldehyde substrate **20** is transformed into three furan products **21**−**23** having three different σ-elements − two-carbon side chains containing two, one, or zero nucleophilic hydroxyl groups. These three compounds **21**−**23** collectively serve as substrates for a skeletal diversity-generating process. A common set of oxidative and acidic reaction conditions is used to transform substrates **21**−**23** into three products having distinct molecular skeletons, a [3.2.1] bicyclic ketal **24** (via oxidative ring-expansion and acid-promoted bicycloketalization), a cyclic hemiketal **25** (via oxidative ring expansion and acid-promoted equilibration of the anomeric center), and a *trans*-enedione **26** (via oxidative furan cleavage and acid-promoted cis → trans isomerization).

Transforming σ-Elements into Distinct Skeletons

To test the feasibility of this plan, we performed solid-phase syntheses of three substrates having two-carbon side chains bearing two, one, or zero nucleophilic hydroxyl groups. A two-step, solid-phase synthesis of model compound 5-(6-hydroxyhexyl)-2-furaldehyde (**30**) was developed as shown in Scheme 1. Commercially available 5-hexen-1-ol was loaded onto 500−560 μm polystyrene-based "macrobeads" using a previously published protocol.[18] Hydroboration of the terminal olefin with 9-BBN followed by $PdCl_2$(dppf)-mediated Suzuki coupling with commercially available 5-bromo-2-furaldehyde (**29**) led to the desired macrobead-bound furaldehyde **30** with high loading (0.679 mequiv/g) and purity (>85% by LCMS).

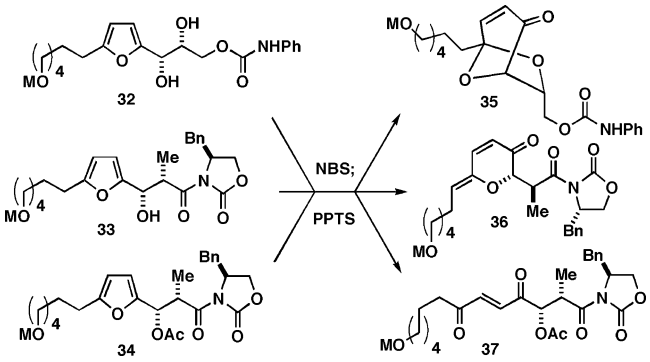
As shown in Scheme 2, this common furaldehyde starting material was then transformed into three furan derivatives **32**, **33**, and **34** having two, one, and zero appended nucleophilic hydroxyl groups, respectively. Horner−Wadsworth−Emmons olefination of aldehyde **30** with allyldiethylphosphonoacetate followed by deallylation, reduction, carbamate formation, and Sharpless asymmetric dihydroxylation[19] generated macrobead-bound diol **32** in >90% purity (LCMS analysis of crude product after cleavage from macrobeads) and 66% ee.[20] Evans' aldol

Scheme 2 [a]



[a] Reagents and conditions:[22] (a) allyldiethylphosphonoacetate, LiOH, THF, room temperature, 25 h; (b) $Pd(PPh_3)_4$, thiosalicylic acid, THF, room temperature, 24 h; (c) isobutylchloroformate, 4-methylmorpholine, *i*-Pr_2NEt, THF, 0 °C, 2 h; $LiBH_4$, *i*-Pr_2NEt, THF, 4 °C, 24 h, 76% (three steps), purity 68%; (d) phenyl isocyanate, pyridine, CH_2Cl_2, room temperature, 24 h; (e) OsO_4, $(DHQD)_2PHAL$, 4-methylmorpholine *N*-oxide, TEAAT, acetone:H_2O 10:1, 4 °C, 48 h, 65% (two steps), purity >90%; (f) (*S*)-(+)-4-benzyl-3-propionyl-2-oxazolidinone, *n*-Bu_2BOTf, Et_3N, CH_2Cl_2, 72 h, −78 to 0 °C; 30% aqueous H_2O_2, pH7 buffer, MeOH, 4 °C, 12 h, >95%, purity >90%; (g) Ac_2O, *i*-Pr_2NEt, DMAP, CH_2Cl_2, room temperature, 28 h, 88%, purity >90%.

Scheme 3 [a]



[a] Reagents and conditions: *N*-bromosuccinimide (NBS), $NaHCO_3$, NaOAc, THF:H_2O 4:1, room temperature, 1 h; PPTS, CH_2Cl_2, 40−45 °C, 20 h, **35**, 33%, purity 64%,[23] **36**, 35%, purity 86%, **37**, 81%, purity >90%.

reaction[21] with aldehyde **30** provided the α-hydroxy furan **33** with >90% purity and >20:1 dr. Acylation of aldol adduct **33** generated the α-acetoxyalkyl furan **34** (>90% purity), which bears no nucleophilic hydroxyl groups.

As shown in Scheme 3, when **32**−**34**, each having a common furan skeleton, were exposed to the same optimized set of "folding conditions" (NBS, $NaHCO_3$, and NaOAc in THF:H_2O 4:1 at room temperature for 1 h followed by PPTS in CH_2Cl_2 at 40 °C for 20 h), they were transformed into products having three different skeletons.

Furan derivatives **32** and **34** were transformed into the anticipated [3.2.1] bicyclic ketal **35** (64% purity[23]) and *trans*-enedione **37** (>90% purity), respectively. However, aldol adduct **33**, having a single nucleophilic hydroxyl group appended to the same furan core, underwent an initial oxidative ring expansion to the expected cyclic hemiketal followed by an unanticipated, acid-mediated dehydration reaction to yield the alkylidene-pyran-3-one **36** (86% purity) as a single geometric isomer.[24] (For an animation of this skeletal diversity-generating

(18) (a) 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. (b) M. Narovlyansky and J. Tallarico are gratefully acknowledged for generously providing all of the macrobeads used in these studies.

(19) (a) Kolb, H. C.; Van Nieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483−2547. (b) For a study of the Sharpless dihydroxylation on solid phase, see: Han, H.; Janda, K. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1731−1733.

(20) In a solution-phase model study, the asymmetric dihydroxylation of a related substrate proceeded with >90% ee. It is unclear why the enantioselectivity was diminished for the solid-phase reaction.

(21) Evans, D. A.; Bartroli, J.; Shih, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 2127−2129.

(22) Yields were determined by mass of recovered products after HF-mediated cleavage from ~75 mg of macrobeads and purification by silica gel chromatography. Purities were determined by LCMS analysis (UV detection at λ_{214}) of crude products following cleavage from macrobeads.

(23) An impurity in the LCMS chromatogram that was ^{1}H NMR-silent and had an isotope pattern in its mass spectrum consistent with an osmium-containing substance was not included in the calculation of the purity of product **35**.

