### General Textbooks of Combinatorial Chemistry


(At present the best and most comprehensive textbook of all aspects of combinatorial chemistry. Especially useful for general aspects, automation, applications in catalysis and material science, and design criteria. Contains a good but not comprehensive section about solid-phase reactions. The quality of the individual chapters differs significantly.)


(A very good book about different aspects of combinatorial chemistry - some chapters are very specialized. Good chapters about on-bead analytics.)


(A short but very instructive introduction in many aspects of Combinatorial chemistry. With a special focus on biological methods.)

### Textbooks of Solid Phase Synthesis


(An excellent and very well written textbook about solid phase synthesis. The main part is composed of a highly useful comprehensive collection of all reactions implemented on solid phase including reaction conditions. Very well referenced. It is definitely the source which should be consulted first before running a solid-phase reaction.)


(A very good and concise introduction into the concepts of solid and solution phase chemistry supplemented by the description of the synthesis of several libraries.)

### Catalogs


(Contains a 35 pages strong section with experimental protocols for general solid phase reactions and monitoring of functional groups. Available for free at [www.peptide.com](http://www.peptide.com).)

Novabiochem **2002/3** Catalog.

(Contains a 176 pages strong section with theoretical background and experimental protocols for general solid phase reactions and monitoring of functional groups. Available for free at [www.novabiochem.com](http://www.novabiochem.com).)

### Web-Portals

http://www.combichem.net/home/index.html
http://www.combinatorial.com
http://www.combichemlab.com/
http://www.5z.com/divinfo/

(All these websites contain useful information, background reading, literature references, links, experimental, lists of suppliers, etc.)
# Articles about Combinatorial Chemistry


(Bruce Merrifield tells in his nobel prize lecture how everything got started.....)


(An insightful collection of personal accounts on the history of Combinatorial Chemistry by the pioneers of this field.)


(Good introduction into solid phase organic synthesis.)


(Good introduction into combinatorial library synthesis.)


(A IUPAC team provides definitions for terms often used in Combinatorial Chemistry.)


(Provides an exhaustive literature overview of linker types primarily sorted by functional groups achieved by cleavage from a linker.)


(An insightful collection of personal accounts on the history of Solid-Phase Synthesis by the pioneers of this field.)


(A comprehensive review of the OBOC strategy.)


(An excellent introduction into the synthesis of DNA and peptide microarrays.)

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# Methodology References


(In these three articles scientists from Organon have collected all solid-phase reactions reported in the literature from 1992 - Nov 1997. The graphical abstract format allows fast browsing of these articles.)


(This review article is highly useful as it deals with an important synthetic problem, for which solutions cannot be found by routine literature search methods.)

# Linker


(Provides an exhaustive literature overview of linker types primarily sorted by functional groups achieved by cleavage from a linker.)


(Provides an exhaustive literature overview of linker types primarily sorted by the type of reagent used for the cleavage of the linker.)


(This article proposes a new classification system of traceless linkers, which it applies for a comprehensive review of this field.)

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References 1-011002
How many Organic Molecules are Conceivable?

Estimation based on the following margins:

- elements: C, H, N, O, S, P, F, Cl and Br
- molecular weight: <500
- stable in air and water
- number of non-H-atoms: <30

$10^{62} - 10^{63}$ molecules

Molecules with desired Properties are scattered in the Chemical Universe like galaxies in the real universe.
MERRIFIELD - Peptide-Synthesis

- Polymeric beads as a protecting group facilitate work-up:

attachment

1) CF₃COOH
2) R₃N
deprotection

iterative cycles

coupling

cleavage

Split-Mix-Synthesis is an efficient strategy to generate many compounds with few reactions:


The power of combinatorial chemistry

Two Examples:

3 sets of building blocks:  
\[ \begin{align*}  
A^1, A^2, A^3 & \\
B^1, B^2, B^3 & \\
C^1, C^2, C^3 & \\
\end{align*} \]
\[ \Sigma: 9 \text{ building blocks} \]

3 chemical reactions:  
\[ \begin{align*}  
A^1, A^2, \ldots, A^{20} & \\
B^1, B^2, \ldots, B^{20} & \\
C^1, C^2, \ldots, C^{20} & \\
\Sigma: 60 \text{ building blocks} \]

Number of coupling products:  
\[ \begin{align*}  
27 \text{ products} & \\
8000 \text{ products} & \\
\end{align*} \]
First publicly known compound from combinatorial libraries entering human clinical trials:

AG7088
(rhinovirus 3C protease inhibitor)

Other clinical candidates:

OC144-093
(modulator of P-glycoprotein-dependent MDR)

RWJ-53308
(fibrinogen receptor antagonist)

IC50 = 0.2 nM
(farnesyltransferase inhibitor)


Asymmetric Strecker Reaction:

\[
\text{R = aryl, alkyl}
\]

<table>
<thead>
<tr>
<th>Step</th>
<th>Reaction Conditions</th>
<th>Product</th>
<th>ee</th>
</tr>
</thead>
<tbody>
<tr>
<td>1)</td>
<td>2 % cat, HCN, -78 °C</td>
<td>R-CN</td>
<td>70-91 %</td>
</tr>
<tr>
<td>2)</td>
<td>TFAA, toluene, 0 °C</td>
<td>C-N</td>
<td>&gt;98 %</td>
</tr>
</tbody>
</table>

Asymmetric Phosphorylation:

\[
\text{R = aryl, alkyl}
\]

<table>
<thead>
<tr>
<th>Step</th>
<th>Reaction Conditions</th>
<th>Product</th>
<th>ee</th>
</tr>
</thead>
<tbody>
<tr>
<td>1)</td>
<td>2 % cat, HCN, -78 °C</td>
<td>R-CN</td>
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</tr>
<tr>
<td>2)</td>
<td>TFAA, toluene, 0 °C</td>
<td>C-N</td>
<td>&gt;98 %</td>
</tr>
</tbody>
</table>


### General Reviews for Solid Supports


(A very important review on all effects making solid phase reactions different from reactions in solution.)


(A nice review of the preparation and properties of polymer supports for solid phase reactions.)


(This review gives an overview of all methods which have been used to study effects associated with reactions on solid supports.)


(An insightful collection of personal accounts on the history of Solid-Phase Synthesis by the pioneers of this field.)


Preparation of Polystyrene Resins

**Ingredients:**

- **Monomer:**
  ![Monomer](image)

- **Cross-linking agent:**
  ![Cross-linking agent](image)

- **Radical Initiator:**
  ![Initiator](image)

- **Additives:**
  - porogen
  - polymeric stabilizer for aqueous phase

**Suspension Polymerization**

1. **Shearing** (comonomer liquid droplets)
2. **Thermal Polymerisation**
3. **Filtration**

**Crosslinked Polystyrene**

Table of swelling properties of resins in different solvents:

Characteristic Number: Volume of swollen, drained resin (1.0 g) in ml

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Wang resin</th>
<th>Merrifield-resin</th>
<th>Rink Amide-resin</th>
<th>Tentagel</th>
<th>MBHA-resin</th>
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</thead>
<tbody>
<tr>
<td>NMP</td>
<td>6.4</td>
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<td>1.6</td>
<td>1.6</td>
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</tbody>
</table>

**Preparation and derivatisation of Merrifield-resin:**

1. ClICH₂CH₃, SnCl₄, 1 h, reflux
2. Potassium phthalimide, DMF
3. KOAc, DMAc, 85 °C
4. Thiourea, dioxane/EtOH, 85 °C
5. DMSO, NaHCO₃, 155 °C, 6 h
6. LiPPh₂, THF

