IV. Combinatorial Chemistry

1. Library synthesis
   a) in solution, parallel synthesis
   b) on solid support
   c) split and combine, one bead one compound

2. Deconvolution and Tagging

3. Dynamic Combinatoric Chemistry

Parallel Library Synthesis

- 12 reactions provide 9 compounds
- The library members are spatially separated, so this technique can be used for solution as well as solid phase synthesis
Split-pool Synthesis

- 6 reactions lead to 9 compounds
- Each library member must be compartmentalized (each compound on its own bead) to allow pooling of the library

An Example of Split-Pool Synthesis

Split-Pool step:

Overview of the Entire Split-Pool Library

Example of the Efficiency of the Split-pool Strategy

- Optimization of 154 reactions affords $10^5$ amplification in the number of compounds

\[
\begin{align*}
\text{18 iodides} & \quad \text{30 alkynes} & \quad \text{62 amines} \\
\text{30+62+62} = 154 \text{ reactions} & \quad 18 \times 30 \times 62 \times 62 = 2.1 \text{ million compounds}
\end{align*}
\]


Structural Characterization: Direct Methods

**Off-bead Analysis**

- Cleavage, then use of analytical techniques used in TOS (e.g. LC, MS, NMR)
- Requires high sensitivity and high throughput format
- Example: LC-UV/ MS
Structural Characterization: Direct Methods

On-bead Analysis I
- Can be used to monitor the progress of a reaction
- MAS-NMR (Magic angle spinning NMR) is necessary due to polymer

![Magic angle rotor (left), rotor spinning at the magic angle (right)]

MAS-NMR spectrum (600 MHz)

On-bead Analysis II
- Example: Single-bead FT-IR microspectrometry
- Can be used to monitor the progress of a reaction

![Beads in IR cell]
Structural Characterization: Indirect Methods

Deconvolution
• Screen as a mixture of compounds then re-synthesize and re-assay possible candidates in active pools

Drawbacks:
• Interference by unwanted properties of other compounds (e.g. cytotoxicity)
• Possible synergistic interaction of multiple compounds
• Sub-library synthesis is cumbersome

Encoding

• Encoding should provide a fast and simple way to identify the structure of all library members
• Classification
  – Spatial encoding: position of the compound provides the information about its structure (possible only in parallel synthesis)
  – Graphical encoding: bar codes or other graphical tags are displayed on the solid support used in the library synthesis
  – Chemical encoding: every reaction used in the library synthesis is recorded on the solid support by the chemical attachment of a tag
    • binary coding (presence or absence of a tag) or polymer based (polypeptide, DNA)
  – Spectrometric encoding: using a spectrometric technique (NMR, MS, Fluorescence microscopy, NMR etc.) to read tags directly from the solid support
  – Electronic encoding: radio frequency memory chip attached to the solid support records and emits coded information
Encoding in Split-pool Synthesis

- Optimization of 6 reactions leads to 9 compounds
- Each library member must be isolated on its own bead to allow pooling of the library

How do you know what compound is on a given bead after a pool step?

Chemical Encoding in Split-Pool Synthesis

- Every diversification reaction is followed by a tagging reaction in which a tag(s) that codes for a particular transformation is covalently attached to the solid support
Decoding

- Every bead has tags that provide information, once cleaved, about the chemical history of that bead.
- Conditions for cleavage of compound and tags have to be orthogonal.

**Binary Chemical Coding**

<table>
<thead>
<tr>
<th>Building blocks</th>
<th>Binary (base 2) codon</th>
<th>Tags</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>00 01</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>01</td>
<td></td>
</tr>
<tr>
<td>011</td>
<td>001</td>
<td></td>
</tr>
<tr>
<td>101</td>
<td>010</td>
<td></td>
</tr>
<tr>
<td>110</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>111</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

- 2 digit codon: $2^2 = 4$ max
- 3 digit: $2^3 = 8$
- 1 digit

$n$ binary tags code for $2^n$ building blocks
Halogenated Aromatics As Tags

- Small amount of tag can be reliably detected (0.5-1 pmol/bead) using easily automated electron capture GC in the mixture of tags based on different retention times
- Inert under most reaction conditions

Attachment and Cleavage of Tags

- Tags are attached using rhodium carbene-insertion chemistry and can be cleaved using (NH₄)Ce(NO₃)₆ (CAN).

Binary Chemical Encoding of a Peptide Library

- A library of decapeptides was synthesized and screened for binding to 9E10 mAb.
- 7 Amino acids were used at each position (S, I, K, L, Q, E, D).
- Every amino acid was assigned a 3 digit binary codon (001=S, 010=I, 011=K, 100=L, 101=Q, 110=E, 111=D) where 1=presence of a tag and 0=absence.
- For each step in the library synthesis there are 3 tags designated nX (total of 18 tags for a library of 117,649 members, maximum encodable is 2¹⁸ = 262,144).
Dynamic Combinatoric Chemistry

Virtual Dynamic Combinatoric Library

Molding a receptor  Casting a substrate
Virtual Dynamic Combinatoric Library - macrocycles

Virtual Dynamic Combinatoric Library – carboanhydrase inhibitors

Virtual Dynamic Combinatoric Library


Virtual Dynamic Combinatoric Library – metal grids

Lehn et al, *PNAS, 2003, 100, 11970*
Virtual Dynamic Combinatoric Library – metal grids

Lehn et al, PNAS, 2003, 100, 11970

Virtual Dynamic Combinatoric Library – neuraminidase inhibitors

Virtual Dynamic Combinatoric Library – neuraminidase inhibitors


Virtual Dynamic Combinatoric Library – acetylcholine esterase inhibitors

Sharpless et al, PNAS, 2004, 101, 1449
Pseudodynamic Dipeptide Library

Protease from *Streptomyces griseus*

Pseudodynamic Dipeptide Library

Scheme 2. Competitive inhibition constants of the library members for the CA-catalyzed hydrolysis of p-nitrophenyl acetate. The non-sulfonamide compounds showed no detectable inhibition at 1 µM. CA = carbonic anhydrase

V. Diversity Oriented Synthesis

Convergent Synthesis – Target oriented synthesis (TOS)
- complexity-generating rxns.
- fragment coupling rxns.

Divergent Synthesis – Diversity oriented synthesis (DOS)
- complexity-generating rxns.
- multicomponent coupling
- diversity-generating rxns.

TOS: Retrosynthetic Analysis of Saframycin A

Pictet-Spengler Isoquinoline Synthesis


Pseudoephedrine as a Chiral Auxiliary for Enolate Alkylation

TOS: A Concise Synthesis of Saframycin A

Why Perform Diversity-Oriented Synthesis?

- To systematize the discovery of molecules with properties that are difficult to design or predict.

- DOS is especially useful in cases where processes are complex and few of the “design” criteria are well understood. For example:
  - Asymmetric catalysis.
  - Small molecules with specific biological functions (protein binding, cellular effects).

- DOS approaches are useful when molecules that act through new mechanisms are desired. This is useful because it may illuminate aspects of chemistry or biology that have not yet been illuminated. It is possible because the screen may make no assumptions about mechanism. For example, a DOS approach could be used to discover Diels-Alder catalysts that work by an unexpected and completely novel mechanism and in the process illuminate new means of promoting cycloaddition reactions.

DOS: Synthesis, Screen, Discover, Study

- Synthesis of diverse molecules: Molecules may be biased towards properties that will achieve the goal (e.g. protein-binding elements or metal binding elements).
- Screen for molecule(s) with desired properties
- Discovery of molecule with desired properties
- Study Mechanism; understand chemistry or biology
DOS vs. TOS in Enantioselective Catalysis (Example 1)

DOS vs. TOS in Enantioselective Catalysis (Example 1)

**Target-Oriented Synthesis Approach**

7 steps; separation by chiral HPLC; not easily modified
S = 43-65 with benzyl alcohols;
S = 14-22 with allylic alcohols


**Diversity-Oriented Synthesis Approach**

16 steps-easily synthesized via amide coupling reactions on solid phase;
structure easily modified;

An Example of DOS: Discovery of New Enantioselective Acylation Catalysts for Kinetic Resolution

**Plan for Discovery of Enantioselective Peptide-Based Acylation Catalysts**

Include Amino Acids with Desirable Properties, but use Approach that is Modular and Variable

**Brightest Beads Contain Most Active Catalysts**
Split-Pool Diversity-Oriented Synthesis of Acylation-Biased Peptides

1 AA in each of 16 different flasks

pool into one flask

FmocNH₂

DMF

split into 16 flasks

HBTU, iPr₂NEt

DMF, 25°C

repeat 6 times

Discovery of Powerful Enantioselective Acylation Catalysts

This peptide-based catalyst would have been impossible to design - DOS leads to a discovery.
Use of a DOS-Catalysis Approach in Complex Molecule Synthesis

S. J. Miller et al. Organic Letters; 2001; 3(18); 2879-2882

Enantiospecific Synthesis of a Mitomycin Core Structure

**DOS vs. TOS in Enantioselective Catalysis (Example 2)**

**Target-Oriented Synthesis Approach**

- 98% ee with wide range of 6-membered rings
- However, low ee for 5 and 7-membered rings; the structure is not easily modified

**Difficult Substrates**

- 10% ee
- 53% ee

Ligand structure optimization is difficult and time consuming

---

**DOS vs. TOS in Enantioselective Catalysis (Example 2)**

**Diversity-Oriented Synthesis Approach**

- 72-98% ee with wide range of 5, 6, and 7-membered rings
- 3 steps-easily synthesized via amide coupling reactions; structure easily modified;

**Difficult Substrates**

- 79% ee
- 72% ee
- 62% ee

Rapid ligand optimization using DOS approach
The DOS approach allows optimization

Difficult Substrates

rapid ligand structure optimization because it was discovered using DOS approach

positional optimization strategy

The positional optimization strategy involves optimizing each AA individually while holding the others constant


Use of DOS to Discover Enantioselective Catalysts of the Strecker Reaction (Example 3)