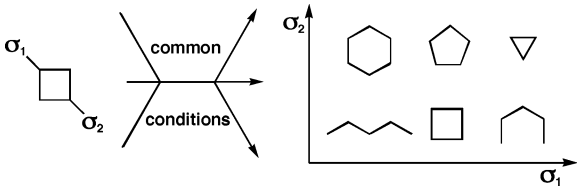
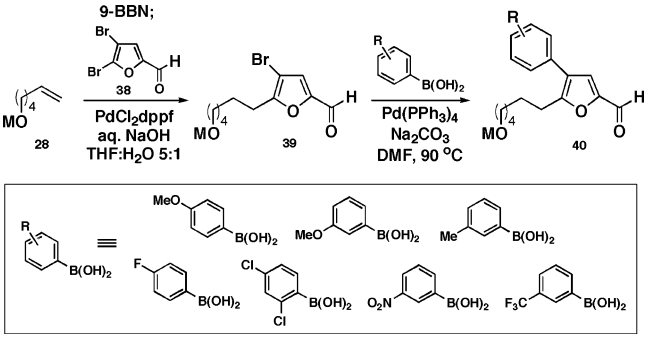


Figure 5. Generating skeletons of small molecules combinatorially.

Scheme 4



folding process, see http://slsiris.harvard.edu/home/animation.html.) Unanticipated reaction outcomes, particularly those involving the formation of new skeletons, can be highly valuable in DOS.

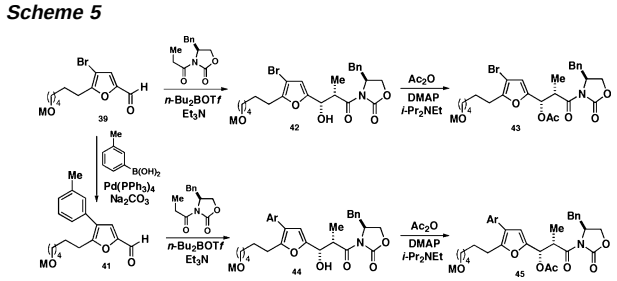
Generating Skeletal Diversity Combinatorially

Expanding the above approach to generate skeletal diversity combinatorially requires the identification of at least two sets of σ-elements that function in combination to pre-encode different molecular skeletons (see Figure 5). This approach has the potential to achieve a multiplicative increase in skeletal outcomes with an additive increase in σ-elements (just as a multiplicative increase in building block combinations is achieved with the one synthesis-one skeleton approach), and thereby to generate skeletal diversity efficiently.

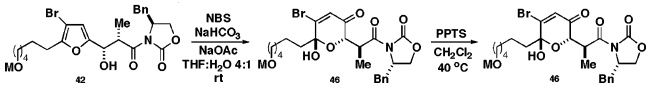
During the course of our studies, it was determined that substituents at the 4-position of furan can also pre-encode different skeletal outcomes. Moreover, we found that substituents appended at the 4-position of furan and those on the α-carbon can function in combination to pre-encode a combinatorial matrix of diverse skeletons.

In model studies originally directed at expanding building block diversity, it was determined that a regioselective Suzuki reaction with commercially available 4,5-dibromo-2-furaldehyde (**38**)[25] could be used to generate macrobead-bound 4-bromo-5-alkyl-2-furaldehyde **39** (see Scheme 4).[26] Competitive β-hydride elimination, observed when using $Pd(PPh_3)_4$ as catalyst in this reaction, was minimized by using $PdCl_2$dppf.[27] No 4,5-bis-alkyl functionalization (via on-bead site−site interactions)[28] was

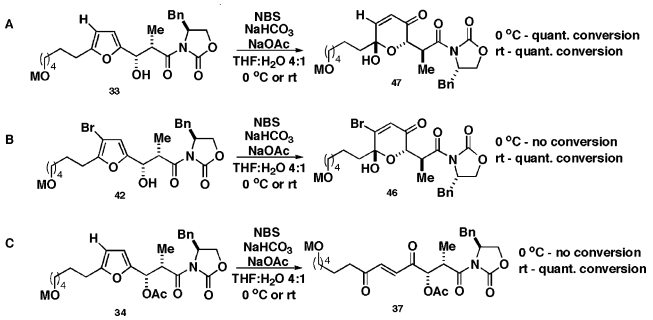
Scheme 5



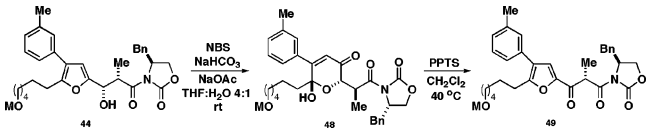
Scheme 6



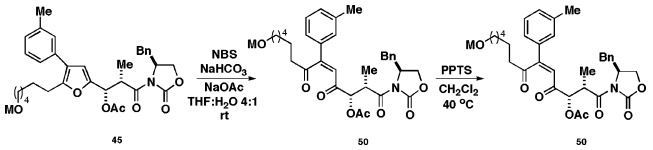
Scheme 7



Scheme 8



Scheme 9



observed. It was also determined that the 4-position of **39** could subsequently be functionalized[26,29] at higher temperatures using $Pd(PPh_3)_4$ as catalyst and a variety of arylboronic acids as coupling agents to yield bis-functionalized furaldehydes **40**.[30]

As shown in Scheme 5, macrobead-bound-4-bromo-5-alkyl-2-furaldehyde **39** and 4-*m*-methylphenyl-5-alkyl-2-furaldehyde **41** were determined to be excellent substrates for the Evans aldol reaction with or without subsequent acetylation (see Scheme 11 below for optimized syntheses of these compounds) to generate model substrates **42**−**45**,[31] each having a distinct combination of substituents at the 2- and 4-positions of the furan ring. Treating these four substrates with the oxidative and acidic conditions described above led to four different skeletal outcomes.

(24) The mechanism for this dehydration presumably involves acid-promoted E1-type elimination via an oxocarbenium ion intermediate. The indicated *cis*-alkene in **36** (stereochemistry assigned with ^{1}H NMR nOe studies) likely represents the thermodynamic product (minimization of sterics).

(25) This reagent was purchased from Moscow MedChemlabs (Moscow, Russia) or synthesized in one step via treatment of 2-furaldehyde with molecular bromine in the presence of aluminum trichloride: Chiarello, J.; Joullié, M. M. *Tetrahedron* **1988**, *44*, 41−48.

(26) Similar regioselective Sonogashira and Stille couplings have been reported: Bach, T.; Krüger, L. *Eur. J. Org. Chem.* **1999**, 2045−2057.