**Derivatisation based on Friedel-Crafts-adduct:**

1. X¹Cl
2. CH₃COCl
3. H₂NWH₂, EtOH
4. LiBH₄, Et₂O
5. KOH, dioxane/EtOH
6. mCPBA, DME

**Resins:**
- Trityl-resin
- Oxim-resin
- Benzhydryl-resin
 Functionalisation of PS-Resins

**Derivatisation via lithiation:**

- Benzophenone
  - THF, RT
  - $\text{Br}_2$
  - FeCl$_3$ or Ti(OAc)$_3$
  - CCl$_4$
  - 1 h reflux

- nBuLi
  - Hexane or toluene
  - 60 °C, 3 h

- B(OMe)$_3$
  - THF, RT
  - 20 h

- CIPPh$_2$
  - THF, RT

- Cl$_2$SiMe$_2$
  - Benzene
  - RT, 45 min

- Ethylenoxide
  - Toluene

**Derivatisation via electrophilic substitution:**

- $\text{Br}_2$
  - THF, RT

- FeCl$_3$ or Tl(OAc)$_3$
  - 1 h reflux

- H$_2$SO$_4$ or ClSO$_3$H
  - HNO$_3$
  - 0 °C - 15 °C

- SnCl$_2$*2 H$_2$O
  - DMF, 100 °C, 6 h

- H$_2$NOH*HCl
  - Pyridine, 90 °C

- Cl$_2$SiMe$_2$
  - Benzene
  - RT, 45 min
Resin Types I

Polyacrylamide resins (Pepsyn) (Sheppard-resin):

- excellent swellability in polar solvents
- very good for peptide synthesis and oligonucleotide synthesis
- due to labile peptide bonds less suitable for small molecule synthesis
- composite resins are mechanically stable (polyacrylamide in pores of kieselguhr: "Pepsyn-K resins"; polyacrylamide in PS: "PolyHIPE")

Poly(ethyleneglycol)-copoly($N,N'$-dimethylacrylamide) (PEGA-resin):

- excellent swellability in non-protic solvents (DCM, DMF) as well as in protic solvents
- very good for peptide synthesis
- due to labile peptide bonds less suitable for small molecule synthesis
- good diffusion properties and accessibility
- on-bead screening with proteins has been performed
Resin Types II

**TentaGel™ resins:**

Preparation through anionic polymerization:

- good swelling properties in protic and polar solvents
- high flexibility of the PEG-chains
- 60-80 wt% of beads refer to PEG-chains
- low loading (0.15-0.4 mmol/g)
- bleeding of the PEG grafts due to strong acids and oxidation
- hygroscopic (difficult to get them water free)

**ArgoGel™ resins:**

- higher loading than TentaGel (0.4 - 0.5 mmol/g)
- more acid stable than TentaGel

**TentaGel-S resins:**

- more stable than normal TentaGel

**Novagel resins:**

- more stable than normal TentaGel

**PEG-crosslinked Merrifield resin:**

- incorporation of PEG crosslinks into the core matrix
- swells in polar and non-polar solvents
- modification of the reactive sites does not alter the swelling properties of the resin
- very good for large peptides
ArgoPore resins:
(Argotech)
(highly crosslinked macroporous polystyrene resin)
- good diffusional properties in protic and polar solvents (water)
- almost no swelling
- good accessibility even at low temperatures
- resistant to osmotic shock
- high loading (0.2-1.8 mmol/g)
- pore size (ca. 90 Angstrom)
- surface area: ca 650 m²/g

Functionalised Silica Gels:
(Aldrich/SiliCycle)
- good diffusional properties in protic and polar solvents (water)
- no swelling
- good accessibility even at low temperatures
- resistant to osmotic shock
- high loading (1-4 mmol/g)
- surface area: ca 500 m²/g
- available with many different functionalities
- very good for scavenger resins

CPG-beads (Controlled Pore Glass)
(porous granules of high silica glass permeated by interconnecting pores of uniform and precisely controlled size)
- good diffusional properties in protic and polar solvents (water)
- best accessibility for biomolecules
- very expensive
- preferred support for DNA-synthesis and chromatography with biopolymers
- low loading (0.1-0.5 mmol/g)
- defined pore size over wide range (75-3000 Angstrom)
- surface area depends on pore size: (7-340 m²/g)

Soluble Polymers:
(polyethylenglykol (PEG))
dendritic glycerol polyether
- soluble in polar solvents, insoluble in apolar solvents
- isolation by ultrafiltration or precipitation
- low loading
- kinetic behaviour like soluble substrates
- useful for immobilization of catalysts or drugs
Influence of bead size:
- absolute amount of generated compounds
- efficiency of diffusion within the polymer

Rules of thumb for compounds with MW = 400:

<table>
<thead>
<tr>
<th>Resin Size</th>
<th>90 µm (TentaGel)</th>
<th>200 µm (PS)</th>
<th>500 µm (PS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75 mmol/g</td>
<td>350 pmol/bead</td>
<td>4.4 nmol/bead</td>
<td>66 nmol/bead</td>
</tr>
<tr>
<td>140 ng/bead</td>
<td>1.8 µg/bead</td>
<td>26 µg/bead</td>
<td></td>
</tr>
</tbody>
</table>

Keywords

- Integral/Non-integral linker
- Preloading
- Classification of Linker types
- Benzyl type linker
- Silyl-type linker
- Ketal-type linker
- Acidic cleavage
- Nucleophilic cleavage
- Metal mediated cleavage
- Photocleavable linker

General Reviews for Linker


(Provides an exhaustive literature overview of linker types primarily sorted by functional groups achieved by cleavage from a linker.)


(Provides an exhaustive literature overview of linker types primarily sorted by the type of reagent used for the cleavage of the linker.)


(This article proposes a new classification system of traceless linkers, which it applies for a comprehensive review of this field.)


(A very well readable account on this topic. Provides an excellent literature overview of linker types sorted by linker types, cleavage conditions, and formed products.)


(A short and eclectic update about recent developments.)
**'Safety-catch' linker:**

Example: Kenner safety-catch linker


Fragmentation/Cycloreversion-cleavage linker:

\[
\begin{align*}
\text{AcCl} + \text{TMSCN} &\rightarrow \text{DCM, 48 h} \\
\text{N}^\text{R1} &\rightarrow \text{LDA, allylbromide} \\
\text{KOH, THF/H}_2\text{O} &\rightarrow \text{N}^\text{R1}
\end{align*}
\]


Traceless linker:

\[
\begin{align*}
\text{HO}_2\text{B} + \text{Pd[PPh}_3\text{]_4} &\rightarrow \text{Cu(OAc)}_2 \text{PrNH}_2 \\
\text{K}_3\text{PO}_4 &\rightarrow \text{DMF/H}_2\text{O, 80 °C, 24 h} \\
\text{Me} &\rightarrow 93 \%
\end{align*}
\]

Example:


additional element of diversity

multiplication of the final library members both in terms of structural and functional diversity

multidirectionality often limits orthogonality in the synthetic sequence

multi-step syntheses require safety-catch-linkers

excess of reagents must be easily removable

Example:

T2 linker:


Keywords

- Photocleavable linker
- Safety-catch-linker
- Traceless linker
- Cyclorelease strategy
- Multidirectional linker
- Amino acids as building blocks
- Peptide coupling

General Reviews for Linker


(Provides an exhaustive literature overview of linker types primarily sorted by functional groups achieved by cleavage from a linker.)


(Provides an exhaustive literature overview of linker types primarily sorted by the type of reagent used for the cleavage of the linker.)


(This article proposes a new classification system of traceless linkers, which it applies for a comprehensive review of this field.)


(A very well readable account on this topic. Provides an excellent literature overview of linker types sorted by linker types, cleavage conditions, and formed products.)