These ligands that are common in asymmetric catalysis are not well suited for a DOS approach since:
1. They do not provide a nonobtrusive site for attachment to the solid-phase
2. They are not conducive to wide structural variations (diversity)
Use of DOS to Discover Enantioselective Catalysts of the Strecker Reaction (Example 3)

These ligands that are common in asymmetric catalysis are not well suited for a DOS approach since:
1. They do not provide a nonobtrusive site for attachment to the solid-phase
2. They are not conducive to wide structural variations (diversity)

Metal-binding library plan

diversity by varying R, R', R'', linkers and amino acid


Parallel Synthesis of Schiff-Base Ligands on the Solid-Phase
# Initial Screening Identifies a Metal-Free Catalyst

**Library 1**

<table>
<thead>
<tr>
<th>Metal</th>
<th>None</th>
<th>Ti</th>
<th>Mn</th>
<th>Fe</th>
<th>Ru</th>
<th>Cu</th>
<th>Zn</th>
<th>Gd</th>
<th>Nd</th>
<th>Yb</th>
<th>Eu</th>
</tr>
</thead>
<tbody>
<tr>
<td>ee</td>
<td>19</td>
<td>4</td>
<td>5</td>
<td>10</td>
<td>13</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>conversion</td>
<td>59</td>
<td>30</td>
<td>61</td>
<td>69</td>
<td>63</td>
<td>68</td>
<td>55</td>
<td>91</td>
<td>95</td>
<td>84</td>
<td>94</td>
</tr>
</tbody>
</table>

Although the library was based upon a Schiff base that would bind metals, the most enantioselective catalyst is the metal-free ligand!

**Library #2:** Screen metal-free ligand but vary amino acid, diamine, and salicylaldehyde

---

# Synthesis and Screening of Metal-Free Catalysts

**Library 2**

library size = 48 compounds (6 salicylaldehydes x 4 amino acids x 2 diamines): made in 48 flasks simultaneously-

parallel diversity-oriented synthesis

![Chemical structures of Library 2](image)

- 5% ee if D-Leu
- 32% ee if R4 = tBu, H, or OMe
- 30-45% ee if better ee without linker
- 45-55% ee if S = NH2, 21% ee
- Thiourea improves ee

![Chemical structures of Library 2](image)
Winning Catalyst Would Not Have Been Found By One-At-A-Time Approach

Library 3

library size = 132 compounds (4 salicylaldehydes x 11 amino acids x 3 diamines): made in 132 flasks simultaneously

parallel diversity-oriented synthesis

A Parallel Combinatorial Approach to Catalyst Optimization

MS Sigman, EN Jacobsen
JACS 1998, 120, 4901
NMR Solution Structure of the Optimized Strecker Catalyst

NMR Solution Structure of the Imine-Catalyst Complex
Enantioselective Synthesis of $\alpha$-Amino Acids

R = wide range of aromatic and aliphatic groups


Catalytic Enantioselective Synthesis of Quaternary Centers

R = aromatic and some aliphatic groups

Split-Mix Diversity-Oriented Synthesis of a 1,3-Dioxane Library

500 µm PS bead

500 µm PS bead

500 µm PS bead

pool then split into 30 vials (each vial contains equal amounts of the three epoxyols)

react with 30 different 2π amines or thiols

S. Sternson, J. Louca, J. Wong, S. Schreiber

JACS 2001, 123, 1740.

Building Blocks Used in the 1,3-Dioxane Library

(a)

(b)

Slit-Mix Diversity-Oriented Synthesis of a 1,3-Dioxane Library

500 µm PS bead

500 µm PS bead

500 µm PS bead

pool then split into 30 vials (each vial contains equal amounts of the three epoxyols)

react with 30 different 2π amines or thiols

S. Sternson, J. Louca, J. Wong, S. Schreiber

JACS 2001, 123, 1740.

Building Blocks Used in the 1,3-Dioxane Library

(a)

(b)
Split-Pool Diversity-Oriented Synthesis of a 1,3-Dioxane Library


Split-Pool Diversity-Oriented Synthesis of a 1,3-Dioxane Library

Building Blocks Used in the 1,3-Dioxane Library

Split-Pool Diversity-Oriented Synthesis of a 1,3-Dioxane Library

1890 compounds; each bead contains one type of molecule

place each bead in a separate well of a 384-well plate

1890 stock solutions in 7 μl of DMSO

Screening an Unbiased DOS Library Uncovers New Molecules to Explore Biology

(a) cyclin B degradation in Xenopus extract

(b) no compound

(c) spherical actin staining phenotype


Binding assay: 1. Activation of Glass Slides

Small Molecule Printing

Split-pool library

Array beads
Cleave compounds
Prepare Stock Solutions

384-well stock plate
Print samples
Probe slides

TAG
Protein-Ligand
Binding

Fluorescent slide reveals binding interactions

Printed Glass Microscope Slide

Small Molecule microarraying robot
Biomimetic Diversity oriented Synthesis

Combinatorial chemistry is mainly used for optimization

valium - a benzodiazepin that binds to the GABA receptor

Benzodiazepin-based library design

Combinatorial chemistry library of natural products analogs


Natural products as reagents to explore biology

Colchicine

Colchicine is used to discover tubulin

Colchicine alters microtubule dynamics

Brefeldin A

Brefeldin reversibly blocks protein secretion by disrupting golgi structure
Biomimetic Diversity oriented synthesis

Amaryllidaceae Alkaloids:
Divergent Biosyntheses and
Diverse Biological Activities

norbelladine

enzyme-mediated
cyclizations

Biomimetic Diversity-Oriented Synthesis

Biosynthesis

norbelladine

galanthamine

natural selection

Discovery of galanthamine-like molecules with biological properties beyond those of the natural product

efficient synthesis of thousands of natural product-like molecules

protein binding screens

cell-based screens

Synthesis of natural product analogs to optimize drug-like properties

**Biomimetic Diversity oriented synthesis**

**Biosynthesis**

4'-O-Methyl norbelladine → P-450 like enzyme → Spiro-dienone

**Biomimetic solid-phase synthesis**

Norbelladine Equivalent

**Diversity-Generating Reactions**

- **Add Building Blocks**: 
  - $R_1OH$, PPh$_3$, DIAD
  - $R_2SH$, nBuLi
  - $R_3CHO$, NaCNBH$_3$
  - $R_3COCl$
  - $R_3NCO$ Acylation

- **Add Building Blocks**: 
  - $H_2N=OR_4$ or $H_2NNHSO_2R_4$

> 80% purity
First Step of the Galanthamine-Based Library

Reductive amination of a tyrosine-derived amine prepares a norbelladine-like starting material on the solid-phase

From tryosine

\[ \text{CH(OCH}_3\text{)}_3, \text{CH}_2\text{Cl}_2 \text{ wash, then NaBH}_3\text{CN, AcOH} \]
\[ \text{MeOH-THF, 23°C} \]

1. \[ \text{Cl O O} \]
2. \[ \text{O O} \]
3. \[ \text{Si iPr iPr} \]

Nature’s starting material for galanthamine biosynthesis

\[ 4’\text{-O-Methyl norbelladine} \]


Solid-Phase Biomimetic Oxidative Cyclization

The oxidant PhI(OAc)₂ promotes a biomimetic oxidative intramolecular biaryl coupling reaction to generate a seven-membered ring

\[ \text{norbelladine equivalent} \]

\[ 4’\text{-O-Methyl norbelladine} \]

Solid-Phase Biomimetic Oxidative Cyclization

The oxidant PhI(OAc)₂ promotes a biomimetic oxidative intramolecular biaryl coupling reaction to generate a seven-membered ring

\[ \text{norbelladine equivalent} \]

\[ 4’\text{-O-Methyl norbelladine} \]

\[ \text{P-450 like enzyme} \]

\[ \text{Spiro-dienone} \]
Deprotection initiates a stereoselective biomimetic cyclization

Pd-catalyzed triple deprotection yields a bisphenol which adds selectively to one of the two diastereotopic enones. The stereochemistry is controlled by a remote stereogenic center.

what is the mechanism of this reaction? how do you account for the stereoselectivity?

The First Diversification Reaction: A Mitsunobu Reaction

Mitsunobu coupling of the solid-phase phenol with five alcohols and a “skip”. The skip refers to a flask where no building block is added so that the phenol is represented in the library.

building blocks that gave >80% yield were used

By products: and Ph₃P=O
The Second Diversification Reaction: Conjugate Addition

A diastereoselective conjugate addition is achieved with seven thiolates and a skip so that the enone is represented in the library.

```
R_2SH, 2,6-lutidine, nBuLi, THF 0°C →

building blocks that gave >80% yield
```

The Third Diversification Reaction: Acylation and Reductive Amination

An example of functional group diversification - introducing different types of building blocks at a single position, leading to diverse functional groups. A library with diverse functionality has diverse properties: in this case amides, ureas, and amines leads to library members which are charged or uncharged at physiological pH.

```
R_3CHO, AcOH, MeOH-THF, then NaBH_3CN in MeOH, 23°C or R_3COCl, 2,6-lutidine, CH_2Cl_2, 23°C or R_3NCO, CH_2Cl_2, 23°C.
```

building block diversity

```
uncharged at pH= 7.4
charged at pH= 7.4
charged at pH= 7.4
```
The ketone is transformed - with functional group diversification - into oximes, hydrazones, and a ketone (via skip)

\[
\begin{align*}
R_1 & \quad R_2 \\
O & \quad S \\
H & \quad Pr
\end{align*}
\]

\[
\text{Building blocks that gave >80% yield}
\]

**Synthesis of a 2946 Member Library**
Synthesis of a 2946 Member Library

Library synthesized as one type of compound per 500 micron bead

Library contains one type of core structure with various appendages.

A challenge is a library with diverse core structures.

Assay of a 2946 member library

Single beads are arrayed in each well of a 384-well plate, 18 total plates. Then, the compounds are cleaved simultaneously with HF-pyridine and re-suspended in DMSO to make 2946 stock solutions.

