Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis

Agenda

1. Introduction: The Drug Discovery and Development Process

2. Lead Discovery and Lead Optimization-Drugability
   - Drugability: Lipinski’s rule of 5
   - Drugability parameters
   - Shape analysis
   - Is there a difference between leads and drugs? the rule of 4
   - Fragments: the rule of 3
   - Privileged structural elements
   - Bioisosteres
   - Unwanted molecular properties

3. Combinatorial and Parallel Synthesis in Medicinal Chemistry
   - Historical background-objective
   - The role of combinatorial chemistry and parallel synthesis in drug discovery
   - Compound mixtures versus single compounds
   - Solid phase synthesis versus synthesis in solution
   - Parallel versus split-mixed synthesis
4. **Combinatorial synthesis of Biopolymers**
   - Linear, modular synthesis of biopolymers
   - Solid-phase synthesis of polypeptides; peptoids; oligosaccharides
   - Parallel synthesis vs combinatorial synthesis: split-mixed synthesis
   - Examples for solid-phase synthesis:
     Split-mixed synthesis; tagging strategies; pin synthesis; tea-bags; photolithography; radiofrequency tags; binary encoding; factor Xa inhibitors; thrombin inhibitors; inhibitors of protein-protein interactions; hot spots and o-rings; synthesis of $\alpha$-helix mimetics; phage libraries
   - Peptide mimetics

5. **Strategies for the Synthesis of Small Molecule Libraries**
   - Library synthesis planning
   - Synthesis strategies
     - Classical multi-component reactions (MCR's)
     - Sequential multi-component reactions (SMCR's)
     - Diversity-oriented synthesis (DOS)
     - Collective synthesis of natural products
     - Fragment-based lead discovery
     - Dynamic Combinatorial Synthesis;
     - Target-guided synthesis (TGS)
     - Disulfide tethering; click chemistry
Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis

Agenda

5. Strategies for the Synthesis of Small Molecule Libraries (cont.)
   - Most important reactions used in parallel and combinatorial synthesis
   - Most important building blocks used in parallel and combinatorial synthesis
   - Parallel and/or combinatorial synthesis
   - Parallel work-up

6. Applications of Parallel Synthesis and Combinatorial Chemistry in Medicinal Chemistry
   - Case studies
   - Drug targets

7. Appendix (Definitions; Reviews; Literature)
Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis

1. Introduction: The Drug Discovery and Development Process

A long road to a new medicine
Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis

1. Introduction: The Drug Discovery and Development Process

The value added chain of pharmaceutical R & D
## Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis

### 1. Introduction: The Drug Discovery and Development Process

<table>
<thead>
<tr>
<th>Phases</th>
<th>Goals</th>
<th>Subjects</th>
<th>Duration</th>
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<tbody>
<tr>
<td>Phase 0</td>
<td>• Also known as Human Micro-dosing studies</td>
<td>10 - 15</td>
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<tr>
<td></td>
<td>• Gather preliminary data on drug pharmacokinetics by single sub-therapeutic dose</td>
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<td>• To enable go/ no go decision</td>
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<tr>
<td>Phase I</td>
<td>• Initial Safety and tolerability(pharmacology)</td>
<td>20 - 80</td>
<td>3 - 6 months</td>
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<td></td>
<td>• Determine safe Dosage Range (MAD, SAD)</td>
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<tr>
<td></td>
<td>• Identify Side-Effects</td>
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<td></td>
<td>• Only about 70 % of the experimental drug passes Phase I Trial</td>
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<tr>
<td>Phase II</td>
<td>• Effectiveness (therapeutic exploratory)</td>
<td>100 – 300</td>
<td>~ 1 year</td>
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<td></td>
<td>• Dose Response</td>
<td></td>
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<td></td>
<td>• Further Evaluation on Safety</td>
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<td>• Only about 35 % of the experimental drug passes Phase I Trial</td>
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1. Introduction: The Drug Discovery and Development Process

**Clinical Trials Phases**

<table>
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<th>Phases</th>
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<th>Subjects</th>
<th>Duration</th>
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<tbody>
<tr>
<td>Phase III</td>
<td>• Effectiveness (therapeutic confirmatory)</td>
<td>1000 – 5000</td>
<td>1-5 years</td>
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<td></td>
<td>• Monitor Side-effects</td>
<td></td>
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<tr>
<td></td>
<td>• Compare to Commonly Used Treatments</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>• Collect information that will allow the drug or treatment to be used safely</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>• Only about 25% of the experimental drug pass Phase III Trial</td>
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<tr>
<td>Phase IV</td>
<td>• Post-Marketing (therapeutic use)</td>
<td>Patient population sample</td>
<td>Ongoing Process</td>
</tr>
<tr>
<td></td>
<td>• Effectiveness in General Population</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Optimizing Drug Use</td>
<td></td>
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1. Introduction: The Drug Discovery and Development Process

Attrition rates in the discovery and preclinical phases

- **Lead Optimisation**
- **Clinical Candidate Selection (CCS) from alternatives** (Candidate Profiling)

- **Lead Identification**: 5-12 months
- **Lead Optimisation**: 12-36 months
- **CCS**: 3-6 months

- **Selecting Leads that are “drugable”**
- **Avoiding problematic templates**

- **Removing the ADMET concerns**

- **Selecting the candidate that provides the best exposure (e.g. unbound concentration at target) without safety concerns**

Of 10 projects starting in Lead Identification <3.5 will reach CCS (..but 5 possible?)
Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis
1. Introduction: The Drug Discovery and Development Process

Attrition rates in the clinical phases

Enabling
Proof-of-Concept
Efficacy
Long term
Safety
Reproductive toxicity
Long term pre-clinical & clinical safety,
carcinogenicity studies.
Final assessment of drug-drug interactions &
of bioavailability of the final marketed
formulation

Ensuring PK, metabolism, exposure,
half-life, safety, in humans are as
expected. Definition of possible human
safety issues and margins.
Reproductive toxicity

4% of marketed cpds withdrawn

Att r i t i o n  i n  D e v e l o p m e n t

EIH Enabling
Phase 1
Phase 2
Phase 3
Registration
NDA

30%
10%
40%*
20%
62%*
50%
45%*
20%
13%*
<5%

0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00

EIH Enabling
Phase 1
Phase 2
Phase 3
Registration
NDA

Winter Semester 15 Daniel Obrecht, Polyphor Ltd
The Drug Discovery Process

HBM New Drug Approvals (U. Geilinger, R. Belleli, C. Barra, July 2013)
1. Introduction: The Drug Discovery and Development Process

**Drug Discovery Process**

- **Target Identification**
  - Assay development capabilities
  - Screening libraries
  - Parallel chemistry
  - Medicinal chemistry

- **Establishing primary screening**
  - Screening capabilities
  - PEM, small molecules, fragments
  - Molecular modeling
  - ADMET properties

- **Hit identification**
  - Genomics; Proteomics; Phage display, Fragments screening; X-ray crystallography

- **Hit-to-lead**
  - Assay development capabilities

- **Lead optimization**
  - Preclinical and clinical development
Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis

1. Introduction: The Drug Discovery and Development Process

Elements of the drug profile

- Integrity
- Lipophilicity
- Solubility
- Permeability
- Blood Brain Barrier (BBB)
- Drugability
- Metabolic Stability
- Metabolite Identification
- Scalable Synthesis
- Stability PhysChem
Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis

1. Introduction: The Drug Discovery and Development Process

Multi-dimensional Lead Optimization

- Potency
- Selectivity

Drug Candidate

- Safety

- Absorption
- Distribution
- Metabolism
- Excretion
- Toxicity

Chemical Process

Formulation
Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis

1. Introduction: The Drug Discovery and Development Process

Drug absorption, distribution and elimination

- **Tissue distribution**
- **Metabolism and biliary clearance of unchanged drug**
- **Dose**
- **Systemic Circulation**
- **Liver**
- **Portal Vein**
- **Gut Wall**
- **Gut Lumen**
- **Dissolution**
- **pH ranges**
  - stomach 1.5-6
  - upper g.i. tract pH 4.4-7.8
  - colon 5
  - effect of food, bile acids etc
- **Metabolism**
- **Renal excretion of drug and/or metabolites**
- **Undissolved dose**
**Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis**

1. Introduction: The Drug Discovery and Development Process

**Conc vs Time Curves**

Absorption phase

Distribution & Elimination Phase

\[ t_{1/2} = 6h, \ \text{k} = \frac{0.693}{t_{1/2}} = 0.12h^{-1} \]

- \( t_{\text{max}} \)
- \( C_{\text{max}} \)
- \( C_{\text{min}} \)

- C mg/L

- C mg/L_Oral
- C mg/L_IV

- Time (h)
Bioavailability of drugs

$$\text{Bioavailability} = \frac{\text{AUC (oral)}}{\text{AUC (injected)}}$$
Drugability: Lipinski’s rule of 5

-Lipinski and colleagues analyzed 2245 compounds from USAN (United States Adopted Name) and the WDI (World Drug Index) which entered phase II clinical trials [1]
-Such compounds are likely to have favorable physico-chemical properties (cell permeability, solubility) or ADMET properties (Absorption, Distribution, Metabolism, Excretion, Toxicity)
-The Lipinski rule of 5 predicts that poor absorption and permeation is more likely when:

there are more than:
-5 H-bond donors
-10 H-bond acceptors
-the MW (molecular weight) is >500
-the CLogP (calculated log P) is >5

-In addition additional parameters determining favorable oral bioavailability are [2]:
-not more than 5 (10) fully rotatable bonds
-polar surface area <120Å²; BBB: <80Å²

Drugability: Lipinski’s rule of 5

Linezolid

- MW: 337.35 (ok)
- cLogP < 5.0 (ok)
- H-bond acceptors: 7 N/O atoms (ok)
- H-bond donors: 1 (ok)
- Rotatable bonds (ok)

Linezolid is a typical small molecule drug with favorable drug-like properties and is orally available.

C\textsubscript{16}H\textsubscript{20}FN\textsubscript{3}O\textsubscript{4} (337.35)

leading references:  
Medicinal Chemistry : Combinatorial Chemistry-Parallel Synthesis
2. Lead Discovery and Lead Optimization-Drugability

Drugability parameters

Additional useful properties:

Flatness:
-The aromaticity of a compound has become increasing attention
-One measure is the fractional sp³ character:
  ratio of sp³-carbons/total number of carbons
  A. Yan et al. QSAR Comb. Sci. 2003, 22, 821-829
-The flatness (sp² content) has increased over time, probably because many good sp²-sp²-bond formation reactions were developed in the eighties and nineties (Suzuki ect.) amenable to combinatorial synthesis

CLogP: calculated logP; measure for lipophilicity
  -partitioning of a compound between octanol and water
  -key parameter impacting on solubility, permeability, hERG binding and BBB penetration

Polar surface area (PSA):
-Over the past 10 years PSA has become increased attention
-Compounds with large PSA may encounter difficulties in transiting biological membranes
-poor cell permeation: PSA <120-140Å²; good BBB penetration: PSA<80-90Å²

„What do medicinal chemists actually make? A 50-year retrospectice
Drugability parameters

Additional useful properties:

**Rotatable bonds:**
- Molecular flexibility is another parameter that is frequently optimized over the course of drug discovery programs.
- Rigidifying a molecule reduces its conformational flexibility (entropy) and often increases affinity and selectivity.
- The number of rotatable bonds in drug candidates increased from 4 (1985) to 5-6 (1990s).

**Hydrogen bonding:**
- Properly placed H-bonds can impart both potency and selectivity of a compound.
- H-bonds are usually hydrated *in vivo*. Too many H-bonds are usually detrimental for good permeation and oral absorption. Membranes are lipophilic.

**Molecular complexity:**
- In the last ten years there was trend to natural product-like scaffolds with higher sp³ content away from flat or linear compounds; in particular macrocyclic natural product-like compounds have become popular: E. M. Driggers et al. *Nature Rev. Drug Discov.* **2008**, 7, 608-624.


„What do medicinal chemists actually make? A 50-year retrospectice
Shape analysis

- The shape analysis introduced by Sauer et al. is a simple and intuitive way to assess the 3D-molecular shape diversity of large combinatorial libraries.
Shape analysis

- During 1995-2005 large small molecule libraries were synthesized exhibiting limited 3D-diversity.
- Large combinatorial libraries have many linear (cigarette-shape) and flat (disc-shape) molecules of limited 3D shape diversity.
- Natural products have been traditionally a rich source for novel leads and drugs and show a higher content of spherical-shape.
- Natural products often require a large and complex multistep synthesis effort. Diversity-oriented synthesis aims at synthesizing natural product-like libraries via common synthetic precursors.
Is there a difference between Leads and Drugs? The rule of 4

To be considered for further development, lead structures should display the following properties:
- Simple chemical features, amenable for chemical optimization
- Membership to an established SAR (structure activity relationship) family
- Favorable patent situation
- Good ADME (absorption, distribution, metabolism, excretion)

Lead structures compared to drugs exhibit, on average (analysis of 96 lead-drug pairs):
- less molecular complexity (less MW, less number of rings, less number of rotatable bonds)
- are less hydrophobic (lower CLogP and logD$_{7.4}$)
- are generally less drug-like

These findings indicate that the process of optimizing a lead into a drug results generally in more complex structures.
Combinatorial libraries are composed of compounds with generally higher lipophilicity, higher MW and lower drug-likeness than leads and drugs

Is there a difference between Leads and Drugs? The rule of 4

Based on this comparison between leads and drugs it was proposed that good leads should be less complex to be good starting points for optimization. Compounds usually tend to get more lipophilic and structurally complex during lead optimization. The rule of 4 applicable for good leads was generated. This rule was also recommended to be applied for the design of screening libraries.

- MW < 400
- Number of H-bond donors < 4
- Number of H-bond acceptors < 8 (N/O atoms)
- CLlogP < 4

The properties of 40 fragment hits identified against a range of targets using high throughput X-ray crystallographic screening technology has been examined. The results indicated that on average fragment hits possessed properties consistent with a **rule of three**:

- MW < 300
- Number of H-bond donors < 3
- Number of H-bond acceptors < 6 N/O atoms
- CLogP < 3

In addition it was noted that:

- The number of rotatable bonds was on average < 3
- Polar surface area was < 60Å²

Privileged structural elements

A single framework or fragments which can bind to different target families in a specific way


The *benzodiazepin scaffold* was the first scaffold termed as privileged. It occurs in valium, librium, in CCK-A antagonists and several more.