(Comprehensive review article about the important class of multidirectional cleavage linker.)
Keywords

- Peptide coupling
- Racemisation
- Coupling reagents
- Reaction monitoring
- Spot tests
- Fmoc determination
- IR-Spectroscopy
- Gel-phase NMR
- MAS-NMR spectroscopy

General Reviews for Peptide Coupling


(A very good book about all aspects of peptide chemistry, which contains an excellent chapter about peptide synthesis.)


(A very useful source for all kind of informations about all aspects of peptide synthesis. Includes many experimental procedures.)


(Especially useful for the coupling of N-alkylated amino acids.)


(Short, but good review about peptide coupling - difficulties, reagents, etc.)


(Two recent reviews which provide an update about current developments in this field.)

General Reviews for Reaction Monitoring


(This article is an excellent compendium of colorimetric assays and spectrophotometric-based quantification methods used in SPOS.)


(A terrific review about on- and off-bead analytical techniques for solid phase synthesis such as NMR, IR, MS and color tests.)
Reactivity towards amines:

- Most reactive:
  - HBTU
  - TBTU
  - BOP
  - PyBOP

- Least reactive:
  - CDI
  - DSC
  - HOSu
  - HOBt
  - HOAt

Coupling reagents:

- Prices based on the 2002 catalogs of Fluka, Novabiochem, and Advanced Chemtech.

- HATU: (1790 €/mol)
- PyBrOP: (3358 €/mol)
- TFFFH: (9300 €/mol)
- HBTU: (910 €/mol)
- TBTU: (770 €/mol)
- BOP: (1060 €/mol)
- PyBOP: (1685 €/mol)
- DCC: (52 €/mol)
- DIC: (100 €/mol)
- EDC: (475 €/mol)
- CDI: (490 €/mol)
- DSC: (953 €/mol)
- HOSu: (52 €/mol)
- HOBt: (88 €/mol)
- HOAt: (3600 €/mol)

Racemisation in Peptide Synthesis

- **Racemisation through Oxazolones:**
  - (mesomeric charge transfer favoured by acyl protecting groups)
  - (deprotonation favoured by strong bases)

- **Racemisation through enolisation:**

- **Factors influencing racemisation**
  - Urethane protecting groups (Boc, FMOC) are less racemisation prone than Acyl-protecting groups (Acetyl-, Benzoyl-, Trifluoracetyl-). Although they also form an oxazolone H-abstraction is less likely.
  - Strong leaving groups X (-Cl, -N₃) favour racemisation.
  - The addition of additives such as HOSu or HOBt also reduce epimerisation.
  - N-alkylated amino acid are less reactive for coupling and more prone for epimerisation and side reactions.
  - The more basic the medium (DMAP, Et₃N, etc...), the more likely racemisation.
Enfuvirtide ("Fuzeon", T20):
- 36 amino acid peptide
- potently and selectively inhibits HIV-1 membrane fusion
- approved for clinical use by the FDA in March 2003
- 180mg/d per patient
- manufactured by Trimeris/Roche


Large Scale Synthesis:
- 2-chloro-trityl-chloride resin
- coupling conditions: 1.5 eq Fmoc - amino acid
  1.5 eq HBTU
  1.5 eq HOBt
  DMF or NMP
- three peptide fragments prepared by SPPS, isolated by precipitation
  (>85% yield, >90% purity, 300-500 kg/batch)
- condensation reactions in DMF
- global side chain deprotection with TFA and scavenger cocktail
- final purification by HPLC
- cycle time: 4 months
- 106 steps
- efficiency: ~30% from loaded resin
- manufactured by Trimeris/Roche

Detection of Primary and Secondary Amines

**KAISER Ninhydrin Color Test**

*Reagents:* Solution I: Ninhydrin in ethanol  
Solution II: phenol in ethanol  
Solution III: KCN in pyridine

*Procedure:* 2 drops of each solution to beads, heated to reflux for a few minutes

*Detection:* blue/purple color indicates primary amine functionality (only slight effect with secondary)

*Colored species:*

![Ninhydrin](image1)

**Bromophenol Blue Test**

*Reagents:* Solution: Bromphenol Blue in acetonitrile

*Procedure:* add solution to swollen beads in acetonitrile

*Detection:* a persistent blue color indicates primary and secondary amines  
(Residual DMF causes fading of the color after 1-2 min, therefore beads should be washed with ACN before performing the color test.)

*Colored species:*

![Bromophenol Blue](image2)

**Trinitrobenzene Sulfonyc Acid (TNBSA) Test**

*Reagents:* Solution: TNBSA in DMF

*Procedure:* add drops of solution to beads in a solution of 10 % DIPEA/DMF

*Detection:* red stained beads indicate primary amines

*Colored species:*

![TNBSA](image3)

**Chloranil Test**

*Reagents:* Solution: saturated solution of chloranil in toluene

*Procedure:* add a few drops of either acetaldehyde (primary amines) or acetone (secondary amines) to beads, followed by one drop of the chloranil solution

*Detection:* dark blue beads indicate primary or secondary amines

*Colored species:*

![Chloranil](image4)

Detection of -CHO, -SH and -OH

**2,4-Dinitrophenylhydrazine (DNP) Test for Aldehydes and Ketones**

**Reagents:** Solution: 2,4-dinitrophenylhydrazine in AcOH/DCM

**Procedure:** add a few drops of solution to resin in DCM

**Detection:** red/orange colour indicates the presence of aldehydes/ketones

(This test can be modified for the quantification of -CHO functionalities. The beads are titrated with DNP. After full conversion the excess of DNP in the supernatant is measured via UV.)

**Colored species:**

\[
\text{O}_2\text{N} - \text{NH} - \text{N}^-\text{R} \]

**ELLMAN Test for Thiols**

**Reagents:** Solution: Ellman's reagent (5,5'-dithiobis(2-nitrobenzoic acid) in pH 8 phosphate buffer

**Procedure:** add a few drops to resin beads swollen in dioxane

**Detection:** an orange red colour is characteristic of the presence of free thiols groups on the resin

**Colored species:**

\[
\text{S} - \text{NO}_2 - \text{CO}_2\text{H} \]

**POMONIS Test for Alcohols**

**Reagents:** 5% TsCl in pyridine/EtOH (1/1)

**Procedure:** 2% in acetone

1M Na2CO3 aq

**Detection:** an orange red colour is characteristic of the presence of free thiols groups on the resin

**Colored species:**

deep blue resin

Application of Spot Tests

- **PNB-stain for alkylhalogenides:**
  
  ![PNB-stain](image)


- **Disperse Red-cyanuric chloride conjugate for -OH groups:**
  
  ![Disperse Red-cyanuric chloride conjugate](image)


Application in Solid Phase Oligosaccharide Synthesis:

- **PNB staining**
  
  ![PNB staining](image)

Keywords

- Parallel Synthesis
- Synthesis on Planar Supports
- Combinatorial Chemistry
- Split-Mix-Synthesis
- Deconvolution
- Positional Scanning
- Self-Encoding
- Chemical Encoding
- Binary Encoding
- Radio-Frequency Tags

General Reviews for Encoding Strategies


(A very well readable account of chemical and physical encoding techniques.)


(An excellent but early review about encoding techniques.)
Parallel Synthesis is the extension of conventional one-flask-one-compound synthesis:

The number of individual vials limits the number of compounds.