Plate 03

384 Well Plate, ~50 nmol/well, 6.7 uL DMSO/well, ~7.5 mM/well
Assay of a 2946 member library

After cleavage from the solid phase, the solutions of library members are transferred using a robotic pin transfer arrayer.

Biochemical pathway

The Secretory pathway is responsible for the transport of newly synthesized proteins from the ER, through the Golgi to either the plasma membrane for secretion or to other cellular compartments.

A GFP is used to screen for exocytosis

Screen of the library at ~7.5mM revealed ER blockers and Golgi blockers
Active compound identified

V. Diversity Oriented Synthesis – more examples

Shikimic acid library

- DOS based upon a complexity-generating reaction (reaction-based)
- Synthesis of a 2.1 million member natural product-like library
- Reaction selection and building block evaluation in DOS
- Quality control in DOS

Benzopyran library

- DOS based upon privileged structure - the benzopyran core
- Scaffold formation during attachment and cleavage
- Directed sorting in library synthesis
- The IRORI NanoKan system
- Further diversification of a library after cleavage
A DOS Library Inspired by a Powerful Complexity-Generating Reaction

- A tandem esterification-[3+2] dipolar cycloaddition reaction gives a complex tricyclic structure, forming 3 bonds, 2 rings, and 3 new stereocenters in high yield and with complete stereocontrol.
- The efficiency of this transformation and the complexity of the product make this reaction a good candidate for the basis of a library.

\[
\text{HO} + \text{MeO} \rightarrow \text{O} \quad \xrightarrow{0.1 \text{ eq TiCl}_4 \ 4\AA \text{ MS}} \quad \text{DCE, rt, 19h} \quad 97\%
\]

\[
\begin{align*}
\text{HO} & \quad \text{O} \\
\text{MeO} & \quad \text{N} \\
\text{O} & \quad \text{O} \\
\text{O} & \quad \text{MeO} \\
\text{N} & \quad \text{O}
\end{align*}
\]

Testing of the Reaction on the Solid Support

- The model tandem reaction was tested on beads, and proceeded successfully with modified conditions.

\[
\begin{align*}
\text{HO} & \quad \text{O} \\
\text{PyBOP, NMP} & \quad \text{rt} > 98\%
\end{align*}
\]

\[
\begin{align*}
\text{HO} & \quad \text{N} \\
\text{O} & \quad \text{O} \\
\text{MeO} & \quad \text{N}
\end{align*}
\]

\[
\begin{align*}
\text{(SCNBu}_2\text{Sn)}_2\text{O} & \quad \text{4Å MS, tol, rt, 12h} > 98\%
\end{align*}
\]


Caproic Acid  Geysen Linker (cleave with hv)  Tentagel resin
The shikimic acid pathway converts simple carbohydrate precursors derived from glycolysis and the pentose phosphate pathway to the aromatic amino acids (Hermann and Weaver, 1999). One of the pathway intermediates is shikimic acid, which has given its name to this whole sequence of reactions. The well-known, broad-spectrum herbicide glyphosate (available commercially as Roundup) kills plants by blocking a step in this pathway. The shikimic acid pathway is present in plants, fungi, and bacteria but is not found in animals. Animals have no way to synthesize the three aromatic amino acids—phenylalanine, tyrosine, and tryptophan—which are therefore essential nutrients in animal diets.

Chiral Template for Library Synthesis

- The starting material for the library was made from (-)-Shikimic Acid:
  - Source of chirality (both enantiomers were synthesized)
  - Provides functional groups for additional building block diversification

(-) shikimic acid

\[
\begin{align*}
(-) \text{ shikimic acid} & \xrightarrow{\text{Amberlite–IR120}} \text{methyl shikimate} \\
\text{OMe} & \xrightarrow{\text{CH}_3\text{CN, 0°C, 90 min, 76%}} \text{Br} \\
\text{LiOH} & \xrightarrow{\text{THF/H}_2\text{O, 0°C, 1 h, 62%}} \text{(-) shikimic acid}
\end{align*}
\]
Chiral Template for Library Synthesis

- The synthesis of the (+) enantiomer of the starting material also proceeded from (-)-Shikimic Acid.

\[ \text{(-)-shikimic acid} \xrightarrow{\text{Amberlite-IR120}} \text{methyl shikimate} \]

\[ \text{BzOH, DEAD, PPh}_3, \text{THF} \quad 0^\circ \text{C} \rightarrow \text{rt}, 12 \text{ h} \quad 88\% \]

\[ \text{LiOH, THF/H}_2\text{O} \quad 0^\circ \text{C}, 1 \text{ h} \quad 59\% \]

\[ \text{PyBOP, DIPEA, NMP, rt, 1 h} \quad >98\% \]

\[ \text{PyBroP, DIPEA, DMAP, CH}_2\text{Cl}_2, 0^\circ \text{C} \rightarrow \text{rt} \quad 3 \times 3 \text{ h}, >98\% \]

Diversification Potential of the Scaffold

- The scaffold offers many potential sites for elaboration and diversification, but they must first be experimentally tested
- Black arrows are transformations that were found to be experimentally viable

![Chemical structure with reactions]

Reaction Selection

- Requirements for a good library reaction:
  - High yield
  - High purity (chemo-, diastereo-, regioselectivity)
  - High conversion
  - Solubility of reagents in solvents compatible with solid-phase synthesis
  - Reaction conditions compatible with solid-phase synthesis
    - Avoid: pressure, extreme temperature, light- and moisture-sensitivity, conditions that may break beads
  - Compatibility with linker used for library synthesis
  - If split-pool synthesis is used, the scope of the reaction is important: the reaction should not be sensitive to sterics or the presence of any functional group used in the library
Examples of Reaction Evaluation

Ritter reaction:
\[
\text{Yb(OTf)}_3, \text{PhCN} \rightarrow \text{neat, rt, 16h, low yield}
\]

Debenzylation/Carbamate formation:
\[
\text{PhOCOCl} \rightarrow \text{DIPEA, DCM, rt, 16h, 50%}
\]

Aminolysis of the lactone:
\[
\text{nBuNH}_2 \rightarrow \text{THF, rt, 95%}
\]

Diversification Sequence Development

- Attempted reactions with the amide were uniformly unsuccessful

\[
\text{nBuNH}_2 \rightarrow \text{THF, rt, 24h, >95% conversion}
\]

\[
\text{Bu}_3\text{Sn} \rightarrow \text{Pd}_2(\text{dba})_3, \text{AsPh}_3, \text{NMP, rt, 30h}
\]

\[
\text{PhB(OH)}_2 \rightarrow \text{Pd(PPh}_3)_4, \text{DMF, rt, 30h}
\]

all reactions proceed in low yield
Diversification Reaction Sequence and Building Block Testing

- Reversal of steps solved the problem.
- Testing of building blocks was conducted on a representative route.

Pathway Development

Building Block Testing

Synthesis and Analysis of a Small Test Library

LC–MS analysis of 8 pools of 64 distinct compounds – 456 of 456 compounds detected
Choosing An Encoding Strategy and Linker

- 90 μm TentaGel copolymer bead
- hν (365 nm) photocleavage
- Photocleavable linker
- CAN oxidative cleavage
- Binary encoding tag
- Library compound
- Chemical genetic assays with engineered cells in nanowells

Synthesis of the Complete Library

- TentaGel with Geysen linker
- 2 spacers + skip 98% → 3 compounds
- 3 iodobenzyl nitrones >98% → 18 compounds
- 62 amines + skip 95 – >98% → 35,154 compounds
- 2 epoxy cyclohexenol enantiomers >98% → 6 compounds
- 30 alkynes + skip 90 – 95% → 558 compounds
- 62 acids + skip 95 – >98% → 2,180,106 compounds
Summary of Shikimic Acid Library Development

1. Identification of a complexity generated reaction
2. Testing and adopting the reaction to the solid phase
3. Reaction pathway development
4. Building block testing
5. Quality control - Synthesis of a test library
6. Encoding
7. Library synthesis

Shikimic Acid Library
Reaction-Based
Split-Pool Synthesis
161 Reactions, 2.1 million compounds

Privileged Structure

• Privileged Structure: a structural pattern which is commonly found associated with a particular property and may contribute to that property

• Example: Biological Activity
  – The benzodiazepine substructure is found in a wide variety of biologically active natural products

  ![Cycloopenin](image1)
  ![Anthramycin](image2)
  ![Asperlicin](image3)

  - Cycloopenin
    Phytotoxic activity

  - Anthramycin
    Antitumor and antibiotic activity

  - Asperlicin
    Human Cholecystokinin antagonist

• Example: Asymmetric Catalysis
  – The binaphthyl substructure is part of many effective chiral ligands

  ![BINAP](image4)
  ![BINOL](image5)
  ![MOP](image6)

  - BINAP
    Asymmetric hydrogenation

  - BINOL
    Enantioselective hydroisilylation

  - MOP
    Asymmetric hydroformylation

Benzopyran as Privileged Structure

• The benzopyran substructure is found in many diverse biologically active natural products

  ![Benzopyran](image7)

  - deguelin
    electron transport inhibitor

  - daleformis
    ICE inhibitor

  - calanolide A
    HIV RT inhibitor

Nicolaou et al. JACS, 2000, 122, 9939-9967 (3 full papers)
Benzopyrans: Privileged Structure?

