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Modular, Parallel Synthesis of an Illudinoid Combinatorial Library

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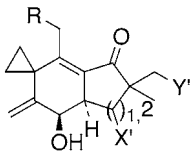
ABSTRACT



A library of 49 illudinoids was prepared via a three-step synthesis using a parallel synthesizer and solid-phase extractive purification. Products could be rapidly prepared for initial biological testing against three cancer cell lines, which revealed library members that inhibit nonsmall cell lung cancer. The activities of the library members are not easily correlated with structure, meaning the empirical approach used here is essential for such nonintuitive discoveries.

While the power of library approaches to the creation of diverse molecules has become abundantly apparent, many of the most intriguing molecular classes (regarding both structure and biological activity), such as natural products, have been relatively inaccessible via these methods. Progress in this field is an important current topic of research.[1] An ongoing challenge is the adaptation to combinatorial and/or solid-phase synthesis of the *many* types of reactions needed to prepare complex targets. Often, this has involved use of a natural product as a template and modifying substituents around its periphery by C-heteroatom bond formation.[2] Reliable combinatorial methods to assemble modified natural product core structures through C−C bond formation are less developed.

In considering a natural product synthesis, convergency[3] has traditionally been accorded high value owing to its ability to permit parallel processing in the generation of advanced intermediates used in late-stage coupling. While convergency is a virtue to most efficiently provide a single target, it impairs efficiency when the goal is preparing a family of structurally variant targets. A more efficient strategy to generate multiple targets involves synthesis of a common advanced intermediate and diversification by the addition of readily available modules. With increasing interest in the use of library methods that form C−C bonds to access natural products and complex natural product-like compounds,[4] this "divergent" or modular approach may become more important in synthesis design.

We have examined these issues in the context of the illudin family (Figure 1). These sesquiterpenes have a broad range of interesting biological activities in cancer that were initially discovered through the natural products illudin M and S from

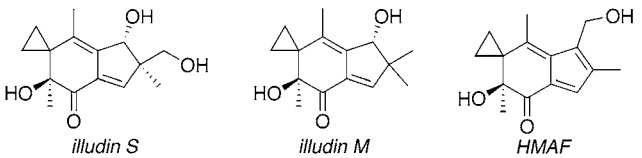


Figure 1. Illudin natural products and a more potent relative.

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(2) Nicolaou, K. C.; Winssinger, N.; Vourloumis, D.; Ohshima, T.; Kim, S.; Pfefferkorn, J.; Xu, J.-Y.; Li, T. *J. Am. Chem. Soc.* **1998**, *120*, 10814−10826.

(3) Velluz, L.; Ralls, J.; Nominé, G. *Angew. Chem., Int. Ed. Engl.* **1965**, *4*, 181−270. Ireland, R. E. *Organic Synthesis*; Prentice-Hall: Englewood Cliffs, 1969; p 29.

(4) Hung, D. T.; Jamison, T. F.; Schreiber, S. L. *Chem. Biol.* **1996**, *3*, 623−39.

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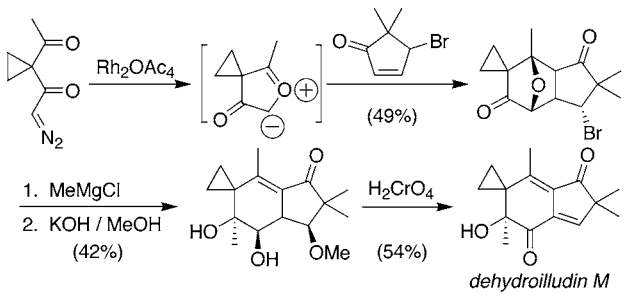
the Jack O'Lantern mushroom (*Omphalotus illudens*) and improved through structural modifications to give HMAF (hydroxymethylacylfulvene).[5] Equally important for our considerations was that brief and modular synthetic routes to the illudins had been developed through the efforts of Padwa and Kinder.[6] These routes involve the dipolar cycloaddition of a carbonyl ylide to an enone (Scheme 1).

