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

Agenda

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 α-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 thethering; click chemistry

Agenda

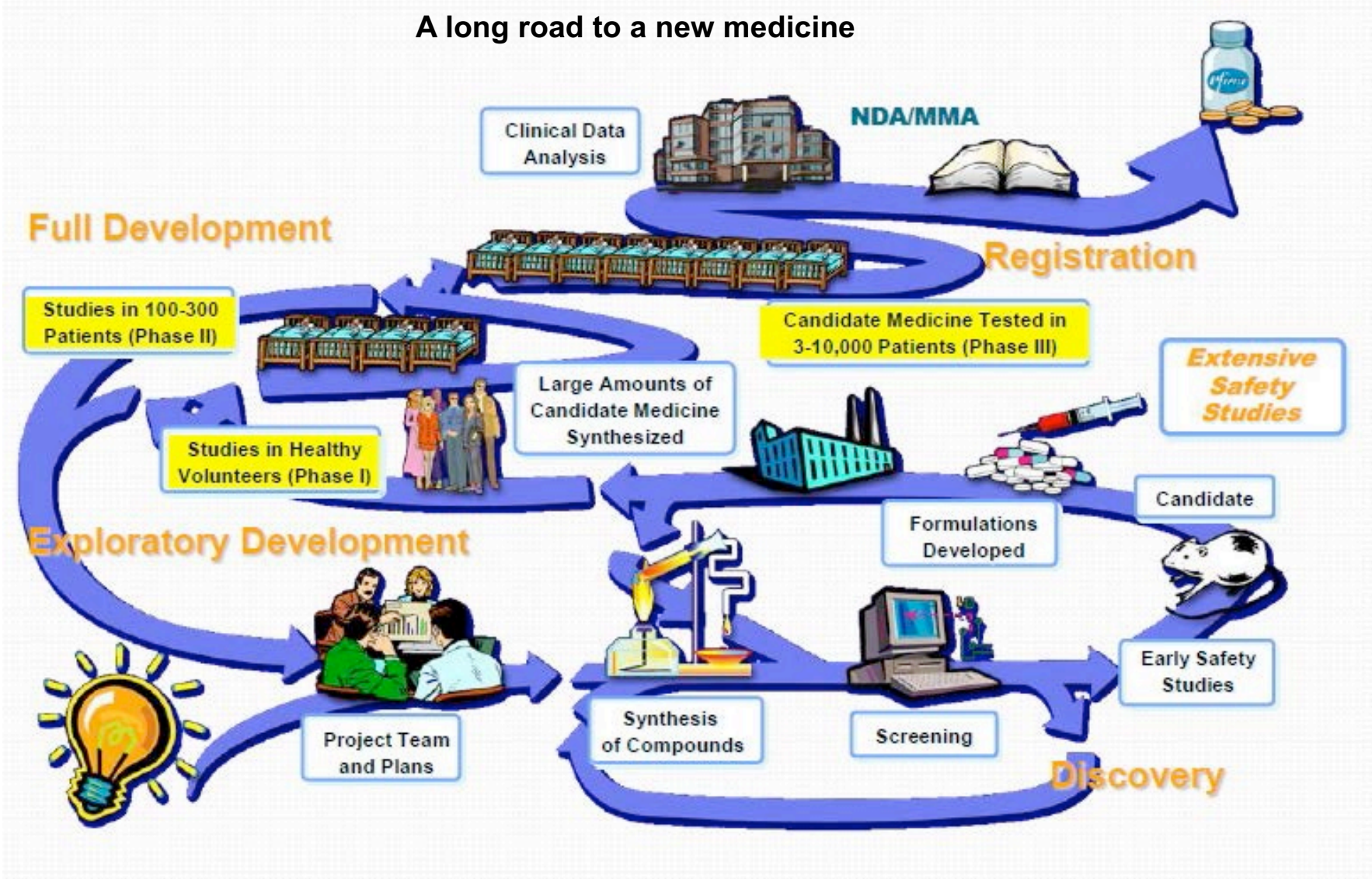
5. **Strategies for the Synthesis of Small Molecule Libraries (cont.)**

   -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

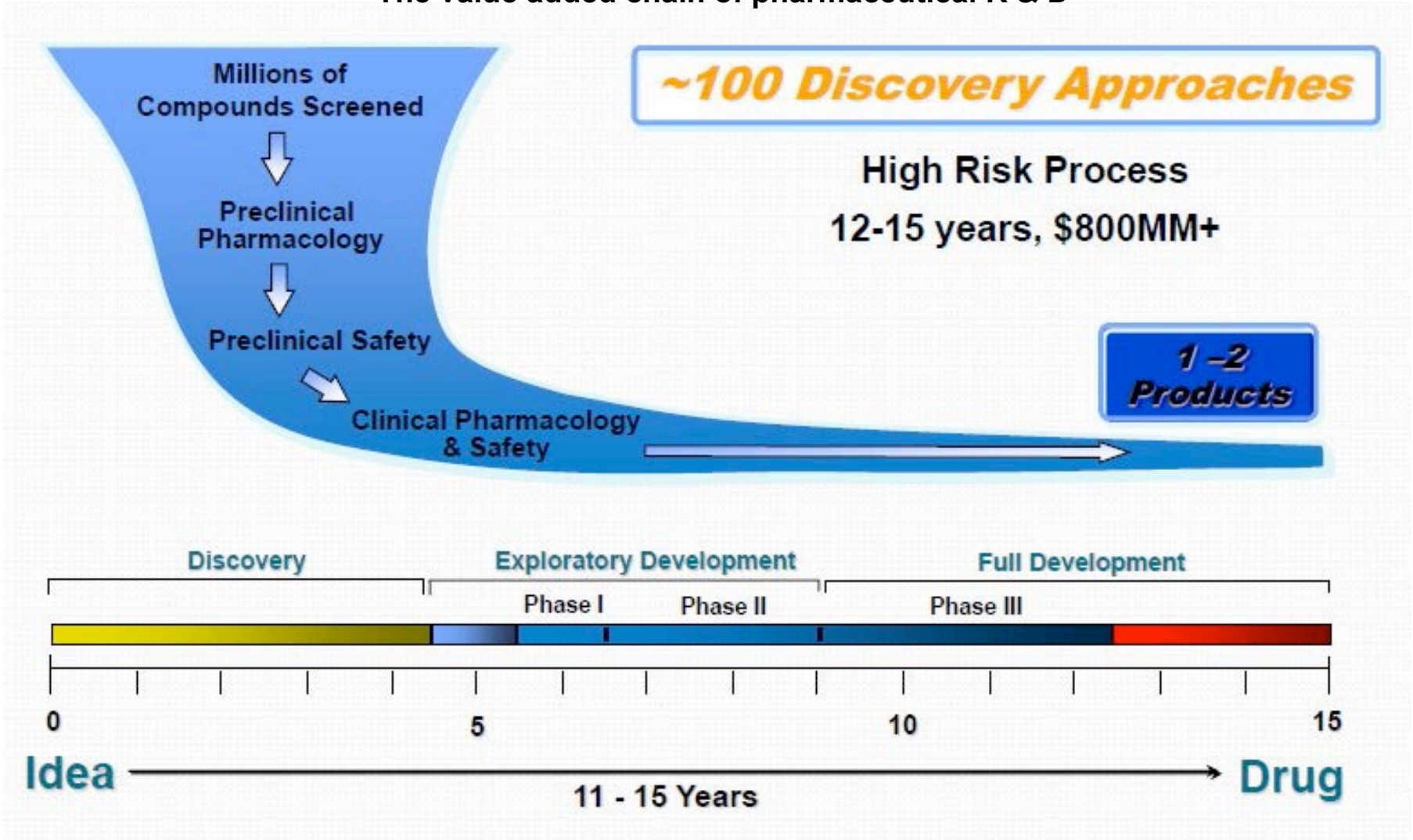
6. **Applications of Parallel Synthesis and Combinatorial Chemistry in Medicinal Chemistry**
   - **-Case studies**
   - **-Drug targets**

7. **Appendix (Definitions; Reviews; Literature)**

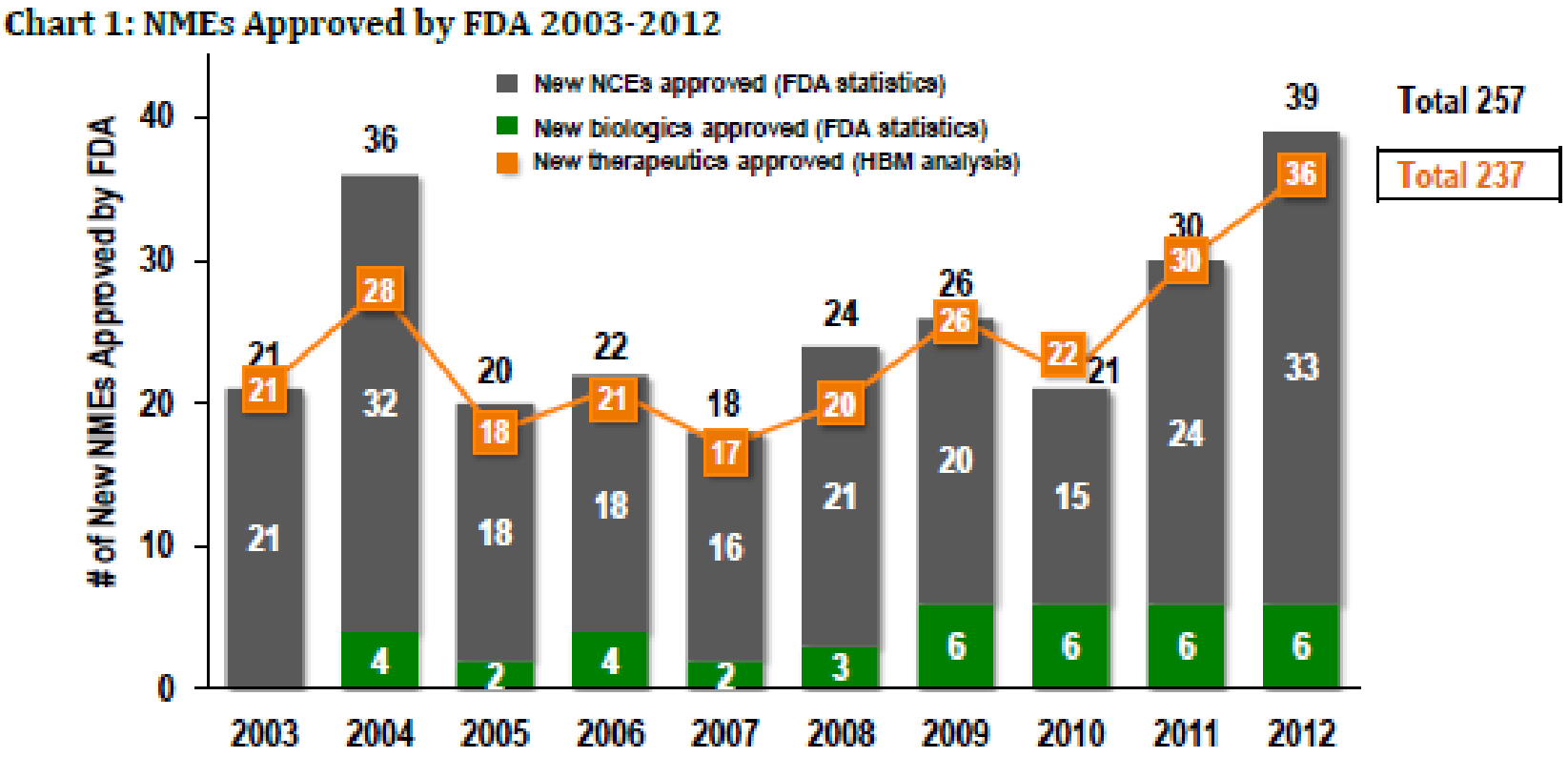
A long road to a new medicine



The value added chain of pharmaceutical R & D

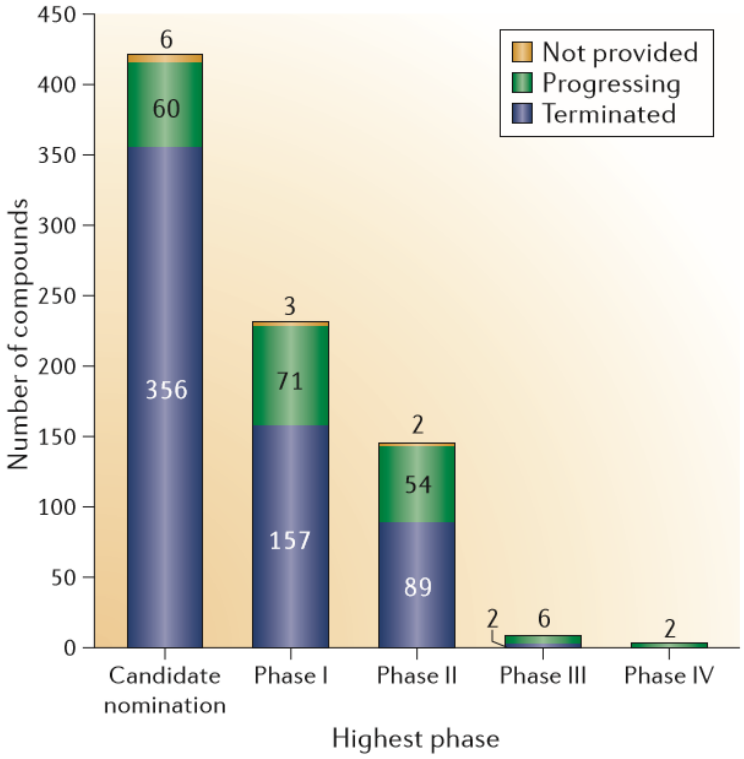


The Drug Discovery Process



HBM New Drug Approvals (U. Geilinger, R. Belleli, C. Barra, July 2013)

Attrition rates



	Candidate nomination	Phase I	Phase II	Phase III	Phase IV
Total	422	231	145	8	2
Ratio of progressing:terminated	0.17	0.45	0.61	3.00	–

-Rationalization/changes of company portfolio

-Biological concept was not tested adequately: levels of drug required for the desired target exposure could not be reached

-Compounds that achieve efficacy at lower concentrations are more likely to progress through toxicological studies

-High confidence in exposure, binding to the desired target combined with a pharmacological response

-Compound lipophilicity has an influence on toxicology: 3/75 rule: calc. logP<3 and TPSA>75Å^2 were 2.5 times more likely to be non-toxic

M. J. Waring et al. *Nat. Rev. Drug Discov*. **2015**, DOI: 10.1038/nrd4609

Reasons for high attrition rates

Table 1 | **Populations of the primary cause of failure categories for terminated compounds***

Termination reason	Overall	Period		Phase		
		2000–2005	2006–2010	Candidate nomination	Phase I	Phase II
Clinical safety	68 (11%)	48 (13%)	20 (8%)	5 (1%)	40 (25%)	22 (25%)
Commercial	40 (7%)	23 (6%)	17 (7%)	26 (7%)	10 (6%)	4 (4%)
Efficacy	55 (9%)	45 (11%)	10 (4%)	10 (3%)	14 (9%)	31 (35%)
Formulation	9 (1%)	4 (1%)	5 (2%)	8 (2%)	1 (0.6%)	0
Non-clinical toxicology	240 (40%)	144 (40%)	96 (40%)	211 (59%)	21 (13%)	7 (8%)
Patent issue	1 (0.2%)	0	1 (0.4%)	1 (0.3%)	0	0
Pharmacokinetics or bioavailability	29 (5%)	19 (5%)	10 (4%)	3 (0.8%)	25 (16%)	1 (1%)
Rationalization of company portfolio	124 (21%)	46 (13%)	78 (32%)	75 (21%)	29 (18%)	19 (21%)
Regulatory	2 (0.3%)	2 (0.6%)	0	1 (0.3%)	1 (0.6%)	0
Scientific	33 (5%)	28 (8%)	5 (2%)	13 (4%)	15 (10%)	5 (6%)
Technical	3 (1%)	3 (1%)	0	2 (0.6%)	1 (0.6%)	0
Other	1 (0.2%)	0	1 (0.4%)	1 (0.3%)	0	0
Total	605	362	243	356	157	89

*Table entries for each column indicate the total number and the percentage in parentheses.

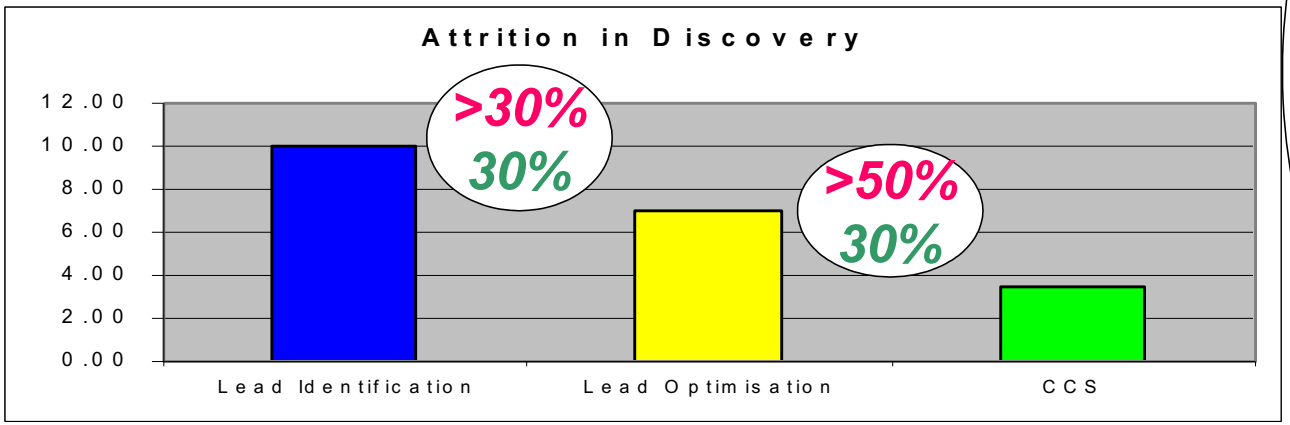
M. J. Waring et al. *Nat. Rev. Drug Discov*. **2015**, DOI: 10.1038/nrd4609

Attrition rates in the discovery and preclinical phases

Lead Optimisation

Clinical Candidate Selection (CCS) from alternatives (Candidate Profiling)

5-12 m | **12-36 months** | **3-6 m**



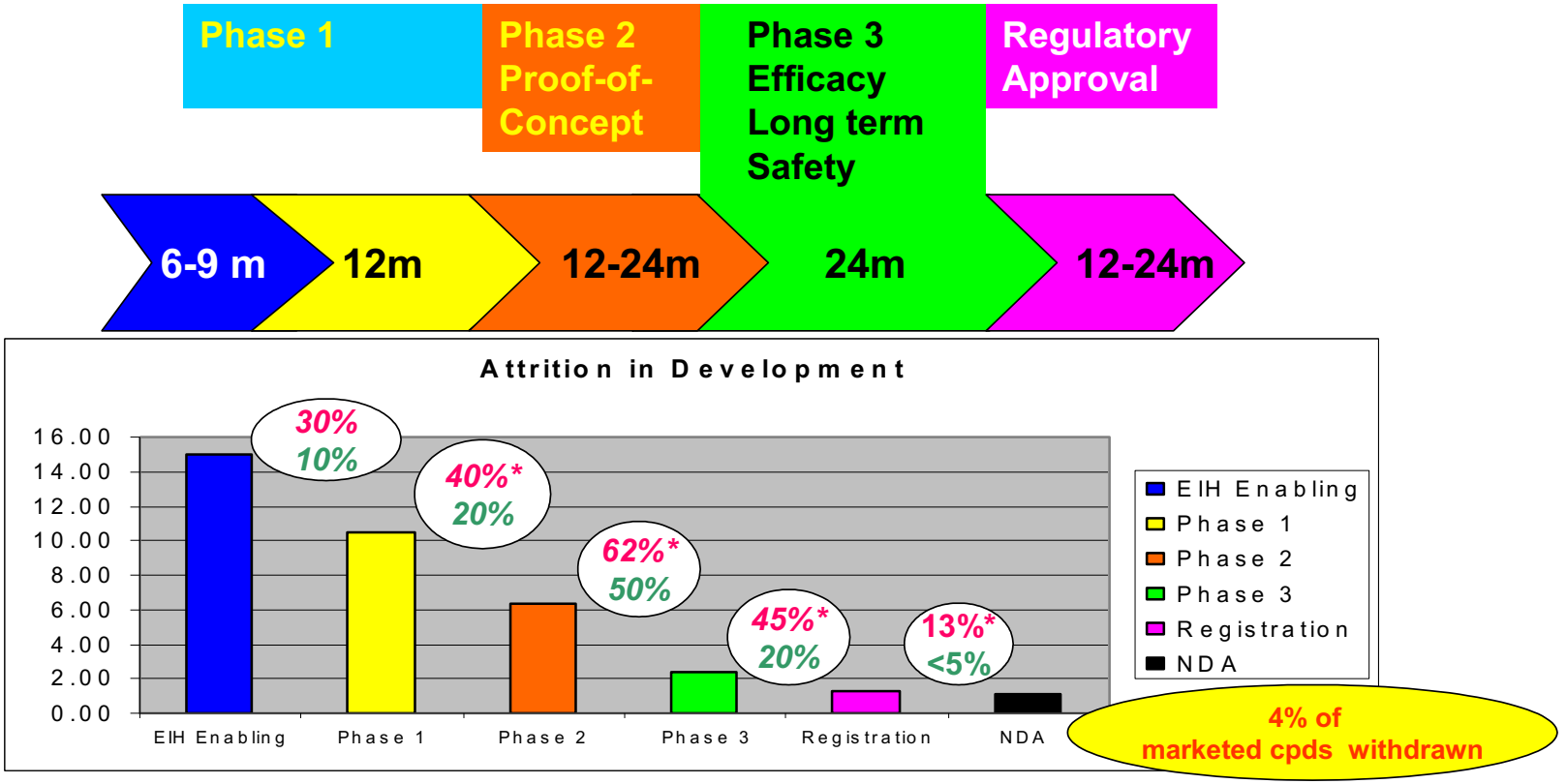
Of 10 projects starting in Lead Identification <3.5 will reach CCS (..but 5 possible?)