Micellaneous biologically active 2,2-dimethylbenzopyran containing natural products

DNA cleaving agent
EC50 = 4.5 μg/mL (KB)

Inhibitor of arachidonate 5-lipoxygenase
IC50 = 0.36 μM

Inhibitor of tyrosinase
IC50 = 4.9 μM

Pharmaceutical ligands containing benzopyran motifs

Inhibitor of aldoketoreductase
IC50 = 0.09 μM

Inhibitor of phosphodiesterase IV
IC50 = 10 μM

Inhibitor of phosphodiesterase IV
IC50 = 5 μM

Benzopyrans: Privileged Structure?
A Plan for a Library of Benzopyrans

- Attachment to the solid phase and cyclization to the dihydrobenzopyran are accomplished simultaneously
- The selenide linker is stable to strong acidic, basic and reductive conditions (and some oxidants), but is cleaved with mild hydrogen peroxide treatment
- Cleavage of the compound from the solid support completes the benzopyran structure

The First Diversification Pathway

- Addition of nucleophiles to an aldehyde scaffold was followed by acylation or Mitsunobu reaction of the resulting secondary alcohol

Nicolaou et al. JACS, 2000, 122, 9939-9967 (3 full papers)
The Second Diversification Pathway

- Reductive amination followed by acylation or sulfonylation of the resulting amine

\[
\begin{align*}
\text{OMe} \quad \text{Me} \quad \text{Se} & \quad \text{Me} \quad \text{OMe} \quad \text{Se} \\
\text{OMe} \quad \text{Me} \quad \text{Se} & \quad \text{OMe} \quad \text{Me} \quad \text{Se} \\
\text{OMe} \quad \text{Me} \quad \text{Se} & \quad \text{OMe} \quad \text{Me} \quad \text{Se} \\
\text{OMe} \quad \text{Me} \quad \text{Se} & \quad \text{OMe} \quad \text{Me} \quad \text{Se}
\end{align*}
\]

9 aldehydes

\[
\begin{align*}
\text{Cl} \quad \text{R}^3 & \quad \text{Et}_3\text{N}, \quad \text{DMAP} \\
\text{Et}_3\text{N}, \quad \text{DMAP} & \quad \text{H}_2\text{O}_2, \quad \text{THF}, \quad 25^\circ\text{C}
\end{align*}
\]

10 R^4\text{SO}_2\text{Cl}

\[
\begin{align*}
\text{H}_2\text{O}_2, \quad \text{THF}, \quad 25^\circ\text{C}
\end{align*}
\]

The Third Diversification Pathway

- Knoevenagel condensation of a benzylic nitrile was followed by cleavage of some library members - and glycosylation of library members containing a protected phenol

\[
\begin{align*}
\text{NC} \quad \text{NC} \quad \text{OMe} \quad \text{Me} \quad \text{Se} \\
\text{NC} \quad \text{OMe} \quad \text{Se} \quad \text{OMe} \quad \text{Me} \\
\text{NC} \quad \text{OMe} \quad \text{Se} \quad \text{OMe} \quad \text{Me}
\end{align*}
\]

9 aldehydes

\[
\begin{align*}
\text{NC} & \quad \text{KOEt, THF, 25^\circ\text{C}} \\
\text{KOEt, THF, 25^\circ\text{C}} & \quad \text{TsOH, THF-MeOH}
\end{align*}
\]

If R^2 = p-\text{OTHP}

\[
\begin{align*}
\text{If R}^2 = p-\text{OTHP} & \quad \text{TsoH, THF-MeOH} \\
\text{TsoH, THF-MeOH} & \quad \text{H}_2\text{O}_2, \quad \text{THF}, \quad 25^\circ\text{C}
\end{align*}
\]

cleave

5 sugars

\[
\begin{align*}
\text{BF}_3\text{Et}_2\text{O}, \quad \text{CH}_2\text{Cl}_2 \\
4\text{A MS, 0^\circ\text{C}}
\end{align*}
\]
Building Blocks Used in the Benzopyran Library

Synthesis Scheme for the Entire Library
The IRORI System: Kan Reactors for Solid Support

- The IRORI system carries each library member on beads contained in a plastic or ceramic capsule ("kan")
- Kans have higher loading levels than beads, and are more mechanically robust
- Handling of the IRORI kans is highly automated

Individual Labeling of Kans Simplifies Decoding

- Each Kan has a laser-readable tag
- The Kans can be read and sorted in a high-throughput machine

Encoding of an IRORI library:
- The path of a Kan through the library synthesis is pre-determined
- At each step, the Kan is sorted into the appropriate reaction vessel by a computerized sorter (2,000 Kans/hour)
- At the end of the library synthesis, the path followed by each Kan is known by the computer, so no decoding of the library is necessary
Construction of the Library with IRORI

- Directed sorting of library members greatly simplifies the execution of a complex library synthesis plan

- Analogous to selective splitting of compounds in a split-pool library
Diversification After Cleavage from the Solid Phase

- The benzopyran library could be elaborated and further diversified after cleavage from the solid phase.

Benzopyran library

Benzopyran Library
Privileged Structure-Based
Directed Sorting
18 reactions, 10215 compounds
Microreactors in Chemical Synthesis

Changes in the way we do chemistry
Chemical dimensions

Surface to volume ratio

\[ \frac{A}{V} \text{ [m}^2\text{ / m}^3\text{]} \]

- **Mikroreaktor**: 4.000 - 20.000
- **Millireaktor**: 1.500 - 4.000
- **Rohrbündelreaktor**: 100 - 400
- **Vollraumreaktor/ Rührkessel**: 4 - 40
Small dimensions = short mixing time

Diffusion coefficient:

\[ D = 1 \times 10^{-5} \text{ cm}^2/\text{s}, \]

for example, water at 20°C

\[ D = 2 \times 10^{-1} \text{ cm}^2/\text{s}, \]

for example, nitrogen at 1 bar and 20°C

\[ \tau = \frac{L^2}{D} \]

Characterestic dimension L

Small dimensions = short mixing time

Injection of substreams

Periodic injection

Splitting and recombination

Injection into a main stream

Decrease of diffusion path

Forced mass transport

Contacting
Small dimensions = efficient heat transfer

Nu = Nusselt number; describes enhancement of heat transfer from a surface in a “real” situation if compared to conductive heat transfer.

Thermal diffusivity:

\[ a = 1.4 \times 10^{-3} \, \text{cm}^2/\text{s}, \]
e.g. water at 20°C

\[ \tau = \frac{L^2}{a \cdot \text{Nu}} \]

Nu = Nusselt number; describes enhancement of heat transfer from a surface in a “real” situation if compared to conductive heat transfer.

Exact control of residence time

Diffusive Wegstrecke, die innerhalb der Verweilzeit \( \tau \) zurückgelegt wird.

Mikrostruktur, Makrostruktur
Safety

Small volumes and better control of reaction conditions reduce risk and open new process windows (reactions at very high temperatures and pressures with very short reaction times).

A Closer Look at Mixing

Reynolds number = \( \frac{\text{density} \times \text{velocity}}{\text{kinematic viscosity}} \)

laminar \(<\ Re = 30 <\ \text{turbulent} \)
Induction of vortices (Strudel, Wirbel) by stirring, moving parts or structured channels.

For all mixing techniques:
Direct simulation of mixing is hardly possible for liquid/liquid systems.
Microreactor apparatus set up

Inlet 1

Inlet 2

Inlet

Outlet

Outlet
Microreactor apparatus set up

Mischer
Wärmetauscher
Reaktoren
Filter Separatoren

Einlass Auslass
Verbindungen
Ventile
Pumpen Sensoren
Integrated units:
- Micromixer
- Delay loop
- Micro heat exchangers

Operating temperature:
-50 to +250 °C
Microreactor apparatus set up

Lab scale reactions

- Diels-Alder
- Diazo couplings
- Grignard reaction
- Carbanion chemistry
- Enol ethers
- Michael additions
- Pyrazole synthesis

- Suzuki
- Heck
- Evans auxiliary
- Enamines
- Ugi 4CC
- Amide synthesis
- Peptide synthesis

- Kumada
- Wittig
- Horner Wadsworth Emmons
- Complexometric reactions
- Dehydration reactions
- Enzyme based reactions
- Aromatic nitration

<table>
<thead>
<tr>
<th>Reaction</th>
<th>In Flask</th>
<th>In Channel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time**</td>
<td>Conversion %</td>
</tr>
<tr>
<td>Suzuki</td>
<td>8 h</td>
<td>70</td>
</tr>
<tr>
<td>Wittig</td>
<td>3 h</td>
<td>48</td>
</tr>
</tbody>
</table>

* Channel residence time (followed by rapid analysis). ** Monitored to point of no further change

Michael reaction

Discontinuous, 'stop-go' flow: increase in residence time and mixing quality

- Standard continuous flow: conversion of 15%
- Discontinuous, 'stop-go' flow (period 2.3 s to 5 s): conversion of 34%
- Discontinuous, 'stop-go' flow (period 10 s): conversion of 100%

Increase in conversion by 22% compared to batch operation


Synthesis of 1,3-Diketones

- 1.0 M enol ether/benzoyl fluoride in THF
- 0.1 M TBAF (catalytic) in THF
  - 100 % conversion

C. Wiles et al., *Chem. Commun.*, 2002, 1034
Synthesis of Tetracyclone

Mixing and reaction at +80°C:

Mixing and reaction at + 90°C:

Aromatic Nitration

Segmented flow
Landolt Reaction - Iodine Clock

\[
\text{IO}_3^- + 6 \text{H}^+ + 3 \text{HSO}_3^- \rightarrow \text{I}^- + 3 \text{HSO}_4^- + 6 \text{H}^+ \\
\text{IO}_3^- + 6 \text{H}^+ + 6 \text{I}^- \rightarrow 3 \text{I}_2 + 3 \text{H}_2\text{O}
\]
**Segmented flow**

Controlled precipitation
In a bubble tube

Forced precipitation of calcium carbonate

\[ \text{Ca}^{2+} + \text{CO}_3^{2-} \rightarrow \text{CaCO}_3 \downarrow \]
**Segmented flow**

**Micro-encapsulation of drugs in polymeric microspheres**

Polylactic acid; polylactic-polyglycolic acid

Lab process for microsphere preparation:

- discontinuous, multiple-step process
- difficult scale-up
- sensitive towards variations
- difficult to be run aseptically