Scheme 1. Padwa−Kinder Approach to Illudin Synthesis



With some obvious limitations, cycloaddition reactions are expected to provide general C−C bond-forming synthetic methods for combinatorial synthesis, as has already been demonstrated.[7]

With this background, the design of a combinatorial approach to illudinoids was straightforward. Families of diazocarbonyl compounds (Figure 2) and enones (Figure 3)

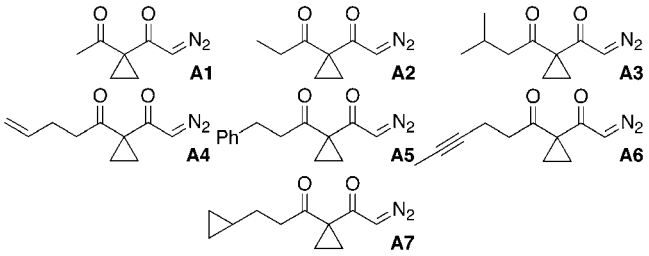


Figure 2. Diazocompound building blocks **A**.

were targeted. The former were prepared by alkylation of the dianion of methyl acetoacetate[8] and application of the literature route[9] to **A1** (21−29% overall yields); the latter were known.[10]

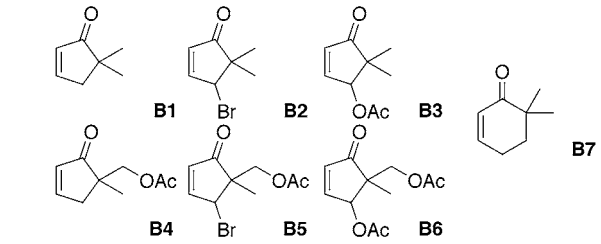
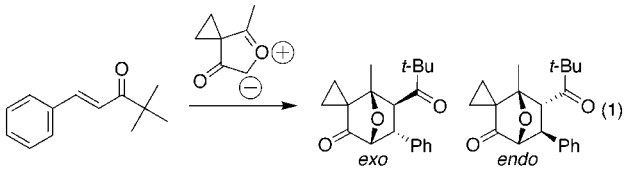


Figure 3. Enone building blocks **B**.

Improvements to the cycloaddition step were sought on the basis of a reaction between **A1** and the model enone (*E*)-4,4-dimethyl-1-phenylpent-1-en-3-one (1.5 equiv, eq 1). Cycloaddition affords two diastereomers differing in the stereochemistry of the ether bridge relative to the dipolarophile carbonyl. Products with the carbonyl group syn to the ether bridge are defined as exo. This stereochemistry is readily assigned on the basis of the absence of ^3J-coupling for the bridgehead hydrogen in the endo isomer because of its 90° dihedral angle with the adjacent methine. The yield and stereoisomer ratio could be directly determined by ^{1}H NMR using an internal standard. Of 14 solvents examined with Rh_2(octanoate)$_4$ as catalyst, ether provided the highest yield (69%) and exo/endo ratio (2.5:1) in this reaction. Good results were also obtained in THF and dioxane. Ethereal solvents are not frequently used for carbenoid reactions. A speculation on their utility in this case is that they slow carbenoid generation by coordination to the catalyst, more closely matching the rate of carbonyl ylide formation to its subsequent cycloaddition and thereby minimizing side reactions.



Using these conditions, cycloadditions of building blocks **A1**−**A7** (0.3 mmol) with **B1**−**B7** were performed as summarized in Scheme 2.[11] Because the diazo compounds were more precious, the enones were used in excess. Polar byproducts were also produced in these reactions. A two-step, high-throughput purification protocol was therefore developed. Solid-phase extraction (SPE) with SiO_2/CH_2Cl_2 removed polar materials, and after solvent exchange, excess enone was removed by a thiophenol scavenging resin,[12] giving $\mathbf{A}_n\mathbf{B}_m$ in ∼70% yields (Table S1, Supporting Informa-

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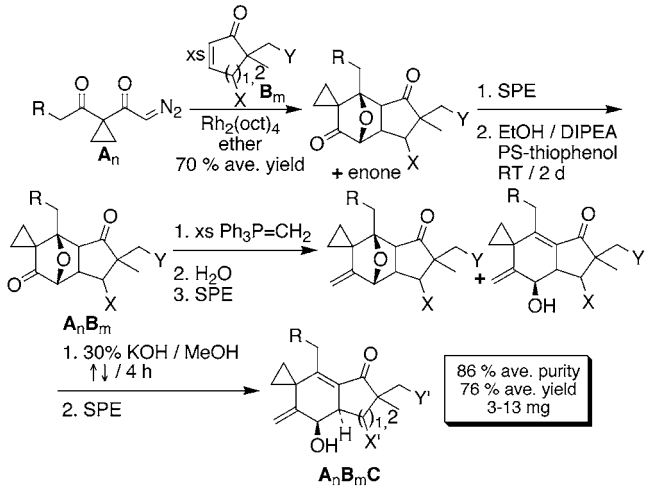
(11) All operations in this synthesis were conducted 20 at a time on a Quest 210 parallel organic synthesizer.