Parallel Synthesis on Planar Supports:

- Light directed synthesis on chips:
  

- Synthesis using Geysen-pins:
  

- SPOT Synthesis on membranes:
  
Split-Mix-Synthesis is an efficient strategy to generate many compounds with few reactions:


The power of combinatorial chemistry

Two Examples:

3 sets of building blocks:

- A1, A2, A3
- B1, B2, B3
- C1, C2, C3

Σ: 9 building blocks

3 chemical reactions:

- 9 chemical operations

Number of coupling products:

- 27 products
- 8000 products
Many useful asymmetric catalytic reactions are ligand accelerated reactions:

- Sharpless-Dihydroxylation
- Sharpless-Epoxidation
- Hydrovinylation
- Jacobsen Hydrolytic Kinetic Resolution
- Zincorganyl-Addition to Aldehydes

Discovery of an epoxidation catalyst:


**Goal:** Identification of an enantioselective epoxidation catalyst using H$_2$O$_2$ as oxidant.

Library Design:

1. Step: Preparation of Ligand Library
   - 4 amino acids: Asp, Cys, His, Met, Ser
   - 4 bridging elements
   - 12 end groups
   - Split-Mix-Library of 192 potential ligands

2. Step: Identification of Metal Source
   Ligand-Library was incubated with 30 different metal sources (20 elements), then washed
   Screening of the metal-libraries for catalytic efficiency (also compared with activity of metal source alone)
   - highest activity with libraries containing FeCl$_2$, FeCl$_3$

3. Step: Parallel Screening of 192 compounds containing FeCl$_2$
   - highest activity with the following compounds

4. Step: Next Generation Library and Catalytic Screening
   - 20 % ee
   - (78 % conversion @ 5 mol% catalyst)
Positional Scanning in Asymmetric Catalysis

R. Breinbauer

**Achiral lead reaction:**

\[
\begin{align*}
\text{Cat:} & \quad \text{Ti(OiPr)}_4 \\
\text{rac} & \quad 12 \% \text{ yield}
\end{align*}
\]

- 10 amino acids varied
- highest enantioselectivity with L-Leu: 56 \% ee

1. Step: Variation of amino acid 1:

2. Step: Variation of amino acid 2:

- 16 amino acids varied
- highest enantioselectivity with Thr(t-Bu): 62 \% ee

3. Step: Variation of aldehyde:

- 13 aldehydes varied
- highest enantioselectivity with 3-Fluorosalicylaldehyde: 89 \% ee

4. Step: Validation in solution:

- 20 mol\% Ti(OiPr)_4
- highest enantioselectivity with 3-Fluorosalicylaldehyde: 89 \% ee

**Discovery of an asymmetric catalyst:**


**Library Design:**

**Early Lead from Exploratory Solution Phase Experiments:**
General Principle

Every diversification step is followed by a tagging reaction in which a tag that codes for a particular transformation is covalently attached to the solid support.

Encoding via double linker strategy

Encoding of a peptide library with oligonucleotide tags:


Encoding via reaction with the support

Encoding of a peptide library with acylcarbene tags:

**General Principle:**

Every diversification step is followed by a tagging reaction in which unique acylcarbene tag are covalently attached to the solid support. After cleavage of the compound the tags are cleaved from the solid support and analysed by GC with an electron-capture-detector (ECD).

**Acylcarbene Tags:**

![Acylcarbene Tags Diagram]

- **linker** (oxidative cleavage)
- **variable region**
- **variable electrophore**
- **determine GC-retention time**

The ECD detector allows the detection of the electrophore in amounts <1 pmol/bead.

**General Reaction Protocol:**


It has been noted that the acylcarbene also reacts in tiny amounts with the small molecule moiety. Although not detectable by conventional analytical methods (NMR, HPLC-MS) this tiny amount is sufficient to provide enough material for decoding, after the tags have been cleaved off the small molecule by treatment with CAN.
Binary Encoding

General Principle:

1) Binary Encoding is based on the presence of a tag ("1") or its absence ("0").

2) Depending on the number of individual building blocks in a set used for a reaction step, the number of required digits (=tags) may vary.

<table>
<thead>
<tr>
<th>Number of building blocks</th>
<th>Required digits</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1 digit</td>
<td>0, 1</td>
</tr>
<tr>
<td>4</td>
<td>2 digits</td>
<td>00, 01, 10, 11</td>
</tr>
<tr>
<td>8</td>
<td>4 digits</td>
<td>000, 001, 010, 011, 100, 101, 110, 111</td>
</tr>
</tbody>
</table>

3) The tags for a set of building blocks used for a certain reaction step are chosen in such a way that they appear in the same retention time in ECD-GC. The fine tuning of the retention time is performed by variation of the tether length and the electrophore.

Example:

Encoding of a library with 2 sets of building blocks using 3 digits each

Signal retention time

Keywords

Target Oriented Synthesis (TOS)
Diversity Oriented Synthesis (DOS)
Diversity
Complexity
Building blocks
Privileged Structures
Natural Product Leads
Design Criteria for DOS

General Reviews for Design Strategies

S. L. Schreiber ("Target-Oriented and Diversity-Oriented Organic Synthesis in Drug Discovery").

(The authoritative paper on this topic, in which the term diversity-oriented synthesis was explained for the first time.)


(An insightful review about design strategies in combinatorial chemistry, especially drug-likeness, privileged structures, diversity assessment, etc.)

Y. C. Martin ("Diverse Viewpoints on Computational Aspects of Molecular Diversity").

(This article contains perspectives of the key players in this field and opens access to the literature.)

J. M. Blaney, E. J. Martin ("Computational approaches for combinatorial library design and molecular diversity analysis").

(This article describes strategies applying computational methods to increase the diversity of combinatorial libraries in its design process.)
What is Diversity?

Diversity is the opposite of similarity, which can be calculated pairwise using one or more descriptors.

Molecular similarity analysis is based on the observation that chemical structure can be correlated with activity and that small changes in structure produce small changes in activity, thus structurally similar molecules are expected to have similar biological activity.

Assignment of Diversity

- Quantitative Measurement of Diversity:

  Computer programmes allow the definition of molecules by 2D- or 3D-molecular descriptors, which can be compared.

- Qualitative Assignment by Intuition:

  These compounds are dissimilar by size, topology, shape, hydrophobicity, functional groups.

Limitations to the Concept of Diversity:

Compounds which are very similar can exhibit contrasting biological activity (often the case with ligands for G-protein coupled receptors).
Complexity of a molecule is a function of:
- size of the molecule
- content of elements
- content of functional groups
- number and arrangement of stereocenters
- cyclic connectivity
- chemical reactivity
- structural instability

Examples

Thromboxan TXA₂

Taxol™

Manzamine A

Haplophytine

Vitamin B₁₂

Palitoxin
Strategy in Synthesis - How to keep the number of steps short

1) Divide the target molecule into two or more pieces of comparable complexity!

2) Fragment couplings have to work!

- Try to install them at sterically less congested parts of the target molecule!

- Frequently used fragment couplings:
  - Pd-catalyzed Cross Coupling (Stille, Suzuki, Heck)
  - Wittig - Reaction
  - Horner - Wadsworth - Emmons - Reaction
  - Hydrazone Coupling
  - Aldol - Reaction
  - Nozaki-Hiyama-Kishi - Reaction
  - Dithiane - Coupling

3) Generate intrinsic instabilities of the target molecule as late as possible!

- Intrinsic instabilities can be caused by functional groups which are sensitive against light (e.g. conjugated double bonds), air, acid, or base. Also certain forms of strain within the molecule scaffold or a special arrangement of functional groups (e.g. Grob fragmentation) might contribute to the instability of a molecule. In such a case the functional groups should be introduced or deprotected at the latest stage.

4) Change the oxidation state of an atom only in one direction!

5) The best protecting group is no protecting group!