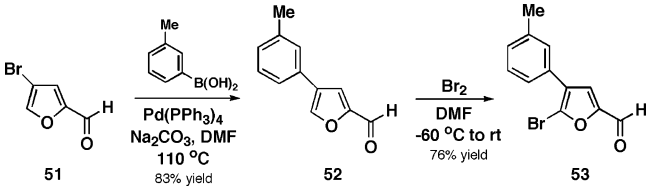
(27) (a) Miyaura, N.; Ishiyama, T.; Sasaki, H.; Ishikawa, M.; Satoh, M.; Suzuki, A. *J. Am. Chem. Soc.* **1989**, *111*, 314−321. (b) Hayashi, T.; Konishi, M.; Kobori, Y.; Kumada, M.; Higuchi, T.; Hirotsu, K. *J. Am. Chem. Soc.* **1984**, *106*, 158−163.

(28) Blackwell, H. E.; Clemons, P. A.; Schreiber, S. L. *Org. Lett.* **2001**, *3*, 1185−1188.

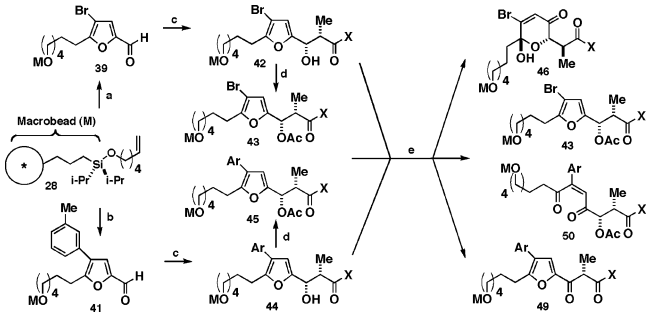
(29) (a) Chang, C. K.; Bag, N. *J. Org. Chem.* **1995**, *60*, 7030−7032. (b) Zhou, X.; Tse, M. K.; Wan, T. S. M.; Chan, K. S. *J. Org. Chem.* **1996**, *61*, 3590−3593.

(30) Efforts to couple a variety of B-alkyl reagents at the 4-position were not fruitful. Efficient coupling was achieved under similar conditions using as coupling agents a variety of *trans*-alkenylboronic acids. Less efficient coupling was achieved with tetramethyltin [using $PdCl_2(Po\text{-}Tol_3)_2$ in DMA at 115 °C, two cycles].

Scheme 10



Scheme 11 [a]



[a] X = (*S*)-4-benzyl-2-oxazolidinone, Ar = *m*-methylphenyl. Reagents and conditions: (a) 9-BBN, THF, room temperature, 5 h; 4,5-dibromofuraldehyde (**38**), $PdCl_2$dppf, NaOH, THF:H_2O 5:1, 65 °C, 18 h, 0.188 mequiv/g; (b) 9-BBN, THF, room temperature, 5 h; 4-*m*-MePh-5-bromofuraldehyde (**53**), $PdCl_2$dppf, NaOH, THF:H_2O 5:1, 65 °C, 22 h, 0.545 mequiv/g; (c) (*S*)-(+)-4-benzyl-3-propionyl-2-oxazolidinone, *n*-Bu_2BOTf, Et_3N, CH_2Cl_2, 72 h, −78 to 0 °C; 30% aqueous H_2O_2, pH 7 buffer, MeOH, 4 °C, 12 h, **42**, >95%, purity >90%, **44**, 95%, purity >90%; (d) Ac_2O, *i*-Pr_2NEt, DMAP, CH_2Cl_2, room temperature, 28 h, **43**, 90%, purity >90%, **45**, 84%, purity >90%; (e) NBS, $NaHCO_3$, NaOAc, THF:H_2O 4:1, room temperature, 1 h; PPTS, CH_2Cl_2, 40−45 °C, 20 h, **46**, 82%, purity 90%, **43**, 88%, purity >90%, **49**, 74%, purity 72%, **50**, 72%, purity 66%.

As shown in Scheme 6, model substrate **42** having a bromine atom at the 4-position of furan and a hydroxyl group on the α-carbon of the 2-substituent underwent quantitative, NBS-mediated oxidative ring expansion to yield the anticipated cyclic hemiketal **46** as a >9:1 mixture of epimers.[32] However, upon exposure to the same acidic conditions (PPTS in CH_2Cl_2 at 40 °C) that effected dehydration with the hemiketal derived from the oxidation of model substrate **33** (having a hydrogen atom at the 4-position of furan, see **33** → **36**, Scheme 3), the bromine-substituted cyclic hemiketal **46** remained unchanged. It seems plausible that formation of an oxocarbenium ion intermediate, presumably required for dehydration to occur, is disfavored both electronically and sterically by the electronegative and large (1,2-type strain) bromine substituent.

In contrast, the 4-bromo-2-α-acetoxyalkyl furan **43** proved completely resistant to NBS-mediated oxidation and was likewise unchanged upon subsequent treatment with PPTS, resulting in preservation of the original α-alkoxyalkyl furan skeleton. As shown in Scheme 7, the deactivating effect of both the 4-bromo and the 2-α-acetoxyalkyl substituents toward furan oxidation was observed independently when the reaction was performed at 0 °C (~1 h reaction time). As a point of reference, the 4-hydrido-2-α-hydroxyalkyl furan **33** was readily oxidized both at room temperature and at 0 °C by NBS (Scheme 7A). However, both the 4-bromo-2-α-hydroxyalkyl furan **42** and the 4-hydrido-2-α-acetoxyalkyl furan **34**, each bearing a single deactivating substituent, were resistant to oxidation at 0 °C (Scheme 7B and C, respectively), even though both substrates were oxidized quantitatively in 1 h at room temperature. By combining these two deactivating substituents into the same substrate (**43**), a distinct skeletal outcome was achieved upon exposure to the same reagents at room temperature, that is, preservation of the initial furan skeleton via complete resistance to oxidation.

The ability of electron-withdrawing substituents to deactivate nearby olefins toward electrophilic reagents is well-known.[33] In early studies of furan oxidation, Clauson-Kaas and co-workers noted that furan derivatives having highly electron-withdrawing substitutents (e.g., furoic acid and α-acetylfuran) were resistant to oxidation with molecular bromine.[34] It seems reasonable that the deactivating effect of the bromine substituent at the 4-position is predominantly due to its electron-withdrawing nature (Pauling electronegativity[35] for −Br is 2.8 as opposed to 2.1 for −H), although steric effects may also contribute.

The deactivating effect of the 2-α-acetoxyalkyl group is likely the result of two factors: (1) the electron-withdrawing nature of the acetoxy group, and (2) the removal of the hydrogen-bond donating hydroxyl group on the α-carbon, which likely facilitates furan oxidation via intramolecular directing of the NBS reagent. This proposed two-fold effect is consistent with the classic studies by Henbest and co-workers on the epoxidation of cyclohexene and its derivatives using the oxidant perbenzoic acid.[36]

As shown in Scheme 8, treatment of 4-aryl-2-α-hydroxyalkyl furan **44** with NBS resulted in oxidative ring expansion to yield the expected aryl-substituted cyclic hemiketal **48**. However, when exposed to acidic conditions (e.g., PPTS), complete conversion into a new product was observed, identified as the α-keto furan **49**.[37]

The basis for this reactivity is not known. A similar type of reaction, involving the Br_2/H_2SO_4-mediated transformation of an α-hydroxy-β-butoxycarbonyl furan into an α-keto-β-butoxycarbonyl derivative, was reported in early studies by Achmatowicz,[6] who proposed the intermediacy of an enediol tautomer.