**Segmented flow**

**Micro-encapsulation of drugs**

**Micro mixer process**

- replaced by pressurized vessel
Resorcin Azocoupling reaction

\[
\text{Resorcin} + \text{nitrosobenzene} \xrightarrow{\text{Base NEt}_3} \text{resorcin azo dye}
\]

Peptide Synthesis

(4-[N-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]-amino] benzyl alcohol) = Dmab-OH

Quantitative conversion

At 0.1 M concentration of reagents - 4.2 % racemisation
At 0.5 M concentration of reagents - 7.8 % racemisation

P. Watts et al., Chem. Commun., 2001, 990
Peptide Synthesis

P. Watts et al., Tetrahedron, 2002, 5427

Suzuki coupling

Photo reactions

Conversion of $^{1}$O$_2$ and α-terpine to ascaridol

- Safe handling of the low volumes of the explosive endo peroxide
- After 5 s only, high conversions


[4+2]-Cycloaddition of cyclopentadiene

- Creation of $^{1}$O$_2$ by bengal rosa
- Pharmaceutical intermediate 2-cyclopenten-1,4-diol accessible

Jahnisch, K., Baerns, M.; Patent filing DD 10257239.9

Combinatorial compound library synthesis

Domino reaction: Knoevenagel condensation and Hetero-Diels-Alder

Combinatorial compound library synthesis

Amide formation and Knorr-Pyrrol Synthesis

Got a few problems going from lab scale up to full commercial scale

Garcia-Egido, E. et al; Lab Chip 3, (2003) 73
Asymmetric synthesis via organoboranes

Synthesis of the chiral allylboranes
Asymmetric synthesis via organoboranes

Synthesis of chiral allylalcohols
### Asymmetric synthesis via organoboranes

<table>
<thead>
<tr>
<th></th>
<th>Literatur* (Batch Operation)</th>
<th>Microplant (Kontinuierlich Operation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperatur</td>
<td>-100 °C</td>
<td>-35 °C</td>
</tr>
<tr>
<td>Ausbeute</td>
<td>66% (gesamt)</td>
<td>90% (gesamt)</td>
</tr>
<tr>
<td>Enantiomeren-Überschuss</td>
<td>≥96-99% (letzte Stufe)</td>
<td>81% (gesamt)</td>
</tr>
<tr>
<td>Selektivität</td>
<td>-</td>
<td>93% (gesamt)</td>
</tr>
<tr>
<td>Verweilzeit</td>
<td>0.5h</td>
<td>ms</td>
</tr>
</tbody>
</table>

**Gesamte Flussrate:** 0.2ml/min

**Maximale kontinuierliche Operation:** 5h

**Kein Produkt erhalten:**
- *bei -100°C* (Schlechte Löslichkeit von Benzaldehyd in Ether)
  - *bei doppelter Flussrate* (Verweilzeit zu kurz)

### Azo pigment synthesis

\[
\begin{align*}
\text{HNO}_2 & \quad \text{Ar} - \text{N=N Y} + \text{RH} \\
\text{Ar} - \text{NH}_2 & \quad \rightarrow \quad \text{Ar} - \text{N} - \text{R} + \text{HY}
\end{align*}
\]

**Coupling**

**Diazotation**

**Pigmenting**

**Graph:**

- Volume fraction per dot [%]
- Equivalent particle diameter [μm]
Azo pigment synthesis

- Realizing a lab scale system
- Process optimization
- Process stability

10 t per year

Production reactor
Azo pigment synthesis

Advantages:

• Constant product properties compared to batch synthesis
• Short development time (18 months) from lab to plant
• Switching between products is fast
• Low operating cost

Summary

Microreactor systems allow a better control of reaction conditions and therefore provide advantages for
• fast reactions
• very exothermic reactions
• handling of dangerous reaction intermediates
• reactions at very high temperatures and pressures
• sequential reactions

A better control of reaction conditions may reduce side reactions.

Optimization of reaction conditions is facilitated and the use of otherwise difficult accessible “process windows” becomes possible.

Simple scale up of a reaction by “numbering up” (parallel use of several microreactors)

Production of t/year in a laboratory environment
Customized production on demand
VI. Complexity Generating Reactions

Molecular Complexity

Defined by Our Intuition
– Complexity is defined in part by the frontiers of chemistry
– Complexity resists definition, but we know it when we see it

• Molecular Size
• Element and Functional Group content
• Cyclic Connectivity
• Stereocenter Content
• Chemical Reactivity
• Structural Instability

Intuitive Definition of Complexity

• Contributors to Complexity
  – **Molecular Size**
    Palytoxin

• 115 carbon chain
• 71 stereogenic elements
• Requires 42 hydroxyl protecting groups!
Intuitive Definition of Complexity

• Contributors to Complexity
  – Element and Functional Group Content
    Vancomycin

Aminodisaccharide → Atropisomerism
Benzylic β-hydroxyls → Carboxamide
Free Acid → Phenols

Intuitive Definition of Complexity

• Contributors to Complexity
  – Cyclic Connectivity
  – Stereocenter Content
    Ginkgolide B

• 6 fused 5-membered rings
• Dense functionality
• 13 of 14 ring carbons are asymmetric
Intuitive Definition of Complexity

- Contributors to Complexity
  - Chemical Reactivity: Neocarzinostatin Chromophore

  \[
  \text{Chemical Structure 1}\quad \text{Chemical Structure 2}
  \]

  - Aglycon decomposes in 1-2 hours

Intuitive Definition of Complexity

- Contributors to Complexity
  - Structural Instability
    - Example: Phomoidride B (CP-263,114)

  \[
  \text{Chemical Structure 3}\quad \text{Chemical Structure 4}
  \]

  - Highly epimerization-sensitive
  - Sensitive pseudoester
  - Reactive maleic anhydride
A Computational Definition of Complexity?

- Using graph theory: Complexity (C) is related to the number of times (n) a given pattern can be mapped onto the molecule

\[
C(n) = 2n \log_2 n - \sum n_i \log_2 n_i
\]

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Atoms</th>
<th>Bonds</th>
<th>Propane</th>
<th>Adjacent Bonds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8.00</td>
<td>15.51</td>
<td>8.00</td>
<td>16.00</td>
</tr>
<tr>
<td></td>
<td>8.00</td>
<td>15.51</td>
<td>15.51</td>
<td>9.51</td>
</tr>
<tr>
<td>Propane</td>
<td>8.00</td>
<td>15.51</td>
<td>43.02</td>
<td>4.00</td>
</tr>
<tr>
<td>Adjacent Bonds</td>
<td>8.00</td>
<td>15.51</td>
<td>43.02</td>
<td>16.00</td>
</tr>
</tbody>
</table>

- These methods are limited to simple connectivity, double bonds, etc. Influence of stereochemistry, heteroatoms, functional groups, reactivity, and stability are not effectively included

Why Complexity in DOS?

- Intuition and the desire to emulate nature
  - Nature makes molecules of a wide range of complexity for a wide range of functions

- Kramerixin: Potent, broad-spectrum antifungal agent
- Distamycin A: Antibiotic by binding to the minor groove of DNA
- Palau’amine: Potent immunosuppressant by unknown mechanism
- Epothilone B: Anti-mitotic by stabilization of microtubules
Challenges of Complexity in DOS

- Length of the Synthetic Route
  - Target-oriented synthesis of a complex natural product often requires 20-40 steps
    - The length of a synthetic route on the solid phase is limited to 5-15 steps by purity considerations

  Ginkgolide B

Corey et al. - 31 linear steps
*JACS, 1988, 110, 649.*

Crimmins et al. - 25 linear steps
*JACS, 1999, 121, 10249.*
*JACS, 2000, 122, 8453.*

<table>
<thead>
<tr>
<th>Avg. transformation efficiency</th>
<th>% of desired B on bead</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>11%</td>
</tr>
<tr>
<td>90%</td>
<td>35%</td>
</tr>
<tr>
<td>95%</td>
<td>60%</td>
</tr>
</tbody>
</table>

Challenges of Complexity in DOS

- Planning a complex library requires reliable reactions
  - Testing of reactions and building blocks is conducted in a “representative” route, but quality control of all reactions in a library synthesis is impractical
  - Density of functional groups and steric crowding can cause unpredictable reactivity

- A complex library requires rapid generation of complexity to minimize the number of steps
Complexity-Generating Reactions

- Efficient and reliable complexity-generating reactions (CGRs) are necessary in DOS to assemble natural product-like molecular frameworks in few steps.

- Reactions which rapidly create rings, stereocenters, carbon-carbon bonds, and functional groups
  - Cycloaddition reactions
  - Multicomponent couplings
  - Tandem reactions - Two or more transformations conducted in sequence in one reaction vessel

Types of Tandem Reactions

- Tandem cascade reactions - no isolable intermediates

- Tandem consecutive reactions - a change in conditions starts the subsequent transformations

- Tandem sequential reactions - requires addition of another component
Design of Tandem Reactions

Thermodynamics
- Keep in mind that reactions must be energetically favorable
  - The Benson additivity rules are useful for the quick calculation of the bond and ring strain energies gained and lost in a reaction
  - The introduction of strain into a molecule is often an effective way of promoting rearrangements

<table>
<thead>
<tr>
<th>σ-bonds</th>
<th>π-bonds</th>
<th>rings</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-Csp$^3$</td>
<td>100</td>
<td>C-C 85</td>
</tr>
<tr>
<td>H-Csp$^2$</td>
<td>103</td>
<td>O-C 84</td>
</tr>
<tr>
<td>H-Csp</td>
<td>125</td>
<td>N-C 79</td>
</tr>
<tr>
<td>H-O</td>
<td>102</td>
<td>Cl-C 82</td>
</tr>
<tr>
<td>H-O$_2$C</td>
<td>112</td>
<td>Br-C 70</td>
</tr>
<tr>
<td>H-N</td>
<td>103</td>
<td>I-C 54</td>
</tr>
</tbody>
</table>