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

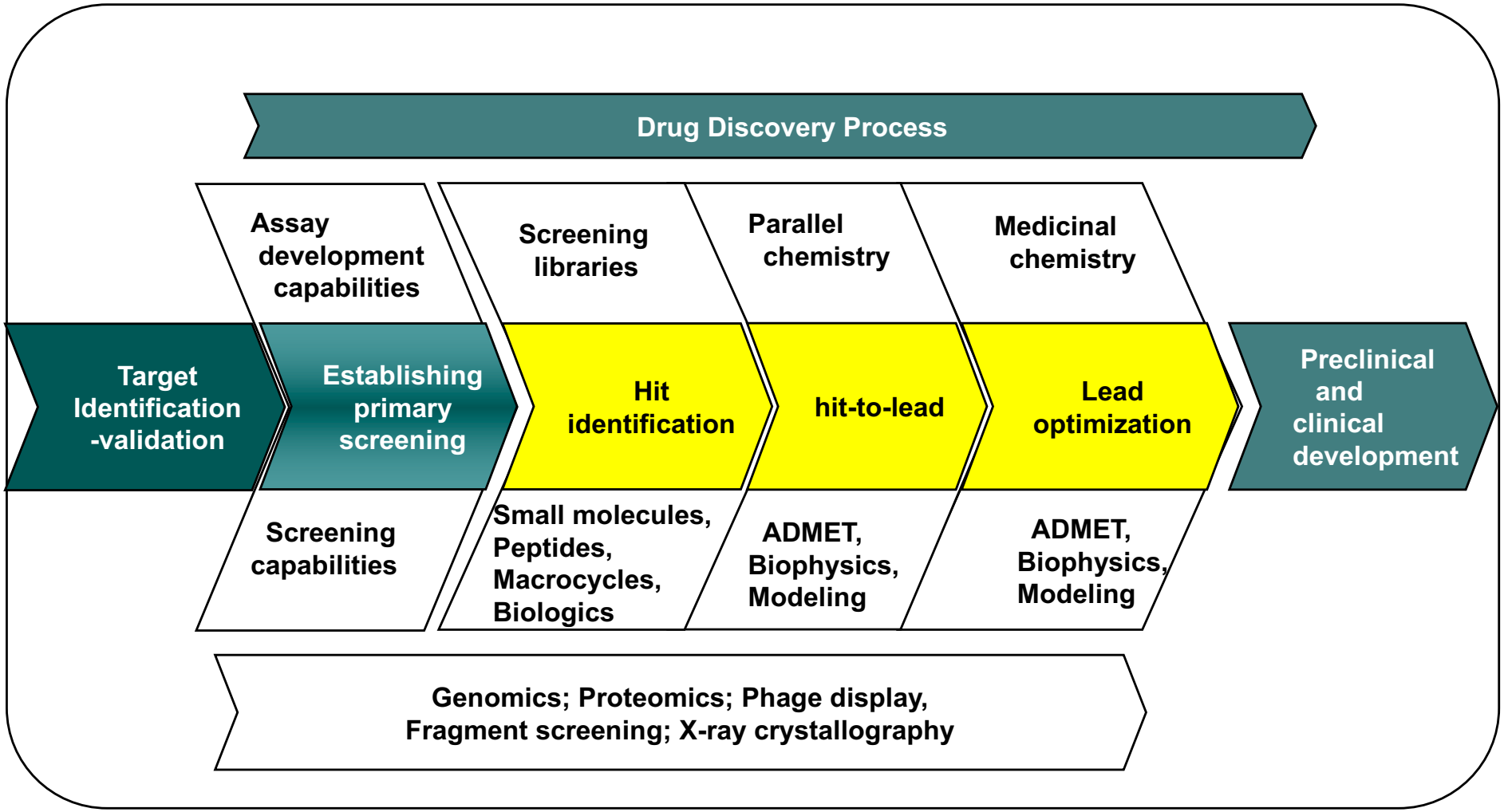
Attrition rates in the clinical phases



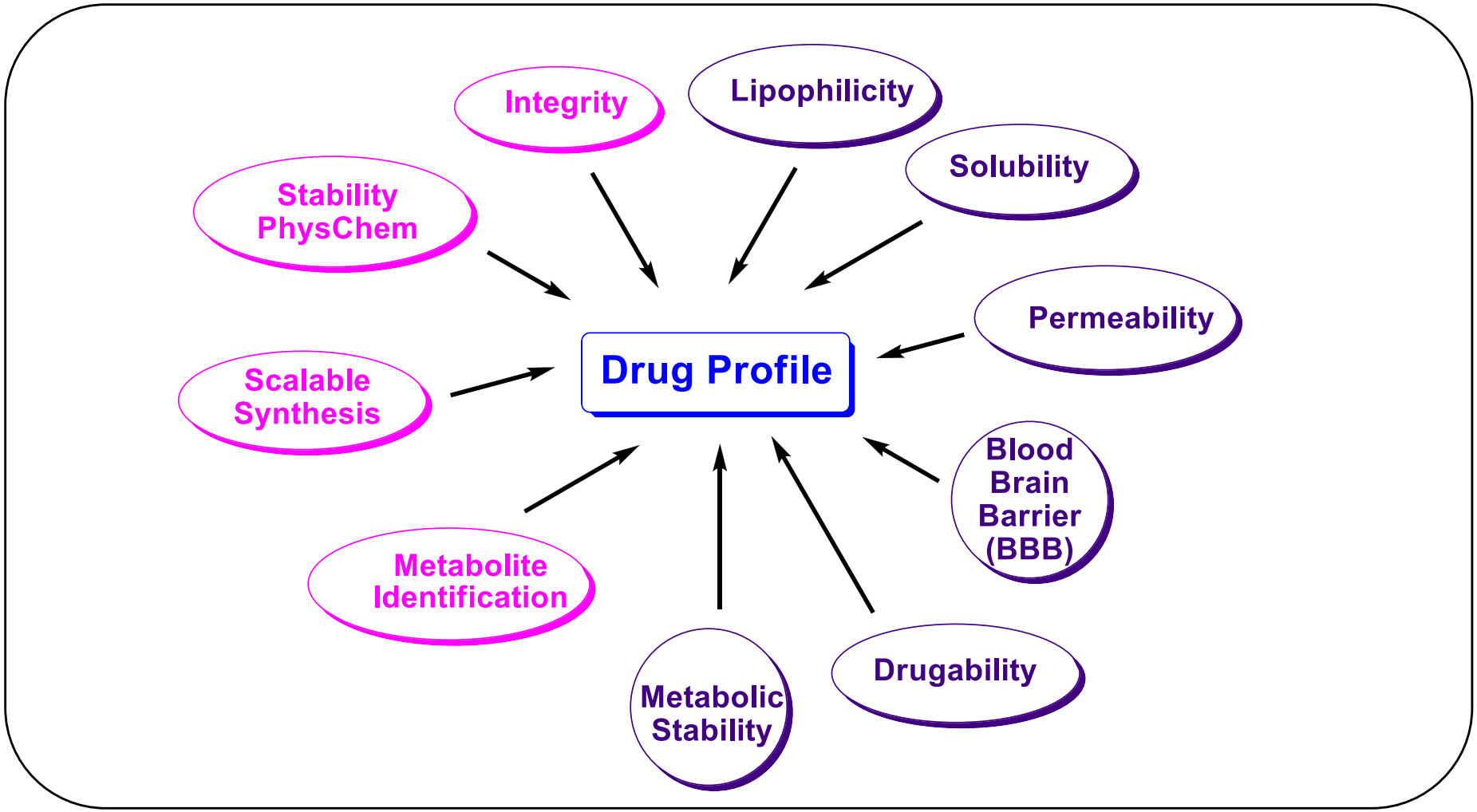
Ensuring PK, metabolism, exposure, half-life,, safety, in humans are as expected. Definition of possible human safety issues and margins. Reproductive toxicity

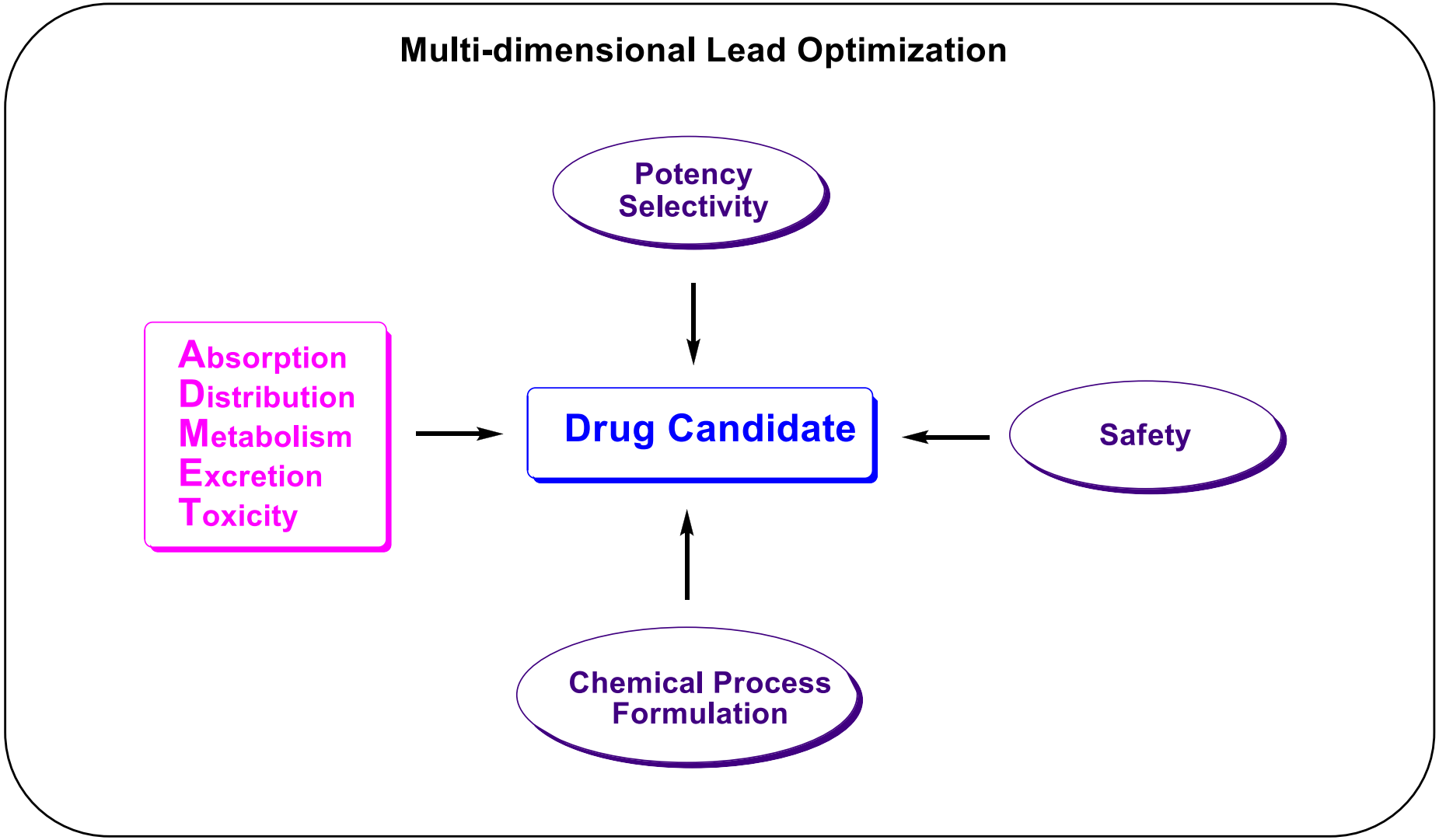
Long term pre-clinical & clinical safety, carcinogenicity studies. Final assessment of drug-drug interactions & of bioavailability of the final marketed formulation

The Drug Discovery Process

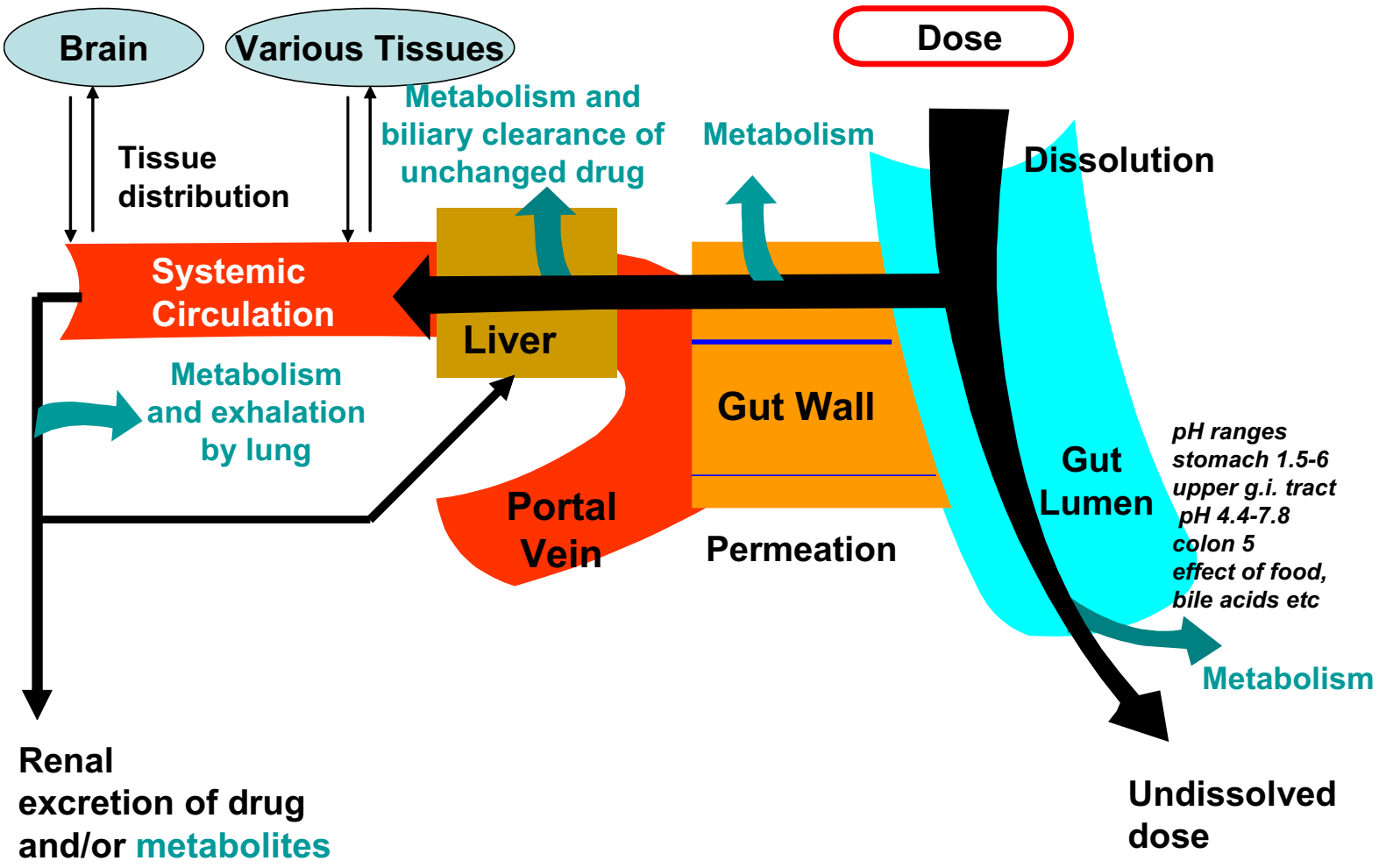


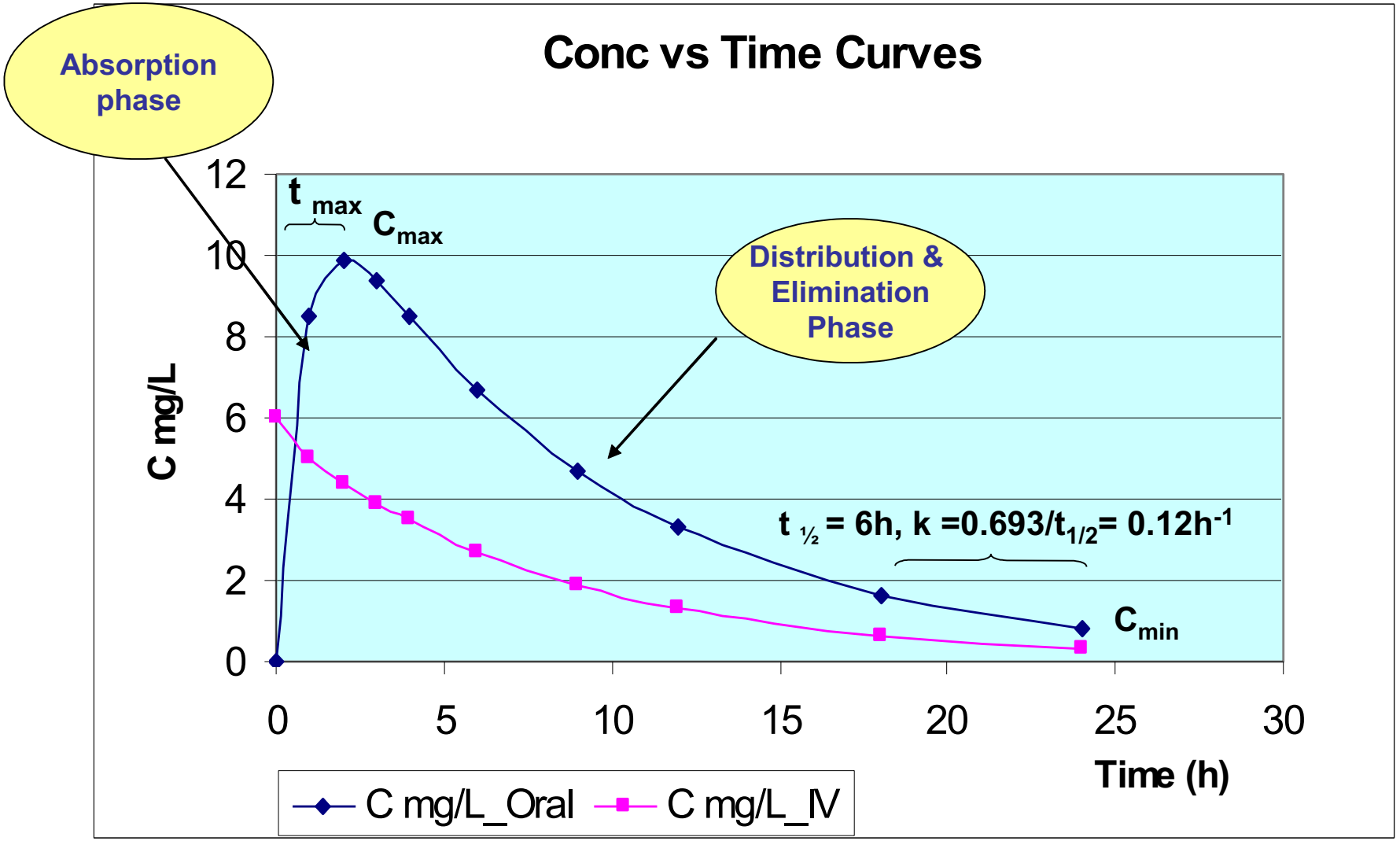
Elements of the drug profile



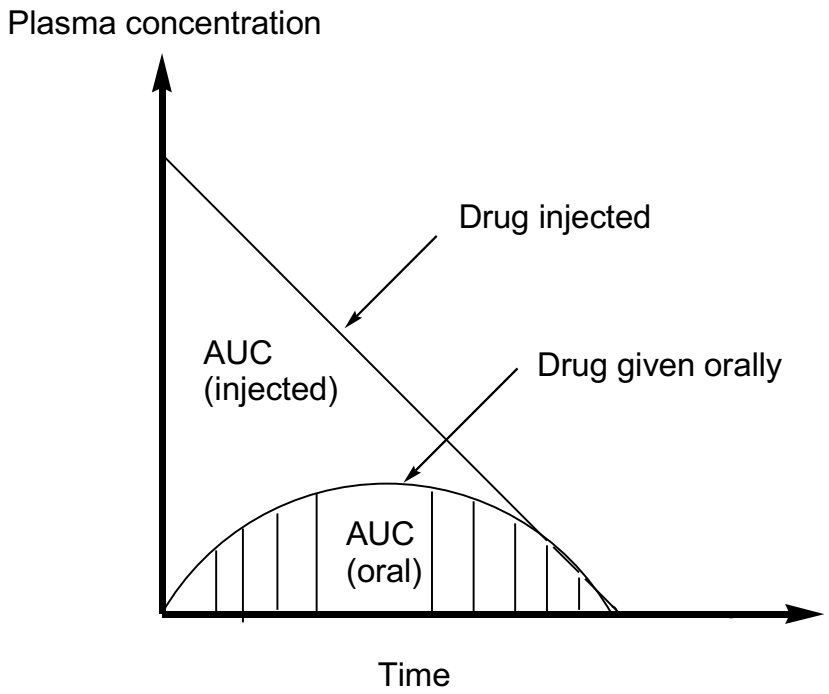

Multi-dimensional Lead Optimization
Potency
Selectivity
Absorption
Distribution
Metabolism
Excretion
Toxicity
Drug Candidate
Safety
Chemical Process
Formulation

Drug absorption, distribution and elimination




Conc vs Time Curves
Absorption phase
Distribution & Elimination Phase
t max
Cmax
Cmin
t ½ = 6h, k =0.693/t1/2= 0.12h-1
C mg/L
Time (h)
0
2
4
6
8
10
12
0
5
10
15
20
25
30
C mg/L_Oral
C mg/L_IV

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:

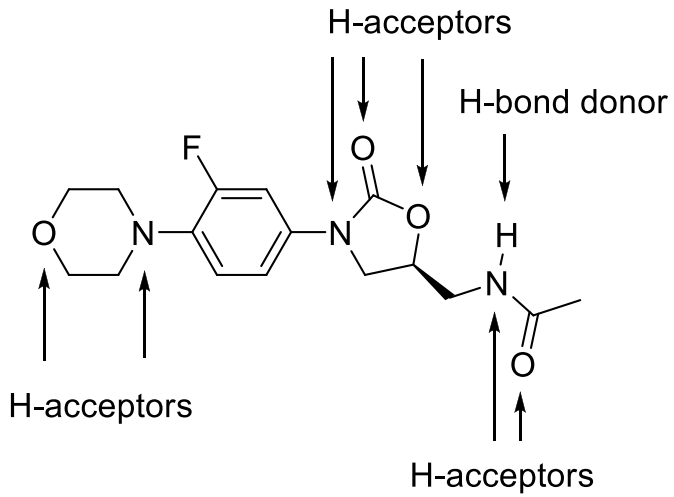
there are more than:
-5 H-bond donors
-10 H-bond acceptors
-the MW (molecular weight) is >500
-the CLogP (calculated log P) is >5

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

leading references: [1] C. Lipinsky et al. *Adv. Drug Delivery Rev*. **1997**, *23*, 2; [2] D. Veber et al. *J. Med. Chem*. **2002**, *45*, 2615-2623

Drugability: Lipinski's rule of 5

Linezolid



$C_{16}H_{20}FN_3O_4$ (337.35)

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

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

leading references: [1] C. Lipinsky et al. *Adv. Drug Delivery Rev*. **1997**, *23*, 2; [2] H. Kubinyi et al. *J. Med. Chem*. **1998**, *41*, 3325; [3] M. Murko et al. *J. Med. Chem*. **1998**, *41*, 3314; ¨[4] J. R. Proud-food, *Bioorg. Med. Chem. Lett*. **2002**, *12*, 1647

Drugability parameters

Flatness:
-The aromaticity of a compound has become increasing attention
-One measure is the **fractional sp^3 character**:
ratio of sp^3-carbons/total number of carbons
A. Yan et al. *QSAR Comb. Sci*. **2003**, *22*, 821-829
-The flatness (sp^2 content) has increased over time, probably because many good sp^2-sp^2-bond formation reactions were developed in the eighties and nineties (Suzuki ect.) amenable to combinatorial synthesis

CLogP: calculated logP; measure for lipophilicity
-partioning of a compound between octanol and water
-key parameter impacting on solubility, permeabilty, 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 <120-140Å^2; good BBB penetration: PSA<80-90Å^2

W. P. Walters et al. et al. *J. Med. Chem*. **2011**, *54*, 6405-6416
„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.