The oxidative ring expansion and acid-promoted rearrangement observed for 4-*m*-methylphenyl-2-α-hydroxyalkyl furan **44** proved to be general for a series of related 4-aryl-2-α-hydroxyalkyl furans that were synthesized and tested. It was observed that this outcome is more preferred with substrates having electron-donating aryl substituents (e.g., *p*-methoxyphenyl) versus electron-withdrawing aryl substituents (e.g., 2,4-dichlorophenyl).

As shown in Scheme 9, when treated with the same oxidative and acidic reagents, 4-aryl-2-α-acetoxyalkyl furan **45** was

(31) The HWE olefination, reduction, functionalization, and dihydroxylation sequence was also explored with these and related substrates; however, the length of the synthesis pathway precluded the generation of the desired macrobead-bound diols with acceptable macrobead integrity and compound purity.

(32) The stereochemistry at the anomeric carbon of cyclic ketal **46** was tentatively assigned as *R* on the basis of two-dimensional NOESY and one-dimensional nOe experiments.

(33) (a) Swern, D. *J. Am. Chem. Soc.* **1947**, *69*, 1692−1698. (b) Swern, D. *Chem. Rev.* **1949**, *45*, 1−68.

(34) Clauson-Kaas, N.; Limborg, F.; Fakstorp, J. *Acta Chem. Scand.* **1948**, *2*, 109−115.

(35) (a) Pauling, L. *The Nature of the Chemical Bond*, 3rd ed.; Cornell University Press: Ithaca, NY, 1960. (b) Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry, Part A: Structure and Mechanisms*, 3rd ed.; Plenum Press: New York, 1990; p 15.

(36) Henbest, H. B.; Wilson, R. A. L. *J. Chem. Soc.* **1957**, 1958−1965.

(37) (a) Epimerization of the potentially labile methyl-bearing stereogenic center in **49** was not observed. This observation is consistent with the lack of epimerization observed with the structurally similar Evans' extended polypropionate (β-ketoimide) reagents. (b) Evans, D. A.; Clark, J. S.; Metternich, R.; Novack, V. J.; Sheppard, G. S. *J. Am. Chem. Soc.* **1990**, *112*, 866−868. (c) Evans, D. A.; Ng, H. P.; Clark, J. S.; Rieger, D. L. *Tetrahedron* **1992**, *48*, 2127−2142.

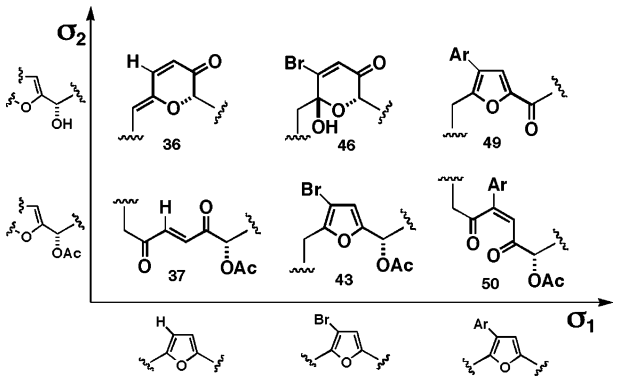


Figure 6. A combinatorial matrix of σ-elements pre-encodes a complete matrix of distinct skeletal outcomes.

transformed into product **50** having a *cis*-enedione skeleton,[38] in contrast to the *trans*-enedione skeleton formed from the (otherwise identical) 4-hydrido derivative **34** (see **34** → **37**, Scheme 3).[39] The selective formation of products having a *cis*-enedione skeleton proved to be general for a series of 4-aryl-2-α-acetoxyalkyl furans similar to **45** that were synthesized and tested. Sterics and increased electron delocalization likely contribute to making the cis isomers thermodynamically more favorable for these compounds.

These *cis*-enedione products, for example, **50** proved to be somewhat sensitive to acid[40] (more than the related *trans*-enedione derivatives, e.g., **37**), and it was observed that this acid-sensitivity correlated positively with the electron-donating capacity of the aryl unit. Therefore, while strongly electron-donating aryl substituents were most effective in promoting rearrangements to α-keto furans (see above), electron-withdrawing aryl substituents were best for producing stable *cis*-enedione products. Studies using various aryl-substituted furan derivatives in both transformations demonstrated that a good balance in reactivity was achieved with furans substituted at the 4-position with the *m*-methylphenyl substituent, making it possible to carry out both transformations using a common set of oxidative (NBS) and acidic (PPTS) reaction conditions.

To summarize these results, it was determined that substitutions at the 4-position of furan (−H, −Br, or −aryl) function as a second σ-element by pre-encoding distinct skeletal outcomes upon exposure to a common set of oxidative and acidic reaction conditions. Moreover, as summarized in Figure 6, it was determined that this second σ-element functions in combination with substituents on the α-carbon (−OH or −OAc) of the 2-substituent to pre-encode a combinatorial matrix of distinct skeletal outcomes ($3 \times 2 = 6$; i.e., a multiplicative increase in skeletal outcomes with an additive increase in σ-elements).

Studies Directed Toward Split-Pool Synthesis

A more concise route (with respect to the number of reactions performed on solid phase) to the 4-*m*-methylphenyl-5-alkyl-2-furaldehyde **41** was developed involving direct coupling of 4-*m*-MePh-5-bromo-2-furaldehyde (**53**) (see Scheme 10). This reagent was prepared in two steps: Suzuki coupling between

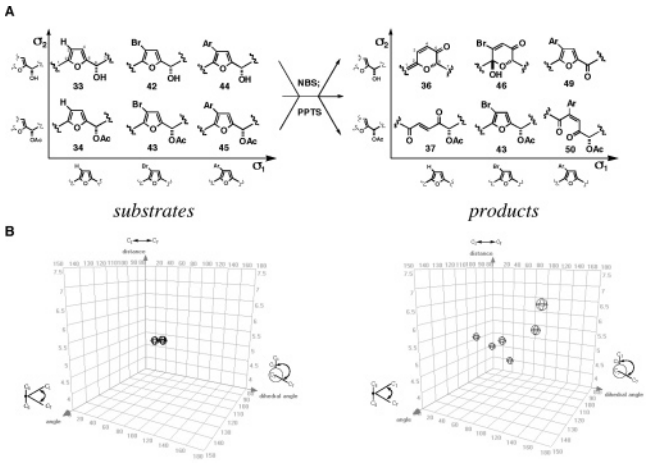


Figure 7. (A) A complete, combinatorial matrix of distinct skeletal outcomes achieved using a common set of reaction conditions. (B) A skeletal diversity metric for quantitating the diversity in the display of chemical information in three-dimensional space.

commercially available 4-bromo-2-furaldehyde (**51**) and *m*-methylphenylboronic acid followed by direct, regioselective bromination of the product **52** using molecular bromine in DMF.[41] Additionally, reaction conditions were optimized for the solid-phase synthesis of model substrates **42**−**45** and their transformation into skeletally distinct products **46**, **43**, **49**, and **50** using a common set of oxidative and acidic reaction conditions (see Scheme 11).