Design of Tandem Reactions

Kinetics
- Intramolecular reactions are greatly accelerated over an analogous intermolecular reaction
- Proximity can be used to enforce the desired reactivity
- Irreversible termination steps can be included if the product would otherwise be a small (uphill) component of an equilibrium
Design of Tandem Reactions

- **Key elements**
  - Recognition of new reactive patterns in the products of a reaction

- **“Programmed” starting materials**
  - Certain functional group patterns lead to cascade reactions
  - e.g. spaced olefination: Heck, radical, cation cascades

![Chemical structure](image)

Keying Elements:
- Enolate
- Leaving group

Complexity-Generating Reactions in DOS

- Tsuge reaction followed by a [2,3]-dipolar nitrile oxide cycloaddition

![Chemical structure](image)

Complexity-Generating Reactions in DOS

- Tandem esterification - nitrone [3+2] cycloaddition approach to a tetracyclic scaffold

\[
\text{OH} \quad \text{N} \\
\text{O} \quad \text{O} \\
\text{N} \\
\text{H} \quad \text{O}
\]

\[\text{PyBroP or HATU} \quad \text{DMAP} \quad \text{iPr}_2\text{NEt}\]


Complexity-Generating Reactions in DOS

- A three-component condensation gives tetrahydroquinolines from simple starting materials

\[
\text{O} \quad \text{O} \\
\text{N} \\
\text{H} \\
\text{O}
\]

Complexity-Generating Reactions in DOS

- An oxidative coupling - hetero-Diels-Alder approach to Carpanone-like molecules

\[
\begin{align*}
\text{OR}_3 & \quad \text{HO} \\
\text{R}_4 & \quad \text{O} \\
\text{R}_3 & \quad \text{O} \\
\text{R}_1 & \quad \text{O} \\
\text{R}_2 & \quad \text{O}
\end{align*}
\]


\[
\begin{align*}
\text{OR}_3 & \quad \text{HO} \\
\text{R}_4 & \quad \text{O} \\
\text{R}_3 & \quad \text{O} \\
\text{R}_1 & \quad \text{O} \\
\text{R}_2 & \quad \text{O}
\end{align*}
\]


Complexity-Generating Reactions in DOS

- A biomimetic oxidative phenolic coupling used to make a library of Galanthamine-like molecules

\[
\begin{align*}
\text{MeO} & \quad \text{OH} \\
\text{NH} & \quad \text{OH}
\end{align*}
\]


\[
\begin{align*}
\text{Br} & \quad \text{O} \\
\text{OH} & \quad \text{O}
\end{align*}
\]

Complexity-Generating Reactions in DOS

- A sequential 1,4 cuprate addition - enolate alkylation approach to a library based on Prostaglandins

1) Bu₃Sn → OTBS
2) TMSCl

MeLi; TIO → CO₂Me


Complexity-Generating Reactions in DOS

- A tandem Knoevenagel-Ene reaction gives complex cyclic compounds

Complexity-Generating Reactions in DOS

- An Ugi - Diels-Alder sequence gives a complex tricyclic core from four simple components


Complexity-Generating Reactions in DOS

- The tricyclic core is subjected to an olefin metathesis cascade to give a rearranged tetracyclic core

Tandem Cycloadditions and Sigmatropic Rearrangements

Why use tandem cycloadditions and sigmatropic rearrangements?
- Both reaction types make use of olefins and generate new olefins that can be reacted further

Other advantages of cycloadditions and sigmatropic rearrangements
- Both reaction types can be employed under similar, mild conditions (thermal promotion, Lewis acid catalysis)
- Cycloaddition reactions are capable of creating multiple C-C bonds, rings, and stereocenters in a single step; high degrees of regio- and stereocontrol are often exhibited
- Sigmatropic rearrangements can stereospecifically translate the stereochemistry of a single center and the geometry of two olefins to multiple new stereocenters in the product. Another result is the stereospecific generation of highly substituted olefins, an area that remains a challenge in organic synthesis
Tandem [4+2]/[4+2] (inter-intra)

Starting material contains both diene components

Second diene unmasked by thermal SO₂ extrusion following initial intermolecular Diels-Alder reaction

\[ \text{ZnCl₂, 25°C, 20 h} \]
\[ 85\% \]

Toluene, Δ

[−SO₂]

Thermal unmasking of latent diene

\[ n=1,3 \]


---

Tandem [4+2]/[4+2] (inter-intra)

- Winkler extended earlier methodology work to a synthesis of the taxane skeleton
  - Dienes are sterically differentiated (no protection)

- Monosubstituted diene reacts first (no sulfolene protection required)

- Neither Lewis Acid catalyzes both Diels-Alder reactions

- Both Diels-Alder are endo selective

Taxol

Tandem [4+2]/[4+2] (intra-intra)
- Tetracyclic structure created from acyclic bis-diene containing an internal acetylenic unit
  - Linear Diene
  - Branched Diene


Tandem [4+2]/[4+2]-Diene Transmissive
Rapid entry into triterpenoid/nor-steroid skeleta using a diene transmissive intramolecular Diels-Alder in tandem with an intermolecular Diels-Alder

Tandem [4+2]/[3+2]

- Denmark has developed a series of tandem [4+2]/[3+2] cycloadditions using nitroalkenes.
  - Intermediate nitronates undergo [3+2] cycloaddition
  - Five stereocenters, three rings created in a single step
  - Use of recoverable chiral auxiliary results in high stereoselectivity

\[
\begin{align*}
\text{MeO}_2C &= \text{Me} \quad \text{OR}^* \\
\text{TiCl}_2(\text{Oi-Pr})_2 &= \text{Me} \quad \text{OR}^* \\
\text{CH}_2Cl_2, -70^\circ C &= \text{MeO}_2C \\
\text{[4+2]} &= 89% \\
\text{Olefin transmitted to } 1,3\text{-dipole by Diels-Alder} \\
\text{MeO}_2C &= \text{Me} \quad \text{OR}^* \\
\text{H}_2/\text{Ra Ni} &= \text{HOI} \\
\text{MeOH} &= \text{Me} \quad \text{R}^* \\
\text{99% ee} &= \text{OCH}_2\text{-Bu} \\
\end{align*}
\]


Tandem [4+2]/[3+2]

- Tandem process results in full vindoline skeleton with all six stereocenters established

\[
\begin{align*}
\text{[4+2]} &= \text{[3+2]} \\
\text{[3+2]} &= \text{Retro [3+2]}: [3+2] \text{ enabled} \\
\end{align*}
\]

Tandem [3,3]/[4+2]

- 11-Deoxydaunomycinone synthesised via a tandem Claisen/Diels-Alder strategy
  - 3 C-C bonds and two rings formed in key step

![Chemical structures and reaction scheme](image)

- Tautomerize
- 11-Deoxydaunomycinone


Tandem [1+2]/[3,3]

- Tandem cyclopropanation-Cope sequence provides formal [4+3] cycloadducts
  - Facile synthesis of seven membered rings
  - Cope synthon (1,5-diene) created in cycloaddition step

![Chemical structures and reaction scheme](image)

- α-diazocarbonyl breaks down to carbene in presence of Rh(II); [1+2] cycloaddition with olefin is facile
- With an appropriately functionalized cyclopropane, a ring-expansive, strain-accelerated Cope will occur

Radical cyclization

Heck Cascades

- Alkyne or 1,1-dialkylalkene groups are positioned three to five carbon apart leading to the formation of 5,6, and 7-membered rings.

1 reaction: 7 new rings, 7 new C-C bonds, and 14 new stereocenters!

**Tandem Heck Reaction**

- Intermolecular Heck reaction followed by intramolecular one
- Syn-Carbopalladation leads to only one geometric isomer

Stable Organo-Pd intermediate without syn β-H


**Tandem Heck Reaction**

- Intermolecular Heck reaction terminated with intramolecular one

Sequential Tandem Heck Reaction

- Two Heck reactions are achieved in one-pot

\[
\begin{align*}
\text{Br} & \quad \text{Br} \\
\text{MeO} & \quad \text{OtBu}
\end{align*}
\]

10 % Pd(OAc)_2, 22 % PPh_3, 60 °C, 60 h.
CH₃CN, DMF, MeO
nBu₄N(OAc)

More Reactive

Syn- Elimination

\[
\begin{align*}
\text{Br} & \quad \text{Br} \\
\text{MeO} & \quad \text{OtBu}
\end{align*}
\]

120 °C

63 %


Stable Neopentyl Organo-Pd-intermediate

- Strained tricyclic system

\[
\begin{align*}
\text{R} & \quad \text{I} \\
\text{OTBS}
\end{align*}
\]

10 % Pd(OAc)_2, 20 % PPh_3,
Ag_2CO_3, THF, reflux

Neopentyl-type

\[
\begin{align*}
\text{R} & \quad \text{PdLn} \\
\text{OTBS}
\end{align*}
\]

> 90 %

Scopadulic acid

Chemistry of Stable Organo-Pd Intermediate

An Opportunity for Diversification

![Diagram of chemical reactions]

Sequential Tandem Heck-Suzuki Reaction

- Three component coupling reaction on solid support
- Stoichiometric amount of Pd used

\[
\begin{align*}
X &= 4\text{-MeO} & R &= 4\text{-MeOC}_6\text{H}_4 \\
&= 4\text{-Me} & = 4\text{-MeC}_6\text{H}_4 \\
&= H
\end{align*}
\]

![Chemical reactions diagrams]

Heck-Carbonylation and intermolecular Nucleophilic addition

- Three component coupling reaction

\[ \text{Pd(0)} \rightarrow \text{LnPd} \rightarrow \text{PdL_n} \]

F. Vöglt. et al “Stimulating concepts in Chemistry” pp 56

Multicomponent Reactions

- Multicomponent reaction: A reaction in which three or more reagents react in one pot to generate a product containing atoms from all reagents.