- Intrinsic instabilities can be caused by functional groups which are sensitive against light (e.g. conjugated double bonds), air, acid, or base. Also certain forms of strain within the molecule scaffold or a special arrangement of functional groups (e.g. Grob fragmentation) might contribute to the instability of a molecule. In such a case the functional groups should be introduced or deprotected at the latest stage.

6) Notoriously low yielding steps should be carried out as early as possible!

- Such reactions might involve reactions with an unfavourable equilibrium constant or kinetic resolutions, which by definition have a maximum yield of 50%.

7) Take advantage of already installed stereogenic centers for the generation of others by diastereoselective methods!

8) Chiral pool can be a valuable source of advanced intermediates

Verbenone
Diversity-Oriented Synthesis (DOS)

In contrast to Target-Oriented Synthesis (TOS), where a convergent synthesis strategy is key for success, in DOS a divergent synthesis strategy is the fundamental principle.

Tenets for Reactions suitable for DOS on Solid Phase:
- high yields with a variety of substrates
- functional group tolerant
- building blocks readily available
- reagents compatible with polymeric support
- moderate temperature range
- soluble reagents and reaction side products
- reagent-based stereocontrol preferred

Efficiency Rules for Library Design

1) The sequence involves a small number of steps which are amenable to solid phase!

Long sequences would require almost perfect yields per step to reach satisfactory purity of the final product. Provision must be made for convenient removal of reagents and byproducts.

2) No more than one variable is introduced in any step!

If several variables are introduced in one step, then separate reactions for each combination are required if substances are to be generated without producing molecular mixtures.

3) Starting materials are readily obtained with a diverse selection of substituents!

Substituents must be compatible with subsequent reaction steps.

4) Ideally, every reaction should increase the diversity of the library!

Batch transformations and protecting group manipulations are less desirable.

5) Cyclic, nonoligomeric structures represent the most interesting targets.

Conformationally constrained molecules are generally more attractive as lead structures from a biological assay, since they provide more information about the three-dimensional requirements for ligand binding.


Keywords
Building blocks
Privileged Structures
Natural Product Leads
Design Criteria for DOS
Bioavailability
Library Synthesis
Multi-Component Reactions

Diversity-Oriented Synthesis
(An excellent and inspiring article about different strategies for the design of diverse compound libraries, such as folding strategies and others.)

Natural Product Libraries
(A very good and comprehensive review of all efforts to create natural products libraries using combinatorial methods.)

(A very well readable review about combinatorial libraries inspired by natural products.)

(This review article discusses the origin of the biological importance of natural products and how it has led to the design of combinatorial libraries.)

Combinatorial Libraries
(In this regularly updated article series all solution and solid phase libraries published in the literature are collected and presented in a graphical abstract format. If available also biological data are given. In addition analysis of recent trends are given in the introduction.)

(An early review about heterocyclic synthesis on solid phase.)

(A review about traceless heterocyclic synthesis on solid phase.)

(This review summarizes the strategies used in the synthesis of most classes of heterocycles.)

(In this article 43 compounds are highlighted which are said to be the only members of combinatorial libraries, which have been identified to achieve "lead compound" status.)
Privileged Structures

Definition:
Privileged structures are defined as a single molecular framework able to provide ligands for diverse protein receptors.

"...judicious modification of such structures could be a viable alternative in the search for new receptor agonists and antagonists"


Benzodiazepines:

- Cholecystokin antagonist
- Opiate receptor
- (NK-1 antagonist)
- K-Secretase inhibitor
- Farnesyl transferase inhibitor

Substructure Analysis of Drugs:

5120 known drugs were analysed for molecular frameworks and side chains:
- 1179 frameworks identified; 32 of these account for half of all drugs
- 20 different side-chains account for 75% of the total


**Library Design:**

Kinases are enzymes which phosphorylate other proteins. They use ATP as a phosphorylation agent, which binds in the "ATP-binding pocket" of the kinase. This pocket is the target of many natural and unnatural kinase inhibitors.

(Natural inhibitor of CDK2/cyclin A with an IC50 ~ 7 µM. Adopts a different orientation in the binding pocket than that observed for ATP).

**Several generations of libraries:**


**Biological studies:**


---

**Synthesis of the 2\textsuperscript{nd} generation library:**

- **R. Breinbauer**

- **Library of Kinase Inhibitors**


- **Biological studies:**

Combinatorial Modification of a Natural Product Scaffold

- **Indolactam V**
- **Erythromycin**

Solid Phase Synthesis of Natural Product Scaffolds:

- **Prostaglandine**
- **Epothilon**
- **Sarcodictyn**
- **Paclitaxel**
- **Galanthamine**
- **Dysidiolide**

**Natural Product Guided Combinatorial Synthesis I**
Natural Product Guided Combinatorial Synthesis II

R. Breinbauer

**Design strategy:**

Following a biomimetic strategy an oxidative cyclisation is applied to build up the complex arrangement of rings.

**Galanthamine**

(Natural product with acetylcholinesterase activity. Is produced on industrial scale by total synthesis and marketed as a drug against Alzheimer's disease (Sanochemia).

**Support and Linker:**

PS beads (500-560 µm)

Silyl-Linker

**Biological Results:**

Phenotypic Screen for inhibitors of protein trafficking:

Secretamine

(blocks protein transport from Golgi apparatus to plasma membrane at 2 µM)

**Solid Phase Synthesis:**

1) CH(OCH₃)₃ then NaBH₃CN, MeOH/THF
2) allylchloroformate, DIPEA
3) piperidine, THF

Ph(OAc)₂

1) Pd[PPh₃]₄, morpholine
2) R¹OH, PPh₃, DIAD

1) R²SH
2) R³CHO, AcOH then NaBH₃CN or R³COCl, 2,6-lutidine

1) R⁴NH₂, AcOH
2) HF*pyridine

2527 compounds

Library Design:

(-) Shikimic acid

Support and Linker:

Tentagel S NH₂ (90 µm)

Geysen-Linker (celavable at 365 nm)

Encoding:

Tagging: Binary encoding

Introduction:

Cleavage: CAN

Library Synthesis:

2 amino acids

Fmoc-AA-OH

PyBOP, DIPEA

NMP, RT, 2 h

H₂N

2 enantiomers

PyBOP, DIPEA

NMP, RT, 1 h

H₂N

3 nitrones

PyBroP, DIPEA, DMAP

DCM, 0 °C to RT, 3 h

H₂N

30 alkynes

(-) Shikimic acid

PyBOP, DIPEA

NMP, RT, 2 h

R₂ R₃

CuI, DIPEA

DMF, RT, 2 h

R₂ R₃

2-HO-pyridine

THF, RT, 14-16 h

R₂ R₃

DIC, DIPEA

then DMAP

DCM, 0 °C to RT, 12 h

R₂ R₃

2.1 Mio compounds

Strategy:

Combination of powerful complexity-generating reactions such as UGI-4C-Reaction and Diels-Alder reaction.