Scheme 2. Parallel Synthesis of Illudin Derivatives



tion). The third, **C**, building block was then introduced. It proved impractical in high-throughput mode to limit methyl Grignard to monoaddition to $\mathbf{A}_n\mathbf{B}_m$ on the basis of stoichiometry. However, methylenetriphenylphosphorane proved very selective for the less hindered, more electronically activated carbonyl groups in $\mathbf{A}_n\mathbf{B}_m$ even when in excess. Partial β-elimination/ring opening of the ether bridge was also promoted by the excess phosphorane. Water converts it to triphenylphosphine oxide, which was removed by SPE (SiO_2/CH_2Cl_2). Completion of the elimination of the ether bridge was conducted with KOH in methanol, which also modifies the bromides and acetates (X → X′, Y → Y′). The products were purified by SPE (SiO_2/3:1 hexanes/ethyl acetate) to provide $\mathbf{A}_n\mathbf{B}_m\mathbf{C}$ in quantities (Table S2, Supporting Information) and of quality sufficient for in vivo screening. All isolated products in this scheme were characterized by ^{1}H NMR, FID GC, and GC/MS.

The stereoselectivity of this sequence was evaluated at two stages, $\mathbf{A}_n\mathbf{B}_m$ and $\mathbf{A}_n\mathbf{B}_m\mathbf{C}$. Exo/endo ratios were 7:1 to >99:1; selectivity for approach to the enone face (for X ≠ H) opposite the X group was 67:33 to 86:14; there was no stereochemical influence from the quaternary stereogenic center (Y = OAc) for **B4**−**B6**. As expected, 1:1 mixtures were produced. The total number of reaction products in this 7 × 7 × 1 library is 49, but includes 119 total compounds due to stereoisomerism. The functional diversity in the enone results in four stereochemical and functional classes of cyclopentane products (Figure 4).

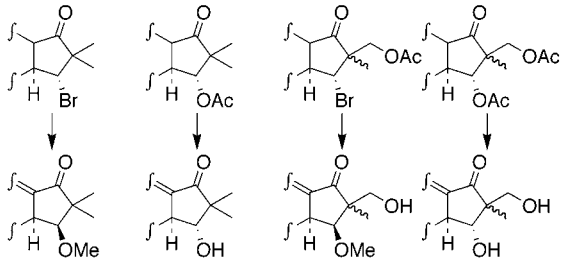
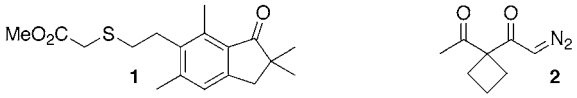


Figure 4. Post-cycloaddition transformations of the cyclopentanone functionality of **B2**−**B3** and **B5**−**B6** introduce additional diversity.

The biological evaluation of the library members was performed at 100 μM in three cancer cell lines (MCF7 breast, H460 nonsmall cell lung, SF-268 CNS). Three products ($\mathbf{A_2B_2C}$, $\mathbf{A_3B_1C}$, $\mathbf{A_3B_4C}$) show complete inhibition of the growth of H460 cells at this concentration. Supporting the idea that this biological activity may reflect the ability of the illudin nucleus to serve as an electrophile,[13] $\mathbf{A_1B_1C}$ was treated with acid and methyl mercaptoacetate to give compound **1**.



A challenge to the use of combinatorial methods for the preparation of diverse core structures is that modifications to the modules that create diversity also often modify their chemical reactivity. The variation in structure that is essential to combinatorial chemistry can frustrate the preparation of structurally diverse targets. For example, the carbonyl ylide derived from **2** is unreactive with cyclopentenones, though earlier studies showed it readily undergoes cycloaddition with quinones.[14] Such considerations place a premium on truly general methods for C−C bond formation that are relatively independent of structure beyond the exact locus of reactivity.

Acknowledgment. We appreciate the provision of biological data by Dr. E. Sausville, NCI. Financial support from NIH AI48521 is gratefully acknowledged.

Supporting Information Available: Table of yields and purity of $\mathbf{A}_n\mathbf{B}_m$. Table of structures, amounts, and MS data for $\mathbf{A}_n\mathbf{B}_m\mathbf{C}$. This material is available free of charge via the Internet at http://pubs.acs.org.

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