![Chemical structures](image-url)
Privileged structural elements

Privileged structures include often favorable conformational arrangements of aromatic/heteroaromatic groups. Planar arrangements of aromatic groups give rise to stacking which results in unfavorable properties such as low solubility and aggregation.

non-planar arrangement of two aromatic rings avoids stacking

COX-II inhibitor (Vioxx)  p38 MAP kinase (SB-218655)  dopamine transporter inhibitor
Privileged structural elements

2-aryl-indole scaffold

NK1 antagonist (0.8nM)

5-HT_{6} (0.7nM)
5-HT_{7} (0.3\mu M)

CCR5 (1.3\mu M)
CCR3 (0.9\mu M)

Privileged structural elements

- Amino-thiazole scaffold

CBS-113A (clinical)
COX, 5-lipoxygenase

BMS-268770 (discovery)
CDK-2 inhibitor

CP-146662 (discovery)
5-HT1A agonist, dopamine uptake

CGS-2466 (discovery)
Adenosin A3 antagonist,
PDE-4, p38 MAP kinase

Ro 61-8048 (discovery)
Kynurenin-3-hydroxylase
Privileged structural elements

Macrocycles are privileged structures:

- A macrocycle provides diverse functionality and stereochemical complexity in a conformationally pre-organized ring structure;

- Macrocycles are semi-rigid compounds. They provide a compromise between structural pre-organization (entropy) and sufficient flexibility to mould to a target surface and maximize binding (induced fit);

- Macrocycles can demonstrate drug-like physicochemical and pharmacokinetic properties such as solubility, lipophilicity, metabolic stability, cell permeability and bioavailability beyond the rule of 5;

- Macrocycles delivered important drugs and clinical stage compounds (cyclosporin A; erythromycin; daptomycin; rapamycin; ramoplanin; geldanamycin; eribulin etc.).
  \textit{(Nat. Rev. Drug Discov. 2008, 7, 606-624);}
Privileged structural elements

Macrocycles are privileged structures: natural products

- Caspofungin (antifungal)
- Daptomycin (Gram-positive antibiotic)
- Eribulin mesylate (Halaven) (anticancer, tubulin)
- Avermectin B1a (nematodes, GABA-gated chloride channel)
- Everolimus (Affinitor) (mTOR)
- Cyclosporin A (immunosuppressive, cyclophilin)
Privileged structural elements

Macrocycles are privileged structures: natural and synthetic products

- Geldanamycin (R=OCH₃)
  17-DMAG (R=NH(CH₂)₃Me₂)  
  (Hsp90)  
  (MW: 523.25)

- Geldanamycin (R=OMe)
  17-DMAG (R=NH(CH₂)₃Me₂)  
  (Hsp90)  
  (MW: 364.47)

- Robotnikinin
  (hedgehog, Shh)  
  (MW: 454.17)

- Epothilone
  (tubulin)  
  (MW: 907.27)

- Rapamycin
  (FKBP12; m-Tor)  
  (MW: 913.59)

- EILN-2081 (Ccl4previr)
  (NS3protease; HCV)  
  (MW: 773.38)
Bioisosteres

- The term **bioisostere** was introduced by Harris Friedman in 1950 who defines it as compounds eliciting a similar biological effect.
- The established utility of bioisosteres is broad in nature, extending to improving potency, enhancing selectivity, altering physicochemical properties, reducing or redirecting metabolism, eliminating or modifying toxicophores, and acquiring novel intellectual property.
- Key bioisostric replacements often used are H to D; H to F, and CH$_3$ to CF$_3$.
- H to F exchange can modulate metabolism (CYP 450 oxidation), modulate basicities, influence conformations, modulate potencies, influence membrane permeability, and BBB penetration.
- Further important bioisosteres for phenols, catechols, carboxylic acids and amides were developed.


Synopsis of some recent tactical application of bioisosteres in drug discovery
Bioisosteres

monovalent bioisosteres
- D and H
- F and H
- NH and OH
- RSH and ROH
- F, OH, NH₂ and CH₃
- Cl, Br, SH and OH
- C and Si

bivalent bioisosteres in which two single bonds are affected
- C=C, C≡N, C=O, C=S
- −CH₂−, −NH−, −O−, −S−
- RCOR′, RCONH₂, RCOOR′, RCOSR′

trivalent bioisosteres in which three bonds are affected
- R₃CH, R₃N
- R₄C, R₄Si, R₄N⁺
- alkene, imine
- −CH=CH−, −S−
- −CH═ and −N=C

-D introduction reduces the rate of metabolism by 50% in 1
-In CTP-347 D introduction preserves CYP 2D6 function

-Introduction of two F atoms in 13 (cholesterol absorption inhibitor) was the critical step toward increased metabolic stability seen in 14 (Zetia)


Synopsis of some recent tactical application of bioisosteres in drug discovery

Winter Semester 15  Daniel Obrecht, Polyphor Ltd
In order to exclude as early as possible compounds with undesired properties from compound libraries several selection criteria (filters) have been developed:

- **chemically reactive compounds**: alkylating agents, Michael acceptors etc.

- **toxic chemical groups** (toxophores)

- **oral bioavailability**

- **aqueous solubility**

- **metabolic clearance**

- **frequent hitters**:
  (O. Roche et al. *J. Med. Chem.* 2002, 45, 137-142)

- the activity of the compound is not specific for the target (promiscuous)
- the compound perturbs the assay or detection method (coloured or fluorescent molecules)
- molecules prone to form polymers (e.g. catechols)
- molecules have a high tendency to form aggregates
Unwanted properties: reactive groups

sulfonyl halides (X: Cl, Br) | acyl halides (X: Cl, Br) | alkyl halides (X: Cl, Br, I) | anhydrides | halopyrimidines
---|---|---|---|---
R\_X | R\_X | R\_X | R\_X | R\_X
aldehydes | imines | α-halo-ketones (X: Cl, Br) | aliphatic esters | aliphatic ketones | trifluoro-ketones
R\_O|R\_O| R\_O | R\_O | R\_O | R\_O | R\_O | R\_O | R\_O
epoxides | aziridines | aliphatic thioesters | sulfonate esters | phosphonate esters | 1,2-dicarbonyl compounds
R\_N|R\_R | R\_S | R\_O | R\_O | R\_O | R\_O | R\_O | R\_O
Michael acceptors
R\_O\_O\_R | R\_S\_S\_R | R\_O\_O\_R | R\_N\_N\_R | R\_N\_N\_R
heteroatom-heteroatom single bond

Unwanted properties: frequent hitters

Examples of frequent hitters (Matthew correlation coefficient: >0.8)


- diethylstilbestrol (1.00)
- dopamine (0.88)
- clofazimine (1.00)
- fenoterol (0.87)

Molecules that form aggregates


Winter Semester 15  Daniel Obrecht, Polyphor Ltd
1. What are the Lipinski’s rules of five and what do they stand for?

2. Please determine number of rotatable bonds, number of H-bond donors and acceptors of the following molecules?

A

B

C

D

H-Lys-Glu-NH₂

3. Describe the difference between drug and lead-like
1961: Ivar Ugi publishes his pioneering paper on his four component reaction: “If, for example, 40 of each different components are reacted with one another, the result is 2,560,000 reaction roducts...”
**Medicinal Chemistry : Combinatorial Chemistry-Parallel Synthesis**

3. Combinatorial and Parallel Synthesis in Medicinal Chemistry

**Historical background-objective**


1967: *J. Fréchet* described a highly loaded trityl resin (2.0mmol/g)


Historical background-objective


1976: Leznoff and Files described bromination and lithiation of insoluble polystyrene, thus pioneering the synthesis of functionalized resins (Can. J. Chem. 1976, 54, 935)

1976: Rapoport and Crowley published a review entitled: Solid-phase organic synthesis: novelty or fundamental concept? This raised three important questions: -degree of separation of resin-bound functional groups; -analytical methods to follow reactions on solid support; -nature and kinetics of competing side reactions (Acc. Chem. Res. 1976, 9, 135)


1977: Wulff et al. Synthesized chiral macroporous resins using carbohydrates as templates for the use of column materials for the separation (Makromol. Chem. 1977, 178, 2799)
**Medicinal Chemistry : Combinatorial Chemistry-Parallel Synthesis**

3. Combinatorial and Parallel Synthesis in Medicinal Chemistry

**Historical background-objective**

1979: Leznoff employed successfully a chiral linker for the asymmetric synthesis of (S)-2-methyl-cyclohexanone in 95% e.e. (Angew. Chem. 1979, 91, 255)


1984: Geysen et al. described the multi-pin technology for the multiple peptide synthesis (Proc. Natl. Acad. Sci. USA, 1984, 81, 3998)


1985: G. P. Smith described in seminal paper the use of filamentous phage for the synthesis of peptide libraries (phage display method, Science 1985, 228, 1315)

1986: Mixtures of activated amino acid monomers were coupled to solid supports for the synthesis of peptide libraries as mixtures; the product distribution depended on the relative coupling rates (Mol. Immunol. 1986, 23, 709)

1991: Fodor et al. described the VLSIPS method (very large scale immobilised polymer synthesis; photolithographic parallel synthesis (Science 1991, 251, 767)
Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis

3. Combinatorial and Parallel Synthesis in Medicinal Chemistry

Historical background-objective


1992: Synthesis of 1,4-benzodiazepines on solid support described independently by S. Hobbs-DeWitt (Diversomer technology, US-Pat. 5324483, 1993) and J. A. Ellman (J. Am. Chem. Soc. 1992, 114, 10997)


1993: Use of multi-cleavable linkers for the synthesis of peptide-like libraries by M. Lebl et al. (Int. J. Protein Res. 1993, 41, 201)


1995: Synthesis of a potent ACE inhibitor by combinatorial organic synthesis on solid support using a 1,3-dipolar cycloaddition reaction by Gallop et al. (WO 95/35278, 1995)
Medicinal Chemistry : Combinatorial Chemistry-Parallel Synthesis

3. Combinatorial and Parallel Synthesis in Medicinal Chemistry

Historical background-objective


1996: Use of the Ugi four component reaction in combination with a 1,3-dipolar cycloaddition reaction of intermediary formed `Munchrones` with electronpoor acetylenes by R. Armstrong et al. (Tetrahedron Lett. 1996, 37, 1149)


3. Combinatorial and Parallel Synthesis in Medicinal Chemistry

Historical background-objective


2008: „**Build-couple-pair**“ strategy as a basis for diversity-oriented synthesis (DOS):


The role of combinatorial chemistry and parallel synthesis in drug discovery

**Aim:**
- Hit identification

**Methods:**
- Combinatorial synthesis on solid support
- High throughput parallel synthesis in solution

**Focused Libraries for Hit Confirmation and Validation**
100 to 1'000 compounds

**Aim:**
- Hit confirmation, validation and exploration of SAR

**Methods:**
- High throughput parallel synthesis in solution

**Focused Libraries for Hit-to-Lead Optimization**
20 to 100 compounds per cycle

**Aim:**
- Hit optimization, SAR, ADMET properties, TPP

**Methods:**
- Medicinal chemistry approaches; parallel synthesis
3. Combinatorial and Parallel Synthesis in Medicinal Chemistry

**Compound mixtures versus single compounds**

**Compound mixtures:**

- Mixtures (most often 10-20 compounds) of purified compounds in equimolar amounts
- Mixtures of products synthesized in one reaction in equimolar ratio:
  
  \[ \text{Mol. Immunol.} \ 1986, \ 23, 709 \]

![Chemical structure](image)

- Most often products originating from a reaction mixture are not formed in equimolar ratio are contaminated with impurities

**Advantage:** compound mixtures can reduce the screening effort in expensive and laborious screens

**Drawbacks:** compounds in mixtures can interfere with one another; prone to false positive hits

**Trend today:** screening of single compounds
Chemical Biology: Combinatorial Chemistry-Parallel Synthesis

3. Combinatorial and Parallel Synthesis in Medicinal Chemistry

Compound mixtures versus single compounds

Single compounds:

- Synthesis on solid supports without final purification:
  requires a lot of development work; allows to make large libraries

- Synthesis in solution using high yielding reactions without further purification:
  limits the scope of reactions that can be used; often used in the context of multi-
  component reactions; useful for large libraries

- Synthesis in solution followed by high-throughput preparative HPLC-purification:
  whole repertoire of organic reactions can be used; is today's standard method for
  the synthesis of focused libraries (hit validation; lead optimization)

Trend:

as screening technologies have increased the throughput, screening of single compound
libraries is more and more becoming the standard

as companies are looking for highly diverse general compound libraries of high quality
(purity, stability) library synthesis has shifted from solid phase synthesis (large libraries)
to solution phase synthesis followed by high-throughput purification (normal and
reverse phase)
Chemical Biology: Combinatorial Chemistry-Parallel Synthesis

3. Combinatorial and Parallel Synthesis in Medicinal Chemistry

Solid phase synthesis versus synthesis in solution

Solution phase chemistry:

++ most reactions and reagents have been studied in solution
+
usually no excess of reagents have to be used
+
solvent effects can be studied and altered readily
++ steric effects are usually less pronounced in solution and can be overcome more easily by using more drastic reaction conditions
++ reaction conditions are usually adapted to a large variety of substituents
-- extensive and time consuming, chromatographic purification procedures are often necessary
-> side products have to be separated and analysed (can also be an advantage in the first exploratory stage of a given project
-- parallelisation and automation usually requires more initial effort
### Solid phase synthesis versus synthesis in solution

**Solid phase chemistry:**

++ excess of reagents can be used to drive reactions to completion

++ purification procedures achieved by simple filtrations which can be easily automated

++ assuming complete spatial separation of the reactive sites on a given solid support, the principle of high dilution ("hyperentropic effect", *Acc. Chem. Res.* 1976, 9, 135) can be used beneficially; e.g. for intramolecular cyclisation reactions

+- overall costs for the synthesis of large libraries (assuming no purification of the final compounds is necessary) can compare favourably with solution synthesis

+- linker molecules have to be designed which are compatible with the polymeric matrix and the chemistry used for library synthesis: labour intense development work; ok for large libraries

-- development of reaction conditions requires more work than in solution reactions on solid support are more sensitive to steric effects: limitations in the design of highly diverse libraries

-- reactions are more difficult to monitor; especially a drawback in the development phase
3. Combinatorial and Parallel Synthesis in Medicinal Chemistry

Solid phase synthesis versus synthesis in solution

**General trends:**

**Solid-Phase chemistry:** *large libraries* (no purification of individual compounds)

- *split mixed approach*

  - *linear approaches:* polypeptides, peptoids, oligosaccharides, oligocarbamates and ureas

    *use of solid support as a protective group:* for guanidines, amidines, hydroxamic acids, carboxylic acids, alcohols...