UGI-4-component reaction:

\[
R^3\text{CHO} + R^2\text{NH}_2 + R^1\text{COOH} + R^4\text{H}_2\text{N}\rightarrow R^1\text{CON}R^2\text{NR}^3\text{NR}^4
\]

ketone, aldehyde, prim. amine, sec. amine, H$_3$NOH, hydrazine, RCOOH, H$_2$N, RCNO, RCNS, CO$_2$ + ROH, R$_2$NH$^+$HCl, Na$_2$S$_2$O$_3$ ("H$_2$S")

Support and Linker:

PS beads (500-560 µm) Silyl-Linker

Solid Phase Synthesis:

1) 2,6-lutidine
2) piperidine

MeOH/THF

 overall yield: 39 %

<table>
<thead>
<tr>
<th>Diversity Oriented Synthesis</th>
<th>Natural Product Guided Combinatorial Synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>- aims to achieve maximum diversity around a spot in the chemistry universe chosen for its chemical accessibility and &quot;natural product&quot;-likeness</td>
<td>- aims to achieve focused diversity around a biologically validated starting point in the chemistry universe - the diversity of these starting points is selected from Nature</td>
</tr>
<tr>
<td>- synthesis route is driven by &quot;powerful&quot; chemical reactions (general, high yielding, stereoselective, bond constructing, functional group tolerant, etc..)</td>
<td>- synthetic methods are primarily chosen to lead to the target core structure, even if this goal requires a considerable amount of optimisation</td>
</tr>
<tr>
<td>- forward diversity oriented synthetic design</td>
<td>- retrosynthetic analysis will be also validated by its potential for diversity generation</td>
</tr>
<tr>
<td>- building blocks should be commercially available in great diversity</td>
<td>- building blocks commercially available or synthesised in solution</td>
</tr>
<tr>
<td>- encoded split-pool synthesis is preferred method</td>
<td>- parallel format or split-pool synthesis</td>
</tr>
<tr>
<td>- large library size</td>
<td>- small to middle library size</td>
</tr>
</tbody>
</table>
Random Walk taken by an oral drug on route to its point of efficacious contact within a human target cell:

(Biological milieu marked with bold letters represent compartments having particularly high metabolic capabilities)

- **Oral Mucosal Cavity (pH ~6-8)**
- **Stomach (pH ~1-3) and Gut Bacterial Flora**
- **Duodenum - Ileum (pH ~5-8) and Gut Bacterial Flora**
- **Intestinal Mucosal Membranes**
- **Heart**
- **Venous Return**
- **Liver**
- **Hepatoportal Circulation (pH 7.4) and Various Blood Components**
- **Bronchopulmonary Circulation (BPC)**
- **Lungs**
- **Bronchopulmonary Circulation (BPC)**
- **Heart**
- **Peripheral Vascular Endothelium or Blood Brain Barrier**
- **Peripheral or Central Blood Flow**
- **Arterial Systemic Circulation**
- **Interstitial Fluid**
- **Target Cell Membrane**
- **Differential Distribution Within Intracellular Fluid and Cellular Organelles**
- **Point of Efficacious Contact**


Lipinski's Rule of Five (Pfizer Rule of Five)

- Analysis of 2245 compounds of the World Drug Index (WDI) having reached Phase clinical trials

Poor Absorption or permeation are more likely when:

- MW is > 500
- > 5 H-bond donors (sum of OH and NH)
- > 10 H-bond acceptors (sum of O and N)
- Log P is > 5 (or MLogP is > 4.15)

Compound classes that are substrates for biological transporters are exceptions to the rule (e.g. cardiac glykosides, vitamins, antibiotics...).


Analysis of molecular properties in correlation with oral bioavailability measurements in rats for over 1100 drug candidates at SmithKline Beecham:

Compounds will have a high probability of good oral bioavailability in rat, if the following two criteria are met:

- 10 or fewer rotatable bonds
- polar surface area equal to or less than 140 \( \text{Å}^2 \)
  (or 12 or fewer H-bond donors and acceptors)

Rotatable bonds: Any single bond, not in a ring, bound to a nonterminal heavy (i.e. non-hydrogen) atom. Excluded from the count were amide C-N bonds because of their high rotational energy barrier.

H-bond donor: any heteroatom with at least one bonded hydrogen.

H-bond acceptor: any heteroatom without a formal positive charge, excluding halogens, pyrrole N, heteroaromatic O and S, and higher oxidation states of N, P, and S, but including the O bonded to them.

The MW cutoff of 500 doesn't appear to be significant, as long as the two criteria are met.

- Predicitive tools for the design of bioavailable compounds


- Determination of bioavailability in model systems:


The aim of HTS is the selection of stable, non-covalent binders (ligands) and elimination of protein-reactive compounds (reagents) from consideration as drug leads at an early stage.


Reactive functional groups responsible for in vitro false positives:

- Sulfonil halides
- Acyl halides
- Alkyl halides
- Anhydrides
- Sulfonate esters
- Phosphonate esters
- α-halocarbonyl compounds
- 1,2-dicarbonyls
- Michael acceptors and β-heterosubstituted carbonyl compounds
- Halopyrimidines
- Aldehydes
- Imines
- Perhalo ketones
- Heteroatom-heteroatom single bonds
Keywords

Supported Reagents
Scavenger Resins
Solution Phase Parallel Synthesis
Dynamic Combinatorial Chemistry
Template Directed Combinatorial Chemistry

Supported Reagents and Catalysts


(A review article about polymer-supported reagents and their use in parallel synthesis.)


(This is an early account by the pioneer of solution-phase combinatorial chemistry.)


(A nice review of recent work done in this field.)


(This review articles focuses on the libraries generated via different solution phase strategies.)


(Good review about applications of dynamic combinatorial libraries in bioorganic chemistry.)

Dynamic Combinatorial Chemistry


(A very well written article about the concept of dynamic combinatorial chemistry.)


(A very illuminating review about the use of solid supported reagents and catalysts, especially on inorganic materials, written by one of the most respected practitioners in homogenous and heterogeneous catalysis.)
Supported Reagents and Catalysts I

■ Polymer supported reagent:

\[ \text{A} \xrightarrow{\text{filtration}} \text{A-B} \]

Examples:

\[
\begin{align*}
\text{Ph} & \quad \text{NMe}_3 \\
\text{P} & \quad \text{BH}_4
\end{align*}
\]

■ Applications:

\[ \text{R} \equiv \text{H} \xrightarrow{\text{filtration}} \text{R} \equiv \text{I} \]

\[ \text{57 - 83 %} \]

\[ \text{R}^1\text{CO}_2\text{R}^2 \xrightarrow{\text{filtration}} \text{R}^1\text{CO}_2\text{N}_2\text{R}^2 \]

\[ \text{68 - 97 %} \]

■ Polymer supported catalyst:

\[ \text{A} \xrightarrow{\text{filtration}} \text{A-B} \]

Examples:

\[
\begin{align*}
\text{Ph} & \quad \text{SO}_2\text{N}_3 \\
\text{N} & \quad \text{Ph}
\end{align*}
\]

\[ \text{R}^1\underset{\text{OH}}{\text{C}}\text{H}2\text{Cl}_2 \]

\[ \text{70 - 99 %} \]

Scavenging reagents:

\[ A + B \rightarrow A-B + X \rightarrow A-B + B \rightarrow A-B \]

(excess)

- Acid chlorides
- Aldehydes
- Isocyanates
- Carboxylic acids
- Alkyl haloformates
- Sulfonyl halides
- Sulfuryl halides
- Amines
- Phenol
- Hydrazines

Examples:

- PhNH2
- N\(\equiv\)C=O
- R1 NH2
- R2 OCl
- N=C=O

Applications:

1) \(\text{HO}_2C\)-NH-NH2

2) N\(\equiv\)C=O

3) H2N

Resin Supported Synthesis of Plicamine

1. 1.1 eq EtOAc, RT, 45 min
2. MeOH/DCM (2/1), 2 h
3. (CF$_3$CO)$_2$O, DCM, 3 eq

91% (over 6 steps)