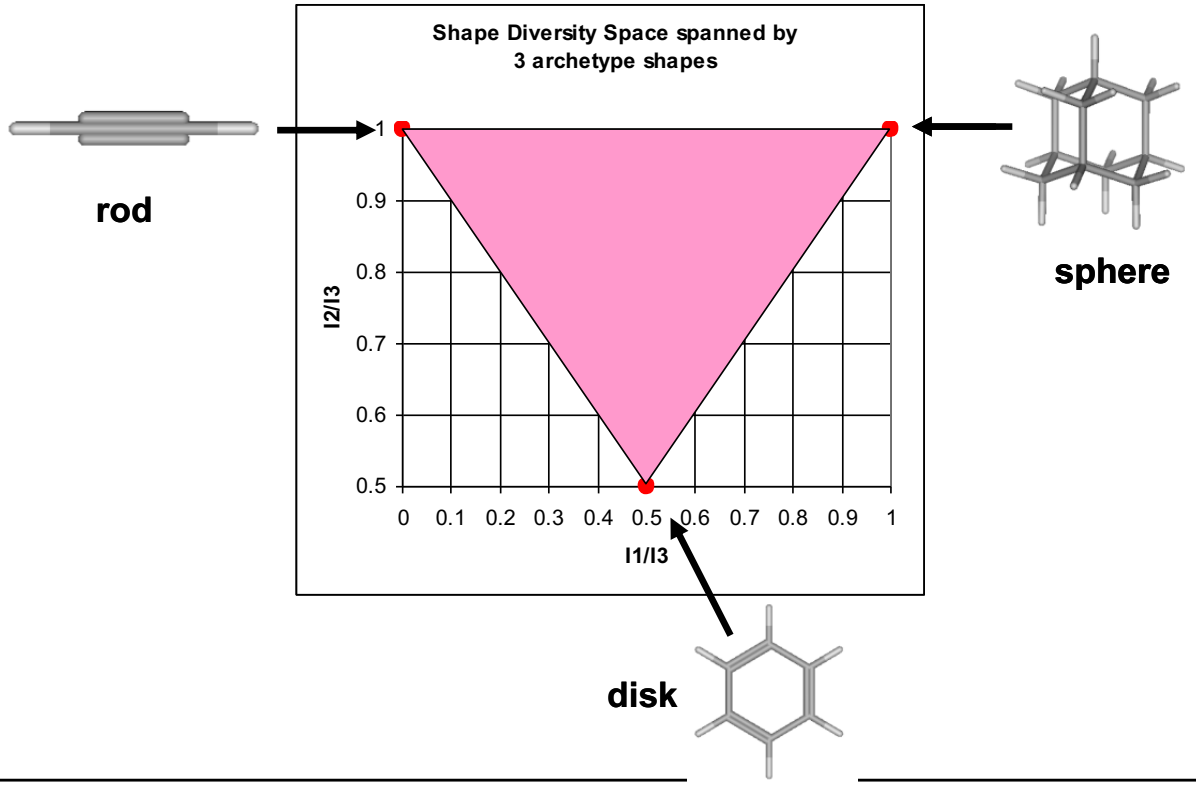
Molecular complexity:
-In the last ten years there was trend to natural product-like scaffolds with higher sp^3 content away from flat or linear compounds; in particular macrocyclic natural product-like compounds have become popular: E. M. Driggers et al. *Nature Rev. Drug Discov*. **2008**, *7*, 608-624

W. P. Walters et al. et al. *J. Med. Chem*. **2011**, *54*, 6405-6416
„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 asses the 3D-molecular shape diversity of large combinatorial libraries
- The shape analysis is based on the principal moments of inertia

(Sauer, W. H. B.; Schwarz, M. K, *J. Chem. Inf. Comput. Sci.*, 2003, *43*, 987-1003)



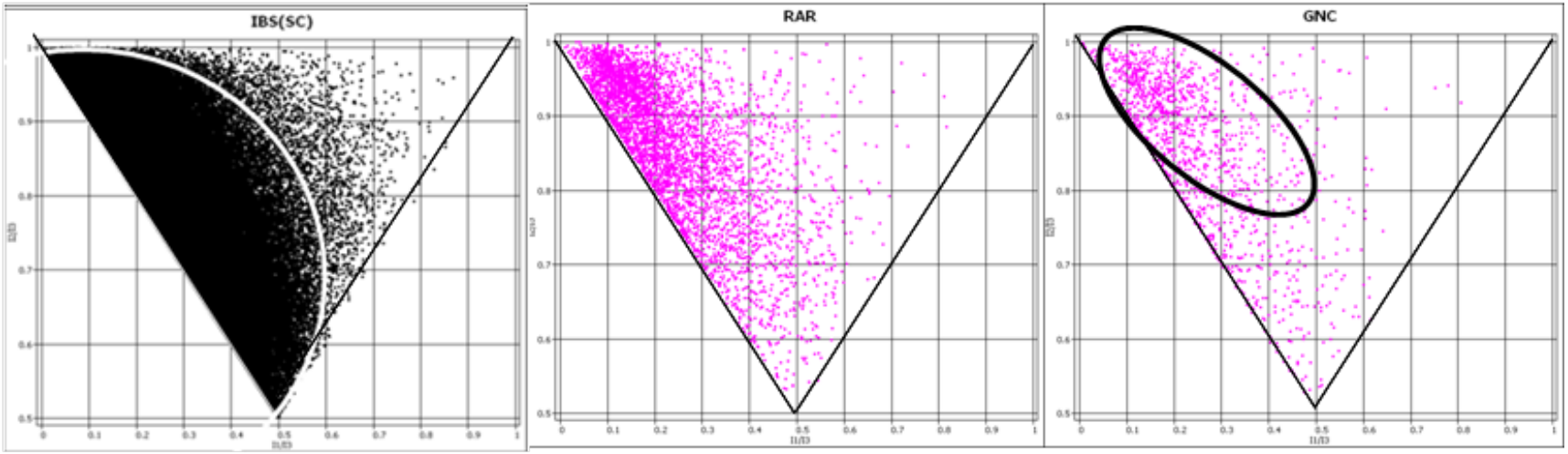
Shape analysis

- During 1995-2005 large small molecule libraries were synthesized exhibiting limited 3D-diversity
- Large combinatorial libraries have many linear (cigare-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 sperical-shape

(A. K. Gosh, *J. Org. Chem*. **2010**, *75*, 7967-7989; D. J. Newman et al., *J. Nat. Prod*. **2007**, *70*, 461-477; E. M. Driggers et al. *Nature Rev. Drug Discov*. **2008**, *7*, 608-624)

- Natural products often require a large and complex multistep synthesis effort. Diversity-oriented synthesis aimes at synthesizing natural product-like libraries via common synthetic precursors

(S. L. Schreiber, *Nature* **2009**, *457*, 153-154)



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

Key features for further development, lead structures should display the following properties:

-Simple chemical features, amenable for chemical optimization
-Membership to an established SAR (structure activity relashionship) 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

T. I. Oprea et al. *J. Chem. Inf. Comput. Sci*. **2001**, *41*, 1308-1315

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

Based on the 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

T. I. Oprea et al. *J. Chem. Inf. Comput. Sci*. **2001**, *41*, 1308-1315; M. Hann, T. I. Oprea, Curr. *Opin. Chem.Biol*. **2004,** *8*, 255-263

Fragments: the rule of 3

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 <60A^2

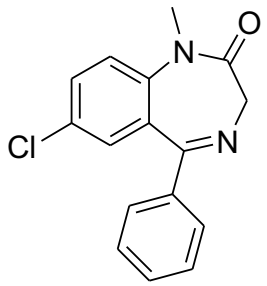
M. Congreve et al. *Drug Discov. Today* **2003**, *8*, 876-77; M. Hann, T. I. Oprea, Curr. *Opin. Chem.Biol*. **2004**, *8*, 255-263

Privileged structural elements

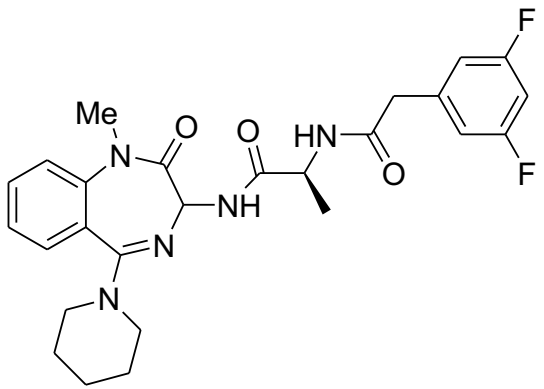
A single framework or fragments which can bind to different target families in a specific way

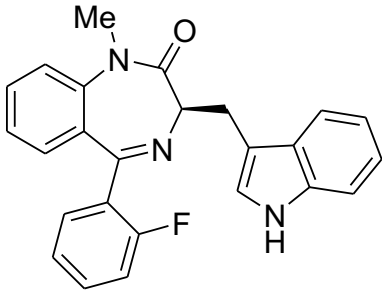
The term privileged structure was first used by Evans et al. (*J. Med. Chem*. **1988**, *31*, 2235-46) on the development of potent, selective, orally active cholecystokinin antagonists

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



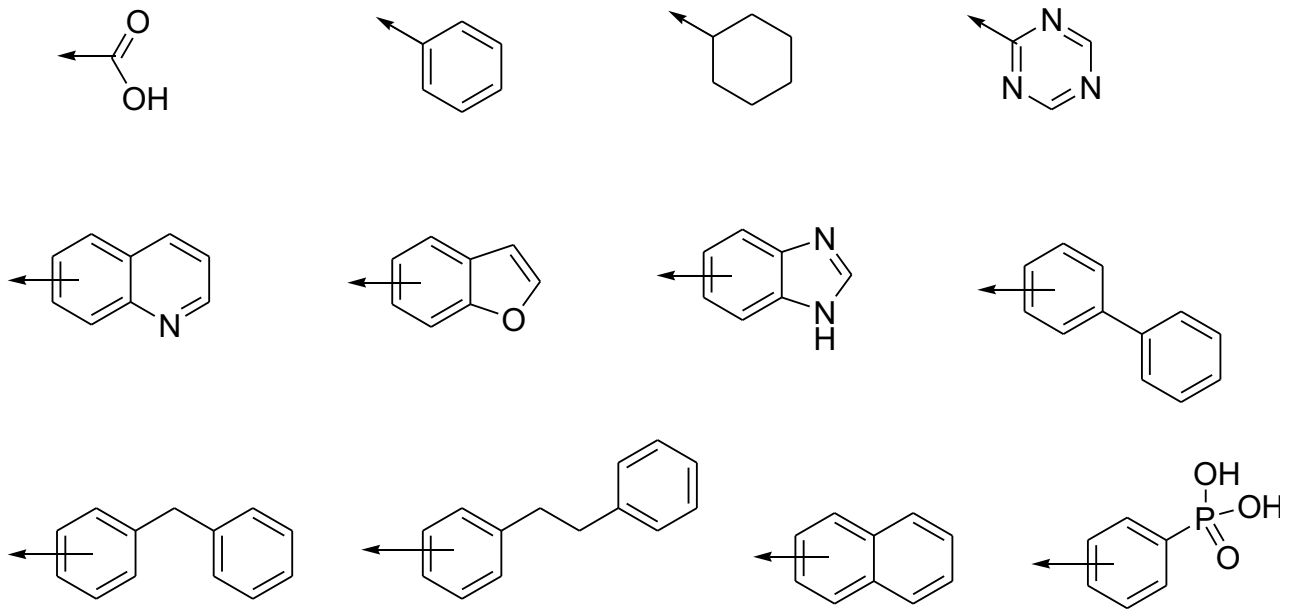
Valium





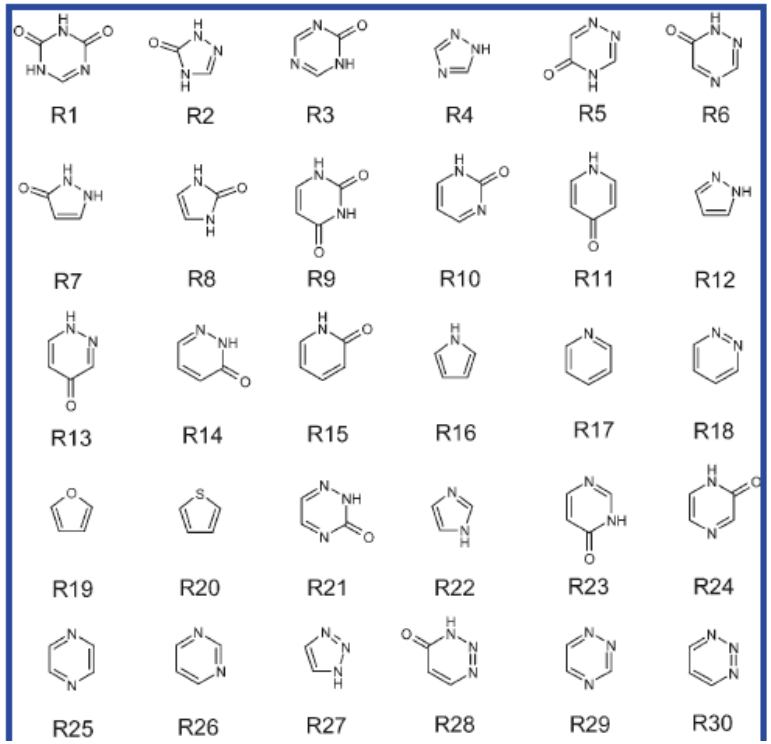
Privileged fragments

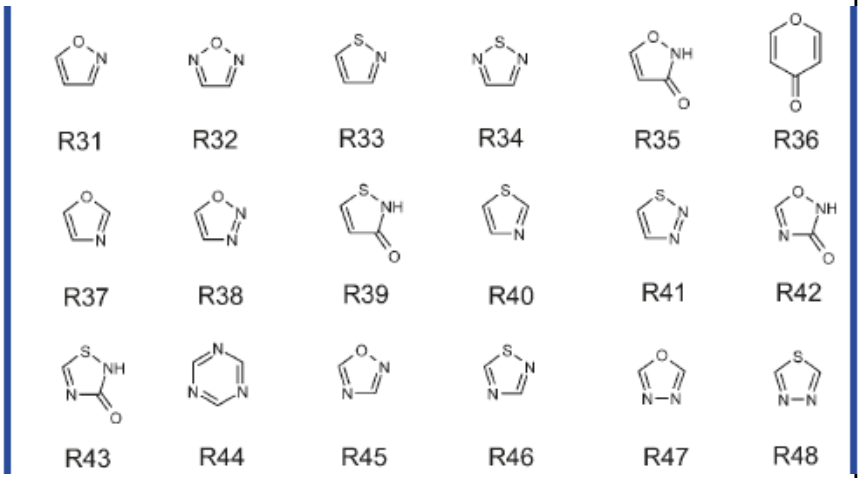
NMR based screening of fragments binding towards a variety of proteins:
Bcl-2 (an antiapoptotic protein), stromeolysin (MMP), VEGF-RBD, p56^{lck} SH2, FK-506 BP and others.
S. W. Fesik et al. *J. Med. Chem*. **2000**, *43*, 3443-47



Privileged structural elements: privileged rings (toolbox)

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

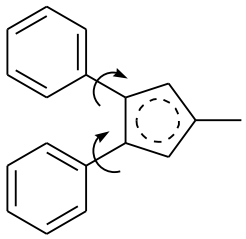




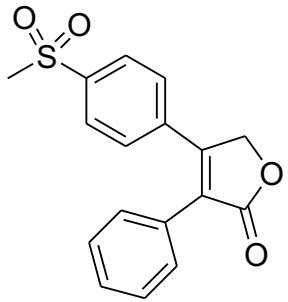
R. Ward et al. *J. Med. Chem*. **2011**, *54*, 4670-4677; S. D. Roughley et al. *J. Med. Chem.* **2011**, *54*, 3451-3479

Privileged structural elements

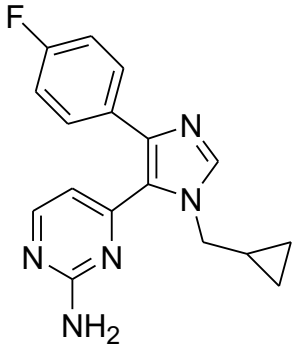
Privileged structures include often *favorable conformational arrangements of aromatic/ heteroaromatic groups*. Planar arrangements of aromatic groups give raise to stacking Which results in unfavorable properties such as low solubility and aggragation.



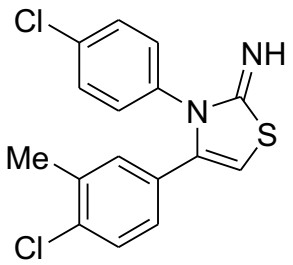
non-planar arrangement of two aromatic rings avoids stacking



COX-II inhibitor (Vioxx)

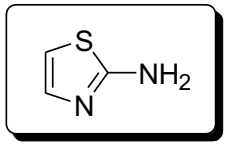


p38 MAP kinase (SB-218655)

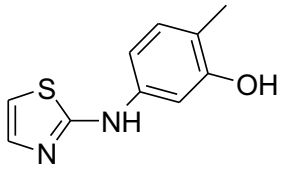


dopamine transporter inhibitor

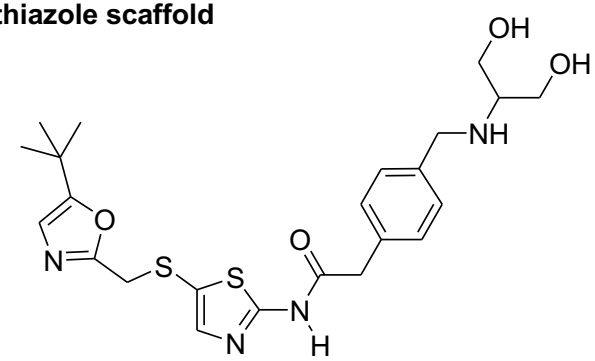
Privileged structural elements



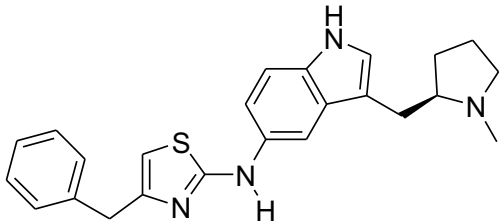
amino-thiazole scaffold



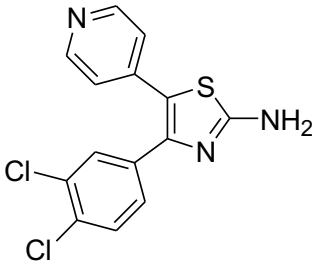
CBS-113A (clinical)
COX, 5-lipoxygenase



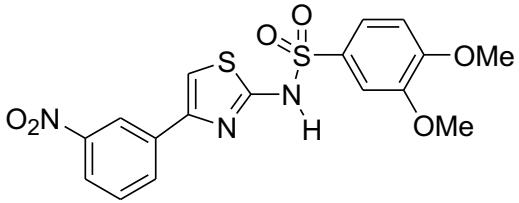
BMS-268770 (discovery)
CDK-2 inhibitor



CP-146662 (discovery)
5-HT_{1A} agonist, dopamine uptake

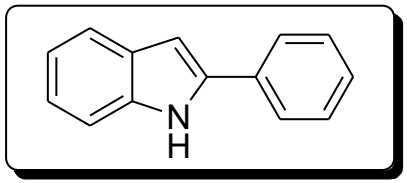


CGS-2466(discovery)
Adenosin A3 antagonist,
PDE-4, p38 MAP kinase

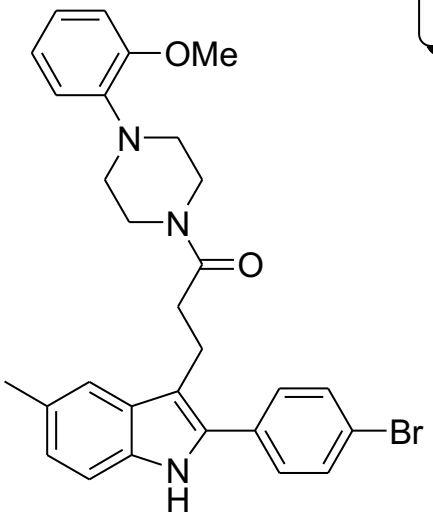


Ro 61-8048 (discovery)
Kynurenin-3-hydroxylase

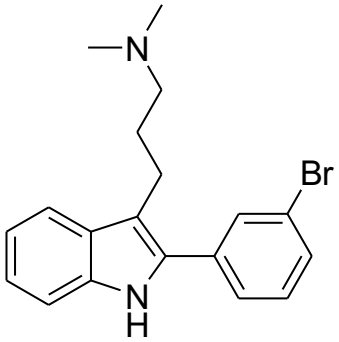
Privileged structural elements



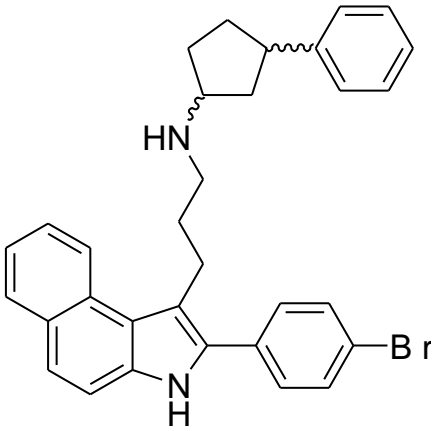
2-aryl-indole scaffold



NK1 antagonist (0.8nM)



5-HT_6 (0.7nM)
5-HT_7 (0.3μM)



CCR5 (1.3μM)
CCR3 (0.9μM)

C. A. Willoghby, *Biiorg. Med. Chem. Lett.* **2002**, *12*, 93-6

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 aquiring 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

N. A. Meanwell, *J. Med. Chem*. **2011**, *54*, 2529-2591
Synopsis of some recent tactical application of bioisosteres in drug discovery

Bioisosteres

monovalent bioisosteres

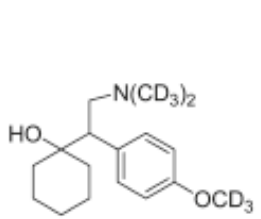
D and H
F and H
NH and OH
RSH and ROH
F, OH, NH_2 and CH_3
Cl, Br, SH and OH
C and Si

bivalent biososteres in which two single bonds are affected

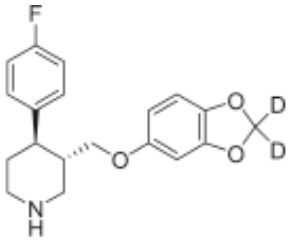
C=C, C=N, C=O, C=S
$-CH_2-$, $-NH-$, $-O-$, $-S-$
RCOR′, RCONHR′, RCOOR′, RCOSR′

trivalent bioisosteres in which three bonds are affected

R_3CH, R_3N
R_4C, R_4Si, R_4N^+
alkene, imine
$-CH=CH-$, $-S-$
$-CH=$ and $-N=C$

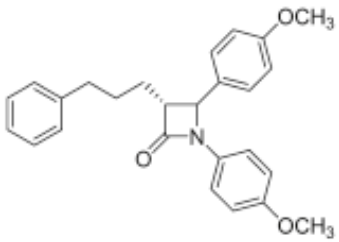


1, SD-254

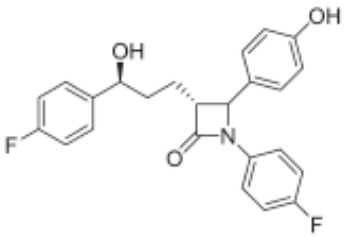


2, CTP-347

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



13: ED_{50} (hamster) = 2.2 mpk



14, ezetimibe: ED_{50} (hamster) = 0.04 mpk

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

N. A. Meanwell, *J. Med. Chem*. **2011**, *54*, 2529-2591
Synopsis of some recent tactical application of bioisosteres in drug discovery