Realizing the combinatorial matrix of molecular skeletons shown in Figure 6 in the context of a split-pool synthesis requires effecting each of the transformations under the same reaction conditions, for example, in one pot. This is because the process of splitting and pooling obscures the identity of the σ-elements (and combinations thereof) appended to the furan substrates attached to each bead. To explore the potential of achieving this aim, we performed the following experiment in triplicate. As shown in Figure 7A, a common reaction vessel was charged with a collection of six macrobead-bound substrates **33**, **34**, and **42**−**45**, collectively representing a complete, combinatorial matrix of σ-elements, that is, −H, −Br, or −Ar at the 4-position of furan combined with −OH or −OAc on the α-carbon. We then exposed this pooled collection of substrates to the same oxidative and acidic reaction conditions (NBS, $NaHCO_3$, NaOAc, THF:H_2O 4:1, room temperature, 1 h; PPTS, CH_2Cl_2, 40−45 °C, 20 h), washed and dried the product beads, separated them into six individual Eppendorf tubes, and performed single-bead compound cleavage followed by LCMS analysis of each of the cleaved products. The results were consistent with the formation of 6/6 of the anticipated molecular skeletons as the major products in all three experiments.

To provide some quantification for the skeletal diversity generated in the collective transformation shown in Figure 7A, a skeletal diversity metric was developed on the basis of the distance, angle, and dihedral angle between common atoms in computationally derived three-dimensional structures. The missing bonds in both the substrates and the products in Figure 7A represent potential attachment sites to which building blocks could be appended. The six substrates, having a common 2-substituted α-alkoxyalkyl furan skeleton, resemble the types

(38) The olefin geometry was determined via nOe studies.
(39) Similar distinct stereochemical outcomes have been observed in the oxidation of an alternative series of furan derivatives: Sayama, S.; Inamura, Y. *Heterocycles* **1996**, *43*, 1371−1374.
(40) Upon prolonged treatment with acidic reagents, slow degradation of **50** to unidentified byproducts was observed (this degradation was more rapid with CSA than with PPTS).
(41) For a related bromination, see: Sessler, J. L.; Hoehner, M. C.; Gebauer, A.; Andrievsky, A.; Lynch, V. *J. Org. Chem.* **1997**, *62*, 9251−9260.

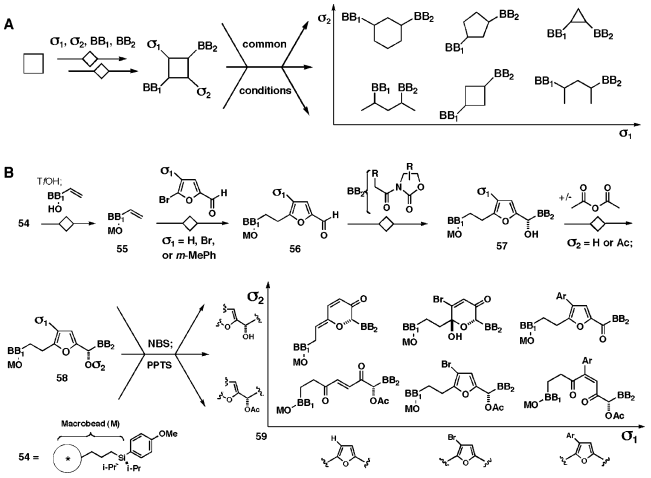


Figure 8. Revised synthetic plan. Efficiently generating overlapping, combinatorial matrices of both molecular skeletons and appended building blocks using split-pool synthesis. The diamond-filled arrows represent appending processes carried out in split-pool format.

of compounds typically derived from the one synthesis-one skeleton approach. Alternatively, the six products represent six molecular skeletons generated combinatorially using the σ-element-based strategy. Comparing and contrasting these two collections (which are almost constitutionally isomeric) can provide a metric for the skeletal diversity generated in this one reaction using a common set of reagents.

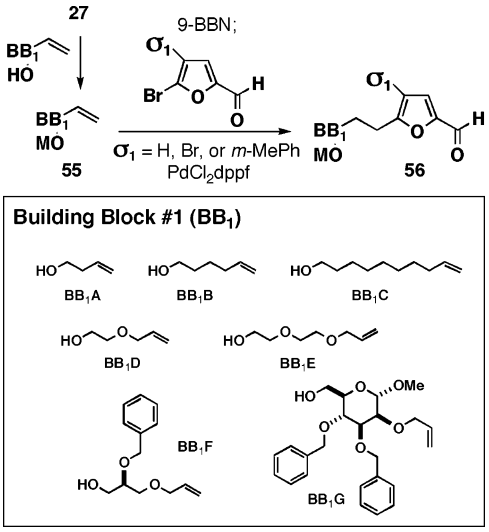
We generated structures in silico in which each of the missing bonds in Figure 7A was "replaced" with a methyl group (or a methylene group for the "left side" of structure **36**) to form a collection of 12 simplified structures sharing in common the seven contiguous carbon atoms labeled C_1-C_7. Using the Spartan software package (Spartan '02, Wavefunction, Inc.), the following two-step calculation was performed on all 12 structures: (1) the equilibrium conformer was determined using the Spartan equilibrium conformer search with semiempirical AM1 calculations, and (2) the equilibrium geometry was determined using the Hartree−Fock method with the 6-31G* split-valence basis set. The positions of every other carbon in the common, contiguous seven-carbon atom stretch were then used to determine the following three parameters (each parameter provides unique information regarding the relative positions of the building block attachment sites, C_1 and C_7, in three-dimensional space): (1) the distance (in angstroms) between C_1 and C_7; (2) the angle C_1−the midpoint between C_3 and C_5−C_7; and (3) the dihedral angle comprising C_1, C_3, C_5, and C_7.

Plotting these parameters for both substrates and products in a three-dimensional plot using the Spotfire graphing package produced the results shown in Figure 7B. The six simplified substrates create a dense cluster (the two lobes correspond to the acetylated and nonacetylated compounds). In contrast, the six skeletally distinct products distribute much more broadly, consistent with a diverse display of chemical information in three-dimensional space.