**Solution-phase chemistry:** *small focused libraries of high chemical diversity* (purified products)

- *parallel synthesis*

- *convergent approaches*
Medicinal Chemistry : Combinatorial Chemistry-Parallel Synthesis
3. Combinatorial and Parallel Synthesis in Medicinal Chemistry

Questions

1. What are the advantages of using mixtures of compounds in the biological screening?

2. What are the disadvantages?

3. What are the advantages of using solid phase chemistry?

4. For which type of molecules is it advantageous to use solid phase chemistry?
Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis

4. Combinatorial Synthesis of Biopolymers

Linear, modular synthesis of biopolymers

Monomer A1 → bond → Monomer A2 → bond → Monomer A3 → bond → Monomer A4

<table>
<thead>
<tr>
<th>Monomers</th>
<th>Bond Formation</th>
<th>Polymers</th>
</tr>
</thead>
<tbody>
<tr>
<td>amino acids</td>
<td>amide bond</td>
<td>peptides, proteins</td>
</tr>
<tr>
<td>nucleotides</td>
<td>phosphoester bond</td>
<td>oligonucleotides</td>
</tr>
<tr>
<td>mono- and disaccharides</td>
<td>glycosidic bond</td>
<td>polysaccharides</td>
</tr>
<tr>
<td>N-alkylated glycines</td>
<td>amide bond</td>
<td>peptoids</td>
</tr>
</tbody>
</table>
Overview: synthesis of polypeptides

Strategies for amide bond synthesis in polypeptides

Medicinal Chemistry : Combinatorial Chemistry-Parallel Synthesis
4. Combinatorial Synthesis of Biopolymers

Solid-phase synthesis of polypeptides

Fmoc-strategy

Boc-strategy

Fmoc-strategy

Boc-strategy

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Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis
4. Combinatorial Synthesis of Biopolymers

Solid-phase synthesis of polypeptides: resins-polymer supports

1. Functionalized polystyrene resins:

- Cross-linked polystyrene resin
- Randomly functionalized cross-linked polystyrene resin
- Selectively functionalized cross-linked polystyrene resin

* Suspension polymerisation: water, free radical catalyst (dibenzoyl peroxide, AIBN), dispergator; particle size depends upon stirring speed, the relative amounts of aqueous and monomer phases, amount and nature of dispergator
Solid-phase synthesis of polypeptides: resins-polymer supports

1. Functionalized polystyrene resins


\[ \text{styrene, DVB} \]  
\[ \begin{array}{c}
\text{Cl} \\
\text{Ph} \\
\text{Ph}
\end{array} \quad \begin{array}{c}
\text{Ph} \\
\text{Ph}
\end{array} \]

\[ \text{Cl} \]

(95:5 para/ortho)

microporous: 1-2% crosslinking  
macroporous: 20% crosslinking

\[ \text{styrene, DVB} \]

\[ \begin{array}{c}
\text{OMe}
\end{array} \]

\[ \begin{array}{c}
\text{Cl}
\end{array} \]

(up to 2.5 mmol/g loading)

i: BCl₃, CCl₄, 0°, 2 h; ii: NaOH, CHCl₃ (or CH₂CH₂Cl₂), BnNEt₃, Cl⁻, SO₂Cl₂, AIBN, 60°; *Macromolecules* **1986**, 19, 2470
Solid-phase synthesis of polypeptides: resins-polymer supports

Swelling properties of Merryfield type microporous resins:

<table>
<thead>
<tr>
<th>Solvent</th>
<th>crosslinked PS (1% DVB)*</th>
<th>crosslinked PS (2% DVB)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>EtOH</td>
<td>1.05</td>
<td>1.0</td>
</tr>
<tr>
<td>AcOH</td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>MeCN</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>pyridine</td>
<td></td>
<td>3.0</td>
</tr>
<tr>
<td>DMF</td>
<td>3.5</td>
<td>2.0</td>
</tr>
<tr>
<td>THF</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>dioxane</td>
<td>4.9</td>
<td>2.5</td>
</tr>
<tr>
<td>Et₂O</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>CH₂Cl₂</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>toluene</td>
<td>5.3</td>
<td>2.8</td>
</tr>
</tbody>
</table>

*swelling capacity: volume of swollen resin/original volume
Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis

4. Combinatorial Synthesis of Biopolymers

Solid-phase synthesis of polypeptides: linkers

2. TentaGel® resins

*Bayer and Rapp; Angew. Chem. Int. Ed. Engl. 1991, 30, 113; contain up to 60-80% of PEG units*

\[
\begin{align*}
\text{aryl} & \quad \text{OH} & \quad \text{aryl} & \quad \text{CH}_2\text{O(CH}_2\text{CH}_2\text{O)}_3\text{CH}_2\text{CH}_2\text{O}^+ & \quad \text{i} & \quad \text{aryl} & \quad \text{CH}_2\text{O(CH}_2\text{CH}_2\text{O)}_{4+n}\text{H} \\
\text{aryl} & \quad \text{ii} & \quad \text{aryl} & \quad \text{Me} & \quad \text{OH} & \quad \text{iii} & \quad \text{aryl} & \quad \text{Me} & \quad \text{O(CH}_2\text{CH}_2\text{O)}_n\text{H}
\end{align*}
\]

i: ethylene oxide; ii: propylene oxide, SnCl₄, CH₂Cl₂; iii: ethylene oxide, KOH, dioxane, 110°

**Good swelling properties in:** water, MeOH, CH₂Cl₂, MeCN, THF and DMF; used preferentially in continuous flow reactors

3. Polyacrylamide resins

pioneered by Sheppard; Bioorg. Chem. 1979, 8, 351

\[
\begin{align*}
\text{basic monomer} & \quad \text{O} & \quad \text{N} & \quad \text{O} & \quad \text{N} & \quad \text{functionalized monomers} \\
\text{crosslinking agent} & \quad \text{O} & \quad \text{N} & \quad \text{H}_{\text{Boc}} & \quad \text{O} & \quad \text{N} & \quad \text{CO}_2\text{Me}
\end{align*}
\]

Persulphate initiated copolymerisation in 66% aqueous DMF, 1,2-dichloroethane and cellulose acetate/butyrates, as emulgar
Solid-phase synthesis of polypeptides: linkers

2. Linkers for releasing carboxylic acids

<table>
<thead>
<tr>
<th>structure</th>
<th>abbreviation</th>
<th>cleavage conditions</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Merryfield resin" /> Cl</td>
<td>Merryfield resin</td>
<td>HF, CF₃SO₂H</td>
<td>J. Am. Chem. Soc. <strong>1963</strong>, 85, 2149</td>
</tr>
<tr>
<td><img src="image" alt="hydroxymethyl-PS" /> OH</td>
<td>hydroxymethyl-PS</td>
<td>HF, CF₃SO₂H</td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Wang resin" /> OH</td>
<td>Wang resin</td>
<td>95% TFA</td>
<td>J. Am. Chem. Soc. <strong>1973</strong>, 95, 1328</td>
</tr>
<tr>
<td><img src="image" alt="Sasrin® resin (Bachem)" /> OMe</td>
<td>Sasrin® resin (Bachem)</td>
<td>1% TFA</td>
<td>Tetrahedron Lett. <strong>1988</strong>, 29, 4005</td>
</tr>
<tr>
<td><img src="image" alt="Rink resin" /> OMe</td>
<td>Rink resin</td>
<td>1% TFA</td>
<td>Tetrahedron Lett. <strong>1987</strong>, 28, 3787</td>
</tr>
<tr>
<td><img src="image" alt="chloro-trityl resin (Barlos)" /> Cl Ph</td>
<td>chloro-trityl resin (Barlos)</td>
<td></td>
<td>Tetrahedron Lett. <strong>1989</strong>, 30, 3943</td>
</tr>
</tbody>
</table>
### Solid-phase synthesis of polypeptides: linkers

<table>
<thead>
<tr>
<th>Linker</th>
<th>Abbreviation</th>
<th>Cleavage Conditions</th>
<th>Reference</th>
</tr>
</thead>
</table>
| ![BHA](image) | BHA (R=H) | HF, CF$_3$SO$_3$H | *J. Org. Chem.* 1985, 50, 5291  
            | MBHA (R=Me) |                      | *Peptides.* 1981, 2, 85 |
| ![Rink resin](image) | Rink resin | 95% TFA | *Tetrahedron Lett.* 1987, 28, 3787 |
| ![PAL resin](image) | PAL resin | TFA | *Int. J. Prot. Pept. Res.* 1987, 30, 206 |
| ![Kaiser oxime resin](image) | Kaiser oxime resin | NH$_3$  
primary and secondary amines  
NH$_2$NH$_2$ x 1H$_2$O | *J. Org. Chem.* 1980, 45, 1295 |

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Solid-phase synthesis of polypeptides: the 20 proteinogenic amino acids

- glycine; Gly; G
- alanine; Ala; A
- valine; Val; V
- leucine; Leu; L
- phenylalanine; Phe; F
- serine; Ser; S
- threonine; Thr; T
- methionine; Met; M
- isoleucine; Ile; I
- tyrosine; Tyr; Y
- aspartic acid; Asp; D
- glutamic acid; Glu; E
- glutamine; Gln; Q
- tryptophan; Trp; W
- lysine; Lys; K
- arginine; Arg; R
- cysteine; Cys; C
- histidine; His; H
### Solid-phase synthesis of polypeptides: coupling reagents

**Uronium salts**

- **X**: PF$_6^-$ HBTU; BF$_4^-$ TBTU

- **X**: PF$_6^-$ HATU

- **X**: PF$_6^-$, BF$_4^-$, (Castro's reagent)

**Azides**

- **PhO-P=N$_3$**
  - DPPA (diphenyl-phosphoryl azide)

- **R-COOH** \(\xrightarrow{\text{base}}\) **R-CON$_3$**

**Carbodiimides**

- **R$^1$-N=C=N-R$^2$, Y=CH, N**
  - **R$^1$=R$^2$=iPr (DIC)**
  - **R$^1$=R$^2$=cyclohexyl (DCC)**
  - **R$^1$=Et; R$^2$=CH$_2$CH$_2$N$^+$/Me$_2$, Cl$^-$ (EDCI)**

**Acid fluorides**

- **R-COOH** \(\xrightarrow{\text{base}}\) **R-COF**
**Medicinal Chemistry : Combinatorial Chemistry-Parallel Synthesis**

4. Combinatorial Synthesis of Biopolymers

---

**Solid-phase synthesis of polypeptides: protective groups**

<table>
<thead>
<tr>
<th>Fmoc strategy:</th>
<th>Cleavage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main chain (backbone) amino groups:</strong> Fmoc</td>
<td>20% piperidine/DMF, rt</td>
</tr>
<tr>
<td><strong>Side chain amino groups (Lys, Orn, Dab):</strong> Boc</td>
<td>TFA, CH₂CH₂, triisopropyilsilane*</td>
</tr>
<tr>
<td><strong>Side chain carboxylic acids (Glu, Asp):</strong> t-butyl esters</td>
<td>TFA, CH₂CH₂ triisopropyilsilane*</td>
</tr>
<tr>
<td><strong>Side chain primary amides (Gln, Ans):</strong> N-trityl</td>
<td>TFA, CH₂Cl₂, triisopropyilsilane*</td>
</tr>
<tr>
<td><strong>Side chain hydroxy(phenol) groups (Ser, Thr, Tyr):</strong> t-butyl ethers</td>
<td>TFA, CH₂Cl₂, triisopropyilsilane*</td>
</tr>
<tr>
<td><strong>Side chain indole and imidazole groups (Trp, His):</strong> N-trityl</td>
<td>TFA, CH₂Cl₂, triisopropyilsilane*</td>
</tr>
<tr>
<td><strong>Side chain guanidine groups (Arg):</strong> Pmc, Pmb</td>
<td>TFA, CH₂Cl₂, triisopropyilsilane*</td>
</tr>
</tbody>
</table>

*other scavengers like thioanisol phenol, H₂O, thiocresol and others are used*
Solid-phase synthesis of peptoids

Peptoids: ideal scaffold for parallel and combinatorial synthesis

- protease stability increased
- number of H-bond donors reduced (can be also disadvantage)
- number of rotatable bonds increased (tertiary amides have lower trans-cis barrier)
- prediction of peptoid backbone conformation quite difficult (flexibility)
- ideally suited for library synthesis: large number of building blocks available available by solid-phase synthesis split-mixed synthesis possible
Solid-phase synthesis of peptoids

**Approach A:** sequential coupling of N-substituted glycines

\[
\begin{align*}
\text{HN} & \text{O} \quad \text{N} \quad \text{Fmoc} \\
\rightarrow & \quad \rightarrow \\
\text{HN} & \text{O} \quad \text{N} \quad \text{Fmoc} \\
\rightarrow & \quad \rightarrow \\
\text{H}_2\text{N} & \text{O} \quad \text{N} \quad \text{R}^1 \quad \text{R}^2 \\
\end{align*}
\]

**Approach B:** sequential coupling of glycine followed by reductive amination with aldehydes

\[
\begin{align*}
\text{HN} & \text{O} \quad \text{N} \quad \text{Fmoc} \\
\rightarrow & \quad \rightarrow \\
\text{HN} & \text{O} \quad \text{N} \quad \text{Fmoc} \\
\rightarrow & \quad \rightarrow \\
\text{H}_2\text{N} & \text{O} \quad \text{N} \quad \text{R}^1 \quad \text{R}^2
\end{align*}
\]

\(i: \text{DBU, DMF}; \ ii: \text{PyBop or PyBroP, R}_2^2\text{NFmocCH}_2\text{COOH}; \ iii: \text{DBU, DMF}; \ iv: \text{RCHO, Na(OAc)}_2\text{BH or NaCNBH}_3, \text{MeOH}; \ v: \text{Fmoc-Gly, PyBop or PyBroP}; \ vi: \text{DIC, DMF, BrCH}_2\text{COOH}; \ vii: \text{R-NH}_2, \text{DMSO}\)
Solid-phase synthesis of peptoids

**Approach C:** coupling of bromo-acetic acid followed by nucleophilic displacement with amines

\[
\begin{align*}
\text{NH}_2 & \xrightarrow{\text{vi}} \text{NH} - \text{Br} \xrightarrow{\text{vii}} \text{NH - R}^1 \xrightarrow{\text{vi, vii}} \text{NH - R}^1 \text{N} - \text{R}^2 \\
\text{R}^1 & \text{N} - \text{R}^2
\end{align*}
\]


i: DBU, DMF; ii: PyBop or PyBrop, R^2_NFmocCH_2COOH; iii: DBU, DMF; vi: RCHO, Na(OAc)_3BH or NaCNBH_3, MeOH; v: Fmoc-Gly, PyBop or PyBrop; vi: DIC, DMF, BrCH_2COOH; vii: R-NH_2, DMSO

Screening 18 pools originated from split-mixed synthesis for [^3H]-DAMGO (μ-specific) binding to opiate receptor.