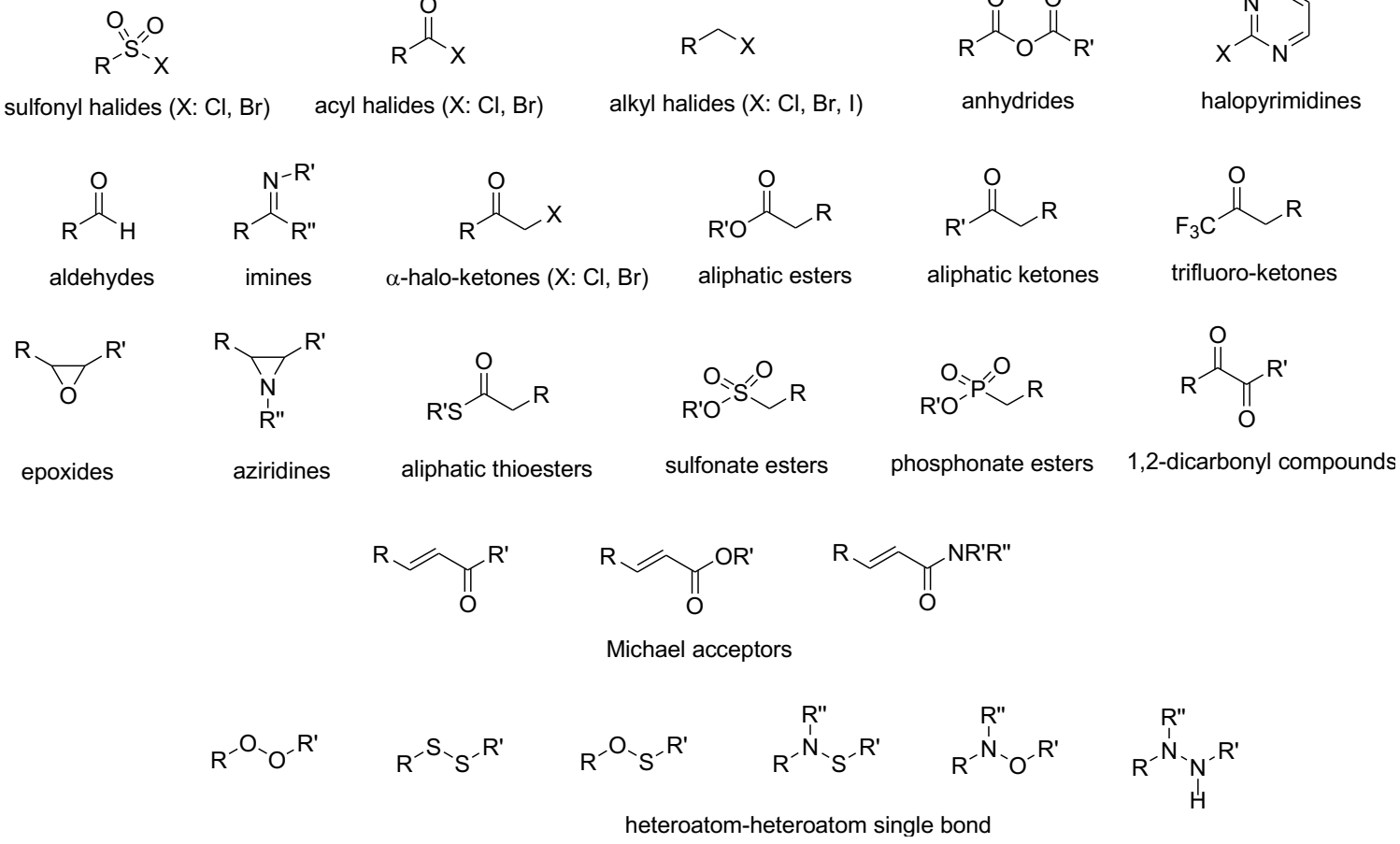
Unwanted properties: frequent hitters

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.
(G. M. Rishton, *Drug Disc. Today*, **1997**, *2*, 382-4)
-**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

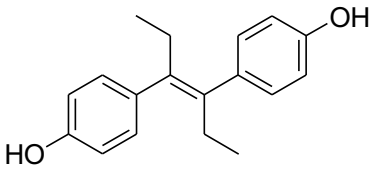


Reactive compounds and *in vitro* false positives in HTS (G. M. Rishton, *Drug Disc. Today*, **1997**, *2*, 382-4)

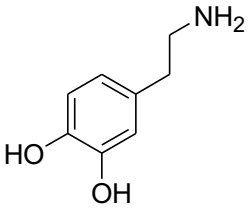
Unwanted properties: frequent hitters

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

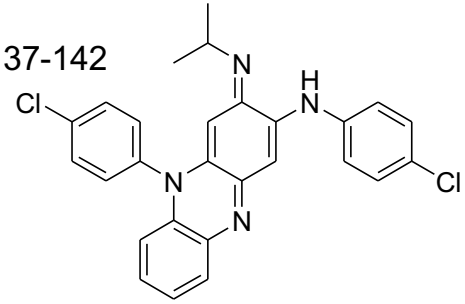
O. Roche et al. *J. Med. Chem*. **2002**, *45*, 137-142



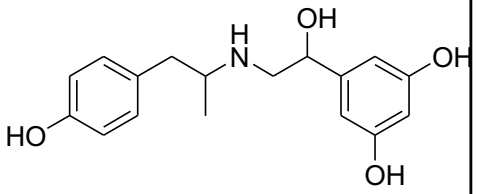
diethylstilbestrol (1.00)



dopamine(0.88)

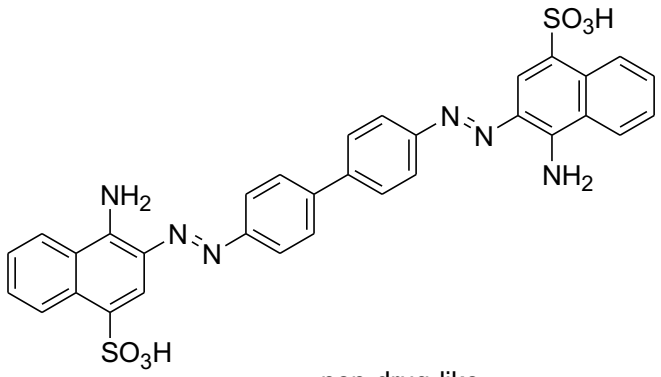


clofazimine(1.00)

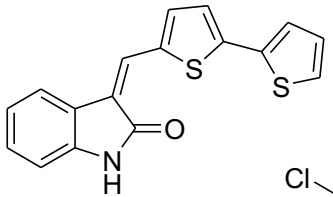


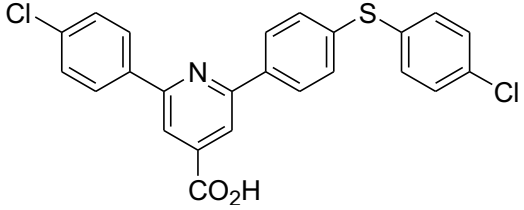
fenoterol(0.87)

molecules that form aggregates



non-drug-like





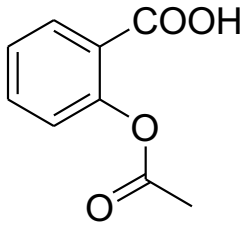
drug-like

G. Müller, *Drug Disc. Today*, **2003**, *8*, 681-91

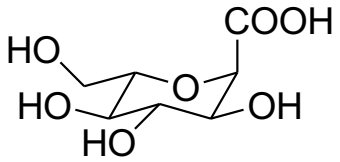
Questions

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?



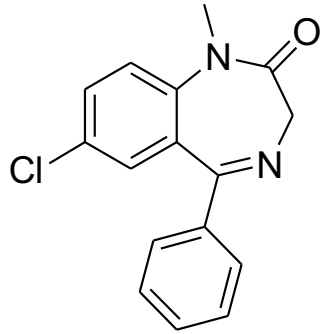
A



B

H-Lys-Glu-NH_2

C



D

3. Describe the difference between drug and lead-like

4. What is the fractional sp^3 character and which characteristics of a molecule does it describe?

The role of combinatorial chemistry and parallel synthesis in drug discovery

Large Screening Libraries for High Throughput Screening

100'000 to 3'000'000 compounds

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:

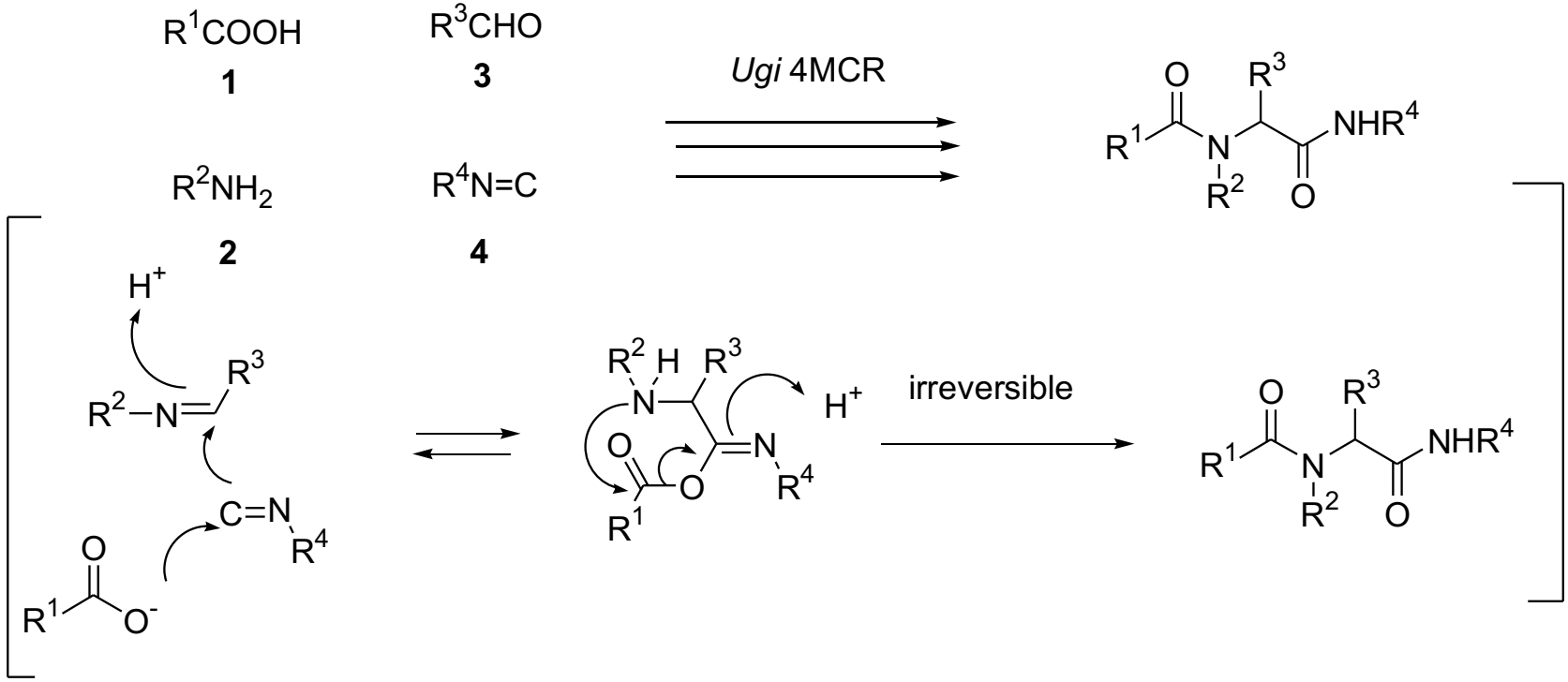
-Hit optimization, SAR, ADMET properties, TPP

Methods:

-Medicinal chemistry approaches; parallel synthesis

Historical background-objective

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 raction roducts...“

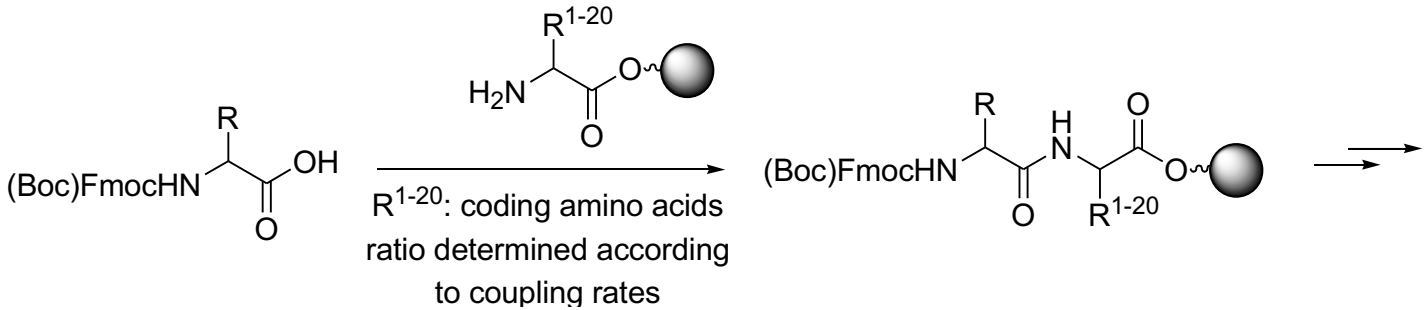


Compound mixtures versus single compounds

Compound mixtures:

-Mixtures (most often 10-20 compounds) of purified compounds in equimolar amounts

-Mixtures of products synthesized in one reaction in equimolar ratio:
***Mol. Immunol*. 1986, *23*, 709**



-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

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 todays 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)

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**

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

Solution-phase chemistry:

-small focused libraries of high chemical diversity **(purified products)**

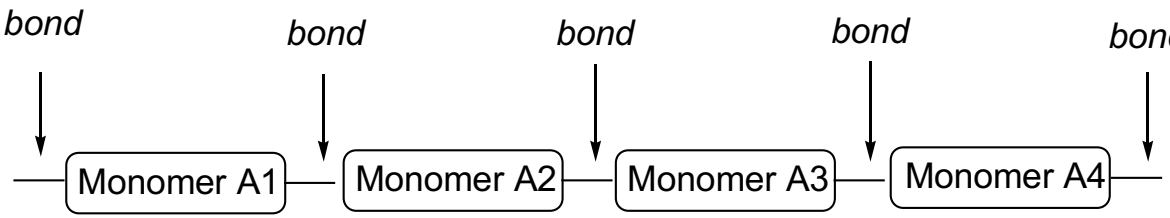
-parallel synthesis

-convergent approaches

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?

Linear, modular synthesis of biopolymers



monomers	*bond formation*	*polymers*
amino acids	**amide bond**	**peptides, proteines**
nucleotides	**phosphorester bond**	**oligonucleotides**
mono- and disaccharides	**glycosidic bond**	**polysaccharides**
N-alkylated glycines	**amide bond**	**peptoids**

Strenghts and weaknesses of peptides as drugs

-Peptides as drugs have a long history and started around 1920 with the discovery of insulin (Banting and Best):

-Insulin, oxytocin, gonadotropin-releasing hormone, vasopressin as highlights

-Nobel laureates:
du Vigneaud, Banting, Macleod, Schally and Guillemin, Sanger, Merryfield

-Polypeptides: contain between 2-50 amino acids (aa‘s)

-Endogenous peptides act as hormones, neurotransmitters, growth factors and anti-bacterial agents (host defense peptides)

-Most messengers of endocrine signaling pathways are peptides

-Most endogenous peptides and most successful peptide drugs are agonists, which generally require lower doses. Peptide antagonists do also exist

A. Henninot et al. *J. Med. Chem*. **2017**: DOI: 10.1021/acs.jmedchem.7b00318

Strenghts and weaknesses of peptides as drugs

Conceived weaknesses:

-Peptides are generally membrane-impermeable

-Peptides are restricted to extracellular and transmembrane targets

-Peptides are usually administered subcutaneously (sc) or intravenously (iv). Orally active peptides are rare (e.g. cyclosporin A)

-Peptides are unable to cross the blood brain barrier (BBB), which precludes targets in the central nervous system (CNS), however, limits also CNS side effects

-Peptides are biologically unstable. Endogenous biologically active peptides (usually agonists) evolved to very effectively activate their cognate receptors via elaborate and highly regulated systems and therefore require short half lives

-The manufacturing costs of peptides is generally higher than for small molecules, however, lower than for therapeutic proteins

A. Henninot et al. *J. Med. Chem*. **2017**: DOI: 10.1021/acs.jmedchem.7b00318

Strenghts and weaknesses of peptides as drugs

-Peptides are usually cleared by proteolytic degradation and by renal filtration, which generally results in short half lives. PK-PD can be optimized by medicinal chemistry optimization

Strenghts:

-Peptides are generally highly potent and selective

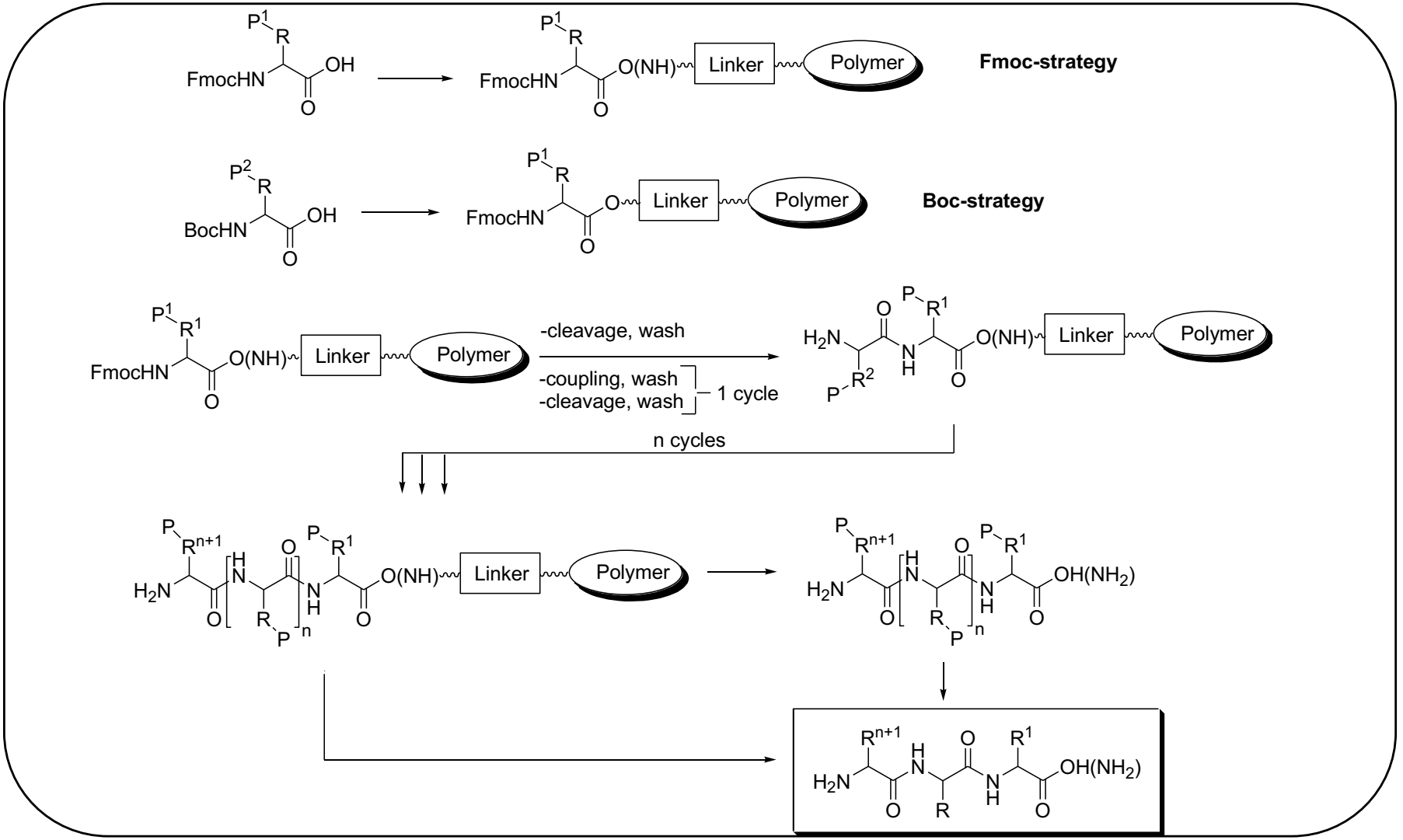
-Most endogenous hormones, neurotransmitters and growth factors are peptide agonists and modulate their cognate receptors in a very short-lived and subtle way

-Most constituents of the innate immune system are peptides (host defense peptides) which have a wide range of biological activities (e.g. antibacterial and immune modulating)

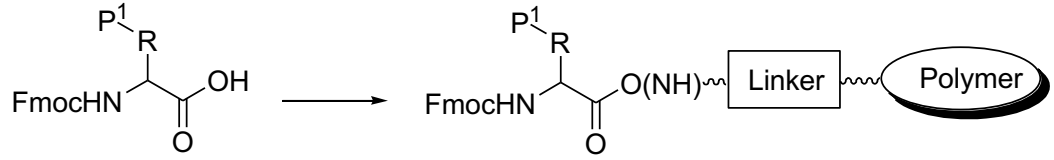
-Low BBB penetration and renal clearance (no Cyp450 inhibition and hepatic clearance) results generally in lower toxicity issues as compared to small molecules

A. Henninot et al. *J. Med. Chem*. **2017**: DOI: 10.1021/acs.jmedchem.7b00318

Solid-phase synthesis of polypeptides

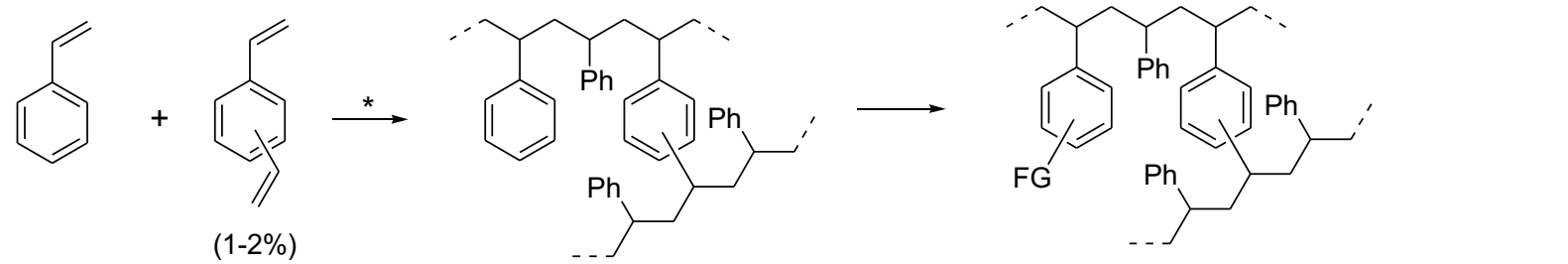


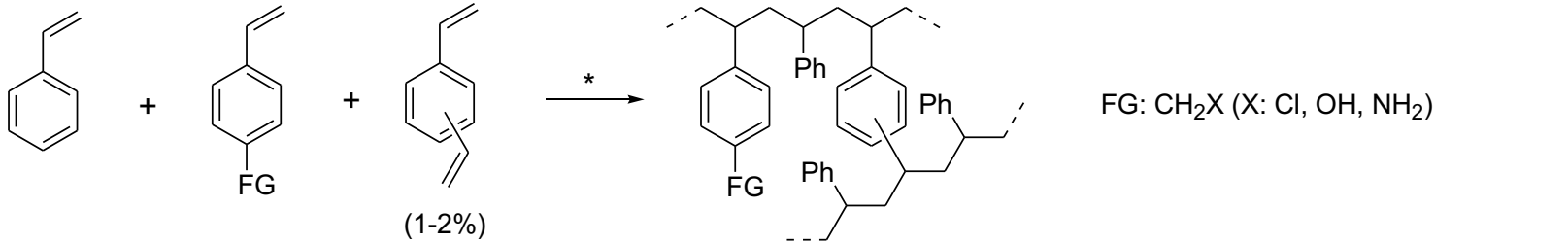
Solid-phase synthesis of polypeptides: resins-polymer supports



Fmoc-strategy

1. Functionalized polystyrene resins:



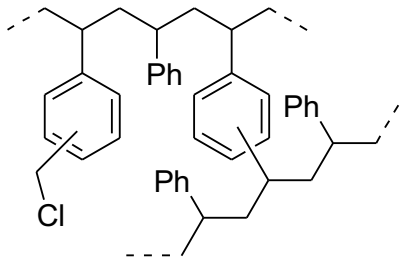


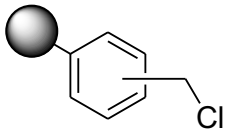
FG: CH_2X (X: Cl, OH, NH_2)