Revised Synthetic Plan: Overlapping, Combinatorial Matrices of Molecular Skeletons and Appended Building Blocks

The discovery of σ-elements that pre-encode a complete matrix of distinct skeletal outcomes stimulated the revised synthetic plan shown schematically in Figure 8A. In this approach, consecutive appending processes are performed in split-pool format to yield a common core structure with both a combinatorial matrix of building blocks and a combinatorial matrix of σ-elements. (The diamond-filled arrow is used in DOS to represent an appending process carried out using the split-pool technique.) These pooled substrates are then exposed to a common set of reaction conditions resulting in a matrix of distinct molecular skeletons, each derivatized with the same matrix of building blocks. Specifically, as shown in Figure 8B, we planned a consecutive series of four appending processes involving macrobead loading, Suzuki reaction, Evans' aldol coupling, and ± acetylation to introduce BB_1, σ_1, BB_2, and σ_2, respectively. The resulting products **58**, representing the complete matrix of building blocks and σ-elements appended to a common α-alkoxy furan core, then serve as substrates for a final NBS- and PPTS-mediated skeletal diversity-generating folding process.

Scheme 12



Realizing this proposed plan required that all of the following questions be answered in the affirmative: (1) Can combinatorial building block diversity be achieved by using diverse coupling partners in both the Suzuki and the Evans' aldol coupling reactions? (2) Can the combinatorial matrix of σ-elements discovered in the previous section prove to be general and effectively pre-encode the same matrix of distinct skeletal outcomes when a diverse collection of building blocks is appended to the same common core? (3) Is this chemistry compatible with an effective encoding strategy that enables the identification of the compounds attached to each macrobead at the end of the synthesis? We therefore set out to answer each of these questions experimentally.

We first explored the building block diversity that might be generated by using different coupling partners in both the Suzuki and the Evans' aldol coupling reactions. For the Suzuki reaction (BB_1), we tested a variety of commercially available compounds, each containing both a primary hydroxyl group and a terminal olefin, and found a diverse set of seven that effectively underwent loading onto macrobeads and subsequent B-alkyl Suzuki coupling with 5-bromofuraldehyde (**29**), 4,5-dibromofuraldehyde (**38**), and 4-*m*-MePh-5-bromofuraldehyde (**53**) (Scheme 12).

We then prepared candidate aldol coupling reagents (BB_2) by generating various combinations of acyl side chains and

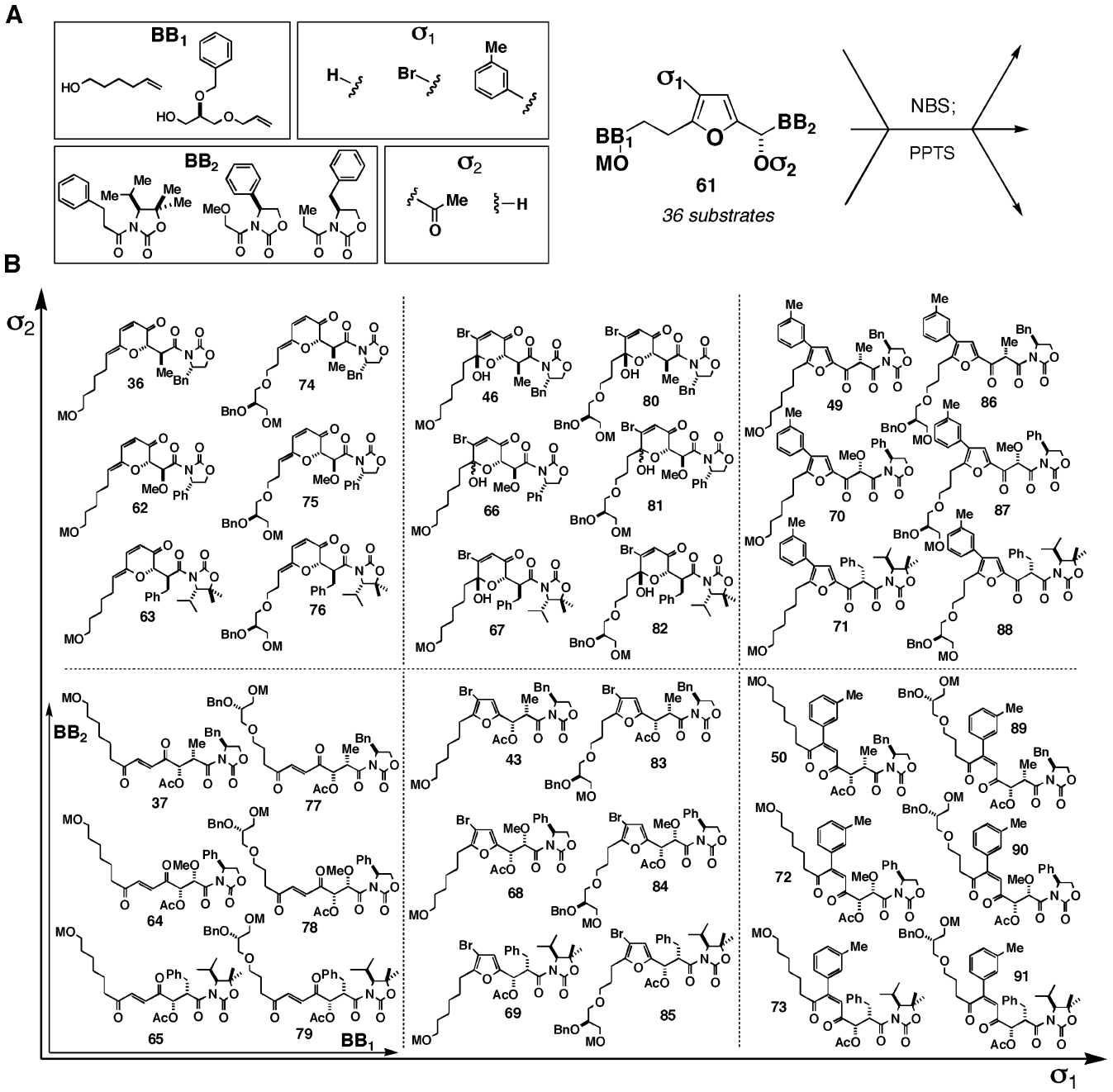
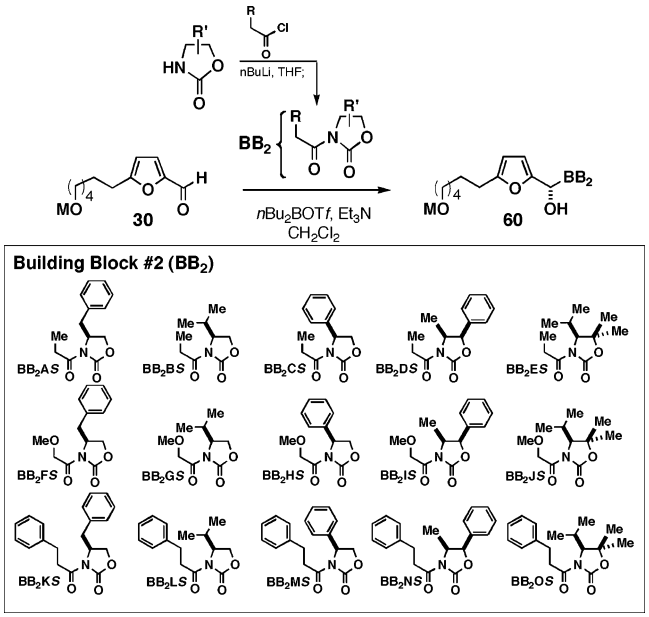