Mannich Reaction:

\[ \text{CHO} \quad \text{H}_2\text{N} \quad \text{CHO} \quad \text{CO}_2\text{H} \quad \text{CaCO}_3 \quad \text{H}_2\text{O}, 25 \degree \text{C} \]

\[ \text{AcO} \quad 0.1 \text{ CpRu}^+ \quad 0.15 \text{ CeCl}_3 \quad \text{DMF}, 60 \degree \text{C} \quad 80\% \]

Interrupted three component reaction
Ru catalyzed coupling followed by Diels-Alder

Advantages of Multicomponent Reactions

- Complexity is generated through the combination of multiple functional groups.
- Diversity is easily incorporated in one step by varying the components.
- Deprotection steps are often avoided.
- One pot reactions are ideal for automation.
- Efficient solid phase or solution phase synthesis is possible.

- Disadvantage: Generally cannot do split-pool synthesis

Two Component vs. Multicomponent Synthesis

- Three component synthesis can make 8 products in 4 fewer reactions than parallel two component synthesis.

Parallel Two Component Couplings

12 reactions

Three Component Couplings

8 reactions
**Multicomponent vs. Split and Pool Synthesis**

- Split-pool two component synthesis can make 8 products in 4 fewer reactions than three component synthesis.

<table>
<thead>
<tr>
<th>Three Component Couplings</th>
<th>Split-Pool Two Component Couplings</th>
</tr>
</thead>
<tbody>
<tr>
<td>A → C → E</td>
<td>A → C → E</td>
</tr>
<tr>
<td>A → C → F</td>
<td>B → C → E</td>
</tr>
<tr>
<td>A → D → E</td>
<td>A → D → E</td>
</tr>
<tr>
<td>A → D → F</td>
<td>B → D → E</td>
</tr>
<tr>
<td>B → C → E</td>
<td>A → D → F</td>
</tr>
<tr>
<td>B → C → F</td>
<td>B → C → F</td>
</tr>
<tr>
<td>B → D → E</td>
<td>B → D → F</td>
</tr>
<tr>
<td>B → D → F</td>
<td>4 reactions</td>
</tr>
<tr>
<td>8 reactions</td>
<td></td>
</tr>
</tbody>
</table>

- Parallel multicomponent synthesis:
  - Requires more steps
  - Does not require tagging
  - Often avoids deprotection steps
  - More easily automated

- Interrupted multicomponent reactions potentially could be used for split and pool synthesis.
The Multicomponent Ugi Reaction

- In the Ugi reaction, an aldehyde, an amine, a carboxylic acid, and an isocyanide couple to form an $\alpha$-amino acylamide.

\[
\begin{align*}
R^1\text{CHO} & \quad H_2N-R^2 \quad R^3\text{COOH} \quad C=N-R^4 & \rightarrow & \quad R_3N-C(=O)NH-R^4 \\
\end{align*}
\]

- Reactions usually are run in methanol or ethanol at high reagent concentrations (0.5 to 2 M).
- Exothermic, ice bath cooling is often required.
- Precondensation of the amine and aldehyde can improve yield.
- Lewis acids can accelerate the reaction.

Preparation of Isocyanides

- Only 12-15 isocyanides are commercially available.
- Isocyanides can be prepared by dehydration of a formamide or derivatization of another isocyanide.

\[
\begin{align*}
\text{CICO}_2\text{CCl}_3 & \rightarrow & \quad \text{R} = 1^\circ, 2^\circ, \text{or } 3^\circ \text{ alkyl, benzyl, aryl, etc.} \\
\\text{NEt}_3, \text{CH}_2\text{Cl}_2 & \quad 0 \text{ °C to } 25 \text{ °C} & \quad 75\text{-}98\% \\
\text{OR:} & \quad \text{R} = \text{N}≡\text{C}⁻ \\
\text{POCl}_3 & \rightarrow & \quad \text{HN(iPr)}_2, \text{CH}_2\text{Cl}_2 & \quad 0 \text{ °C to } 25 \text{ °C} & \quad 53\text{-}89\%
\end{align*}
\]
Isocyanide Chemistry

- The isocyanide reacts carbene-like by the $\alpha$-addition of both a nucleophile and electrophile.

Mechanism of the Ugi Reaction

- The coupling occurs by isocyanide trapping of a reversibly formed iminium and carboxylate, followed by an acyl migration.
Ugi Reactions in TOS

- The Ugi reaction gives more than twice the yield of the dipeptide fragment than does linear peptide coupling.
- Analog synthesis is also simplified.

\[
\begin{align*}
\text{MeOH} & \rightarrow \text{hexanes} \\
25^\circ\text{C} & \rightarrow 59\%
\end{align*}
\]

Motuporin
subnanomolar protein phosphatase inhibitor

S. Bauer and R. Armstrong  
\textit{JACS} \textbf{1999}, \textit{121}, 6355.

Stereoselective Ugi Reaction

- ZnCl\textsubscript{2} forms a rigid chelate with the imine to control the stereoselectivity of the Ugi reaction.

\[
\begin{align*}
\text{MeOH} & \rightarrow -75^\circ\text{C} \\
85\% & \rightarrow >99\% \text{ ds}
\end{align*}
\]

I. Ugi et al.  
Ugi Reaction in DOS

- Ugi Reactions are highly utilized for DOS:
  - High generation of diversity
    - All four components can be varied.
    - Components are commercially available.
    - Reaction variations access diverse structures
  - Compatible with large library size
    - The reaction is amenable to automated parallel synthesis.
    - Reactions can be run in solution or on the solid phase.

Solid Phase Ugi Reaction

- A library of 96 sialyl Lewis x mimetics was synthesized on the solid phase by a four component Ugi reaction.

Fluorous Phase Ugi Reaction

- Fluorous tagged Ugi products can be isolated from excess reagents by fluorous phase extraction.

\[
\text{(R}_{\text{fh}}\text{)}_3\text{Si} \quad \text{R}_{\text{fh}} = \text{CH}_2\text{CH}_2\text{C}_{10}\text{F}_{21}
\]

\[\begin{align*}
\text{1. CF}_3\text{CH}_2\text{OH} & \quad 90 \, ^\circ \text{C} \\
\text{2. extraction} & \quad \text{with FC-72 (fluorocarbon)} \\
\text{3. wash} & \quad \text{with benzene}
\end{align*}\]

\[84\% > 95\% \text{ purity}\]


Ugi Variation: Bifunctional Reagents

- Linking two of the reaction components results in the formation of cyclic products.

Ugi Variation: Cyclization Strategies

- Ugi products can be converted to a diverse range of structures using various cyclization strategies.

\[
\text{R}^1\text{CO}_2\text{H} \quad \text{R}^2\text{NH}_2 \quad \text{R}^3\text{CHO}
\]

\[
\text{Ugi} \rightarrow \text{R}^1\text{N}\text{R}^2\text{R}^3\text{N}\text{H}
\]


Ugi Variation: Cyclization Strategies

- An Ugi, ring-closing metathesis sequence can be used to make β-turn mimetics.

\[
\text{CH}_2\text{Cl}_2, \text{MeOH} \quad 25^\circ\text{C}
\]

**Passerini, Wittig**

- The Passerini three component reaction is like an Ugi reaction but without the amine.

![Passerini Reaction Scheme](https://example.com/passerni_reaction.png)


---

**Multicomponent Cycloadditions**

- A dienophile traps the reversibly formed diene for a three component Diels Alder reaction.

![Multicomponent Cycloaddition Scheme](https://example.com/multicomponent_cycloaddition.png)

VII. Chemical Diversity

Hypothesis Based Research

Problem: Find a molecule that can block protein trafficking. Only hydrophobicity, polarity and hydrogen bonding capacity have an influence on the molecule’s ability to block protein trafficking.

- Success depends on the quality of the hypothesis
- Enormous successes have been achieved using this approach
- As the complexity of the problem increases our ability to make the initial hypothesis and to use results to make new hypotheses diminishes
Diversity Based Research

Hypothesis Based Research

- Hypothesis based research
- Success depends on the quality of the hypothesis, which in turn depends on the information available about the problem and the complexity of the problem

- Many problems in chemistry and biology are multi-variable problems for which it is difficult to make an accurate (productive) initial hypothesis
- Introducing a hypothesis into diversity based search (e.g. privileged structure) can significantly reduce the dimensionality or size of the space that should be covered

Diversity Based Research

- Success depends mostly on the diversity of the library that is screened for a solution

Diversity is a measure of how well the available space is covered and is independent of the complexity of a problem

Diversity

Building block diversity

Stereochemical diversity

Functional group diversity

Molecular framework diversity
Diversification Strategies

- The number of diversity positions used contributes to the total number of compounds in the library more than the number of building blocks used to diversify each of them.

\[ \text{200 building blocks at each diversity position} \rightarrow 40,000 \text{ compounds (}200^2\text{)} \]

\[ \text{6 building blocks at each diversity position} \rightarrow 46,656 \text{ compounds (}6^6\text{)} \]

Building Block Selection

- Tools for building block selection
  - Databases of commercially available compounds that allow substructure search, e.g. find all primary alcohols
  - Computer programs that choose a subset of n compounds from a set of commercially available ones, retaining the diversity of the original set

- Desirable properties of building blocks
  - Commercially available
    - Building blocks should be introduced using reactions that require the presence of a common functional group
  - Compatible with the synthesis plan
    - A building block must contain only functional groups that are compatible with reactions that will be used after the building block is introduced
    - Order in which diversity positions are elaborated can alleviate some of the constraints
Building Block Selection

- Diverse physical and chemical properties
  - hydrophobicity and hydrophilicity
  
  ![hydrophobic and hydrophilic molecules]

  ![hydrophobic and hydrophilic molecules]

- Hydrogen bond donors and acceptors
  
  ![donor and acceptor molecules]

  ![donor and acceptor molecules]

- Acidic and basic groups
  
  ![acidic and basic molecules]

  ![acidic and basic molecules]

- Size
  
  ![size examples]

  ![size examples]

Building Block Selection

- Biasing elements
  - metal binding elements
    
    ![metal binding elements]

    ![metal binding elements]

  - reactive groups
    - nucleophiles
      
      ![nucleophilic examples]

      ![nucleophilic examples]
    - electrophiles
      
      ![electrophilic examples]

      ![electrophilic examples]
Functional Group Diversity

- Functional groups can be a source of diversity

![Functional group diversity elements:](image)

Sulfonyl hydrazone

oxime

ketone

urea

amine

amide

Diversity Potential of a Functional Group

- **TOS** - One functional group is not *a priori* superior to any other
- **DOS** - Diversity potential of a functional group is a part of a library design

![All transformations are achieved in one step](image)
Stereochemical Diversity

Stereochemistry can be used as a source of diversity

Stereochemical diversity elements:
- stereogenic center
- plane of chirality
- axis of chirality
- double bond

Why is Stereochemical Diversity Important?