* **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



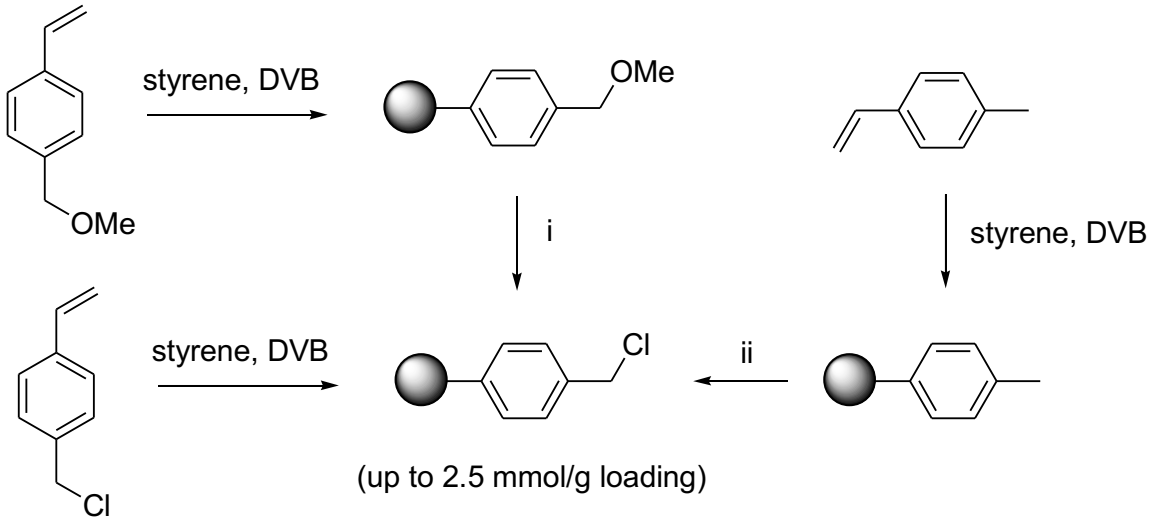


(95:5 para/ortho)

microporous: 1-2% crosslinking

macroporous: 20% crosslinking

chloromethyl-polystyrene resin (Merryfield resin: *J. Am. Chem. Soc*. **1963**, *85*, 2149)



(up to 2.5 mmol/g loading)

i: BCl_3, CCl_4, 0°, 2h; ii: NaOH, $CHCl_3$ (or $ClCH_2CH_2Cl$), BnN^+Et_3, Cl^-, SO_2Cl_2, AIBN, 60°; *Macromolecules* **1986**, *19*, 2470

Solid-phase synthesis of polypeptides: resins-polymer supports

Swelling properties of Merryfield type microporous resins:

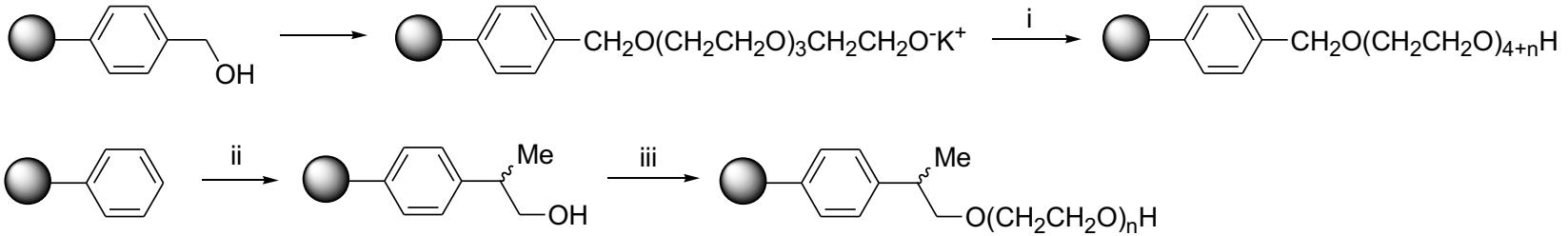
Solvent	crosslinked PS (1% DVB)*	crosslinked PS (2% DVB)*
MeOH	0.95	
EtOH	1.05	1.0
AcOH		1.0
MeCN	2.0	
pyridine		3.0
DMF	3.5	2.0
THF	5.5	
dioxane	4.9	2.5
Et_2O	2.6	
CH_2Cl_2	5.2	
toluene	5.3	2.8

*swelling capacity: volume of swollen resin/original volume

Solid-phase synthesis of polypeptides: linkers

2. TentaGel^{R} resins

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

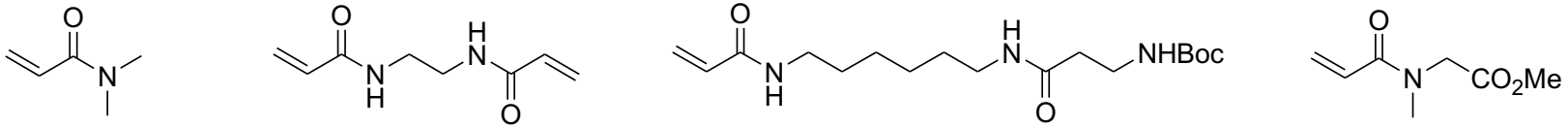


i: ethylene oxide; ii: propylene oxide, $SnCl_4$, CH_2Cl_2; iii: ethylene oxide, KOH, dioxane, 110°

Good swelling properties in: water, MeOH, CH_2Cl_2, MeCN, THF and DMF; used preferentially in **continous flow reactors**

3. Polyacrylamide resins

pioneerd by *Sheppard*: *Bioorg. Chem.* **1979**, *8*, 351

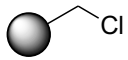
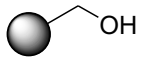
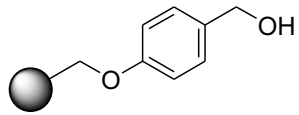
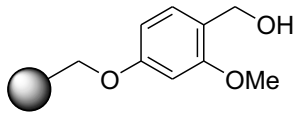
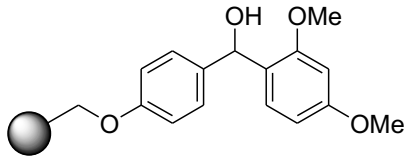
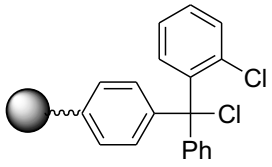


basic monomer | crosslinking agent | functionalized monomers

Persulphate initiated copolymerisationin 66% aqueous DMF, 1,2-dichloroethane and cellulose acetate/butyrate as emulgator

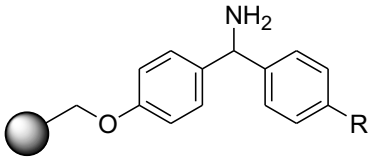
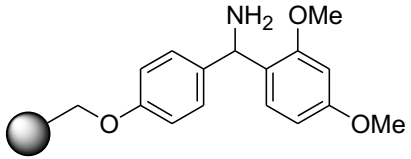
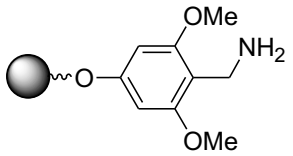
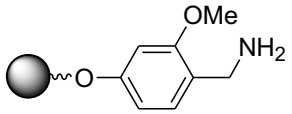
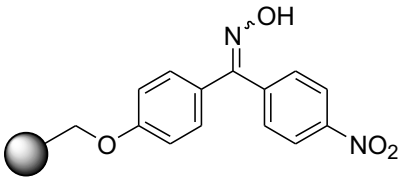
Solid-phase synthesis of polypeptides: linkers

2. Linkers for releasing carboxylic acids

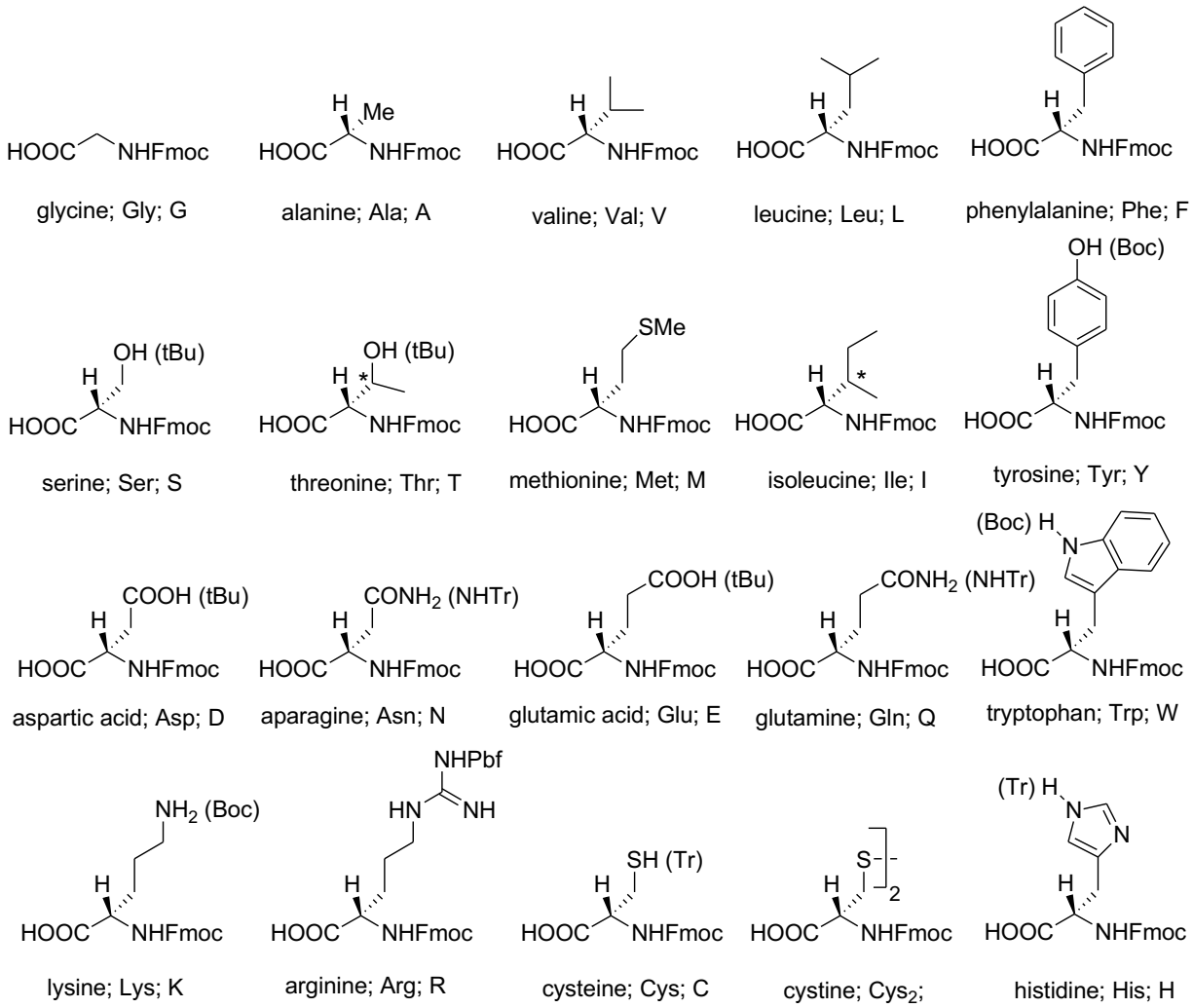
structure	abbreviation	cleavage conditions	reference
	*Merryfield* resin	HF, CF_3SO_3H	*J. Am. Chem. Soc.* **1963**, *85*, 2149
	hydroxymethyl-PS	HF, CF_3SO_3H	
	*Wang* resin	95% TFA	*J. Am. Chem. Soc.* **1973**, *95*, 1328
	Sasrin^R resin (Bachem)	1% TFA	*Tetrahedron Lett.* **1988**, *29*, 4005
	*Rink* resin	1% TFA	*Tetrahedron Lett.* **1987**, *28*, 3787
	chloro-trityl resin (Barlos)		*Tetrahedron Lett.* **1989**, *30*, 3943

Solid-phase synthesis of polypeptides: linkers

2. Linkers for releasing amides

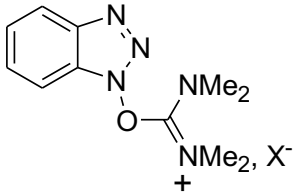
structure	abbreviation	cleavage conditions	reference
	BHA (R=H) MBHA (R=Me)	HF, CF_3SO_3H	*J. Org. Chem.* **1985**, *50*, 5291 *Peptides.* **1981**, *2*, 85
	*Rink* resin	95% TFA	*Tetrahedron Lett.* **1987**, *28*, 3787
	PAL resin	TFA	*Int. J. Prot. Pept. Res.* **1987**, *30*, 206
		TFA	*Tetrahedron Lett.* **1997**, *38*, 7325
	*Kaiser* oxime resin	NH_3 primary and secondary amines NH_2NH_2 x $1H_2O$	*J. Org. Chem.* **1980**, *45*, 1295

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



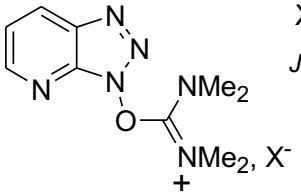
Solid-phase synthesis of polypeptides: coupling reagents

uronium salts



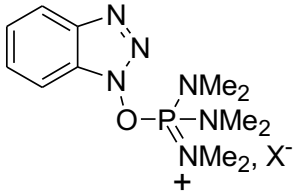
X^-: PF_6^- **HBTU**; BF_4^- **TBTU**

Tetrahedron Lett. **1989**, *30*, 1927



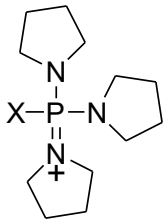
X^-: PF_6^- **HATU**

J. Chem. Soc., Chem. Commun. **1994**, 201



X^-: PF_6^- , BF_4^-, (*Castro's* reagent)

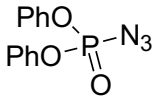
Tetrahedron Lett. **1975**, 1219



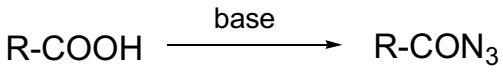
,PF_6^- (X=Cl, **PyCloP**)
(X=Br, **PyBroP**)

J. Org. Chem. **1994**, *59*, 2437

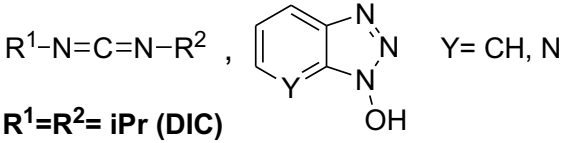
azides



DPPA (diphenyl-phosphoryl azide)



carbodiimides

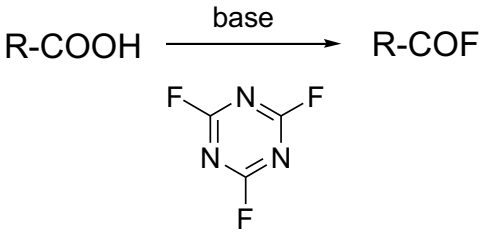


R^1=R^2= iPr (DIC)

R^1=R^2=cyclohexyl (DCC)

R^1=Et; R^2=$CH_2CH_2N^+Me_2$, Cl^- (EDCI)

acid fluorides

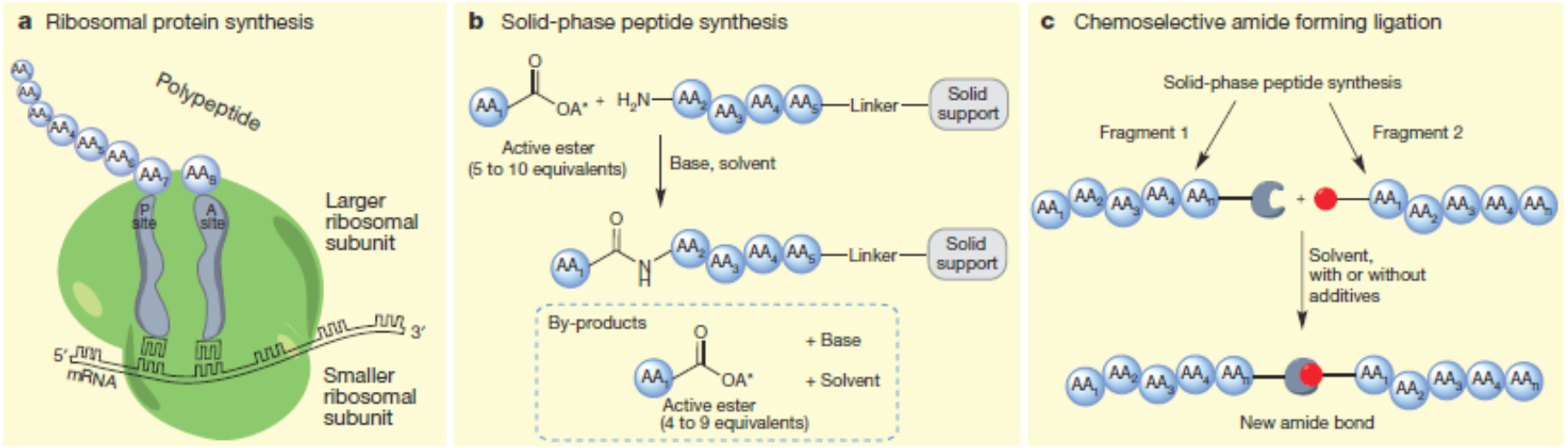


Solid-phase synthesis of polypeptides: protective groups

Fmoc strategy:	**Cleavage**
Main chain **(backbone) amino groups: Fmoc**	20% piperidine/DMF, rt
Side chain **amino groups (Lys, Orn, Dab): Boc**	TFA, CH_2CH_2, triisoprpoylsilane*
Side chain **carboxylic acids (Glu, Asp): t-butyl esters**	TFA, CH_2CH_2 triisopropylsilane*
Side chain **primary amides (Gln, Ans): N-trityl**	TFA, CH_2Cl_2, triisopropylsilane*
Side chain **hydroxy(phenol) groups (Ser, Thr, Tyr): t-butyl ethers**	TFA, CH_2Cl_2, triisopropylsilane*
Side chain **indole and imidazole groups (Trp, His): N-trityl**	TFA, CH_2Cl_2, triisopropylsilane*
Side chain **guanidine groups (Arg): Pmc, Pmb**	TFA, CH_2Cl_2, triisopropylsilane*
	*other scavengers like thioanisol phenol, H_2O, thiocresol and others are used

Overview: synthesis of polypeptides

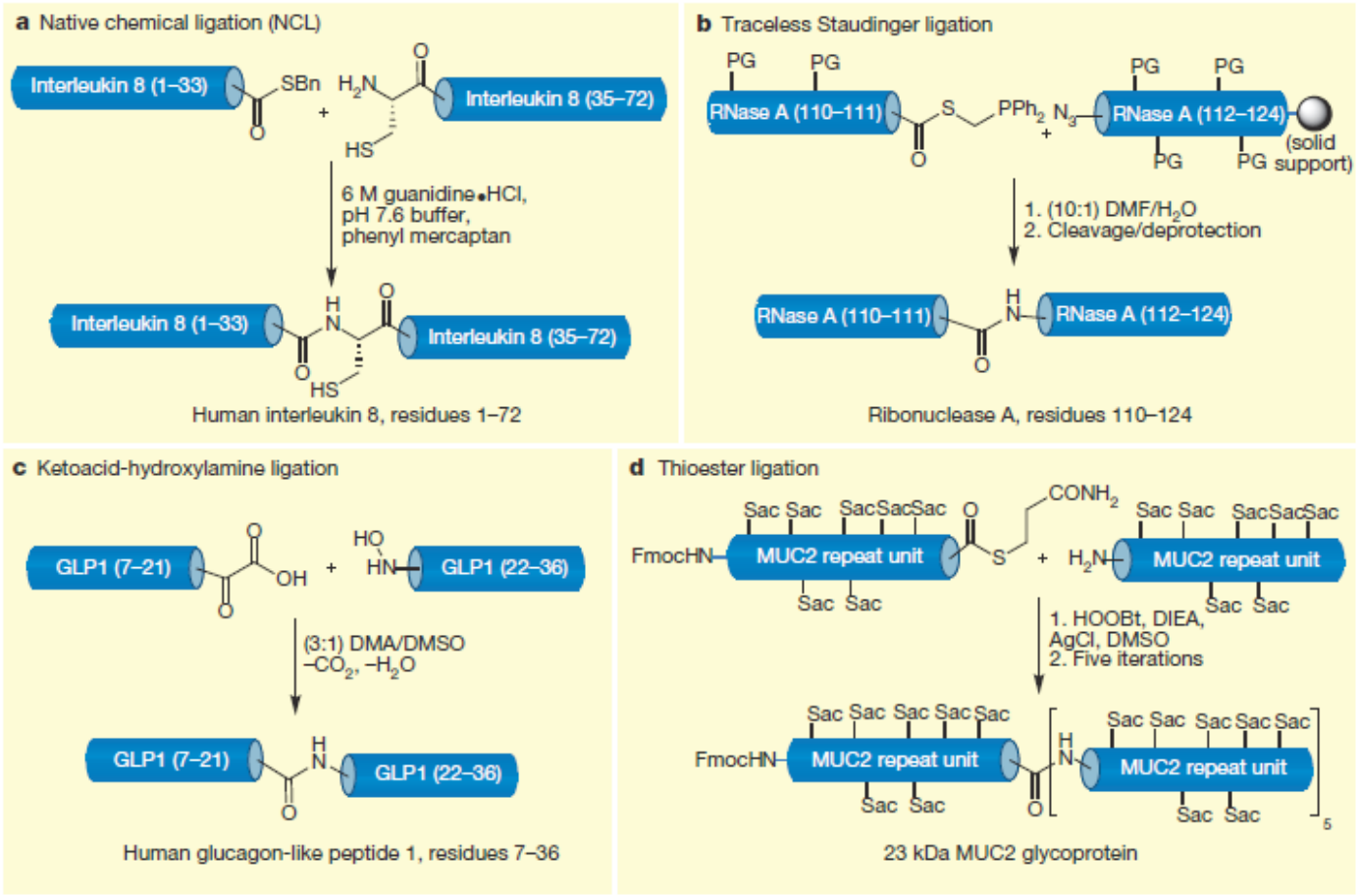
Strategies for amide bond synthesis in polypeptides



from V. R. Pattabiraman et al. *Nature* **2011**, *480*, 471-479

Overview: synthesis of polypeptides

Strategies for amide bond synthesis in polypeptides



from V. R. Pattabiraman et al. *Nature* **2011**, *480*, 471-479

Strenghts and weaknesses of peptides as drugs

Peptide optimization:

Highly potent peptide hits and leads have to be optimized for *selectivity*, *stability*, *solubility* and *minimal toxicity*. Some recepies:

-Determine the minimal sequence

-Identify the critical residues (pharmacophore) by positional scanning: Ala scan, scan with a diverse set of amino acids

-Protection from degradation at the N- and C-termini by N-acylation (e.g. N-acetyl) and C-amidation (e.g. -$CONH_2$)

-Identification of sites of proteolysis: determination of proteolytic degradation products in biological fluids and tissues

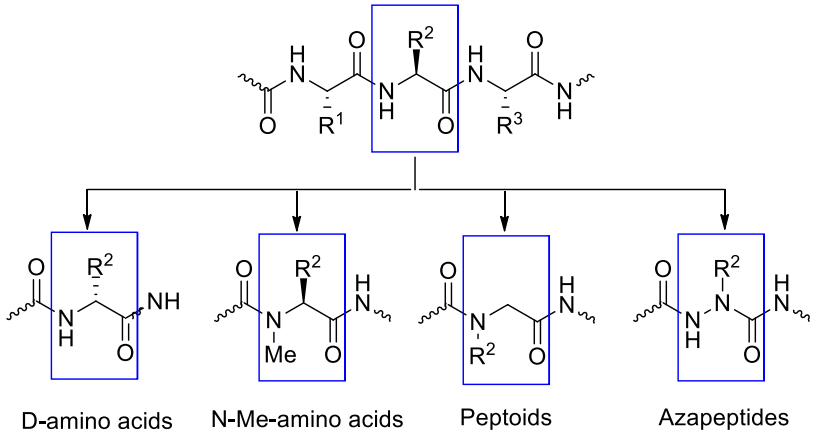
A. Henninot et al. *J. Med. Chem*. **2017**: DOI: 10.1021/acs.jmedchem.7b00318