Figure 9. (A) Parallel synthesis of 36 substrates **61a**−**jj** representing all possible combinations of a matrix of building and a matrix of σ-elements appended to the same α-alkoxy furan core. (B) Transformation of this collection of 36 relatively simple and similar substrates into a collection of 36 more complex and diverse products − a set of complete, overlapping matrices of molecular skeletons and appended building blocks.

commercially available chiral oxazolidinones. From these studies, we identified five distinct commercially available chiral oxazolidinones and three different acyl side chains (ethyl, methoxymethyl, and phenylethyl) that in all possible combinations ($5 \times 3 = 15$) yielded acyl oxazolidinones that underwent efficient and diastereoselective coupling with macrobead-bound furaldehyde **30** (Scheme 13). The 15 enantiomeric acyl oxazolidinones were also prepared, allowing us to take advantage of reagent-based stereocontrol to prepare both sets of enantiomeric (or diastereomeric when BB_1 is chiral) products.

Although two collections of candidate building blocks (BB_1 and BB_2) had been identified, it remained to be seen whether different combinations of these building blocks would be compatible. To achieve the combinatorial matrix of distinct skeletal outcomes described above, the same matrix of σ-elements would need to pre-encode effectively distinct skeletal outcomes in the presence of diverse combinations of building blocks. To explore these questions efficiently, we selected representative Suzuki and Evans' aldol coupling partners (Figure 9A). We then used parallel synthesis to prepare all possible combinations of these representative building blocks and σ-elements appended to the same α-alkoxy furan core [(2×3) building blocks $\times$ (3×2) σ-elements $= 36$ individually synthesized compounds, **61a**−**jj**, see Figure 9A]. For each of these 36 compounds, we cleaved ∼5 mg of resin and characterized the crude products by ^{1}H NMR, LCMS, and HRMS. These data revealed successful formation of the anticipated compounds for 36/36 cases (HRMS

Scheme 13



error < 5 ppm), and, by LCMS analysis, 35/36 (97%) of these compounds were determined to be ≥70% pure.

Each of these macrobead-bound substrates **61a**−**jj** was then exposed to the same set of oxidative (NBS) and acidic (PPTS) reaction conditions in 36 parallel experiments. A portion of macrobeads (~5 mg) from each reaction was then cleaved, and the crude products were analyzed using ^{1}H NMR, LCMS, and HRMS. These three forms of characterization were consistent with the formation of the anticipated functionalized molecular skeleton in 36/36 cases (HRMS error < 5 ppm) (Figure 9B). Moreover, 26/36 (72%) of these products were determined to be ≥70% pure by LCMS. This series of parallel experiments demonstrated high compatibility between combinations of representative building blocks and generality for the skeletal diversity-generating combinatorial matrix of σ-elements.

We tested the compatibility of this chemistry with an optimized protocol for the Still chemical encoding methodology.[42,43] We performed the solid-phase synthesis of compounds **36** and **37** (see Scheme 3) using the five-step route proposed in Figure 8B, while incorporating after each synthetic step the minimum number of polychlorinated aromatic chemical tags required to generate a nonredundant binary code for the anticipated number of appendages to be used in the planned split-pool synthesis [step 1, 7 BBs, 3 tags; step 2, 3 σ-elements, 2 tags; step 3, 15 BBs × 2 enantiomers, 6 tags; step 4, 2 σ-elements (±acetylation), 1 tag]. After each tagging step, the compound and chemical tags were orthogonally cleaved [using HF-pyridine and ceric ammonium nitrate (CAN), respectively] from a portion (~5 mg) of macrobeads and were analyzed separately (compounds were analyzed by LCMS, and tags were analyzed by GC as described previously).[42] It was determined that the synthesis plan and the tagging methodology were compatible, resulting in no significant decreases in the conversions achieved in the solid-phase reactions or the purities of the cleaved products. Encouraged by these results, we set out to realize the synthetic plan outlined in Figure 8 in the context of a five-step, fully encoded split-pool synthesis.

Split-Pool Synthesis

As shown in Figure 10, we ancipated the formation of 1260 distinct compounds representing all possible combinations of the following variables: [(7 BB_1) × (3 σ_1) × (15 BB_2) × (2 enantiomers) × (2 σ_2) = 1260]. We therefore started our synthesis with ~10 400 macrobeads **54** (2 g, ~5.2 macrobeads/g), representing >8 copies of the complete, theoretical matrix.[44] In the first step, the pooled collection of macrobeads was divided evenly into seven portions, and each was subjected to a loading reaction with a unique BB_1 (see Scheme 12). Each portion was tagged with polychlorinated aromatic tags T1A−T3A in a unique combination[42] using rhodium triphenylacetate. The resulting seven portions were pooled and thoroughly mixed. The pooled collection of macrobeads **55** was divided into three equal portions, and to each was coupled one of three different 5-bromofuraldehyde derivatives, each bearing a distinct σ-element (σ_1) in the 4-position. After the three parallel Suzuki reactions were completed, to each portion of product macrobeads was coupled a unique combination of tags 4A and 5A. The resulting three portions of resin were then combined and thoroughly mixed (**56**), and then split evenly into 30 portions. To each of these 30 portions was then coupled a unique acyl oxazolidinone (BB_2) (see Scheme 13) followed by a unique combination of tags T6A−T11A. These 30 portions were then pooled to yield the collection of aldol adducts **92**. In the final split-pool step, this pooled collection of macrobeads **92** was divided evenly into two portions (1.08 g each).[45] One portion was subjected to an acetylation reaction followed by tagging with tag T13A, and the other portion was left unreacted to yield the collection of macrobeads **93**, representing theoretically all possible combinations of BB_1, σ_1, BB_2, and σ_2, in both enantiomeric and diastereomeric (when BB_1 is chiral) forms. Prior to pooling these two portions, 30 individual macrobeads were removed from each portion for analysis. The synthesized compound and corresponding chemical tags were orthogonally cleaved from each individual macrobead and analyzed using LCMS and GC, respectively. These data revealed that the structure predicted by the chemical tags matched the LCMS of the cleaved products in 60/60 cases, and 55/60 (92%) of the products were determined to be ≥70% pure.