Chir 4531: 6nM
Solid-phase synthesis of oligosaccharides

\[ \text{Ph} \quad \text{O} \quad \text{O} \quad \text{N}_3 \quad \text{S} \quad \text{Ph} \quad \text{O} \quad \text{O} \quad \text{N}_3 \quad \text{S} \quad \text{Ph} \]

\[ \text{BnO} \quad \text{O} \quad \text{OBn} \quad \text{i} \]

\[ \text{Ph} \quad \text{O} \quad \text{O} \quad \text{N}_3 \quad \text{Ph} \quad \text{O} \quad \text{Piv} \quad \text{OPiv} \quad \text{Ph} \]

\[ \text{BnO} \quad \text{O} \quad \text{OBn} \quad \text{ii} \]

\[ \text{Ph} \quad \text{O} \quad \text{O} \quad \text{N}_3 \quad \text{Ph} \quad \text{O} \quad \text{Piv} \quad \text{OPiv} \quad \text{Ph} \]

\[ \text{BnO} \quad \text{O} \quad \text{OBn} \quad \text{iii} \]

\[ \text{Ph} \quad \text{O} \quad \text{O} \quad \text{N}_3 \quad \text{Ph} \quad \text{O} \quad \text{Piv} \quad \text{OPiv} \quad \text{Ph} \]

\[ \text{BnO} \quad \text{O} \quad \text{OBn} \quad \text{iii} \]

\[ \text{Ph} \quad \text{O} \quad \text{O} \quad \text{OH} \quad \text{Ph} \quad \text{O} \quad \text{Piv} \quad \text{OPiv} \quad \text{Ph} \]

\[ \text{BnO} \quad \text{O} \quad \text{OBn} \quad \text{iii} \]

\[ \text{i: Cs}_2\text{CO}_3, \text{Merryfield resin;} \quad \text{ii: Tf}_2\text{O}, 2,6-di-tert-butyl-4-methylpyridine, CH}_2\text{Cl}_2, -60 \text{ to } -20^\circ; \quad \text{iii: Hg(OCOCF}_3)_2, \text{CH}_2\text{Cl}_2, \text{H}_2\text{O, r.t.} \]

Medicinal Chemistry : Combinatorial Chemistry-Parallel Synthesis

4. Combinatorial Synthesis of Biopolymers

Questions

1. Name at least three different types of solid supports?

2. Give at least two different ways to synthesize chloro-methyl polystyrene?
Medicinal Chemistry : Combinatorial Chemistry-Parallel Synthesis
4. Combinatorial Synthesis of Biopolymers

Examples for libraries synthesized on solid-phase: parallel synthesis of single compounds

800 products

40 reaction vessels

40 times couple and cleave

40 individual products

800 reaction vessels

40 times couple and cleave

800 individual products
Examples for libraries synthesized on solid-phase: parallel synthesis of mixtures

400 products

2 reaction vessels

2 times couple* and cleave

mixture of 20 products

*cocktail of 20 amino acids

mixture of 400 products
Examples for libraries synthesized on solid-phase: one bead-one compound/split-mixed/couple-divide
Examples for libraries synthesized on solid-phase: peptides

Parallel synthesis of compound mixtures:

++ high-throughput with little synthetic manipulations
-- difficult interpretation of screening results (synergistic and non-synergistic effects)
-- resynthesis of individual compounds necessary
    generally not used anymore

Parallel synthesis of single compounds

++ clear screening results
++ identification of structure unambiguous
++ resynthesis generally not necessary; repurification required
-- many parallel synthetic steps and reaction vessels required; usually expensive robotic equipment required
    method of choice for relatively small compound libraries

Split mixed synthesis of mixtures (one bead- one compound):

++ usually clear screening results can be obtained; on bead or in solution
++ large libraries with few synthetic steps can be obtained in real combinatorial fashion
-- only small amounts are usually obtained and structure of hits have to be determined by cleavage and MS or deconvolution or tagging (binary codes or radio-frequency labels) startegies
    method of choice for large combinatorial libraries
Solid-phase synthesis of polypeptides

- Peptides synthesized as individuals or as mixtures on solid supports (polystyrene, polyacrylamide, polyacrylamide-polystyrene co-polymers) and cleaved to be assayed in solution


*Mixtures of peptides can be obtained by by using two different strategies:*

- As true mixtures where a peptide coupling step involves the coupling of a mixture (typically the 20 coding amino acids) of side-chain protected Boc- or Fmoc-protected amino acids (D or L) in a predetermined molar ratio which compensates for the different coupling rates.

Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis
4. Combinatorial Synthesis of Biopolymers

Examples for libraries synthesized on solid-phase: peptides

Parallel synthesis of single compounds


Spatially separated reaction compartments, where peptides can be synthesized by capitalizing on the fact that all washing, neutralisation and deprotection steps can be performed simultaneously. For parallel synthesis the bags are separated before the coupling steps.


Spatially separated parallel synthesis of compounds in microtiter format
Examples for libraries synthesized on solid-phase: peptides: photolithography


Spatially separated multiple parallel synthesis using photocolavable protective groups such as the N-nitroveratrylcarbonyl group (NVOC), allows the controlled synthesis of (peptide) libraries by the spatially controllable addition of specific reagents to specific locations.
1. What are the advantages of a split-mixed approach over a parallel synthesis approach and for which types of molecules will you apply this technology? Please discuss.
Examples for libraries synthesized on solid-phase: peptides: split-mixed technology

![Diagram showing synthetic steps and peptide pools](image-url)

**Step 1**
- **Couple**
- **Dipeptides**
  - Pool 1A → Pool 2A (7)
  - Pool 1B → Pool 2B (7)
  - Pool 1C → Pool 2C (7)
  - Pool 1D → Pool 2D (7)
  - Pool 1E → Pool 2E (7)
  - Pool 1F → Pool 2F (7)
  - Pool 1G → Pool 2G (7)

**Step 2**
- **Mix, cleave, split**

**Step 3**
- **Couple, cleave**
  - Pool 3A (49) → Pool 4A (49)
  - Pool 3B (49) → Pool 4B (49)
  - Pool 3C (49) → Pool 4C (49)
  - Pool 3D (49) → Pool 4D (49)
  - Pool 3E (49) → Pool 4E (49)
  - Pool 3F (49) → Pool 4F (49)
  - Pool 3G (49) → Pool 4G (49)

Total: 342 tripeptides on bead

7^3 = 343 tripeptides
Examples for libraries synthesized on solid-phase: peptides: split-mixed technology


Screening reveals in which of the *Pools* 4A to 4G are the most active compounds; determines most active building block in the 3rd step (position): assumption it is B; *Pools* 2A to 2G are resynthesized but not mixed and coupled with building block B in the third step. The compounds are retested and this determines the favoured building block in the second step (position): assumption it is G. Now the initial 7 resins are coupled with G (2nd step) and B (3rd step) and the resulting compounds tested again. The most active tripeptide is now identified: assumption it is A-G-B.

**Recursive deconvolution** *(e.g. Nat. Acad. Sci, USA 1994, 91, 11422)*

By using this technique samples of the initial resins as well as *Pools* 2A-2G and *Pools* 4A-4G are stored away for resynthesis of sublibraries similarly to the iterative deconvolution procedure.

**Positional scanning** *(e.g. Nat. Acad. Sci, USA 1994, 91, 11422; Life Sci. 1993, 52, 1509)*

**Indexed or orthogonal libraries** *(e.g. Chem. Biol. 1995, 2, 621; Tetrahedron Lett. 1997, 38, 491)*

**Binary encoding** *(e.g. W. C. Still et al. Proc. Nat. Acad. Sci, USA 1993, 90, 10922)*

Examples for libraries synthesized on solid-phase: peptides; split-mixed technology: binary encoding

Binary encoding
(e.g. W. C. Still et al. *Proc. Nat. Acad. Sci, USA* 1993, 90, 10922)

![Diagram of binary encoding process]

- 7 building blocks
- 3 steps
- Requires 9 tags

**Step 1:**
- Building block A: 1 0 0 tag 1
- Building block B: 0 1 0 tag 2
- Building block C: 0 0 1 tag 3
- Building block D: 1 1 0 tags 1 + 2
- Building block E: 1 0 1 tags 1 + 3
- Building block F: 0 1 1 tags 2 + 3
- Building block G: 1 1 1 tags 1 + 2 + 3

**Step 2:**
- Building block A: 1 0 0 tag 4
- Building block B: 0 1 0 tag 5
- Building block C: 0 0 1 tag 6
- Building block D: 1 1 0 tags 4 + 5
- Building block E: 1 0 1 tags 4 + 6
- Building block F: 0 1 1 tags 5 + 6
- Building block G: 1 1 1 tags 4 + 5 + 6

**Step 3:**
- Building block A: 1 0 0 tag 7
- Building block B: 0 1 0 tag 8
- Building block C: 0 0 1 tag 9
- Building block D: 1 1 0 tags 7 + 8
- Building block E: 1 0 1 tags 7 + 9
- Building block F: 0 1 1 tags 8 + 9
- Building block G: 1 1 1 tags 7 + 8 + 9

*Variations of Ar and n give rise to different tags, which can be detected in minute amounts by GC/MS*
Examples for libraries synthesized on solid-phase: peptides: radio-frequency tags

Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis

4. Combinatorial Synthesis of Biopolymers

Examples for libraries synthesized on solid-phase: peptides: split-mixed technology

Application of the split-mixed method for discovery of Factor Xa inhibitors

Factor Xa is implicated in the blood coagulation cascade: inhibitors of Factor Xa could be potentially useful as anti-thrombotic agents (Biochemistry 1998, 37, 1053-1059; Drug Discovery Today 1998, 3, 223))

octa-peptide library (split-mixed technology)

on-bead screening

H-Tyr-Ile-Arg-Leu-Ala-Ala-Phe-Thr-NH₂ (SEL1691) → SEL2602
Examples for libraries synthesized on solid-phase: peptides: split-mixed technology

Application of the split-mixed method for discovery of Factor Xa inhibitors

$\textit{Blood coagulation factor Xa}$ is implicated in $\textit{hemostasis}$ (bloodcoagulation)

Thrombosis: pathological form of hemostasis:
- myocardial infarction (arterial thrombosis)
- pulmonary embolism (venary thrombosis)
- infection by gram-negative organisms

\[
\begin{align*}
\text{XII} & \rightarrow \text{XII} \\
\text{XI} & \rightarrow \text{Xla} \\
\text{IX} & \rightarrow \text{IXa} \\
\end{align*}
\]

Factor Xa inhibitors

\[
\begin{align*}
\text{Prothrombin} & \rightarrow \text{Thrombin} \\
\end{align*}
\]

Thrombin inhibitors

\[
\begin{align*}
\text{Fibrin} & \rightarrow \text{XIIa} \\
\end{align*}
\]

*Intrinsic pathways

*Extrinsic pathways

*\textit{tissue factor}

\[
\begin{align*}
\text{Fibrinogen} & \rightarrow \text{Thrombin} \\
\end{align*}
\]
Examples for libraries synthesized on solid-phase: peptides: split-mixed technology

Application of the split-mixed method for discovery of Factor Xa inhibitors

Current anti-thrombotic therapies include: aspirin

**Thrombin inhibitors**: heparin (sulphated poly-saccharide); heparin analogues; hirudin; small molecular weight thrombin inhibitors (not on the market yet)

high levels of thrombin inhibition necessary; unacceptable bleeding

**Factor Xa inhibitors**: trypsin-like serine protease

current molecules in clinical trials

*Cor-Therapies* (IC50 factor Xa: 0.65nM)
(IC50 thrombin: 10.0μM)

*Yamanouchi* (IC50 factor Xa: 1.3nM)
(IC50 thrombin: >100μM)
Examples for libraries synthesized on solid-phase: peptides: split-mixed technology

Application of the split-mixed method for discovery of Factor Xa inhibitors

Synthesis of a octa-peptide library by split-mixed synthesis and colorimetric assay on bead:

AP: alkaline phosphatase de-phosphorylates 5-bromo-4-chloro-3-indolyl phosphate forming a blue precipitate, which stains the beads.
Examples for libraries synthesized on solid-phase: peptides: split-mixed technology

Application of the split-mixed method for discovery of Factor Xa inhibitors

Synthesis of an octa-peptide library by split-mixed synthesis and colorimetric assay on bead:

All active compounds contained Tyr-Ile-Arg at the N-terminus

H-Tyr-Ile-Arg-Leu-Ala-Ala-Phe-Thr-NH$_2$ (SEL1691; IC$_{50}$: 4-15µM)  \( \rightarrow \)

SEL2316 (IC$_{50}$: 80nM)

Drug Discovery Today 1998, 3, 223

\[ \text{SEL2489 (IC$_{50}$: 25nM; half-life in rats and rabbits 8 to 10 minutes)} \]
Examples for libraries synthesized on solid-phase: peptides: split-mixed technology

Application of the split-mixed method for discovery of Factor Xa inhibitors

SEL2316 \((\text{IC}_{50}: 80\text{nM})\)

SEL2489 \((\text{IC}_{50}: 25\text{nM}; \text{half-life in rats and rabbits 8 to 10 minutes})\)