Strenghts and weaknesses of peptides as drugs

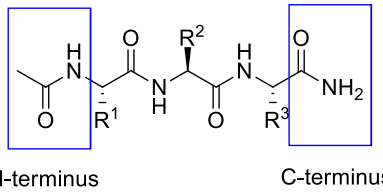
Peptide optimization:

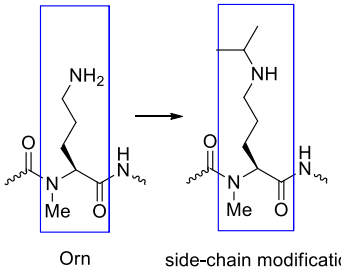
-Stabilization of proteolytic degradation by back-bone modifications:

-incorporation of: D-amino acids; α-methylated amino acids; N-methylated amino acids; β-amino acids; peptoids, and aza-peptides



-Stabilization of proteolytic degradation by N-and C terminal and side-chain modifications:

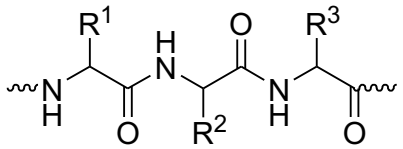




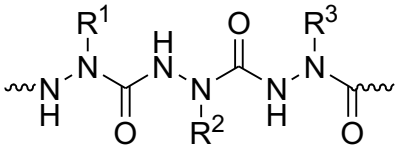
A. Henninot et al. *J. Med. Chem.* **2017**: DOI: 10.1021/acs.jmedchem.7b00318

Solid-phase synthesis of peptoids

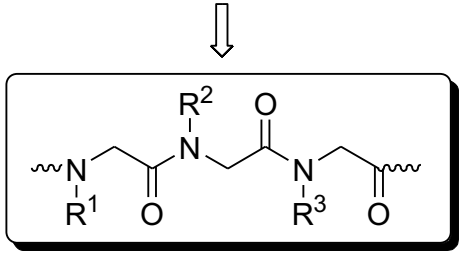
Peptoids: ideal scaffold for parallel and combinatorial synthesis



peptide backbone



azapeptide backbone



peptoid backbone

-*protease stability* increased

-number of *H-bond donors* reduced (can be also disadvantage)

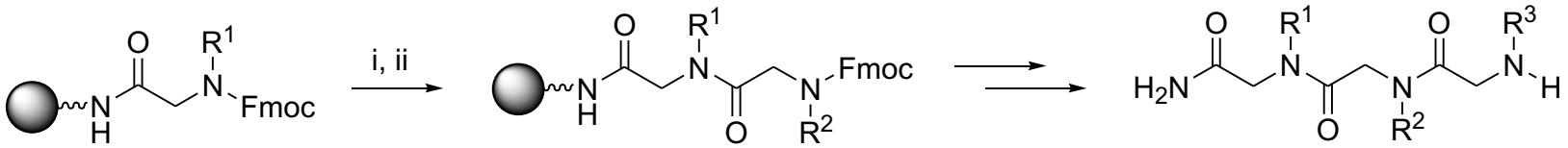
-number *of rotatable bonds* increased (tertiary amides have lower trans-cis barrier)

-prediction of *peptoid backbone conformation* quite difficult (flexibility)

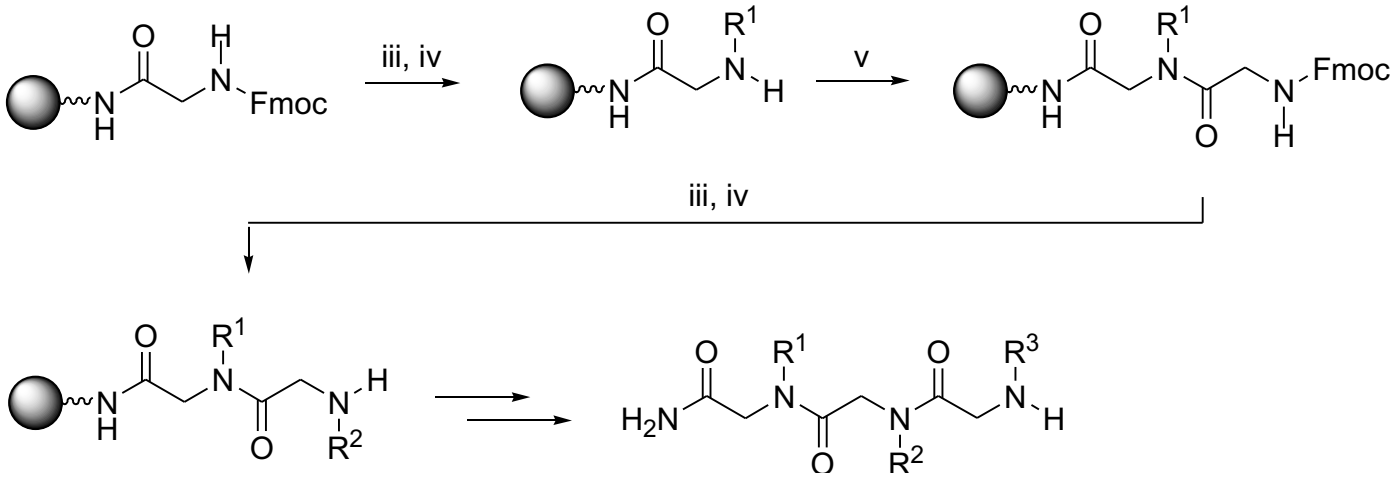
-ideally suited for *library synthesis*: large number of building blocks available
available by solid-phase synthesis
split-mixed synthesis possible

Solid-phase synthesis of peptoids

Approach A: sequential coupling of N-substituted glycines



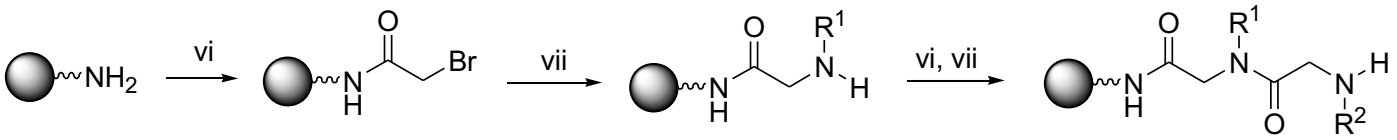
Approach B: sequential coupling of glycine followed by reductive amination with aldehydes



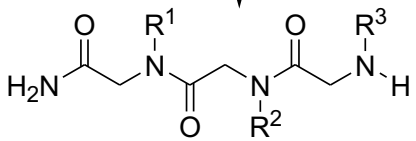
i: DBU, DMF; ii: PyBop or PyBrop, $R^2NFmocCH_2COOH$; iii: DBU, DMF; vi: RCHO, $Na(OAc)_3BH$ or $NaCNBH_3$, MeOH; v: Fmoc-Gly, PyBop or PyBrop; vi: DIC, DMF, $BrCH_2COOH$; vii: $R\text{-}NH_2$, DMSO

Solid-phase synthesis of peptoids

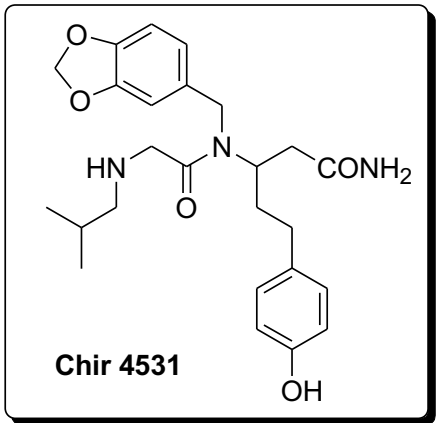
Approach C: coupling of bromo-acetic acid followed by nucleophilic displacement with amines



Proc. Nat. Acad. Sci. USA **1992**, *89*, 9367



i: DBU, DMF; ii: PyBop or PyBrop, $R^2NFmocCH_2COOH$; 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

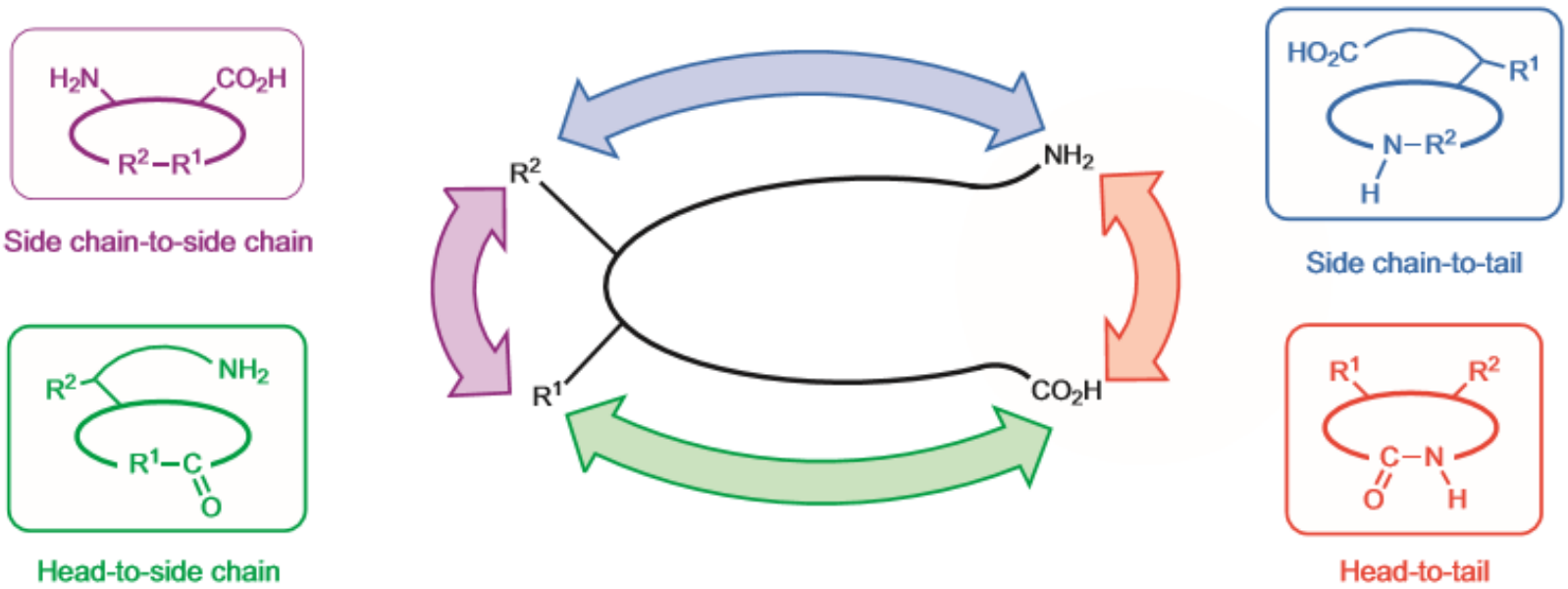


Screening 18 pools originated from split-mixed synthesis for [^{3}H]-DAMGO (μ-specific) binding to opiate receptor.
Chir 4531: 6nM

Strenghts and weaknesses of peptides as drugs

Peptide optimization:

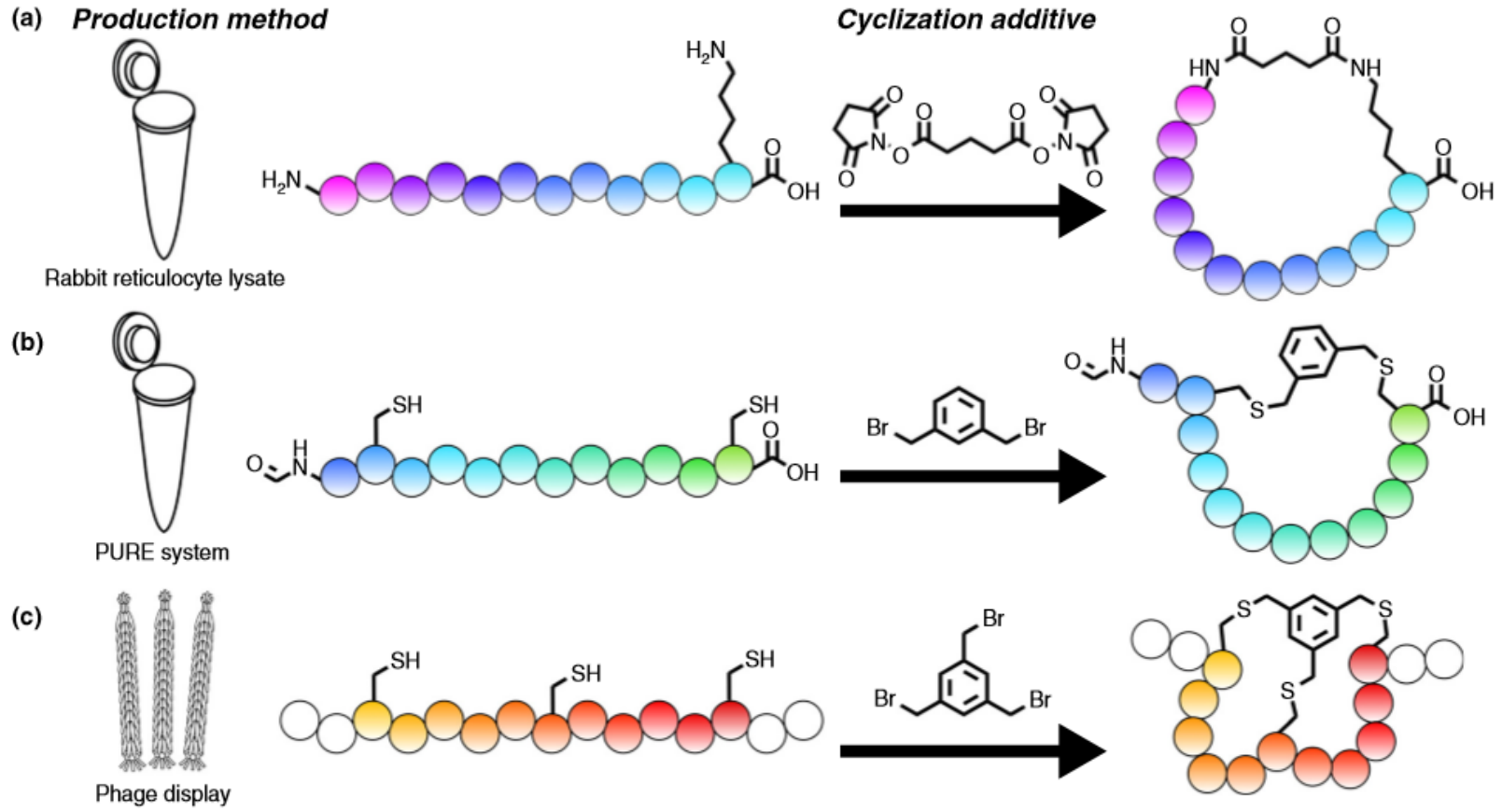
-Stabilization of proteolytic degradation by cyclization:



C. J. White et al. *Nat. Chem.* **2011**, *3*, 509-524

Overview: synthesis of polypeptides

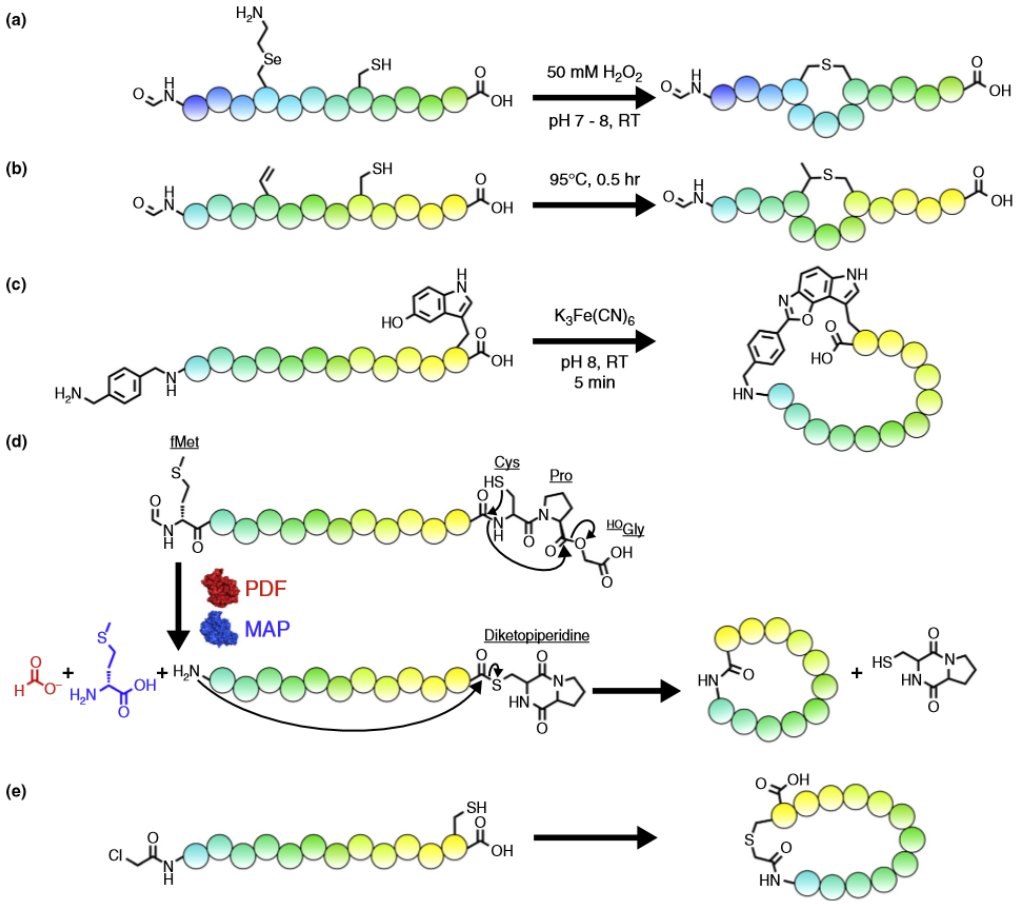
Strategies for polypeptide cyclization using in vitro display startegies



N. K. Bashiruddin et al. *Curr. Opin. Chem. Biol.* **2015**, *24*, 131-138

Overview: synthesis of polypeptides

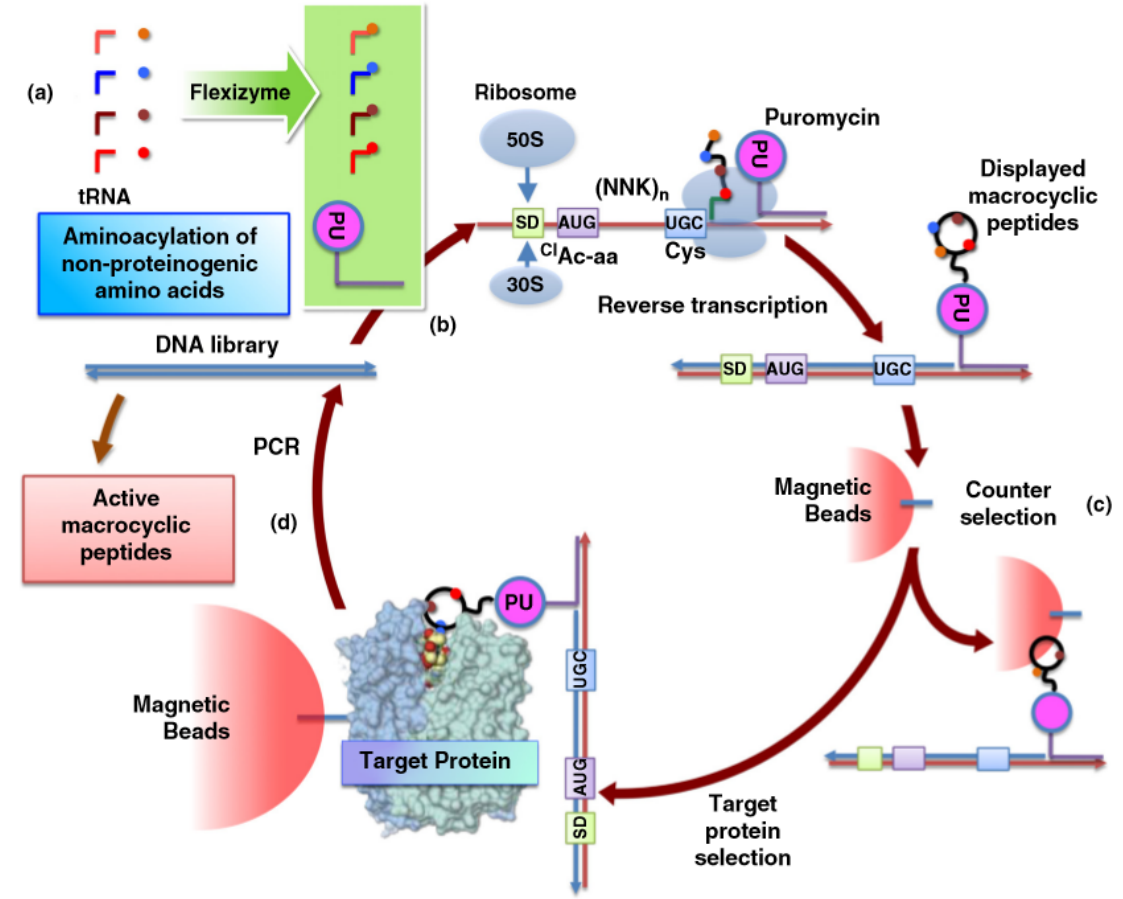
Strategies for polypeptide cyclization using in vitro display startegies



N. K. Bashiruddin et al. *Curr. Opin. Chem. Biol.* **2015**, *24*, 131-138

Overview: synthesis of polypeptides

Strategies for polypeptide cyclization using in vitro display startegies



N. K. Bashiruddin et al. *Curr. Opin. Chem. Biol.* **2015**, *24*, 131-138

Peptide mimetics

α-helix (3.6_{10} helix)
ϕ = -57 °, ψ = -47 °

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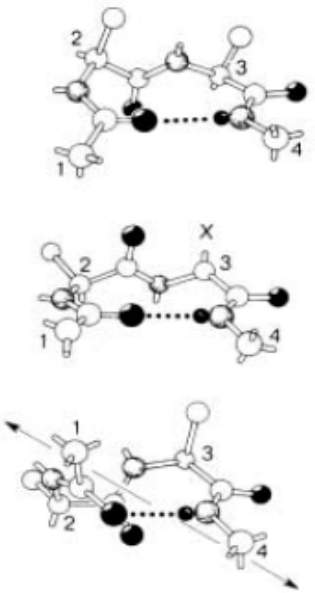
Peptide Secondary Structure Motifs