Different stereoisomers can have dramatically different properties. Examples from asymmetric catalysis and chemical biology:

Thalidomide

S-thalidomide: teratogen, causes severe birth defects
R-thalidomide: safe anti-nausea agent

This compound was originally administered as a racemic mixture
Stereochemical Diversity: TOS vs. DOS

- Palytoxin has 64 stereogenic centers. $2^n$ stereoisomers are possible, where $n$ is the number of stereogenic centers. For palytoxin there are $1.85 \times 10^{19}$ possible stereoisomers!


**DOS**: The goal could be to synthesize all stereoisomers in one synthesis, but not as a mixture of stereoisomers. In split-pool synthesis, each bead must contain one stereoisomer.
Synthesis of L-Hexoses

- Challenge: Develop one synthesis route that provides access to all stereoisomers of the hexoses
- This could be an example of DOS

**Common Starting Material**


---

Total Synthesis of L-Hexoses

- Sharpless epoxidation is used to establish the stereochemistry of the two stereogenic centers
- The Payne rearrangement allows functionalization of the terminal carbon atom

Total Synthesis of L-Hexoses

• The aldehyde was generated from the sulfoxide by a Pummerer rearrangement

\[
\begin{align*}
\text{RO} & \text{OH} \quad \text{SPh} \\
\xrightarrow{\text{MeO} \xrightarrow{\text{POCl}_3} (\text{cat.})} \quad \text{RO} \quad \text{SPh} \\
\xrightarrow{\text{mCPBA} \quad \text{DCM}, -78^\circ \text{C}} \quad \text{RO} \quad \text{SO} \quad \text{Ph} \quad \text{O}^- \\
\xrightarrow{\text{NaOAc} \quad \text{Ac}_2\text{O} \quad \text{heat}} \quad \text{RO} \quad \text{SO} \quad \text{Ph} \quad \text{O}^- \\
\end{align*}
\]

93% over 3 steps

Pummerer rearrangement

Total Synthesis of L-Hexoses

• The stereochemistry at C-4 was diversified by establishing two pathways for hemithioacetal hydrolysis, only one of which provides an opportunity for epimerization to the thermodynamically favored configuration at C-4

\[
\begin{align*}
\text{RO} & \text{SO} \quad \text{Ph} \\
\xrightarrow{\text{K}_2\text{CO}_3, \quad \text{MeOH}, \quad \text{rt}} \quad \text{RO} \\
\xrightarrow{\text{DIBAL-H} \quad \text{DCM}, -78^\circ \text{C}} \quad \text{RO} \\
\end{align*}
\]

no epimerization under the reaction conditions

thermodynamic product
Total Synthesis of L-Hexoses

1. Ph₃PCHCHO (E:Z>20:1)
2. NaBH, MeOH
SAE, (+) DIPT 76%
SAE, (-) DIPT 84%

1. NaOH, PhSH
H₂O - t-BuOH
2. 2,2 methoxy propane, POCl₃

Example of Reagent-Based Stereocontrol in DOS

A = 1. mCPBA
2. AcOH, NaOAc
3. DIBAL-H
B = 1. mCPBA
2. AcOH, NaOAc
3. NaOMe, MeOH
C = 1. TFA - H₂O
2. H₂, Pd-Ç
**Molecular Framework Diversity**

- Molecular framework (MF) is the largest structural element present in all (or a subset of all) the members of the library

**Why is Molecular Framework Diversity Important?**

Different molecular frameworks impart different activities

- **paeoniflorin**
  - Anti-inflammatory activity

- **Me-CBS catalys**
  - Catalytic enantioselective ketone reduction

- **rapamycin**
  - Immunosuppressant
Molecular Framework Diversification Strategies

- MF transformations: convert a single MF to another one
  \[ \circ \rightarrow \square \]
  - Trans-annular reactions - intra-framework bond making
    - Trans-annular reactions in TOS
  - Fragmentation reactions - intra-framework bond breaking
    - Fragmentation reactions in TOS
  - Fragmentation-trans-annular reaction sequences in TOS
  - MF transformations in DOS
- Diverse MF synthesis: synthesis of a large number of MFs
  \[ \rightarrow \square \square \rightarrow \]
  - Linear vs. scaffold based library synthesis
  - Substrate controlled MF synthesis-folding pathways
  - Reagent controlled MF synthesis-branching pathways

Transannular Reactions in TOS

- Transannular Michael reaction

\[ \begin{align*}
\text{O} & \text{H} \\
\text{H} & \text{H}
\end{align*} \]

0.1 eq PhSNa
THF, reflux, 12h
93%

\[ \begin{align*}
\text{O} & \text{H} \\
\text{H} & \text{H}
\end{align*} \]


1 bond
3 stereocenters
vicinal chiral quaternary centers
**Fragmentation Reaction**

- Molecular framework diversification

E. Winterfeldt et al., *Chimia* 1993, 47, 39

**Fragmentation Reactions in DOS**

- Molecular framework diversity

175 compounds

5250 compounds

5250 compounds
Examples of Diversity from Biosynthesis

Terpene Diversity

- Wide structural diversity and topological complexity in terpenoid molecules originate with isopentenyl pyrophosphate (IPP) and dimethallyl pyrophosphate (DMAPP)

- Cholesterol
- Gibberellic acid
- Taxol
- Ginkolide B
- Proto-Daphniphylline

\[ \text{IPP} + \text{DMAPP} \]
Biosynthesis of IPP and DMAPP I

- All terpenes derived from IPP and DMAPP
  - Isopentenyl pyrophosphate (IPP) and dimethallyl pyrophosphate (DMAPP) biosynthesized from acetyl coenzyme A

\[
\text{acetyl coenzyme A} \xrightarrow{\text{acetyl-CoA thiolase}} \text{MeSO} \xrightarrow{\text{HMG-CoA synthetase}} \text{MeS-CoA} \xrightarrow{\text{Aldol}} \text{HMG-CoA} \\
\text{MeS-CoA} \xrightarrow{\text{Claisen}} \text{MeS-CoA} \xrightarrow{\text{NADPH HMG-CoA reductase}} \text{MeS-CoA} \xrightarrow{\text{ATP}} \text{MeS-CoA} \xrightarrow{\text{[-CO}_2\text{] [-OPP]}} \text{IPP} \\
\]

Biosynthesis of IPP and DMAPP II

- Stereospecific removal of the pro-R proton in IPP results in all-trans polyisoprenylated chains

\[
\text{IPP} \xrightarrow{\text{isomerase}} \text{DMAPP} \xrightarrow{\text{ionize}} \text{PPO}^\theta \xrightarrow{\text{prenyl transferase(s)}} \text{geranyl pyrophosphate} \xrightarrow{\text{TERPENES}} \\
\]
Diversity Via Multiple Cyclization Pathways

- Diverse and complex terpene structures are obtained via a wide range of cyclization/rearrangement pathways
  - Terpene cyclase proteins mediate the cyclization process

![Diversity Diagram](image)

Taxol Biosynthesis

- Taxol skeleton produced by taxadiene synthase mediated cyclization of geranylgeranyl pyrophosphate (diterpene)
  - Initial folding/cyclization events leading to the formation of the cationic macrocycle are enzyme mediated (reagent control). Subsequent intramolecular proton transfer is under substrate control

![Taxol Diagram](image)

Modular Polyketide Synthases

- To make chains of non-repeating units, modular PKSs have a separate set of FAS-like domains (modules) dedicated to each chain extension.

```
LOAD MODULE 1

1) The AT of the load module loads the KS of module 1 with propionyl CoA
2) The ACP is loaded with methyl malonyl CoA by the AT of module 1
3) Decarboxylation and attack on the KS-bound propionate gives the extended β-ketothioester
4) The KR reduces the β-ketothioester
5) No more reductive modules are present, so the chain is transferred to module 2
```

Erythromycin Biosynthesis

- Three large modular PKS enzymes (DEBS 1-3), encoded by eryAI, eryAII, and eryAIII, assemble the polyketide chain that forms the core of erythromycin.

```
• each module performs one chain extension
```

Erythromycin: DEBS Engineering - Deletions

- Normal:

- 80 aa deletion in the module 5 ketoreductase of DEBS 3:

- Mutation in the NADPH-binding site of the module 4 enoylreductase of DEBS 2:


Erythromycin: DEBS Engineering - TE Transposition

- Normal:

- Fusion of TE (from DEBS 3) onto DEBS 1:

- Fusion of TE (from DEBS 3) onto DEBS 2:

Erythromycin Biosynthesis

- A sequence of tailoring enzymes further functionalizes 6-DEB to give erythromycin A

6-Deoxyerythronolide B

Selective hydroxylation

D-desosamine + L-mycarose

Glycosyl-transferases

Erythromycin A


Construction of Polypropionates - Nature, PKS

- 4 reagents
- Complete reagent (enzyme)-based control

recombinant PKS enzymes

Premonensin

reductions

claisen condensations

Zincophorin

Rifamycin S
(aliphatic fragment)
Construction of Polypropionates - Laboratory

- Many reactions
- Heavy reliance on substrate-based control makes each molecule a new problem

Sigma-Aldrich Catalog