1) 1.5 eq TFE, -5°C
2) MeOH/DCM (1/1), 2 h

82% (+)-Plicamine

<table>
<thead>
<tr>
<th><strong>Solid Phase</strong></th>
<th><strong>Solution Phase</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Advantages:</strong></td>
<td><strong>Advantages:</strong></td>
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<tr>
<td>◎ Reagents can be used in excess without causing subsequent separation problems. Reaction can be driven to completion.</td>
<td>◎ In principle all organic reactions can be applied.</td>
</tr>
<tr>
<td>◎ Work-up of reactions by simple washing and filtration.</td>
<td>◎ Well-established reaction protocols exist.</td>
</tr>
<tr>
<td>◎ Simple automation of reaction sequences.</td>
<td>◎ No additional steps for attachment and cleavage.</td>
</tr>
<tr>
<td>◎ Split-pool-Synthese possible (OBOC-strategy).</td>
<td>◎ No limitations to scale.</td>
</tr>
<tr>
<td>◎ Pseudodilution</td>
<td>◎ Automation-techniques for work-up and purification are emerging.</td>
</tr>
<tr>
<td><strong>Disadvantages:</strong></td>
<td><strong>Disadvantages:</strong></td>
</tr>
<tr>
<td>◎ Attachment to and cleavage from resin require additional reaction steps.</td>
<td>◎ Reagents used in excess usually cause subsequent separation problems (can be addressed by scavenger-resins).</td>
</tr>
<tr>
<td>◎ Support and linker impose limitations to reactions (stability, solvents).</td>
<td>◎ No split-pool-strategy possible.</td>
</tr>
<tr>
<td>◎ No reactions with heterogeneous reagents or catalysts possible.</td>
<td>◎ Automation of reaction sequences more difficult than in solid-phase.</td>
</tr>
<tr>
<td>◎ Standard protocols only for some reactions accessible. Many reactions still require development and optimisation.</td>
<td>◎ Solvent change in multi-step syntheses requires separation steps.</td>
</tr>
<tr>
<td>◎ Monitoring of reactions very difficult.</td>
<td></td>
</tr>
<tr>
<td>◎ Longer reaction times than in solution-phase.</td>
<td></td>
</tr>
<tr>
<td>◎ Limitations in scale.</td>
<td></td>
</tr>
</tbody>
</table>
Principle:

A ligand for a protein cavity is casted from reversibly connecting components. The protein stabilizes those ligands which can bind and helps to shift the equilibrium.

Basic requirements for dynamic combinatorial chemistry:

1) Selection of a satisfactory set of components.
2) Search for reversible processes to connect the components.
3) Procedures for quenching these processes so as to lock-in irreversibly the constituents expressed.

Synthesis of a carbonic anhydrase inhibitor:

Step 1: Reaction without template

```
\[ \text{starting materials} \xleftrightarrow[buffer, RT]{1 d} \text{equilibrium mixture} \rightarrow \text{NaBH}_3\text{CN} \rightarrow \text{HPLC-1} \]
```

Step 2: Repetition of step 1 in the presence of carbonic anhydrase

```
\[ \text{starting materials} + 3 \text{eq carbonic anhydrase} \xleftrightarrow[buffer, RT]{14 d} \text{equilibrium mixture} + \text{NaBH}_3\text{CN} \rightarrow \text{HPLC-2} \]
```

Step 3: Comparison of HPLC-patterns

Differences in the amount of remaining starting materials and formed product identified one lead structure.

Step 4: Resynthesis of lead structure in isolable form:

```
\[ K_d = 1.1 \text{ nM} \]
```

Principle:

A ligand for a protein cavity is casted by using an irreversible reaction between building block precursors. The protein acts as a template to accelerate the reaction between those building blocks which are colocalized in the protein cavity. This can be done either in a parallel format or under an environment in which building blocks have to compete against each other.

Synthesis of an AChE-inhibitor via "click"-chemistry:

Inhibitors are built by 1,3-dipolar cycloaddition of azides with alkynes in the presence of acetylcholine esterase, an enzyme which plays a key role in neurotransmitter hydrolysis.

\[
R_1^1 - N \equiv N \quad + \quad R_2^2 \equiv H 
\]

\[ \xrightarrow{0.03\text{ eq AChE}} \]

\[ \text{pH 7.4 buffer} \quad \text{RT} \]

\[ R_1^2 - N \equiv N \quad + \quad R_2^2 \equiv H \]

syn

anti

Building blocks:

azides: alkynes:

\[ \text{98 potential binders} \]

\[ \text{49 parallel reactions} \]

(best inhibitor known today)

K_d = 80 fM

Synthesis of a Carbonic anhydrase-inhibitor via competition of building blocks:


Keywords

- Tethering
- Combinatorial Biosynthesis
- Phenotypic Screening
- Chemical Genetics

Combinatorial Biosynthesis


(A very good introduction into the combinatorial biosynthesis of peptides, polyketides and carbohydrates. Also the combinatorial biology of proteins and DNA are discussed.)


(Three eminent scientists of this field provide a very well understandable introduction.)

Chemical Genetics


(In this award lecture manuscript Schreiber describes his journey from total synthesis to chemical genetics.)


(A very good introduction into several aspects of Chemical Genetics.)


(A very good review about Chemical Genetics and applications of small molecules in Chemical Biology.)


(An excellent very well structured review article.)


(A very recent, authoritative review discussing all aspects of Chemical Genetics.)

Tethering


(A comprehensive review article written by the inventors of this method.)
**Principle:**


Tethering allows for the identification of small-molecule fragments that bind to specific regions of a protein target. These fragments can then be elaborated, combined with other molecules, or combined with one another to provide high-affinity drug leads.

**Thymidylate Synthase**

Thymidylate Synthase (TS) plays a critical role in the nucleotide synthesis pathway in all organisms especially during cell division. It is therefore a prominent cancer target.

Using the active site Cys a library of 1200 disulfide containing fragments was screened. The following compound was identified, which was combined with elements of the cofactor methylentetrahydrofolate to deliver a more active compound.

![Thymidylate Synthase](image)

**Interleukin-2**

The cytokine interleukin-2 is a critical component in the immune response. Tethering was used to improve a known inhibitor of the protein-protein interaction between IL-2 and its receptor.

![Interleukin-2](image)
Polyketides: Natural products built from acyl-coenzyme A (CoA) monomers. They are produced by huge modular enzyme complexes (MW >1 Mio. Da) called Polyketide Synthases (PKS).

Polyketide Synthases: Each module of the PKS is responsible for one cycle of polyketide chain elongation and associated functional group modifications. Within each module is a carrier protein domain (ACP) to which the growing polyketide chain is covalently tethered.

Biosynthesis of Polyketides:

Biosynthesis of Erythromycin-Aglycon:

**Polyketides:**

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**Biosynthesis of Polyketides**

**Biosynthesis of Erythromycin-Aglycon:**

AT acyl transferase domain
ACP acyl carrier protein domain
KR keto reductase domain
KS ketosynthase domain
DH dehydrase domain
ER enoyl reductase domain
TE thioesterase domain

Deoxyerythronolide B

Combinatorial Manipulation:

There are four degrees of freedom in polyketide biosynthesis that can be independently manipulated by genetic engineering, taking advantage of the natural modularity of polyketide biosynthesis:

1) length of the polyketide chain
   (determined by the number of modules that comprise the polyketide synthase)

2) choice of primer and extender units
   (controlled by gatekeeper acyl transferase (AT) domains)

3) degree of reduction of the polyketide backbone
   (determined by the set of enzyme domains present in each module)

4) stereochemistry of centers carrying alkyl and hydroxyl substituents
   (locally controlled by enzyme domains that are responsible for generating the stereocenters in question)

For the success of this approach it is essential that the manipulation of the modularity of the biosynthetic apparatus does not cause severe problems such that downstream modules in a PKS may not accept or process efficiently the anomalous product of an engineered upstream module.