Finally, 853 mg of macrobeads **93** (~4410 macrobeads, multiplicative factor = 3.5)[44] was pooled and collectively exposed to the same oxidative (NBS) and acidic (PPTS) reaction conditions described above to yield the final collection of products **94**. Next, 120 of these product macrobeads were cleaved and analyzed as above. The chemical structures predicted by the chemical tags were consistent with LCMS analysis of the cleaved products for 120/120 macrobeads.

(42) (a) Nestler, H. P.; Bartlett, P. A.; Still, W. C. *J. Org. Chem.* **1994**, *59*, 4723−4724. (b) 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.

(43) Jennifer Raggio, Leticia Castro, and John Tallarico are gratefully acknowledged for providing encoding reagents, and for cleaving and analyzing chemical tags.

(44) Statistical calculations and computer simulations suggest that a multiplicative factor of 3.1 is required to provide 99% confidence of achieving 95% coverage of the complete, theoretical combinatorial matrix for a split-pool synthesis involving four split-pool cycles with 10 pools per cycle: Burgess, K.; Liaw, A. I.; Wang, N. *J. Med. Chem.* **1994**, *37*, 2985−2987.

(45) Throughout the course of the synthesis, the total mass of macrobeads increased somewhat, reflecting the added mass of the attached compounds. There was also some loss of intact macrobeads throughout the split-pool synthesis due to bead-breakage.

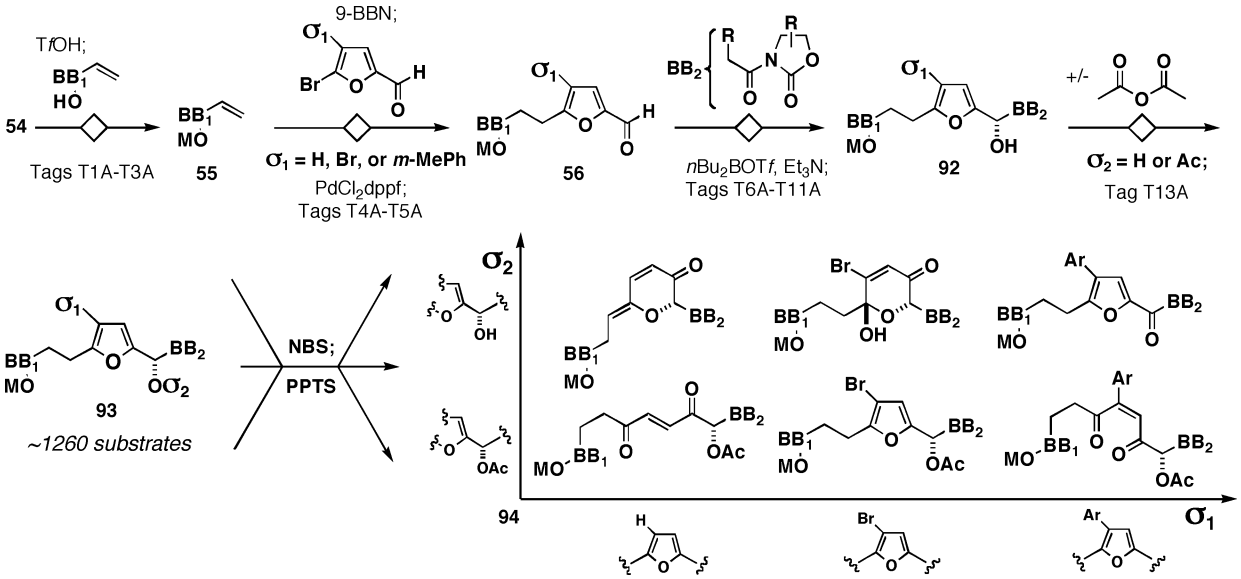


Figure 10. A five-step, fully encoded split-pool synthesis of a collection of ~1260 compounds **94** representing a complete, combinatorial ($3 \times 2 = 6$) matrix of molecular skeletons, each derivatized with a complete, combinatorial ($7 \times 15 = 105$) matrix of building blocks in both enantiomeric and diastereomeric forms ($6 \times 105 \times 2 = 1260$).

Moreover, 84/120 (70%) of these final products were determined by LCMS to be ≥70% pure. These results are consistent with the plan to generate combinatorially a complete set of overlapping matrices of molecular skeletons and appended building blocks in both enantiomeric and diastereomeric forms.

Summary and Conclusions

We have developed and implemented a synthesis strategy for generating skeletal diversity in DOS. The approach is conceptually analogous to the natural process of protein folding. It involves using common reaction conditions to transform relatively simple and similar substrates having different appendages that pre-encode skeletal information (σ-elements) into more complex and diverse products having distinct molecular skeletons.

An attractive feature of this approach is that skeletal diversity can be pre-encoded into substrates combinatorially, thus making it possible to generate a complete matrix of molecular skeletons in an efficient manner. This strategy also permits the formation of diverse skeletons late in the synthesis pathway, which has two theoretical advantages. First, maintaining relative structural similarity in the early stages of a synthesis facilitates the identification of products = substrates relationships and the use of the powerful technique of split-pool synthesis. Second, forming new skeletons late in a synthesis can facilitate the generation of functionalized skeletons that might be otherwise difficult to access in DOS, such as those having building blocks coupled via carbon−carbon bonds at stereogenic quaternary carbon centers (e.g., **46**) and/or potentially unstable molecular skeletons (e.g., acid-sensitive enediones **37** and **50**).

As demonstrated in the split-pool synthesis in Figure 10, this strategy can provide access to a collection of compounds potentially representing all possible combinations of building block, stereochemical, and skeletal diversity elements. Such collections will likely prove to be valuable in efforts to understand the roles each of these three diversity elements plays in small-molecule protein interactions. Systematic screening efforts designed to address this specific issue are now underway.

Acknowledgment. We wish to thank M. Narovlyansky and J. Tallarico for providing macrobeads, J. Raggio, L. Castro, and J. Tallarico for providing encoding reagents and for cleaving and analyzing chemical tags, Q. Liao, P. Wang, and J. Dudek for high resolution mass spectroscopic analysis, and W. Collins and S. Huang for assistance with NMR instrumentation. Additionally, Y.-K. Kim is gratefully acknowledged for helpful discussions regarding the transformation of **30** → **31**. Financial support for this work was provided by the NIGMS and the NCI. The Keck Foundation, Merck KGaA, Merck & Co., and the NCI are gratefully acknowledged for their past support of Harvard's ICCB, and the NCI is additionally acknowledged for support of the Initiative for Chemical Genetics. M.D.B. was a Howard Hughes Medical Institute predoctoral fellow, E.M.B. was an HHMI undergraduate fellow, and S.L.S. is an Investigator at the HHMI.

Supporting Information Available: Experimental procedures and analytical data for model compounds, building block testing, parallel synthesis, and fully encoded split-pool synthesis. This material is available free of charge via the Internet at http://pubs.acs.org.

JA0457415