SEL2602 \((\text{IC}_{50}: <25\text{nM}; \text{improved half-life})\)

SEL2602 \((\text{IC}_{50}: 285\text{nM})\)
Examples for libraries synthesized on solid-phase: inhibitors of protein-protein interaction

Characteristic of large surface protein-protein interactions

• Fundamental to the functioning of biological systems
  – many proteins function as part of complexes
  – cell to cell signalling
  – cell adhesion
  – long distance communication (hormones)
• Specific inhibition offers important therapeutic potential:

• Generally form across a large area of interacting surfaces: 700-1300 A² average
• High binding energy
• Difficult to inhibit with small molecules? Small molecule discovery approaches have largely failed
• Antibodies and fusion proteins (biopharmaceuticals) have emerged as important drugs:
  however, these act only on extracellular targets
• Slow to mature: initial binding is thought to occur through “hotspots” in selected areas
6.5. Examples for libraries synthesized on solid-phase: inhibitors of protein-protein interaction

average contact surface area in protein-protein interactions: 600-900 Å²


Hotspots

O-Rings

topology of the hotspots determine specificity
Examples for libraries synthesized on solid-phase: inhibitors of protein-protein interaction

Extracellular GH-receptor

$K_d \sim 0.3 \text{ nM}$

Buried surface on each protein

$\sim 1300 \text{ Å}^2$

Examples for libraries synthesized on solid-phase: inhibitors of protein-protein interaction

Petidic $\alpha$-helix mimetics as inhibitors of protein-protein interactions

Dr. Sjoerd Wadman

•~40% of all HTS campaigns in GSK were targeted to find small PPI inhibitors in 1998
•Very low success rate
•Many assays suitable for HTS developed

•Most were “shelved” during portefolio review
•Addressed one important target with full resource
Examples for libraries synthesized on solid-phase: inhibitors of protein-protein interaction

**Oncostatin M**
- 4-Helical Cytokine
- Pro-inflammatory hormone
- Therapeutic applications:
  - Rheumatoid Arthritis
  - Asthma
- Interacts with 7TM receptor
- Part of a large family of important proteins
### Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis

#### 4. Combinatorial Synthesis of Biopolymers

Examples for libraries synthesized on solid-phase: four helix bundle proteins

<table>
<thead>
<tr>
<th>Family</th>
<th>Long Chain 4-helix bundle</th>
<th>Short Chain 4-helix bundle</th>
<th>Dimeric-dimeric 4-helix bundle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth Hormone</strong></td>
<td>Growth Hormone</td>
<td>IL-2</td>
<td>IL-10</td>
</tr>
<tr>
<td>Prolactin</td>
<td>Prolactin</td>
<td>IL-4</td>
<td>IFN-G</td>
</tr>
<tr>
<td>IL-6</td>
<td>IL-6</td>
<td>IL-13</td>
<td>IFN-B</td>
</tr>
<tr>
<td>IL-3</td>
<td>IL-3</td>
<td>IFN-a</td>
<td></td>
</tr>
<tr>
<td>IL-7</td>
<td>IL-7</td>
<td>IL-5</td>
<td></td>
</tr>
<tr>
<td>LIF</td>
<td>LIF</td>
<td>GM-CSF</td>
<td></td>
</tr>
<tr>
<td>OSM</td>
<td>OSM</td>
<td>M-CSF</td>
<td></td>
</tr>
<tr>
<td>CNTF</td>
<td>CNTF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDF</td>
<td>CDF</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Examples for libraries synthesized on solid-phase: four helix bundle proteins
Examples for libraries synthesized on solid-phase: four helix bundle proteins

Note side-on interactions of $\alpha$-helices
Examples for libraries synthesized on solid-phase: four helix bundle proteins

-α-helices cluster with hydrophobic residues pointing at the inside (red) whereas hydrophilic residues (yellow) are located at the outside.

-challenge:
  inhibit formation of 4-helix bundle formation by interacting with the helical monomers.
Examples for libraries synthesized on solid-phase: four helix bundle proteins

- Helix side-chains are arranged like the steps on a spiral staircase
- Regular distance
- Regular angle
- Model potential antagonists and pick the ones that fit the model best
- Aimed to antagonise “side-on” \( \alpha \)-helix interactions through 3 side-chains
- Large - 100k compounds
- Non-peptidic
- Split - mix synthesis on solid phase
- Fully Encoded / Partial Release Technology
- 384 screening format
Examples for libraries synthesized on solid-phase: four helix bundle proteins

Amino acid-like side-chains

Spacers hold side chains in correct orientation
Examples for libraries synthesized on solid-phase: four helix bundle proteins

1. Propose Connectivity and potential monomers
2. Model compounds proposed library
3. Take best connectivity and best monomers
4. Measure fit against Helix Vector Model
Examples for libraries synthesized on solid-phase: four helix bundle proteins

Amines  Amino acids  α-Amino acids  Amino acids  Amines
14        7           14         7             14

Tags required
4         3           4          3             3

Total number of compounds: 134'456
Examples for libraries synthesized on solid-phase: four helix bundle proteins

amines
neutral

acidic

basic

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6.5. Examples for libraries synthesized on solid-phase: four helix bundle proteins

Core 1 amino acids

- HOOC\text{NH\text{Fmoc}}\text{H}_2\text{C}_5\text{H}
- HOOC\text{H}_2\text{C}_6\text{H}
- HOOC\text{H}_2\text{C}_7\text{H}
- HOOC\text{H}_2\text{C}_8\text{H}
- HOOC\text{NH\text{Fmoc}}\text{H}_2\text{C}_9\text{H}
- HOOC\text{NH\text{Fmoc}}\text{H}_2\text{C}_{10}\text{H}_2\text{O}_2\text{Me}
- HOOC\text{NH\text{Fmoc}}\text{H}_2\text{C}_{11}\text{H}_2\text{O}_2\text{Me}
- HOOC\text{NH\text{Fmoc}}\text{H}_2\text{C}_{12}\text{H}_2\text{O}_2\text{Me}

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Examples for libraries synthesized on solid-phase: four helix bundle proteins

\(\alpha\)-Amino acids

- Gly
- Ala
- Val
- Leu
- Phe
- Tyr
- Ser
- Thr
- Met
- Asp
- Glu
- Arg
- Hyp (hydroxyproline)
Examples for libraries synthesized on solid-phase: four helix bundle proteins

Core 2: Diacids (Anhydrides)
Examples for libraries synthesized on solid-phase: four helix bundle proteins

Major conformers closely match \( \alpha \)-helix in side-chain display vectors
Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis

4. Combinatorial Synthesis of Biopolymers

Examples for libraries synthesized on solid-phase: four helix bundle proteins

Concepts

- Split Mix synthesis
- Library encoding
- Differential release
- Single Bead screening

<table>
<thead>
<tr>
<th>Building Blocks</th>
<th>Products in Pools</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>27</td>
<td>9</td>
</tr>
</tbody>
</table>

1 bead = 1 compound
Examples for libraries synthesized on solid-phase: four helix bundle proteins

Codes for each building block  Building Blocks
Examples for libraries synthesized on solid-phase: four helix bundle proteins

Affimax encoding strategy

- Codes are different amines
- Cleaved with cHCl
- Dansylate and analyse by hplc

Product on acid- or photolabile linker
Examples for libraries synthesized on solid-phase: four helix bundle proteins

Differential release

50% on acid labile linker  Product can be released twice at different times
50% on photolabile linker

Product
Examples for libraries synthesized on solid-phase: four helix bundle proteins

**Single bead screening**

1. Acid cleave
2. Screen pools

Active pool

3. Plate out individual beads
4. Photocleave
5. Screen single beads

Active bead

**Cleave Tag from bead** → Identify active molecule
Examples for libraries synthesized on solid-phase: four helix bundle proteins

Single bead screening

- Compounds prepared on Tentagel
- Reactions done on an ACT synthesis robot
- All building blocks were “rehearsed”
- Analysis throughout
  - 1st stage by magic angle nmr
  - Later stages by lc/ms and tag reading
  - Lc/ms aided using “analytical constructs”
- All done by one chemist in 5 months
Examples for libraries synthesized on solid-phase: four helix bundle proteins

Resin differentiation

- Amino Tentagel
- BocNH
- FmocNH
- DIC, HOBT, DMF
- FmocNH
- NHBoc
- DIPEA, DMF
- H2N
- NH
- Code1
- Aloc
- Code1
- 95% TFA
- Photolinker
- Acid Linker
- Aloc
- Code1
- Aloc
- Code1
- DIPEA, HATU, DMF
- Photolinker
- Acid Linker
- DIPEA, HATU, DMF
- 95% TFA
Examples for libraries synthesized on solid-phase: four helix bundle proteins
Examples for libraries synthesized on solid-phase: four helix bundle proteins

Reductive amination

\[
\text{Me}_4\text{NBH(OAc)}_3, \text{AcOH, DMF}
\]
Examples for libraries synthesized on solid-phase: four helix bundle proteins

1) Split/mix
2) Code deprotection
3) Codecoupling
4) Fmoc deprotection
5) Pyridine
Examples for libraries synthesized on solid-phase: four helix bundle proteins

Library synthesis 2

1) Split/mix
2) Code deprotection
3) Codecoupling
4) pyridine, DMF
5) DIPEA, DMF
6.5. Examples for libraries synthesized on solid-phase: four helix bundle proteins
Examples for libraries synthesized on solid-phase: four helix bundle proteins

**Screening of library**

- Primary screen: 168 x 96 wells / ~30 beads per well
- 42 plates in 384 format
- Half of acid-cleaved material used
- Screening concentration ~ 2mM/component

**Histogram of 1ry screening data for GL1495 in OSM**
Examples for libraries synthesized on solid-phase: four helix bundle proteins

Screening results

-21 sub-micromolar hits re-made as discretes
-5 Compounds potent and selective
-17 also inhibit TNF in same cell line: signalling inhibitors?

<table>
<thead>
<tr>
<th>Source</th>
<th>Number</th>
<th>Hits</th>
<th>Leads</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSK compound collection</td>
<td>250.000</td>
<td>3134</td>
<td>0</td>
</tr>
<tr>
<td>Natural product extracts</td>
<td>70.000</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Aptamers</td>
<td>2000.000</td>
<td>78</td>
<td>13</td>
</tr>
<tr>
<td>Apha helix library GL1495</td>
<td>134.456</td>
<td>21</td>
<td>5</td>
</tr>
</tbody>
</table>
Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis
4. Combinatorial Synthesis of Biopolymers

Examples for libraries synthesized on solid-phase: four helix bundle proteins

Acknowledgements

-Chemistry: Helen Jenkins
-Biology: Paul Life, John Spaul
-Screening: Liz Clarke, Sandra Arpino
-Modelling: Darren Green

+ many others

And Dr. Sjoerd Wadman
Library synthesis planning

Steps required for the design and synthesis of a library

1. **Planning** (literature search and retrosynthetic analysis of the problem)

2. **Synthesis strategy** (linear, convergent, multicomponent reactions, tandem reactions...)

3. **Building blocks** (commercial or self-made)

4. **Parallel or combinatorial synthesis** (in solution; in solution by aid of solid-supported reagents; on solid supports)

5. **Parallel work-up** (two phases: aqueous, organic, fluoruos; solid-phase extraction)

6. **Purification**: parallel flash chromatography; high-throughput HPLC coupled to MS on normal and reversed phase

7. **Analysis, stability and storage of products**
5. Strategies for the Synthesis of Small Molecule Libraries

Synthesis strategies: introduction

- **Exit vectors**: determine the relative orientation of the high and low variation substituents and thus the overall shape of the final molecule.

  - **Scaffold**: MG~290; for the substituents remain MG~210

- **Diversity associated with scaffolds**: "vertical diversity"; diversity associated with substituents: "horizontal diversity"

- The synthetic strategies generally do not permit simultaneous high variation of substituents \( R^1 - R^3 \); rather sub-libraries (e.g. A and B) are planned; also SAR data often show that not all substituents are equally important for biological activity.
Synthesis strategies: convergent, multi-step

Multi-step synthesis of advanced building blocks (scaffold) by linear or convergent synthetic strategies and parallel conversion into final products.
5. Strategies for the Synthesis of Small Molecule Libraries

Synthesis strategies: classical multi-component approach

Synthesis of advanced building blocks (scaffold) using multi-component reactions and parallel conversion into final products

\[
\begin{align*}
R^1\text{NH} & + R^2\text{N}=\text{C}=\text{S} & \rightarrow & \text{building blocks} & \rightarrow & \text{final products} \\
\text{NH}_2 & + & & & & \\
\text{R}^3\text{O} & \text{Br} & & & & \\
\end{align*}
\]
Synthesis strategies: classical multi-component approach

Classical multi-component reactions (MCR’s) are have in common that components (e.g. A, B, C) react in a reversible way to a reactive intermediate, which reacts in an irreversible way to the product. Thus, the sequence by which the components are added does not affect product formation.

- The best known MCR’s are the following: Ugi, Passerini, Biginelli, Strecker, Hantzsch, Mannich etc.