Torsional Angles

ω: N_{i+1} - C_i

ψ: C_i - C_i^{α}

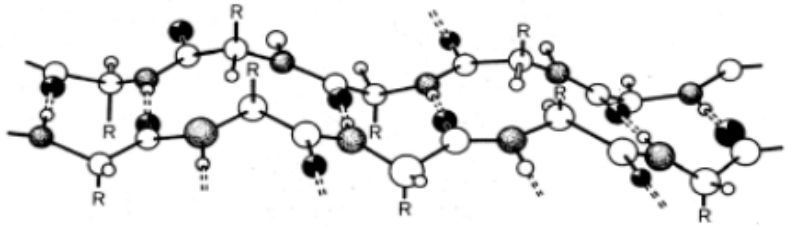
ϕ: C_i^{α} - N_i

χ: C_i^{α} - C_i^{β}

β-turns	ϕ_2	ψ_2	ϕ_3	ψ_3
I	-60	-30	-90	0
II	-60	+120	+80	0
III	-60	-30	-60	-30

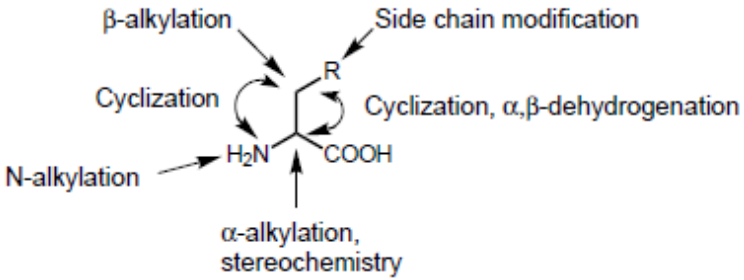
Adapted from *Adv. Drug. Res.* **1997**, *29*, 1-78

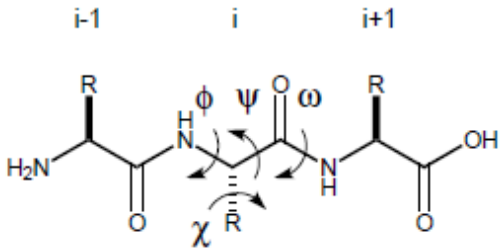
β-sheet
ϕ = -139 °, ψ = +135 °



Peptide mimetics

Conformational Constraints

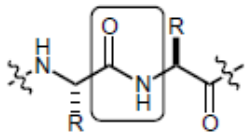




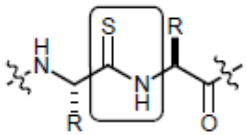
Modification	Conformational effect
1. Backbone *N*-alkylation	ϕ, ψ, χ are constrained, facilitates cis-trans amide bond isomerism
2. Backbone C_α-alkylation	ϕ, ψ are constrained to a helical or extended linear structure
3. D-Amino acid/proline substitution	Favors formation of β-turn structures
4. Peptide bond isosteres	ω can be fixed at 0 or 180° (olefins), or allowed greater freedom of rotation (i.e. -CH_2 S-)
5. Cyclic amino acids	ω can be biased to 0 or 180°, ϕ, ψ are biased towards formation of β-turns or γ-turns, χ can also be affected
6. Dehydroamino acids	Fix χ at 0 or 180°
7. β-alkylation	Constrain χ, may also affect backbone conformation

Peptide mimetics

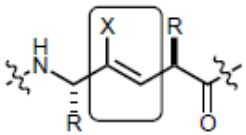
Common Amide Bond Isosteres



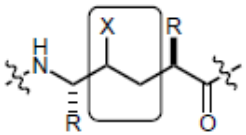
Peptide



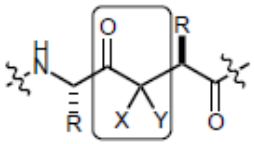
Thioamide isostere



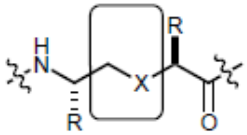
Trans-olefin isosteres
X = H, F



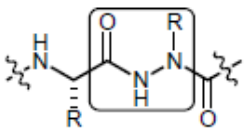
Ethylene isosteres
X = H, OH



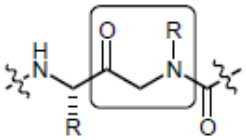
Ketomethylene isosteres
X = Y = H or F
X = H, Y = OH



Methylene isosteres
X = S, S(O), O



Azapeptide isostere

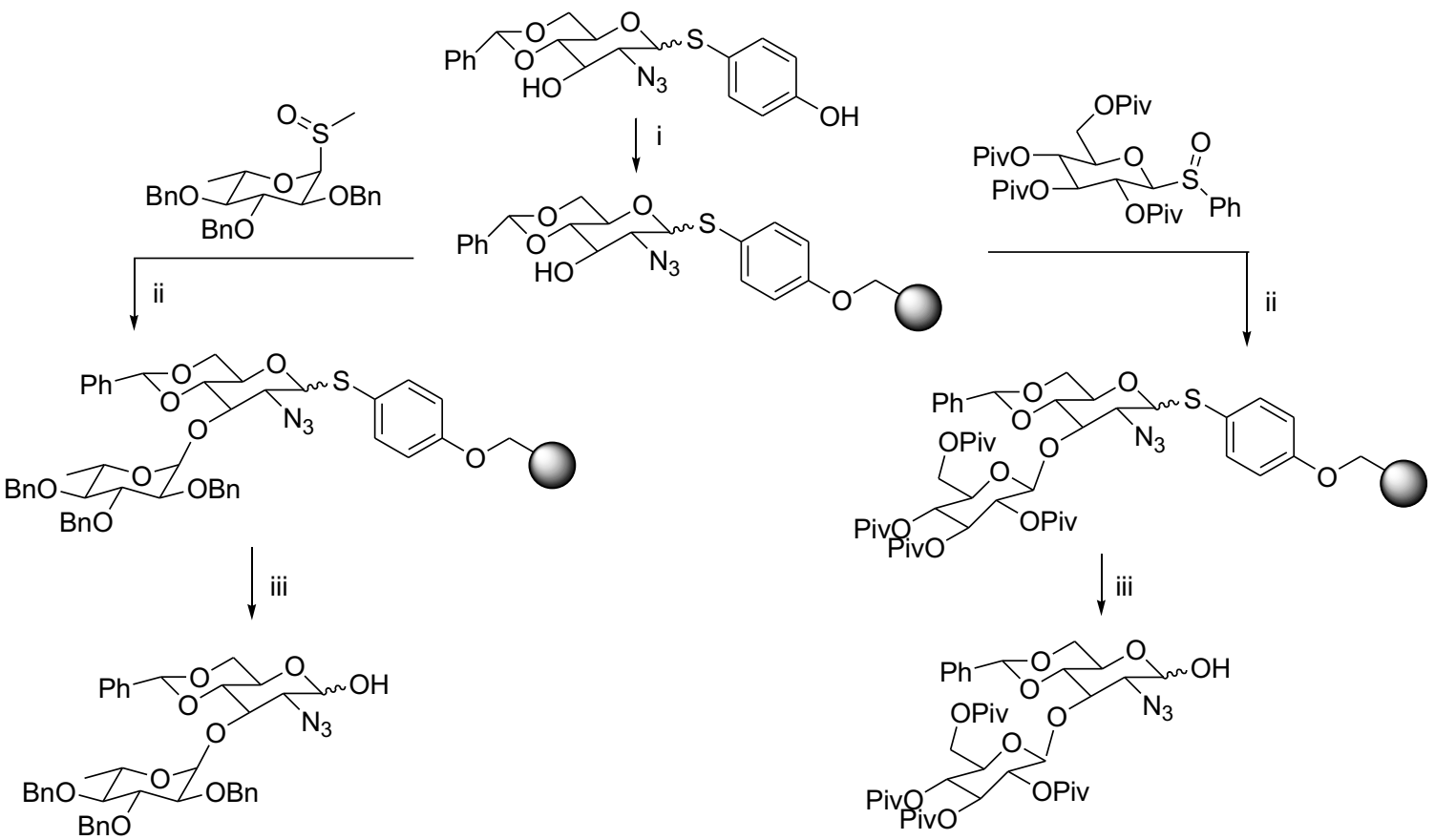


Peptoid isosteres

For a comprehensive review, see:
- Rieger, Evans Group Seminar, 1991
- Goodman *Burger's Medicinal Chemistry and Drug Discovery*. Ed. M. E. Wolff. New York, John Wiley & Sons, Inc., **1995**, 803-861.

3-dipeptide isosteres 4/12/01 10:39 AM

Solid-phase synthesis of oligosccharides



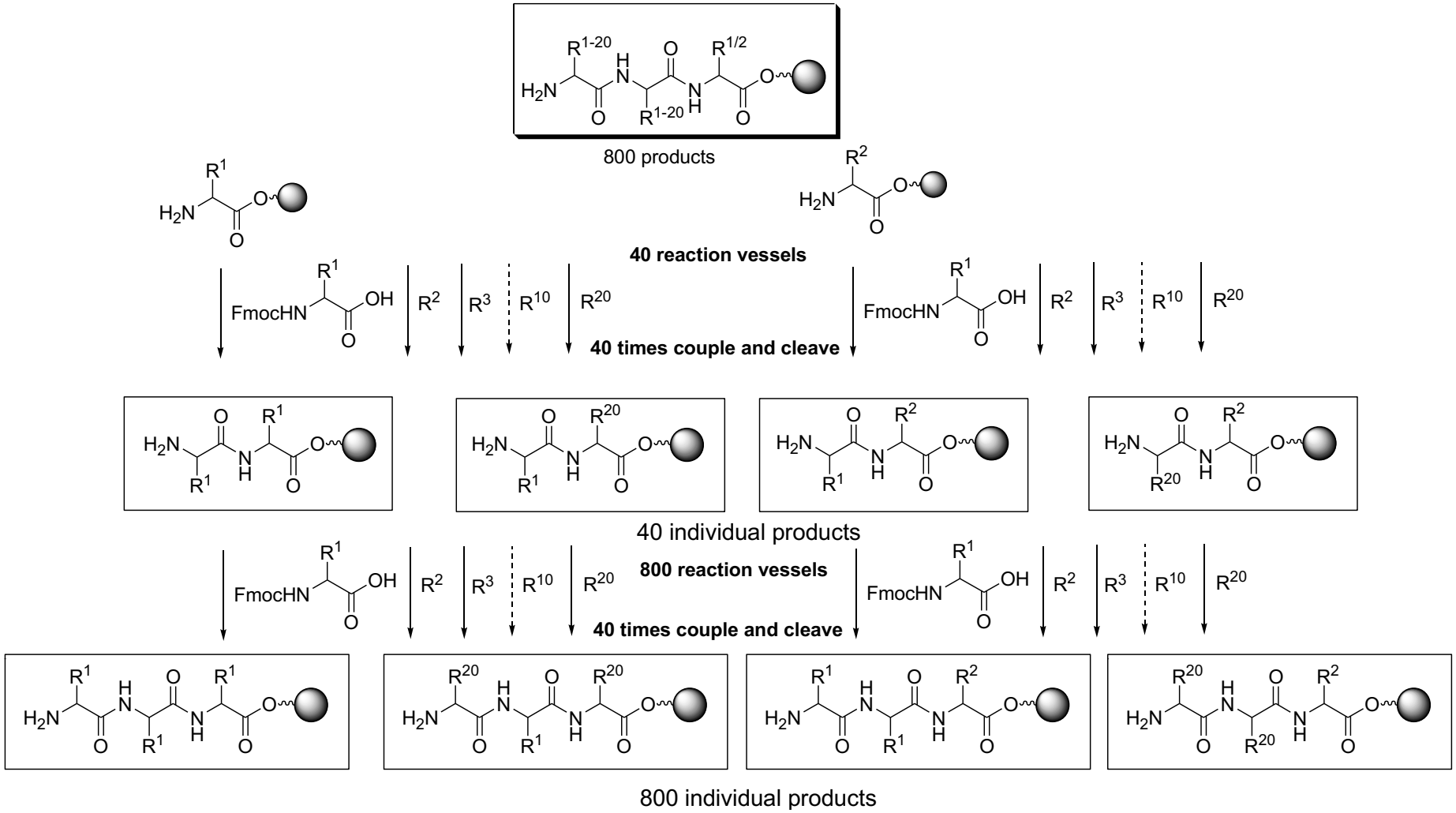
i: Cs_2CO_3, Merryfield resin; ii: Tf_2O, 2,6-di-tert-butyl-4-methylpyridine, CH_2Cl_2, -60 to -20°; iii: $Hg(OCOCF_3)_2$, CH_2Cl_2, H_2O, r.t.

D. Kahne et al. *J. Am. Chem. Soc*. **1994**, *116*, 6953; ibid *J. Am. Chem. Soc*. **1994**, *116*, 1766; ibid *J. Am. Chem. Soc*. **1989**, *111,* 6881

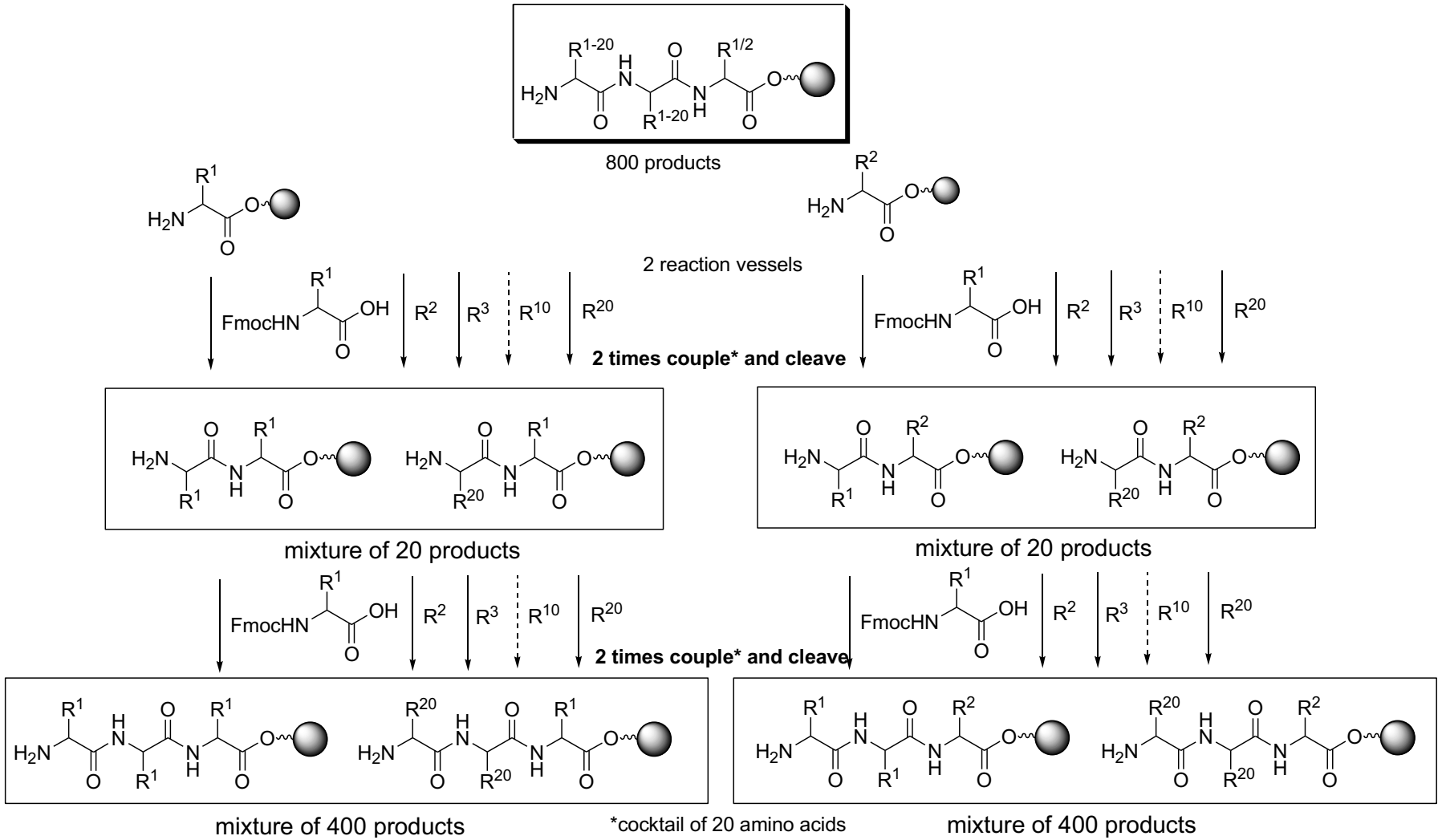
Questions

1. Name at least three different types of solid supports?
2. Give at least two different ways to synthezise chloro-methyl polystyrene?

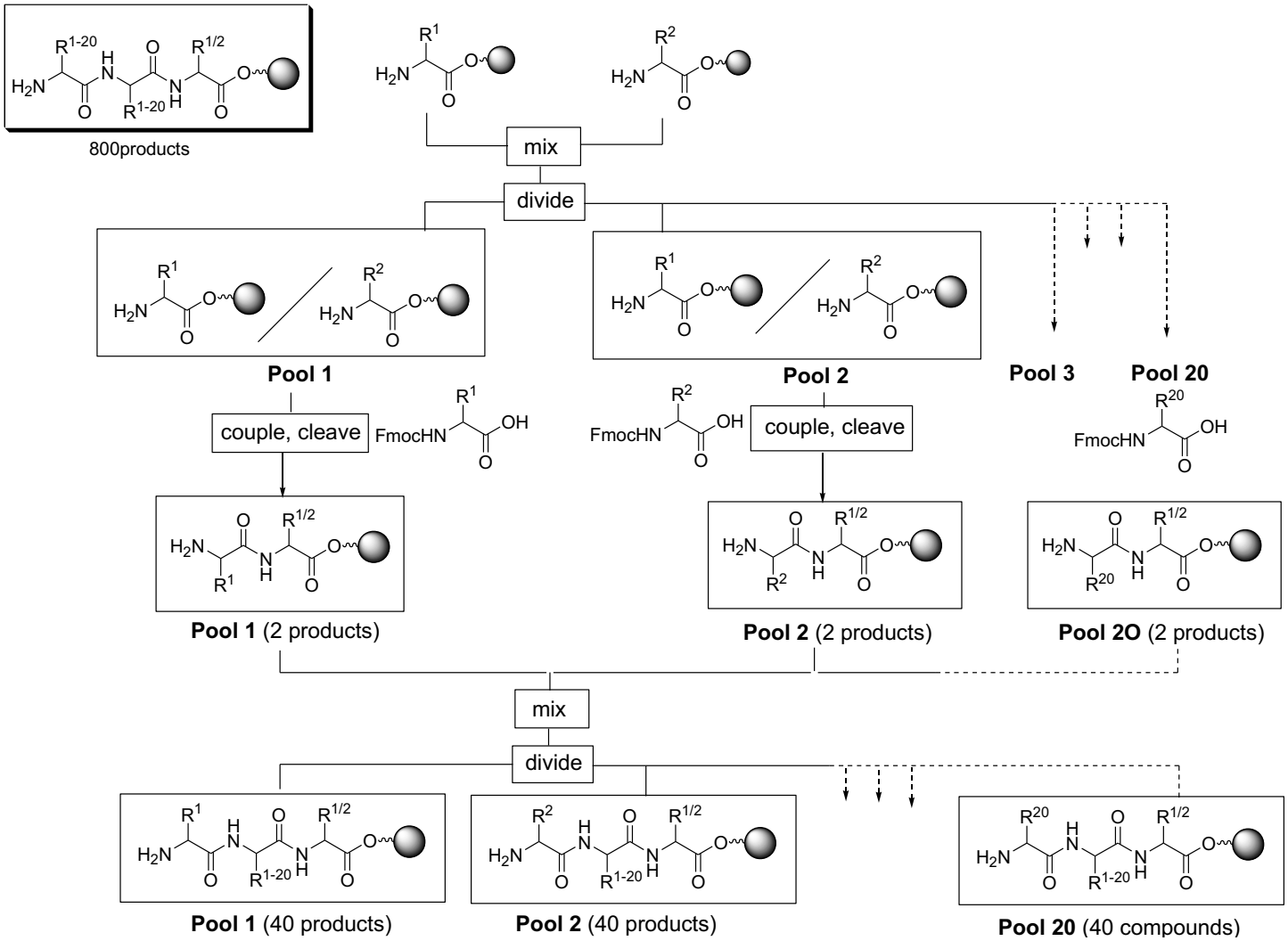
Examples for libraries synthesized on solid-phase: parallel synthesis of single compounds



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



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

-Peptides synthesized and assayed as individuals or as mixtures on solid supports such as *pins* (H. M. Geysen et al. *Mol. Immunol*. **1986**, *23*, 709)**, *resin beads*** (K. S. Lam et al. *Nature* **1991**, *354*, 82)**, *cotton*** (R. A. Houghton et al. Biochemistry 1993,32, 11035), ***microchips*** (S. P. A. Fodor et al. *Science* **1991**, *37*, 481), or ***cellulose membranes*** (A. Kramer et al. *Pept. Res*. **1993**, *6*, 314)

-Peptides synthesized on the surface of a filamentous phage: *Phage display technology*
(G. P. Smith et al. Meth. Enzymol. 1993, 217, 228; J. K. Scott et al. Curr. Opin. Biotechnol. 1994, 5, 40)

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

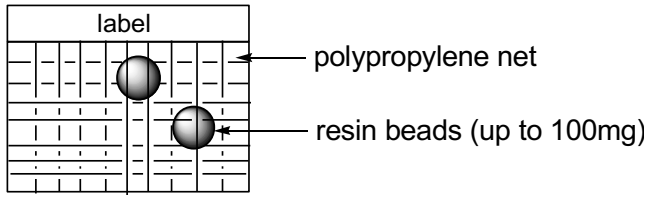
-As true mixtures where a peptide coupling step involves the coupling of a mixture (typically the 20 coding amino acids) of side-chain protected Boc- or Fmoc- protected amino acids (D or L) in a predetermined molar ratio which compensates for the different coupling rates.

-as mixtures of resin beads which resulted from synthesis: `one bead-one compound concept`
`portioning-mixing` (A. Furka et al. *Int. J. Protein Res.* **1991**, *37*, 487)
`couple and recombine` (R. A. Houghton et al. *Nature* **1991**, *354*, 84)
`split synthesis` (V. Hruby et al. *Nature* **1991**, *354*, 82)

Examples for libraries synthesized on solid-phase: peptides

Parallel synthesis of single compounds

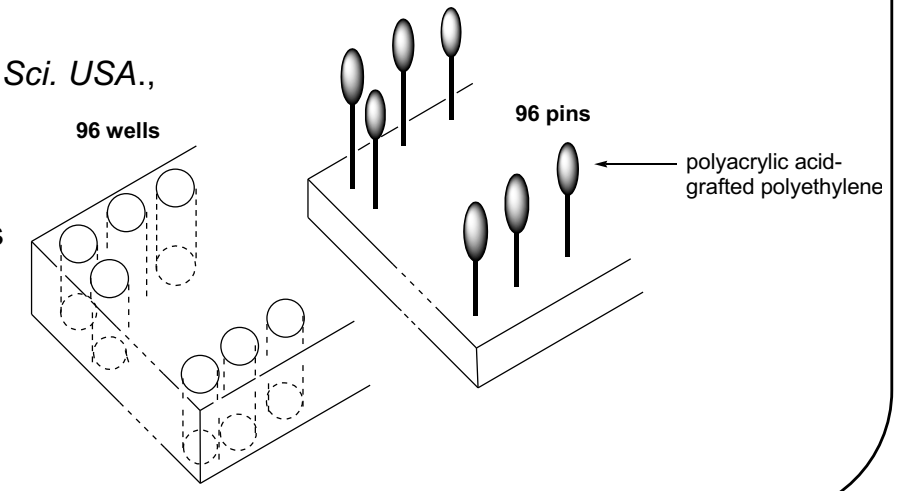
-tea bags: e.g. R. A. Houghton et al. *Proc. Natl. Acad. Sci. USA*., **1985**, *82*, 5131; G. Jung et al. *Pept. Res*. **1991**, *4*, 88



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.

