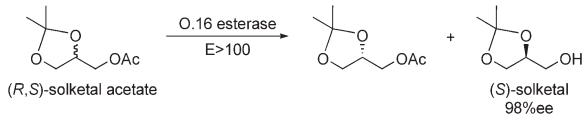

Figure 1. Kinetic Resolution of Racemic Solketal Acetate with O.16 Esterase Proceeded with the Highest Enantioselectivity Reported So Far in the Literature

level of structural and functional complexity than other known esterases.

In conclusion, the work by Ferrer and coworkers does not only add new enzymes to the number of available biocatalysts. It substantially expands our knowledge about enzyme functions and exemplifies Nature's ability to evolve remarkable biocatalysts with no similarity to known enzymes and bearing striking biochemical properties, which would have been rather impossible to discover without the metagenome approach.

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Drugging the Undruggable

In this issue of *Chemistry & Biology*, Li and Lawrence [1] report an iterative synthesis/selection process to identify chemically modified peptide ligands possessing high affinity and selectivity for the SH3 domain of Fyn, a member of the Src kinase family.

Protein-protein interactions within families of highly related macromolecular structures have proven to be among the most challenging assignments for development of suitable pharmacologic reagents. Associations between flat, complementary surfaces which are characteristic of protein-protein interactions involve discontinuous protein epitopes whose binding determinants are difficult to interrupt or mimic with small molecules. High-throughput screening (HTS) of small-molecule libraries has also proven to be largely unsuccessful, presumably because most compound collections tend to be structurally biased to conventional targets such G protein-coupled receptors and kinases. Furthermore, this approach is subject to a higher-than-usual occurrence of artifacts and other false positives [2].

SH3 domains are small, highly conserved binding regions that modulate the activity of Src tyrosine kinases in both normal and disease states [3]. These findings have heightened the level of interest in the functional and mechanistic aspects of SH3 signaling and increased the potential medicinal value of selective modulators in SH3-mediated pathology [4, 5]. Chemically based inhibitors, unlike genetic knockouts, lend themselves to dose-dependent investigation of SH3 targets across a broad spectrum of in vitro and in vivo pharmacology. However, high-affinity small-molecule reagents that selectively bind SH3 domains have been difficult to obtain [6, 7], and a general strategy for their acquisition has yet to emerge. The relatively small binding face of a representative SH3 binding domain combined with the appreciable homology among related structures renders this target class a sizable challenge to conventional optimization technology. Additionally, the transient SH3 binding complex offers few clues to guide intelligent design. Consequently, the majority of reported SH3 peptide ligands bind with micromolar affinity and poor selectivity [8].

Within the Src family kinases, Fyn and the closely related Lck have been shown to play a key role in initia-

tion of signaling through the T cell antigen receptor [9]. While the function of Lck in mediating T cell development and differentiation has been clearly defined using Lck-deficient cell lines, the importance of Fyn is less well understood. Recent evidence, however, indicates that Fyn is critical to the activation and proliferation of naive T cells [10], making this protein an attractive target for pharmacological control.

While no diversity- or knowledge-based approach has successfully emerged for targeting SH3 complexes, approaches derived from the study of other difficult targets have been applied to SH3 domains. Two of these reports utilize structure-based fragment selection guided by either NMR [11] or X-ray crystallography [12] to generate libraries of weak binding fragments. Such fragments are then optimized for affinity and selectivity as part of a larger molecular scaffold or by iterative rounds of structural modification. The Li and Lawrence report [1], unlike these prior approaches, is based entirely on iterative library construction and deconvolution.

The authors utilized the consensus peptide RALPPLP, with only micromolar affinity for Fyn and no selectivity for the related kinases, as a starting point for optimization. Introduction of an optimized diversity element through acylation of the N terminus with a diverse set of carboxylic acids yielded a derivative with binding affinity roughly 10-fold greater than the starting peptide, but with no apparent selectivity. Amplification of this lead in the second optimization round by introducing diversity at the C terminus produced a further improvement in potency as well as a hint of selectivity. In the last optimization round, which focused upon the second amino acid in the peptide (Ala), the authors were able to identify an even more potent ligand which exhibited approximately a ten-fold selectivity for Fyn over the other kinases, including Lck. Finally, the authors were able to demonstrate functional capability of this novel optimized ligand by disruption of a WASp/Fyn signaling complex.

The report by Li and Lawrence represents an important step forward in the development of methodology for identification and optimization of reagents for broad-based pharmacological study. The work highlights the unique role chemical reagents serve in the elucidation of specific macromolecular targets within biological signaling pathways [2]. The most noteworthy contribution of this report resides in the systematic approach by which one of the most vexing problems in chemical biology, the disruption of protein-protein interactions, is overcome. The stepwise modification of a consensus peptide with a diverse collection of carboxylic acids yielded an appreciably potent and selective Fyn SH3 inhibitor in a straightforward manner.

To place this work in a historical context, the authors' reference to "an easily automated methodology" invites acknowledgment of the cornerstone of this methodology. Solid-phase synthesis from its original conception by Merrifield [13] to its more recent applications in chemical biology remains an enabling force in scientific investigation. This is most apparent in the assignment of function to structure within the human genome where the application of genetic deletion or "knockout" strategies has proven insufficient or equivocal. As a result, traditional pharmacology represents a reliable and complementary approach to determining gene function.

The present work exemplifies the broad and unanticipated utility of the tools developed in the context of combinatorial chemistry [14–17] and subsequently refined in the course of its evolution into diversity-oriented synthesis [18]. The speed and simplicity of these methods enables a broader segment of the scientific community to participate in the identification and characterization of functional aspects of the genome.

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