- Reactions can be ideally performed in a matrix format

- Classical MCR’s generally yield generally the same scaffold
The classical multi-component reactions are ideally suited for parallel synthesis, however, they yield generally the same scaffold (limited scaffold diversity)
Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis

5. Strategies for the Synthesis of Small Molecule Libraries

Synthesis strategies: classical multi-component approach

**Passerini 3-MCR**

\[
R^1\text{COOH} + R^2\text{R} + R^4\text{N}=C \rightarrow \begin{array}{c}
\text{O} \\
\text{O} \\
\text{R}^1 \\
\text{R}^2 \\
\text{R}^3 \\
\text{R}^4 \\
\text{H}
\end{array}
\]


**Strecker synthesis**

\[
\text{RCHO} + \text{NH}_3 + \text{HCN} \rightarrow \begin{array}{c}
\text{NH}_2 \\
\text{R} \\
\text{CN}
\end{array}
\]


**Bucherer-Bergs** variation of the Strecker synthesis

\[
R^1\text{CO}\text{R}^2 + \text{KCN} + (\text{NH}_4)_2\text{SO}_4 \rightarrow \begin{array}{c}
\text{N} \\
\text{R}^1 \\
\text{R}^2 \\
\text{H}
\end{array}
\]

Synthesis strategies: mechanism of the *Passerini* 3-MCC reaction

\[
\begin{align*}
R^1\text{COOH} & \quad R^2\text{CHO} & \quad R^3\text{N=C} \\
A & \quad B & \quad C
\end{align*}
\]

\[
\text{Passerini 3MCR}
\]

\[
R^1\text{O} - \text{O} - R^2\text{H} + R^3\text{N} - \text{H}^+
\]

**reactive intermediate**
Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis
5. Strategies for the Synthesis of Small Molecule Libraries

Synthesis strategies: classical MCRs

Modified Passerini 3-MCR

Synthesis strategies: classical MCRs

Hantzsch MCR's

\[
\begin{align*}
R^1\text{C}O\text{NH}_2 + R^2\text{Br}R^3 & \rightarrow R^1\text{S}R^2\\
R^1\text{N}S\text{NH}_2 + R^2\text{Br}R^3 & \rightarrow R^1\text{N}R^2\\
R^1\text{COOR}^2 + \text{NH}_3 + R^3\text{C}O\text{X} & \rightarrow R^1\text{P}R^2\\
2 \times R^1\text{COOR}^2 + \text{NH}_3 + R^3\text{CHO} & \rightarrow R^1\text{OOC}R^2
\end{align*}
\]

Synthesis strategies: Classical MCR’s

Erlemeyer azlactone synthesis

\[
\text{R}_1^\text{N}C\text{O}_\text{HCOOH} + \text{Ac}_2\text{O} + \text{R}_2^\text{CHO} \xrightarrow{\text{NaOAc}} \text{R}_1^\text{N}C\text{O}_\text{O} \]

3-MCR involving a ü1,3'-dipolar cycloaddition

\[
\text{R}_1^\text{CHO} + \text{MeOOCC}_{\text{N}}\text{H}_{\text{N}}\text{MeOOC}_{\text{O}} + \text{N} \xrightarrow{} \text{N} \]

Synthesis strategies: classical MCRs

**Mannich 3-MCR**

\[
\begin{align*}
R^1NHR^2 + CH_2O + R^3CONR^4 \rightarrow R^3CONR^2 + R^1NHR^2 + CH_2O + \text{Indole} \rightarrow \text{Indole}
\end{align*}
\]

C. Mannich et al. *Arch. Pharm.* **1921**, 250, 647

**Biginelli 3-MCR**

\[
\begin{align*}
\text{H}_2\text{NCONH}_2 + R^1\text{CHO} + \text{RCOOR}^3 \rightarrow \text{R}^1\text{COOR}^3 + \text{R}^2\text{NHCONH}_2 + \text{RCOOR}^4 \rightarrow \text{R}^2\text{NHCONH}_2 + \text{R}^3\text{COOR}^4
\end{align*}
\]


**Biginelli 3-MCR (Atwal variation)**

\[
\begin{align*}
\text{R}^1\text{S}NH \ + R^2\text{CHO} + \text{RCOOR}^4 \rightarrow \text{R}^2\text{COOR}^4 + \text{R}^1\text{S}NH \ + R^3\text{CHO} + \text{RCOOR}^3 \rightarrow \text{R}^3\text{COOR}^3
\end{align*}
\]
Synthesis strategies: classical MCR’s

Grieco’s 3-MCR

\[
\begin{align*}
\text{COOH} & + \text{CHO} + \text{H}_2\text{C} = \text{C} = \text{H} & \rightarrow & \text{COOH} \\
\text{NH}_2 & + \text{CH} = \text{CH} & \rightarrow & \text{COOH}
\end{align*}
\]

[4+2]


Pauson-Khand MCR

\[
\begin{align*}
R^1 & \equiv R^2 + R^3 & \equiv R^4 & + \text{CO} & \rightarrow & \text{R}^1 & \equiv R^2 & \equiv R^3 & \equiv R^4
\end{align*}
\]

Synthesis strategies: classical MCRs: applications

- **Nifedipine** is a widely used anti-hypertensive drug (is off patent now). It belongs to the Ca\(^{2+}\) channel blockers (other include: **Verapamil-type**, **Dilthiazem-type**)

- It can be produced in a **Hatsch**-type 3-MCR in a very efficient and cheap way.
Medicinal Chemistry : Combinatorial Chemistry-Parallel Synthesis
5. Strategies for the Synthesis of Small Molecule Libraries

Synthesis strategies: application of the Ugi 4-MCR: genetic algorithm

\[
\begin{align*}
R^4\text{COH} & + R^2\text{H} \\
R^3\text{NH}_2 & + R^1\text{N}=C
\end{align*}
\rightarrow
\begin{align*}
R^4\text{N} & \text{R}^2\text{NH}^R^1 \\
\text{R}^3\text{NH}^R^1 & + \text{R}^2\text{H}
\end{align*}
\]


**Genetic algorithms** constitute an interesting approach for efficient optimization of multiparameter systems

*Parameters*: inputs acids, isocyanates, aldehydes, amines; biological activity (inhibition of thrombin)

*Genetic operations*: replication, mutation and crossover

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Medicinal Chemistry : Combinatorial Chemistry-Parallel Synthesis
5. Strategies for the Synthesis of Small Molecule Libraries


\[
\begin{align*}
4 (40) + R^3NH_2 (10) + R^1N=C (10) & \rightarrow 5 (160'000) \\
\text{bit pattern} & \\
& + 4 \text{ amines}
\end{align*}
\]

1\textsuperscript{st} generation: random selection of 20 bit patterns: synthesis

2\textsuperscript{nd} generation: generated by entering first 20 bit patterns into the genetic algorithm which by means of crossover and mutations generated the next 20 bit patterns: synthesis and biological testing of all 40 compounds

3\textsuperscript{rd} generation: the 20 most active compounds (bit patterns) were again entered into the genetic algorithm which generated the next generation: synthesis and testing

after 16 cycles, the average effective inhibitory concentration (EC\textsubscript{50}) of the 20 best compounds was submicromolar
Synthesis strategies: application of the Asinger-Ugi 6-MCR: Penicillin derivatives

1. Synthesis of precursor molecules
   - Pht\(_{\text{O}}\)CHO
   - Br\(_{\text{CH}}\)CHO
   - NaSH

2. Asinger reaction
   - 1) Asinger
   - 2) Hydrolysis

3. Ugi reaction
   - C\(_{6}H_{11}\)N=C

4. Product: Penicillin derivatives
Synthesis strategies: application of the Ugi reaction: Inhibitors of IAPs

- Apoptosis (programmed cell death) is an essential part of normal homeostasis (self-regulation). Evasion of apoptosis by cells is one of the hallmarks of cancer (D. Hanahan et al. *Cell* 2000, 100, 57-70);
- Inhibitors of apoptosis (IAP’s) are a family of proteins (8 members in human) that inhibit caspases, important proteases which are involved in apoptosis.
- The second mitochondria-derived activator of caspases (Smac) protein is an endogenous dimeric proapoptotic antagonist of XIAP, which is important in melanoma. A tetrapeptide sequence Ala-Val-Pro-Ile of Smac binds to XIAP (via a BIR domain) and inhibits important caspases. Mimetics of Ala-Val-Pro-Ile have been designed and synthesized as potential anti-cancer agents.

**Ala-Val-Pro-Ile motif**

**Genentech (GDC-0152)**

**Genentech (Smac mimetic)**


M. Vamos et al. *ACS Chem. Biol.* 2013, 8, 725-732
Medicinal Chemistry : Combinatorial Chemistry-Parallel Synthesis
5. Strategies for the Synthesis of Small Molecule Libraries

Synthesis strategies: application of the Ugi reaction: Inhibitors of IAPs

\[ \text{1} \xrightarrow{\text{i}} \text{2} \rightarrow \text{3} \xrightarrow{\text{ii}, \text{iii}} \text{4} \]

\[ \text{5} \xrightarrow{\text{i}: 1, 2, 3, 4, TFE, microwave, 80^\circ; \text{then TFA 8-10 equiv.), DCM, 32^\circ; \text{ii}: Boc-N-Me-Ala, HOBt, EDC, NMM, THF; \text{iii}: TFA 8-10 equiv.), DCM, 32^\circ}} \]

M. Vamos et al. ACS Chem. Biol. 2013, 8, 725-732
Questions

1. Please name three classical multi-component reactions (MCR‘s)?

2. Give possible products of the following MCR‘s

\[ \text{a) } \begin{align*} &\text{COOH} \\
&\text{NO}_2 \\
&\text{CHO} \\
&\text{NH}_2 \end{align*} + \begin{align*} &\text{C}_{\text{H}} \\
&\text{O} \end{align*} + \begin{align*} &\text{N=C} : \end{align*} \]

\[ \text{MeOH} \rightarrow 50^\circ \]

\[ \text{b) } \begin{align*} &\text{Cl} \\
&\text{NH}_2 \\
&\text{CHO} \end{align*} + \begin{align*} &\text{S} \\
&\text{C} \end{align*} + \begin{align*} &\text{C}_{\text{H}} \end{align*} \]

\[ \text{CF}_3\text{COOH} \rightarrow \text{CH}_2\text{Cl}_2 \]
Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis
5. Strategies for the Synthesis of Small Molecule Libraries

Synthesis strategies: sequential multi-component reactions (SMCRs)

- In sequential multi-component reactions (SMRC’s) components (e.g. A, B, C) are added in a sequential way to the reaction mixture. Thus, reaction of A + B form irreversibly intermediate A-B which is subsequently reacted with C to form the product A-B-C. By changing the sequence of component addition theoretically 6 different product types (scaffolds) can be obtained.

- The SMCR’s offer the same advantages as the classical MCR’s, but in addition they have the potential to generate different scaffolds.
Medicinal Chemistry : Combinatorial Chemistry-Parallel Synthesis
5. Strategies for the Synthesis of Small Molecule Libraries

Synthesis strategies: sequential multi-component reactions

Sequential multi-component reactions (MCR’s) offer the same advantages as the classical MCR’s: in addition several different scaffolds can be obtained employing the same set of building blocks

**Medicinal Chemistry : Combinatorial Chemistry-Parallel Synthesis**  
5. Strategies for the Synthesis of Small Molecule Libraries

**Synthesis strategies: sequential multi-component reactions**

<table>
<thead>
<tr>
<th>bis-donors</th>
<th>bis-acceptors</th>
<th>acceptor-donors</th>
<th>electrophiles</th>
<th>nucleophiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂N⁻S⁻NH⁺R¹</td>
<td>Cl⁻O⁻N⁺C⁻O⁻</td>
<td>R⁷⁻N⁺C⁻S⁻</td>
<td>R⁹⁻X</td>
<td>R¹⁰⁻NH₂</td>
</tr>
<tr>
<td>H₂N⁻S⁻NH⁻NH₂</td>
<td>Br⁻O⁻C⁻O⁻</td>
<td>R⁸⁻N⁺C⁻O⁻</td>
<td>(X: Cl, Br, I)</td>
<td></td>
</tr>
<tr>
<td>H₂N⁻S⁻NH⁻NH⁻NH⁻M</td>
<td>O⁻H⁻O⁻</td>
<td>R⁹⁻X</td>
<td>(X: Cl, Br, I)</td>
<td></td>
</tr>
</tbody>
</table>

(X: Cl, Br)
Medicinal Chemistry : Combinatorial Chemistry-Parallel Synthesis
5. Strategies for the Synthesis of Small Molecule Libraries

Synthesis strategies: SMCRs: diversity-oriented synthesis (DOS)

Synthesis strategies: SMCRs: diversity-oriented synthesis (DOS)

Synthesis strategies: SMCRs: diversity-oriented synthesis (DOS)
**Synthesis strategies: Sequential multi-component reactions**

1. **Reagents and Conditions:**
   - **i:** DIPEA, DMF
   - **ii:** m-CPBA, CH₂Cl₂
   - **iii:** R·NH₂ (15), dioxane, 80-100°C [8].

2. **References:**
### Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis

5. Strategies for the Synthesis of Small Molecule Libraries

**Synthesis strategies: SMCRs: diversity-oriented synthesis (DOS)**

<table>
<thead>
<tr>
<th>bis-donors</th>
<th>bis-acceptors</th>
<th>acceptor-donors</th>
<th>electrophiles</th>
<th>nucleophiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (\text{H}_2\text{N} \text{S} \text{NH}R^1)</td>
<td>4 (\text{R}^+ \text{S}^{\text{+}} \text{NH}_2 \text{X}^-)</td>
<td>7 (\text{Cl}^\text{N} \text{C} = \text{O})</td>
<td>12 (\text{R}^7 \text{N} \text{C} = \text{S})</td>
<td>(\text{R}^{10} \text{N} \text{H}_2)</td>
</tr>
<tr>
<td>2 (\text{H}_2\text{N} \text{S} \text{NNNH}_2)</td>
<td>5 (\text{R}^+ \text{S}^{\text{+}} \text{NNNH}_2)</td>
<td>8 (\text{Br} \text{CO} \text{R}^2)</td>
<td>13 (\text{R}^8 \text{N} \text{C} = \text{O})</td>
<td></td>
</tr>
<tr>
<td>3 (\text{H}_2\text{N} \text{S} \text{NNN})</td>
<td>6 (\text{R}^+ \text{S}^{\text{+}} \text{NNN})</td>
<td>9 (\text{OH} \text{CO} \text{R}^3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>((\text{X}: \text{Cl, Br}))</td>
<td></td>
<td>10 (\text{R}^4 \text{C} = \text{C} \text{R}^5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>11 (\text{R}^6 \text{COOMe})</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Medicinal Chemistry : Combinatorial Chemistry-Parallel Synthesis
5. Strategies for the Synthesis of Small Molecule Libraries

Synthesis strategies: SMCRs: diversity-oriented synthesis (DOS)

\[ R^2 \text{NH}_2, X^- \quad \text{+} \quad \text{R}^7\text{N}=\text{C}=\text{S} \quad \text{i} \quad \text{[R-S]} \quad \text{NH} \quad \text{S} \quad \text{NH} \quad \text{R}^7 \quad \text{Br} \quad \text{O} \quad \text{R}^2 \quad \text{-RCH}_2\text{SH} \]

\[ \text{DBU, DMF, 0\degree; then DBU and 8, r.t.} \]

D. Obrecht, P. Ermert, 5th International conference on Synthetic Organic Chemistry (ECSOC-5); [www.mdpi.org/ecsoc-5/]
September 1-30, 2001, [B0005]
Synthesis strategies: Diversity-oriented synthesis (DOS)