Engineering of the 6-deoxyerythronolide B synthase (DEBS)

- DNA manipulation were performed in Escherichia coli XL1
- Streptomyces coelicolor or lividans were used as the host for the production of polyketides
- DEBS multienzyme complex consists of 3 large subunits (>300 kDa), each containing two modules (in total 28 catalytic domains)
- 50 compounds produced
  yields: 0.005 - 0.7 % (relative to 6dEB under the same conditions)
  e. g.

- theoretically the DEBS gene cluster could be permuted with the result of $10^7$ different compounds

Nonribosomal polypeptides are a class of natural products built from amino acid monomers. They are produced by huge modular enzyme complexes (MW > 1 Mio. Da) called Nonribosomal Peptide Synthetases (NRPS).

Nonribosomal polypeptide synthases: Each module of the NRPS is responsible for one cycle of polypeptide chain elongation and associated functional group modifications. Within each module is a carrier protein domain (PCP) to which the growing polypeptide chain is covalently tethered.

Penicillins

Cyclosporin A

Biosynthesis of Nonribosomal Polypeptides

Chemical Genetics

The goal of Chemical Genetics is the investigation of cellular biology and the understanding of signaling pathways for particular gene products by using small molecules as modulating ligands.


Advantages of Chemical Genetics vs. Knockout Organisms

- The effect of small molecules is rapid.
- In most cases the effect is reversible (due to metabolism and clearing), allowing temporal control of protein function.
- The effect is tunable, enabling grades of phenotypes by varying concentration.
- The effect is conditional, because it can be introduced at any point of development. A knock-out which is lethal for the embryonic development of an organism cannot be studied for an adult organism.
- Knock-out studies cannot dissect the role of different protein-forms derived from the same gene, whereas a small molecule should in principle be able to differentiate among these functions.
- The effect can be studied by everyone who has access to the compound.

Disadvantages of Chemical Genetics vs. Knockout Organisms

- First of all a compound which exhibits an effect has to be identified.
- The identification of the biological target of the small molecule can be cumbersome.

Three-step Procedure of Chemical Genetics Screens

1) Assembling a set of mutation equivalents (= ligands capable of altering protein function)
2) Doing a High-Throughput Screen for ligands that affect a biological process of interest
3) Identifying protein targets of these active ligands


Hierarchical Levels of Biological Interference

DNA → mRNA → PROTEINS
- gene knockout
- antisense
- small molecules
- DNA-binder
- RNAi

Three-step Procedure of Chemical Genetics Screens

1) Assembling a set of mutation equivalents (= ligands capable of altering protein function)
2) Doing a High-Throughput Screen for ligands that affect a biological process of interest
3) Identifying protein targets of these active ligands


**Colchicine:**

- isolated from *Colchicum autumnale*
- allowed the identification of tubulin proteins
- targets the \(\alpha\)-tubulin/\(\beta\)-tubulin protein-protein interface of microtubules
- inhibitor of mitosis; causes polyploidy

**FK506:**

- isolated from fungi
- enabled the identification of the FK506 binding protein (FKBP12)
- by formation of the ternary complex FKBP12-FK506-calcineurin the phosphatase activity of calcineurin is inhibited
- led to the elucidation of the calcium-calcineurin-NFAT signalling pathway
- clinically used as an immunosuppressivum
- allowed the development "small molecule dimerizers", for the small-molecule regulation of transcription and signaling pathways

**Tetrodotoxin:**

- isolated from the tetrodon pufferfish (fugu)
- very potent neuronal sodium channel blocker
- helped to develop the potential of neuronal sodium channel blockers in CNS-related disorders
- used for biological experiments described in >6000 publications

**Okadaic Acid:**

- isolated from marine sponges
- selective inhibitor of Ser/Thr phosphatase PP2A (IC50 = 1 nM)
- used for biological experiments described in >3000 publications
Phenotypic Screen of Mitotic Arrest

Library: 16320 small molecules

Screening:
1st round screen: cytoblot assay against phosphonuclein → 139 compounds
2nd round screen: in vivo staining of microtubuli and chromosomes (fluorescence microscopy)

Compound identified:

![Chemical Structure of (S)-Monastrol]

Biological Target: novel allosteric site in the motor domain of the kinesin protein Eg5


---

Phenotypic Screening with Zebra Fish (*Danio rerio*)

Library: 1100 small molecules

Screening: zebrafish eggs in 96-well plates.
- Compounds added to embryo buffer (concentration 1 µM)
- Visual screening by dissecting microscope for changes in the central nervous system, the cardiovascular system, pigmentation and the ear.

Compound identified: 1% of all compounds affected a specific aspect of one of the system screened.

**Ear Development:**

![Chemical Structure of 31N3]

31N3 alters prevents otolith formation between 14 to 26 hours after fertilization.


**Cardiovascular Development:**

![Chemical Structure of Concentramide]

Concentramide alters the global organization of the zebra fish heart (ED50 = 2 nM). It exhibits the same phenotype as the heart-and-soul mutation, but addresses a different, still unknown biological target.


Keywords
Combinatorial Chemistry in Homogeneous Catalysis
Combinatorial Chemistry in Heterogeneous Catalysis
Combinatorial Chemistry in Material Science
Directed Evolution
Phage Display
SELEX

Directed Evolution

Fascinating article about a directed evolution approach increasing the enantiomer selectivity of a lipase and the structural consequences of the mutations.


Very good review which discusses the application of directed evolution for the performance enhancement of enzymes.

Paksonal Relationships
Phage Display

Technical review by the inventor of phage display.

SELEX

Authoritative review about all aspects of aptamer generation.

Combinatorial Chemistry in Catalysis

Very good and comprehensive article about combinatorial applications in homogeneous and heterogeneous catalysis.


Very good review but already a little bit out of date.


Very good article which also discusses the important issue of high-throughput screening assays for enantioselectivity.


Good review about combinatorial aspects in heterogeneous catalysis.


This Angewandte Highlight article provides an update of recent developments in the HTS for enantioselectivity.

Combinatorial Chemistry in Material Science:

Very good and comprehensive article about combinatorial applications in material science.

Virtual Screening

A short but illustrative review about virtual screening.


An insightful review about docking and virtual screening.
Permanent Protecting Groups:

Protecting Groups of the Nucleotide Building Blocks are cleaved during the final treatment with methanolic ammonia solution (as well as the CNE-protecting group of the phosphates). In addition the protecting groups increase the solubility of the building blocks and as they are acyl groups they decrease the electron density of the nucleobases.

Only Thymidin doesn't have to be protected in the oligonucleotide synthesis.

Temporary Protecting Groups:

In solid phase DNA synthesis the 3'-OH is protected by the solid support resp. the growing DNA-oligonucleotide, therefore only the 5'-OH group has to be protected. In order to provide orthogonality to the base labile protecting groups of the nucleobases, the acid labile Dimethoxytrityl-group is used, which can be cleaved in 3% TCA/DCM within minutes, conditions which are mild enough to prevent depurination. The orange colored trityl cation allows photometric monitoring of the solid phase reaction.

Oligonucleotide Synthesis:

1) Detritylation

2) Phosphoamidit-Coupling

3) Capping

4) Oxidation

next cycles cleavage

NH₃/MeOH

DNA
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<thead>
<tr>
<th><strong>Compound library development</strong></th>
<th><strong>Suppliers of resins</strong></th>
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<tr>
<td>Pharmacopeia</td>
<td><a href="http://www.pharmacopeia.com">www.pharmacopeia.com</a></td>
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<tr>
<td>Arqule</td>
<td><a href="http://www.arqule.com">www.arqule.com</a></td>
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<td>Discovery Partners International</td>
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<td>Evotec OAI</td>
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<td><strong>Combinatorial catalysis and material sciences</strong></td>
<td><strong>Suppliers of building blocks</strong></td>
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