- During 1995-2005 large small molecule libraries were synthesized exhibiting limited 3D-diversity.
- Large combinatorial libraries have many linear (cigarette-shape) and flat (disc-shape) molecules of limited 3D shape diversity.
Synthesis strategies: Diversity-oriented synthesis
Fragment-based domain shuffling approach

Synthesis strategies: Diversity-oriented synthesis
Fragment-based domain shuffling approach

Scheme 3. Parallel solution-phase synthesis of pyran-containing macrocycles ABC-5 and ACB-5.
E. Comer et al. PNAS 2011, 108, 6751-6756
Synthesis strategies: Fragment-based lead discovery

A. Identification of fragments:
Weak binders mM to 30μM are identified (e.g. F1-3)

B: Fragment evolution:
- An initial fragment is optimized (e.g. F1 to F1’ and F2 to F2’)

C. Fragment linking:
- Two or more fragments, which bind to proximal parts of the active site, are joined together
  - very challenging

Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis

5. Strategies for the Synthesis of Small Molecule Libraries

Synthesis strategies: Dynamic Combinatorial Synthesis

\[
\text{H}_2\text{N}^\prime\text{NH}_2\text{NHAc} + \text{R}^1\text{R}^2\text{O} \rightleftharpoons \text{R}^1\text{R}^2\text{N}^\prime\text{NH}_2\text{NHAc}
\]

neuraminidase

\[
\text{R}^1\text{R}^2\text{N}^\prime\text{NH}_2\text{NHAc} \rightleftharpoons \text{NaCNBH}_3
\]

"amplifications"

amplification = 84
Ki = 700nM

amplification > 30
Ki = 85nM (strongest binder)

Synthesis strategies: Dynamic Combinatorial Synthesis: disulfide tethering

**best R series:** $S^2-S-O-N^+A-B-A-B-C$  
A, B, C: H, CO$_2$H, CO$_2$Me or MeO

improve design of a known inhibitor with tethering "hit"

existing inhibitor  
$IC_{50} = 3 \ \mu M$

improved inhibitors  
$IC_{50} = 0.2 \ \mu M$

Synthesis strategies: Click chemistry

Click Chemistry: Diverse chemical function from a few good reactions


Development of a set of powerful reactions for the rapid synthesis of useful new compounds and combinatorial libraries through heteroatom links (C-X-C); an approach called Click Chemistry.

Reactions that have a high thermodynamic driving force, usually greater than 20 kcal/mol

- Cycloadditions ([1,3]-dipolar additions; Diels-Alder reactions)
- Nucleophilic Substitution reactions on strained heterocyclic electrophiles
- Carbonyl Chemistry of the non-Aldol-type: synthesis of ureas, thioureas, aromatic heterocycles, oxime ethers
- Addition reactions to C-C carbon multiple bonds: epoxidations, aziridinations, dihydroxylations
Synthesis strategies: Click chemistry

Synthesis strategies: Click chemistry

[1,3]-Dipolar additions of acetylenes and azides

\[
\text{Cu (turnings)} \quad (\text{ca 1g}) \\
\text{H}_2\text{O/tBuO (2:1)} \\
\text{RT, 24h} \\
\text{CuSO}_4 (\text{cat.}) \\
\text{(10 Mol\%)} \\
\rightarrow \\
3.7g \ (95\%) \quad \text{white solid}
\]

Synthesis strategies: application of Click chemistry

\[
\begin{align*}
\text{Cu (turnings)} & \quad \text{(ca 1g)} \\
H_2O/tBuO(2:1) & \quad \text{(50ml)} \\
\text{RT, 24h} & \\
\text{CuSO}_4(\text{cat.}) & \quad \text{(10Mol\%)}
\end{align*}
\]

Ki: 62nM; inhibition of fucosyl transferase
cancer metastasis; lymphocyte trafficking


Dramatic rate acceleration of the azide-alkyne cycloaddition by sequestering the two components inside the host structure (enzyme or receptor)
Emerging resistance in clinical isolates of bacteria render existing antibiotics such as Neomycin and Ciprofloxacin inactive. Enzymes such as aminoglycoside 3′-phosphotransferases inactivate 3′ position in aminoglycoside antibiotics by phosphorylation. Combination of two antibiotics has emerged as a valuable strategy to overcome rapid resistance mechanisms.
Synthesis strategies: application of Click chemistry

- Biological activities (MICs) depended significantly on the variable spacer groups X and Y
- Best combinations were X = -(CH$_2$)$_2$- and Y = -CH$_2$OCH$_2$-
- MIC (minimal inhibitory concentration):
  - E. coli (R477-100): 3μg/ml
  - E. coli (ATCC 25922): 3μg/ml
  - E. coli (AG100A): 0.38μg/ml
  - B. subtilis (ATCC 6633): 0.75μg/ml
Synthesis strategies: application of Click chemistry

Summary of fragment-based approaches:

-fragment libraries are smaller: few hundreds to thousands

-screening effort smaller; however, weak binders have to be detectable

-leads derived from fragments are often smaller; allows more extensive optimization

-fragments can be assembled in a thermodynamically or kinetically controlled fashion: dynamic combinatorial synthesis

-fragments can be assembled using click chemistry

-finding the appropriate linkers to assemble fragments is a big challenge
Most important building blocks (toolbox) used in parallel and combinatorial synthesis

Systematic enumeration of key heteroaromatic reagent classes from commercially available sources which have been used in medicinal chemistry programs

Most important reactions used in parallel and combinatorial synthesis

**Formation d’amides et d’urées:**

- $\text{BB} \text{COOH} \rightleftharpoons \text{BB} \text{CONH}(\text{R})\text{RHV}$
- $\text{BB} \text{NH}_2 \rightleftharpoons \text{BB} \text{NH}_2\text{CONH}(\text{R})\text{RHV}$

**Couplage Suzuki:**

- $\text{BB} \text{X} \rightleftharpoons \text{BB} \text{Ar}$

**Réduction au diborane:**

- $\text{BB} \text{CONH}(\text{R})\text{RHV} \rightleftharpoons \text{BB} \text{NRH}^\text{HV}$

**Amination réductrice:**

- $\text{BB} \text{CHO} \rightleftharpoons \text{BB} \text{CONH}(\text{R})\text{RHV}$

**Alkylation du groupe thiol:**

- $\text{R}^1\text{-SH} + \text{Br}\text{CH}_2\text{R}^2 \rightarrow \text{base} \rightarrow \text{R}^1\text{S}\text{CH}_2\text{R}^2$
Chemical Biology: Combinatorial Chemistry-Parallel Synthesis
2.5. Parallel reactions

Most important reactions used in parallel and combinatorial synthesis

Substitution nucleophilique:

\[
\begin{align*}
BB & \\
\text{N} & \\
\text{X} & \\
\end{align*} \\
\begin{align*}
\text{N} & \\
\text{NH(R)} & \\
\text{NH(R)} & \\
\end{align*}
\]

Alkylation de NH activés:

\[
\begin{align*}
R^1 & \\
\text{H} & \\
\text{N} & \\
\text{R} & \\
\end{align*} \\
\begin{align*}
\text{R} & \\
\text{R}^2 & \\
\text{N} & \\
\text{O} & \\
\end{align*} \\
\begin{align*}
\text{R} & \\
\text{O} & \\
\text{N} & \\
\text{H} & \\
\end{align*} \\
\begin{align*}
\text{R} & \\
\text{R}^2 & \\
\text{R}^3 & \\
\end{align*}
\]

Réaction de Mitsunobu:

\[
\begin{align*}
R^1 & \\
\text{OH} & \\
\text{R} & \\
\end{align*} \\
\begin{align*}
\text{OH} & \\
\text{R} & \\
\end{align*} \\
\begin{align*}
\text{N} & \\
\text{R} & \\
\end{align*} \\
\begin{align*}
\text{O} & \\
\text{R} & \\
\end{align*} \\
\begin{align*}
\text{N} & \\
\text{O} & \\
\end{align*} \\
\begin{align*}
\text{R}^1 & \\
\text{OH} & \\
\text{R} & \\
\end{align*} \\
\begin{align*}
\text{OH} & \\
\text{R} & \\
\end{align*} \\
\begin{align*}
\text{N} & \\
\text{R} & \\
\end{align*} \\
\begin{align*}
\text{O} & \\
\text{R} & \\
\end{align*} \\
\begin{align*}
\text{N} & \\
\text{O} & \\
\end{align*}
\]

Réaction de Mannich:

\[
\begin{align*}
R^1 & \\
\text{OH} & \\
\text{R} & \\
\end{align*} \\
\begin{align*}
\text{OH} & \\
\text{R} & \\
\end{align*} \\
\begin{align*}
\text{N} & \\
\text{R} & \\
\end{align*} \\
\begin{align*}
\text{O} & \\
\text{R} & \\
\end{align*} \\
\begin{align*}
\text{N} & \\
\text{O} & \\
\end{align*} \\
\begin{align*}
\text{R}^1 & \\
\text{OH} & \\
\text{R} & \\
\end{align*} \\
\begin{align*}
\text{OH} & \\
\text{R} & \\
\end{align*} \\
\begin{align*}
\text{N} & \\
\text{R} & \\
\end{align*} \\
\begin{align*}
\text{O} & \\
\text{R} & \\
\end{align*} \\
\begin{align*}
\text{N} & \\
\text{O} & \\
\end{align*}
\]
Questions

1. Please name five efficient reactions that can be used for final parallel derivatization?

2. Please name potential advantages of fragment-based lead discovery over screening large combinatorial libraries?

3. What is the rule of 3?
Parallel work-up procedures

Extractions: principle

Liquid-liquid extractions

Solid-phase extractions

Solid-supported scavengers

Ion-exchange resins

Fluorous phase extractions
Parallel work-up procedures: principle

1. Two phase extractions: manual extraction

- Upper phase: contains product (EtOAc or fluorous phase): separated manually
- Lower phase: contains impurities (aqueous phase)

2. Two phase extractions: robotic system (style Tecan)

- Upper phase: contains impurities (aqueous phase): separated by robot
- Lower phase: contains product (CHCl$_3$ or CH$_2$Cl$_2$): dried and evaporated
Parallel work-up strategies: liquid-liquid extractions

1. Two phase extractions: solubilize impurities in the aqueous phase

\[ \text{Me}_2\text{N(CH)}_n\text{NH}_2 + \text{Br}_2\text{C}R^2\text{O} \rightarrow \text{Me}_2\text{NH} \text{HR}^1 \rightarrow 3 \]

1. MeOH, 60°
2. 4, 60°
3. aq.NaOH

\[ \text{CH}_2\text{Cl}_2 \]

Products of type 5 are soluble in the basic aqueous phase

Parallel work-up strategies: solid-phase extractions

Solid phase extractions/filtrations

**Solid phase**: one or several solid phases are filled into a polypropylene syringe or cartridge

**Solid phases**: $\text{SiO}_2$; $\text{Al}_2\text{O}_3$; "ion exchange resins (basic, acidic and mixed bed)"; Kieselguhr; $\text{MgSO}_4$; polymère functionalisé: $\text{-NH}_2$, $\text{-SH}$, $\text{-PPh}_2$, $\text{COOH}$, $\text{CHO}$, $\text{CH}_2\text{OH}$, isothiourée, $\text{N}_3$...;

The organic phases are passed through these cartridges in order to get rid of impurities which are adsorbed onto the solid phase. They can be applied manually or by a robotic system (Tecan)
Parallel work-up strategies: solid-supported scavengers

\[ \text{R}^1\text{-NH}_2 \quad \text{excess} \quad \text{NH}^2 \quad \text{NHCONHR}^2 \quad \text{NHCO}^3 \quad \text{NH}SO_2R^4 \]
Parallel work-up strategies: solid-supported scavengers

Parallel synthesis in solution using polymer-bound reagents

1) CbzHN\_COOH

i

H\_N\_CO\_Cl

DMF, -5°

2) CH\_2\_N\_2/DCM

-10°, 1h

85-90%

(5-10% methylester)

R

\text{CbzHN\_COHN\_N\_N\_2}

\text{R}

\text{CbzHN\_COBr}

\text{R}

\text{CbzHN\_CONR\_R\_2}

or

\text{CbzHN\_CO\_SR\_R\_2}

Parallel work-up strategies: solid-supported scavengers; intermediate catch

Parallel synthesis in solution using polymer-bound reagents
("intermediate catch" or "resin capture"

\[
\begin{align*}
R^3 &- \text{COOH} \\
+ & R^1 \text{NH}_2 \\
+ & R^2 \text{CHO} \\
+ & R^4 \text{N} = \text{C} \\
\text{DCM, 0}^\circ & \rightarrow [\text{structure}] \\
\text{wash} & \rightarrow [\text{structure}] \\
\text{toluene, 60}^\circ & \rightarrow [\text{structure}] \\
\end{align*}
\]

A. Chucholowski, D. Heinrich, B. Mathis, C. Müller, *Generation of benzodiazepin and benzodiazocin libraries through resin capture of Ugi-4CC*, conference: 214th ACS national meeting, Las Vegas, **1997**
What are the prime biological targets?

- Kinases: 22%; market: 2 drugs
- GPCR: 15%; “: 30%
- Ion channels: 5%; “: 7%
- Ser proteases: 4%; “: 1 drug
- Phosphatases: 4%
- Zn proteases: 2%; “: ACE inhibitors
- Nuclear receptors: 2%; “: 4%
- others*: 44%

*Many targets involving large surface protein-protein interactions

-despite the fact that kinases, GPCR’s and ion channels constitute only about 42% of all targets of therapeutic interest, the pharmaceutical industry is devoting about 90% of their resources to those targets; it is believed that these targets can be addressed with small molecules.
- The number of biologicals (antibodies, fusion proteins, peptides) reaching the market is increasing. These molecules target mainly large surface protein-protein interactions.
Targets hit by current drugs

*Drugs, their targets and the nature and number of drug targets*


1. Number of drug targets:

- Marketed drugs hit 482 targets; human genome suggests 100'000 proteins

2002: J. Burgess et al.
- after sequencing of human genome:
  ~8000 targets
  ~5000 hit by known drugs: 2400 by antibodies; 800 by proteins

- on the basis of ligand binding studies:
  399 targets, which belong to 130 target families
  ~3000 targets amenable to small molecules

*Bottom line:* 300-500 targets hit by current drugs; 3'000-8'000 drugable targets
Medicinal Chemistry : Combinatorial Chemistry-Parallel Synthesis

6. Case studies

Kinase inhibitors


-Three families of kinases:
  -Serine-threonine kinases (S/TKs)
  -Tyrosine kinases (TKs)
  -Dual function kinases (DFKs)

-Roughly 2000 kinases known in the human genome

-Kinases phosphorylate serine, threonine and tyrosine and are ATP dependent

\[
\begin{align*}
\text{OH} & \quad \text{TKs)} \\
\text{O} & \quad \text{ATP} \\
\text{phosphatases} & \quad \text{TKs)} \\
\text{ATP} & \\
\text{phosphatases} & \quad \text{R}^+\text{OH} \\
\end{align*}
\]
Kinase inhibitors on the market

**Sutent**
KIT; PDGF and VEGFR

**Lapatinib**
EGFR; ERBB2

**Dasatinib**
Src family; ABL1; KIT; PDGFR; Eph

**Sorafenib**
KIT; PDGFR; B-Ref; VEGFR2

**Imatinib (Gleevec)**
Bcr-Abl; KIT; PDGFR;
GPCR’s: introduction

50% of all drugs target G-Protein-Coupled Receptors (sales in 2001: ~50 billion USD)

G-protein: guanine nucleotide-binding protein

- 240 receptors with known ligands from which only ~30 are currently investigated by pharmaceutical companies
- An additional 160 receptors with unknown ligands (orphan receptors) are known

Family 1: rhodopsin-like or adrenergic-like GPCR’s

constitute the largest family; contain a short N-terminus and amino acid residues in the trans-membrane domain are highly conserved

Family 2: glucagon receptor-like or secretin receptor-like GPCR’s

Family 3: metabotropic glutamate receptors


GPCR’s: introduction

G-protein-coupled receptors

Extracellular

Cytoplasmic

TM1 TM2 TM3 TM4 TM5 TM6 TM7

i1 i2 i3

COOH-

NH2

-S-S-

e1 e2 e3
Medicinal Chemistry : Combinatorial Chemistry-Parallel Synthesis
6. Case studies

GPCR’s: some best-selling drugs

**Claritin** (Schering-Plough, H₁ antagonist allergies, 3.1 billion USD, 2001)

**Zyprexa** (Ely Lilly, D₂/D₄/5-HT₂ allergies, 2.35 billion USD, 2001)

**Neurontin** (Pfizer, GABA B-agonist neurogenic pain, 2.35 billion USD, 2001)

**Serevent** (Glaxo, β₁ agonist asthma, 0.91 billion USD, 2001)

**Diovan** (Novartis, AT1 antagonist hypertension, 0.8 billion USD, 2001)
Case study 3: Parallel synthesis of analogues of antibiotic GE2270 A

Parallel synthesis starting from a natural product-derived building block

GE2270 A

active against many gram positive pathogens
MIC 0.06-1.0 μg/ml; low solubility in aqueous solvents
J. W. Jacobs et al. (Versicor), 40th annual ICAAC conference, Toronto, Canada, september 17-20th, 2000, Poster 2193 and 2194

1
inhibitor of elongation factor EF-TU
Medicinal Chemistry : Combinatorial Chemistry-Parallel Synthesis
6. Case studies

Case study 3: Parallel synthesis of analogues of antibiotic GE2270 A

Parallel synthesis starting from a natural product-derived building block

J. W. Jacobs et al. (Versicor), 40th annual ICAAC conference, Toronto, Canada, september 17-20th, 2000, Poster 2193 and 2194
Case study 2: Parallel synthesis of analogues of antibiotic GE2270 A

Parallel synthesis starting from a natural product-derived building block

J. W. Jacobs et al. (Versicor), 40th annual ICAAC conference, Toronto, Canada, September 17-20th, 2000, Poster 2193 and 2194
Case study 3: Parallel synthesis of analogues of antibiotic GE2270 A

Parallel synthesis starting from a natural product-derived building block

GE2270 D2

J. W. Jacobs et al. (Versicor), 40th annual ICAAC conference, Toronto, Canada, September 17-20th, 2000, Poster 2193 and 2194
Case study 3: Parallel synthesis of analogues of antibiotic GE2270 A

Parallel synthesis starting from a natural product-derived building block

J. W. Jacobs et al. (Versicor), *40th annual ICAAC conference*, Toronto, Canada, september 17-20th, 2000, Poster 2193 and 2194
Case study 3: Parallel synthesis of analogues of antibiotic GE2270 A

Parallel synthesis starting from a natural product-derived building block

GE2270 A

R¹: \(-\overset{\cdot}{\text{C}}=\overset{\cdot}{\text{O}}\text{H}\)  0.44
R²= CH₃

R¹: \(-\overset{\cdot}{\text{C}}=\overset{\cdot}{\text{O}}\text{H}\)  0.41
R²= CH₃

R¹: \(-\overset{\cdot}{\text{C}}=\overset{\cdot}{\text{O}}\text{H}\)  >2.0
R²= \(-\overset{\cdot}{\text{C}}=\overset{\cdot}{\text{O}}\text{H}\)

Solubility (mg/ml)
<0.0001

Steps:
i: R¹NH₂, DMF, DIEA; ii: H₂O; then precipitation + wash; iii: Ts₂O; CH₂Cl₂; DIEA; iv: R2SH, DMF/aq, K₂CO₃; then precipitation + wash; v: TFA/CH₂Cl₂ (1:1); Et₂O; then precipitation + wash; then dry

J. W. Jacobs et al. (Versicor), 40th annual ICAAC conference, Toronto, Canada, september 17-20th, 2000, Poster 2193 and 2194
Case study 3: Parallel synthesis of analogues of antibiotic GE2270 A

Parallel synthesis starting from a natural product-derived building block

J. W. Jacobs et al. (Versicor), 40th annual ICAAC conference, Toronto, Canada, September 17-20th, 2000, Poster 2193 and 2194
**Some useful definitions in medicinal chemistry**

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC50</td>
<td>Effective dose for a 50% of maximal response</td>
</tr>
<tr>
<td>Dose</td>
<td>In mg/kg: mg of compound per kg of body weight; e.g. 1mg in a 25g mouse is the equivalent of 2g dose in a 50kg (small) adult.</td>
</tr>
<tr>
<td>SAR</td>
<td>Structure activity relationship. Correlation between chemical structure and biological activity.</td>
</tr>
</tbody>
</table>

**Phase I:**
In phase I clinical trials a compound is dosed to healthy volunteers and three main questions are asked:
1. Is the compound safe at the proposed dose?
2. What are the limiting side effects likely to be?
3. How long does the compound stay in the system?

**Phase II:**
Phase II clinical trials aim at showing efficacy of the compound in a sample of patients having a particular disease. If there are signs that the compound is active enough it can be promoted to next phase.

**Phase III:**
Phase III clinical studies are big and comprise many patients. The key issues are the following:
- How well does the drug work?
- What are its side effects at the proposed efficacy doses?
- What kind of a dosing schedule is optimal?
- How does it interact, favorably or unfavorably, with other drugs for the same or related conditions?

**Success:**
At least 25000 compounds have to be made in order to get one drug expenses are around 500 million USD with a lead time of 7-10 years.
Targets: Up to now only about 200 discrete molecular targets have been explored. Around 50% of these belong to the GPCR’s (e.g. histamine, dopamine or serotonin receptors). With decoding of the human genome it is believed that 30'000 targets will be unveiled.

Protein structure: - primary sequence: genomics
- sequence alignment with known proteins: conserved residues are characteristic for function
- gene knockout can reveal importance of a target for a certain disease
- expression and purification
- 3D structural determination by X-ray or NMR techniques
- mutagensis studies (site directed mutagenesis) can reveal important residues in receptors or ligands

Protein kinases: transfer the g phosphate of ATP to side chain hydroxyls of substrate proteins. It is estimated that about 2000 kinases exist in the human genome
- Serine/threonin kinases (S/TK’s)
- Tyrosine kinases (TK’s)
- Dual function kinases (DFK’s)

Protein phosphatases: cleave phosphate groups from substrate proteins
ADMET: Adsorption, Distribution, Metabolism, Excretion(Elimination) and Toxicity

In vitro ADMET experiments:

- **Cytotoxicity assay** on different cancer cell lines
- **Stability in plasma**: rodents (mouse, rat), human
- **Caco 2 cell passage** of compounds: indicator for oral absorption
- **Passage of compounds through artificial membranes** (PAMPA)
- **Metabolism studies in liver microsomes**: first pass metabolism
- **Protein binding** (binding to serum albumin): indicates availability of compound in plasma
Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis
7. Appendix (Definitions; Reviews; Literature

Targets hit by current drugs

2. Target classes:  
-2.1. Enzymes  
-2.2. Substrates, metabolites and proteins  
-2.3. Receptors  
-2.4. Ion channels  
-2.5. Transporter proteins  
-2.6. DNA/RNA and the ribosome  
-2.7. Targets of monoclonal antibodies  
-2.8. various  
-2.9. unknown

2.1. Enzymes:  
- Oxidoreductases (e.g. MAO-B, aromatases etc.)  
- Transferases (kinases, phosphatases, DNA polymerase  
- Hydrolases (serine proteases, metalloproteases etc.)  
- Lyases (DOPA decarboxylase, carbonic anhydrase et  
- Isomerases ((DNA gyrase, topoisomerases etc.)  
- Ligases (dehydropteroate synthase, mTOR etc.)
Targets hit by current drugs

2.3. Receptors:  
- Direct ligand-gated ion channels (GABAA, acetylcholine, glutamate R)  
- GPCR’s (class 1, class 2 (secretin-like), others)  
- Cytokine receptors  
- Integrin receptors  
- Receptors associated with TK  
- Nuclear receptors

2.4. Ion channels:  
- Voltage-gated Ca\(^{2+}\) channels (L- and K-type)  
- K\(^{+}\) channels (epithelial, voltage-gated)  
- Na\(^{+}\) channels (epithelial voltage-gated)  
- RIR-CaC  
- TRP-CC  
- Cl- channels

2.5. Transporter proteins:  
- Cation-chloride cotransporter (CCC)  
- Na\(^{+}\)/H\(^{+}\) antiporters  
- Proton pumps  
- Eukariotic sterol transporters  
- Neurotransmitter Na\(^{+}\) symporter  
- Noradrenaline/Na\(^{+}\) symporter  
- Dopamine/Na\(^{+}\) symporter
Targets hit by current drugs

2.6. DNA/RNA and the ribosome:
- Nucleic acids
- RNA (16S-rRNA; 23S-rRNA)
- Spindle (tubulin, kinesins)
- Ribosome (30S subunit; 50S subunit)

2.7. Targets of monoclonal antibodies:
- Vascular endothelial factor (VEGF; e.g. bevacizumab; Avastin)
- Lymphocyte function-associated receptor (LFA-1; efalizumab)
- Epidermal growth factor receptor (EGFR; e.g. cetuximab)
- h-EGFR-2 (e.g. trastuzumab; Herceptin)
- Immunoglobulin E (IgE; e.g. omalizumab Xolair)
- CD-3
- CD-20 (Rituximab; Mabthera)
- CD-33 (Gemtuzumab)
- CD-52 (Alemtuzumab)
- TNFα (Adalimumab; infliximab; Enbrel)
Targets hit by current drugs

**G-Protein Coupled Receptors (GPCR’s):**

- **Acetylcholin receptors** (muscarinic receptors MCR 1-4)
- Adenosin receptors
- Adrenoreceptors ($\alpha_1$, $\alpha_2$, $\beta_1$)
- Angiotensin receptors
- Calcium-sensing receptors
- Cannabinoid receptors (CB1, CB2)
- Cysteinyi-leukotriene receptors
- Dopamine receptors
- Endothelin receptors
- GABA$\beta$ receptors
- Glucagon receptors
- Glucagon-like peptide-1 receptor (GLP-1R)
- Histamin receptors (H1, H2)
- Opioid receptors ($\mu$, $\kappa$, $\delta$)
- Neurokinin receptors (NK1, NK2, NK3)
- Prostanoid receptors
- Prostamide receptors
- Purinergic receptors
- Serotonin receptors ($5-HT_{1A}$, $5-HT_{1B/1C}$, $5-HT_{2A}$, $5-HT_3$, $5-HT_4$)
- Vasopressin receptors (V1, V2, OT)
### Targets hit by current drugs

**Cytokine receptors:**
- Growth hormone receptor
- Erythropoietin receptor (EPO)
- Granulocyte colony stimulating factor receptor
- Interleukin-1 receptor (IL-1R)
- Interleukin-2 receptor (IL-2R)
- Tumour necrosis factor α (TNFα)

**Integrin receptors:**
- Glycoprotein IIb/IIIa receptor (GPIIb/IIIa)

**Receptors associated with TK:**
- Insulin receptor

**Nuclear receptors:**
- Mineralcorticoid receptor
- Glucocorticoid receptor
- Progesteron receptor
- Oestrogen receptor
- Androgen receptor
- Vitamin D receptor
- ACTH receptor
- Retinoic acid receptor (RXR)
- Peroxisome-proliferator-activated receptors (PPAR; α)
- Thyroid hormone receptor
4. Appendix-Reviews


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Very recent reviews:


Medicinal Chemistry : Combinatorial Chemistry-Parallel Synthesis

7. Appendix (Definitions; Reviews; Literature

4. Appendix-Reviews


Medicinal Chemistry: Combinatorial Chemistry-Parallel Synthesis

7. Appendix (Definitions; Reviews; Literature)

4. Appendix-Reviews


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4. Appendix-Reviews

- Hot spots: A review of the protein-protein interface determinant amino acid residues  
  I. S. Moreira et al. Proteins 2007, 68, 803-812


- A question of library design; P. Hayduk, Nature 2011, 470, 42

- Systemic enumeration of heteroaromatic ring systems as reagents for use in medicinal chemistry;  


- Active methylene-based multicomponent reactions under microwave heating; B. Jiang et al. Chimia 2011, 925

4. Appendix-Reviews


-Synopsis of some recent tactical applications of bioisosteres in drug design; N. A. Meanwell, J. Med. Chem. 2011, 54, 2529-2591