-multi pins: H. M. Geysen et al. *Proc. Natl. Acad. Sci. USA*., **1984**, *81*, 3998

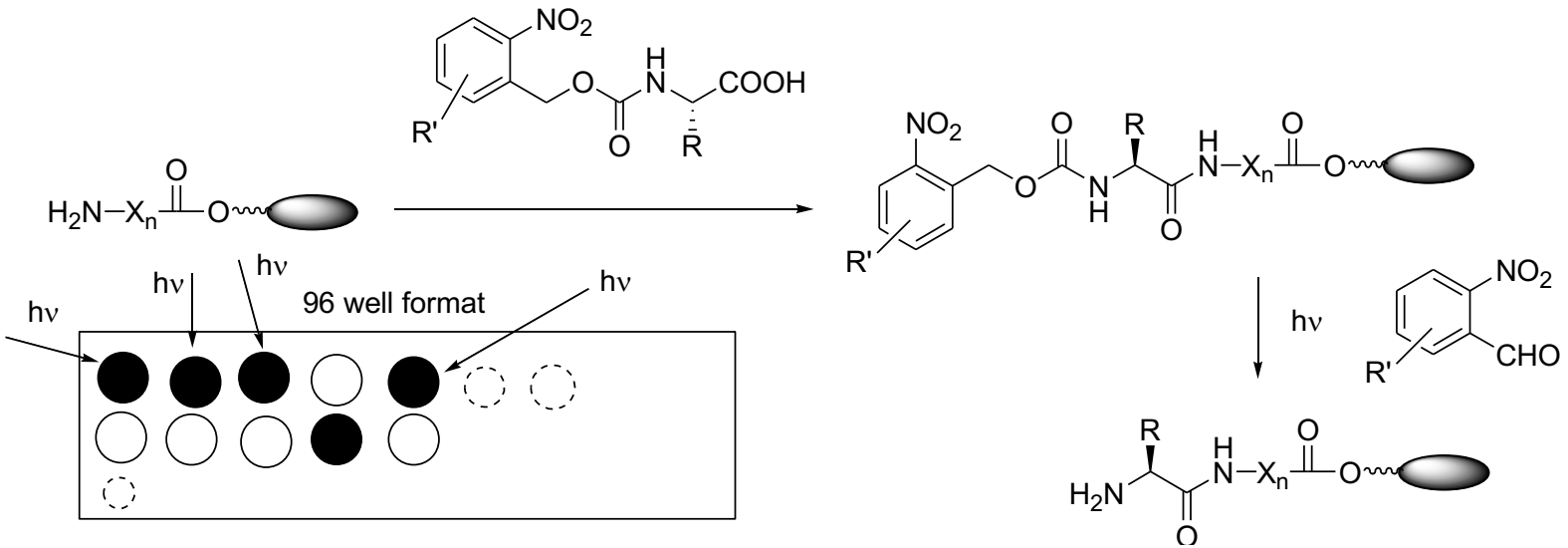
Spatially separated parallel synthesis of compounds in microtiter format



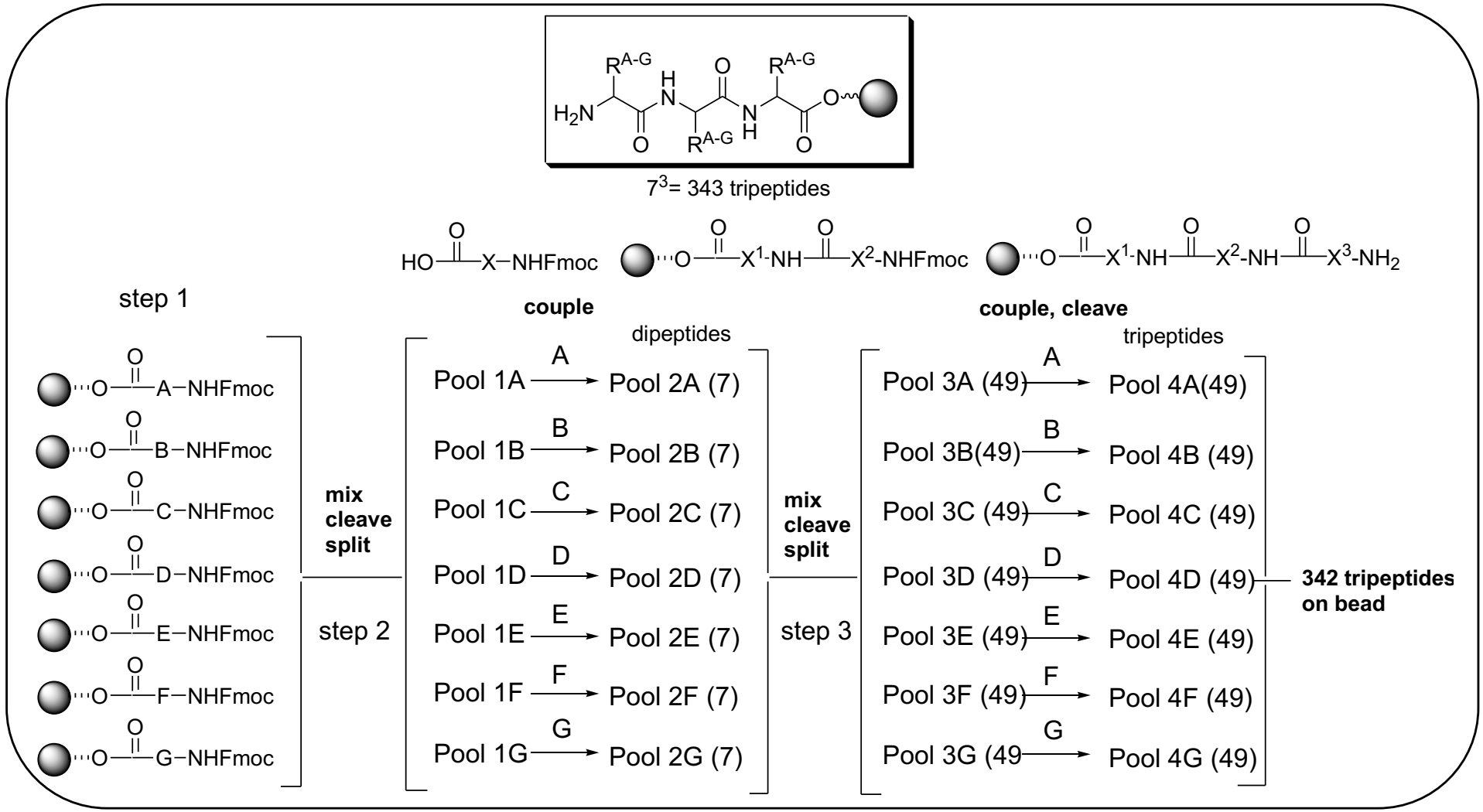
Examples for libraries synthesized on solid-phase: peptides: photolithography

Photolithography: *light-directed combinatorial synthesis* (S. P. A. Fodor et al. *Science* **1991**, *251*, 767)

Spatially separated multiple parallel synthesis using photocleavalbe protective groups such as the N-nitro-veratrylcarbonyl group (NVOC), allows the controlled synthesis of (peptide) libraries by the spatially controllable addition of specific reagents to specific locations.



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



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

Iterative deconvolution (*Nature* **1991**, *354*, 84; *Science* **1994**,*266*, 2019; *Proc. Nat. Acad. Sci, USA* **1993**, *90*, 10811)

Sreening 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)

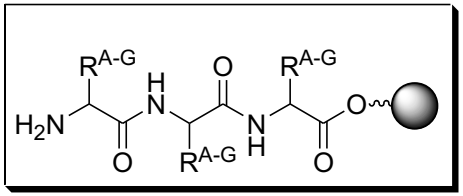
Indexed or orthogonal libraries (e.g. *Chem. Biol*. **1995**, *2*, 621; *Tetrahedron Lett*. **1997**, *38*, 491)

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

Radio-frequency tags (*Irori* system): (*J. Am. Chem. Soc*. **1995**, *117*, 10787; *J. Org. Chem*. **1997**, *62*, 6092)

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)

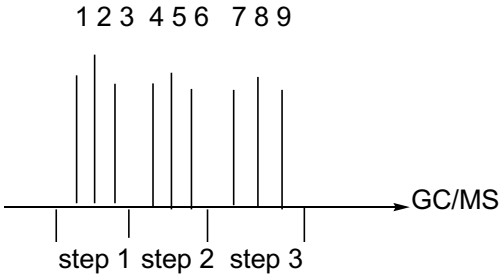


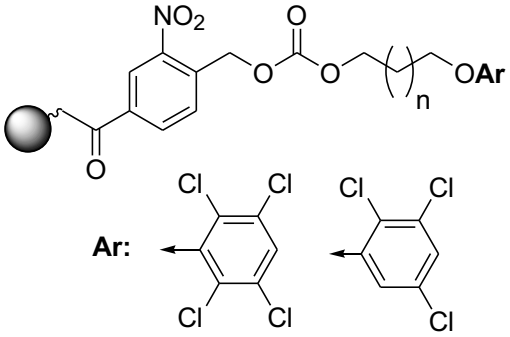
7 building blocks

3 steps

requires 9 tags

7^3= 343 tripeptides



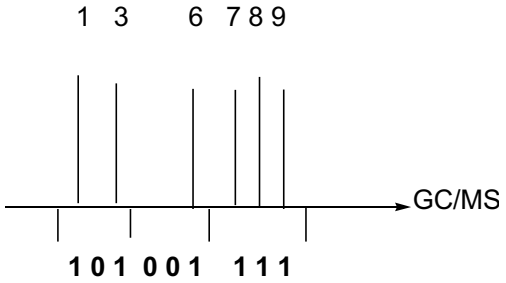


step 1: building block A: 1 0 0 tag 1
B: 0 1 0 tag 2
C: 0 0 1 tag 3
D: 1 1 0 tags 1 + 2
E: 1 0 1 tags 1 + 3
F: 0 1 1 tags 2 + 3
G: 1 1 1 tags 1 + 2 + 3

step 2: building block A: 1 0 0 tag 4
B: 0 1 0 tag 5
C: 0 0 1 tag 6
D: 1 1 0 tags 4 + 5
E: 1 0 1 tags 4 + 6
F: 0 1 1 tags 5 + 6
G: 1 1 1 tags 4 + 5 + 6

step 3: building block A: 1 0 0 tag 7
B: 0 1 0 tag 8
C: 0 0 1 tag 9
D: 1 1 0 tags 7 + 8
E: 1 0 1 tags 7 + 9
F: 0 1 1 tags 8 + 9
G: 1 1 1 tags 7 + 8 + 9

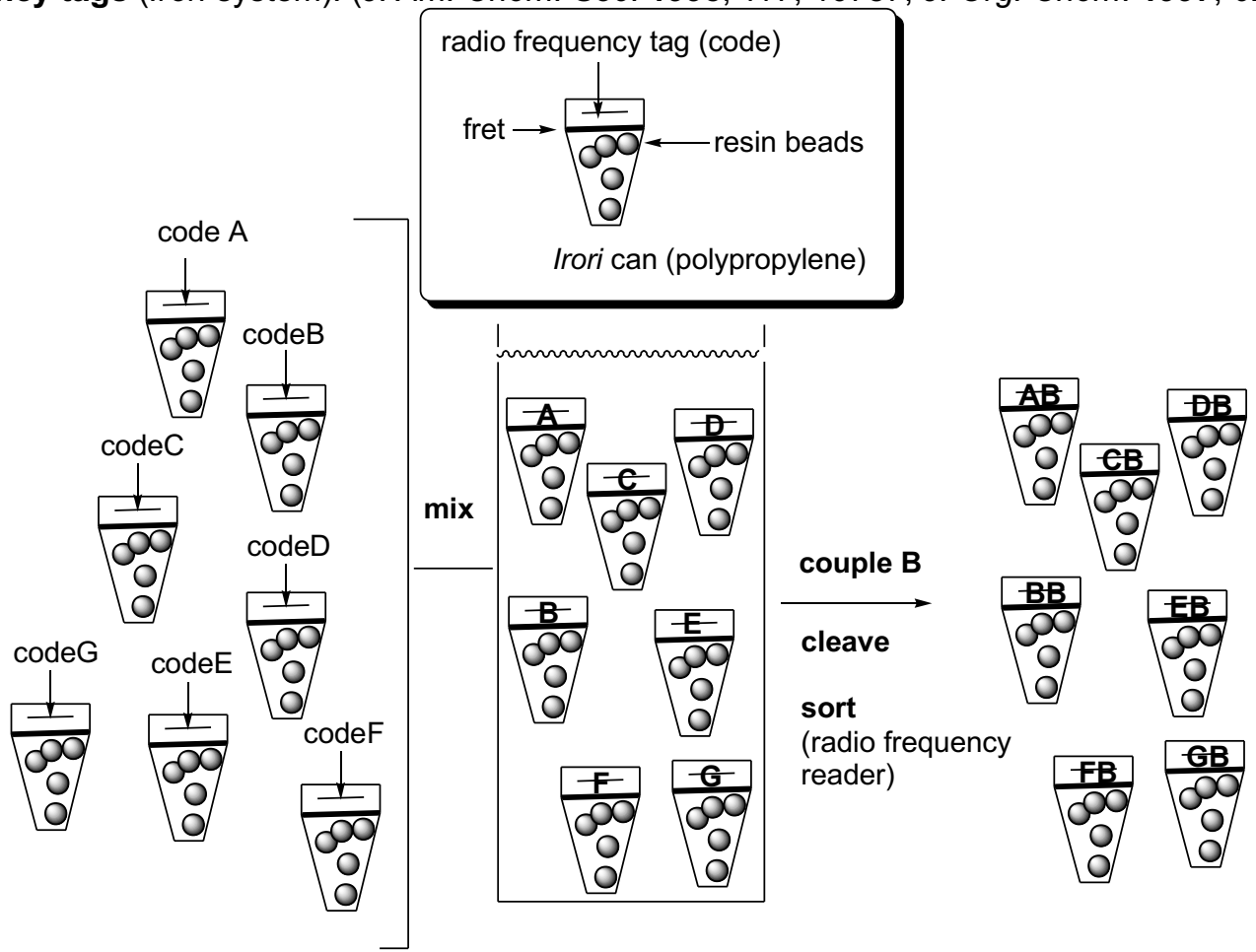
for tripeptide **E-C-G:**



n: 0-x *variations of **Ar** and **n** gives rise to the different tags, which can be detected in minute amounts by GC/MS*

Examples for libraries synthesized on solid-phase: peptides: radio-frequency tags

Radio-frequency tags (*Irori* system): (*J. Am. Chem. Soc*. **1995**, *117*, 10787; *J. Org. Chem*. **1997**, *62*, 6092)



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

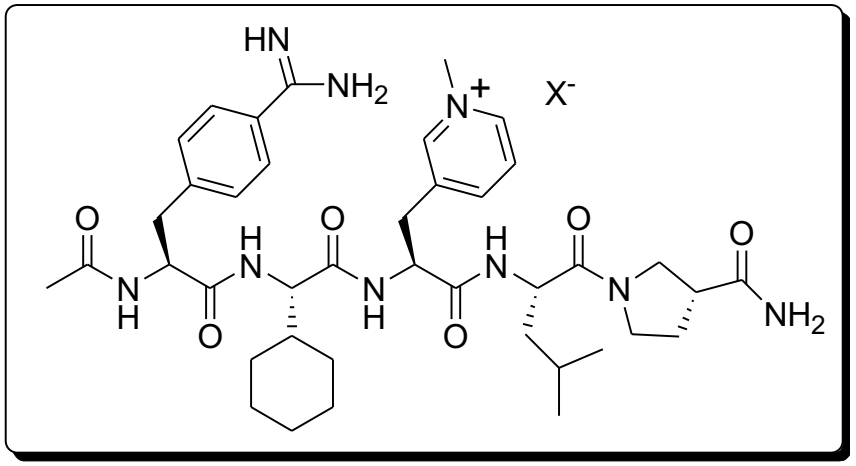
Application of the split-mixed method for discovery of *Factor Xa* inhibitors

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-NH_2 (SEL1691) ⟹



SEL2602

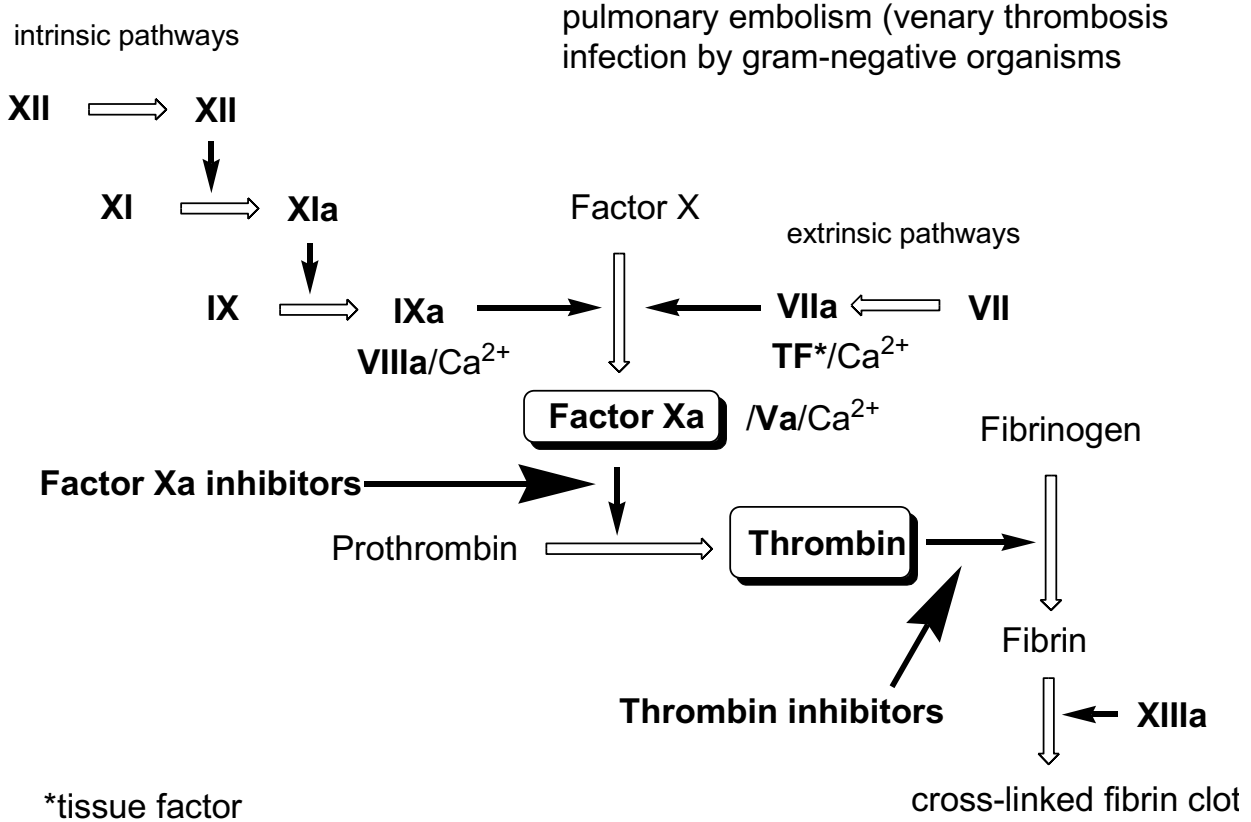
Examples for libraries synthesized on solid-phase: peptides: split-mixed technology

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

Blood coagulation factor Xa is implicated in *hemostasis* (bloodcoagulation)

Thrombosis: pathological form of hemostasis:

myocardial infarction (arterial thrombosis)
pulmonary embolism (venary thrombosis
infection by gram-negative organisms



*tissue factor

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

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

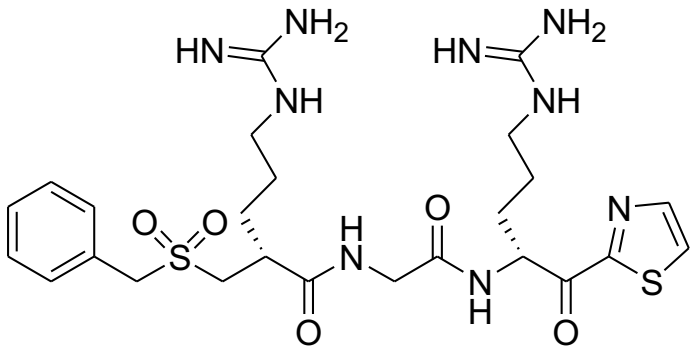
Current anti-thrombotic therapies include: aspirin

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

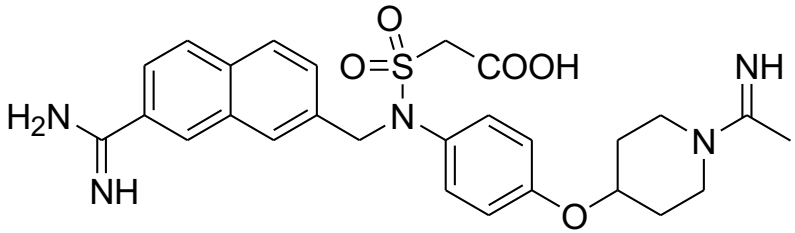
high levels of thrombin inhibition necessary; unacceptable bleeding

Factor Xa inhibitors: *trypsin-like serine protease*

current molecules in clinical trials



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

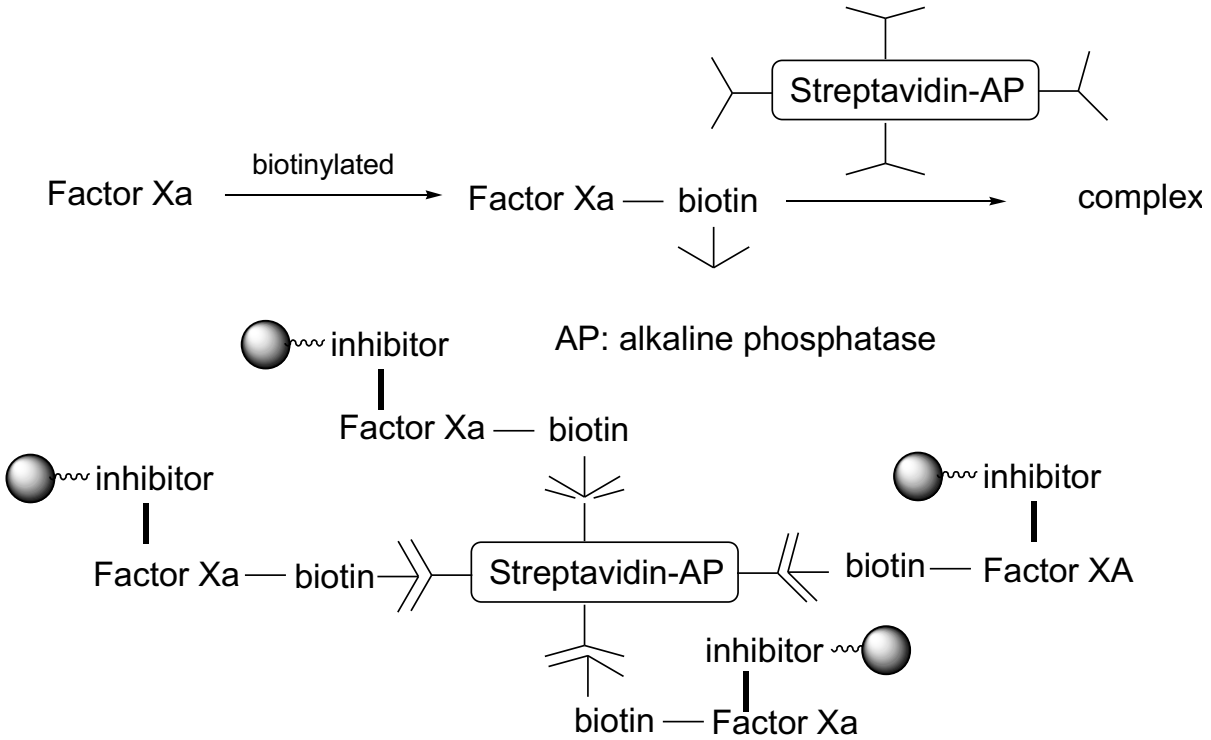


Yamanouchi (IC50 factor Xa: 1.3nM)
(IC50 thrombin: >100μM)

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

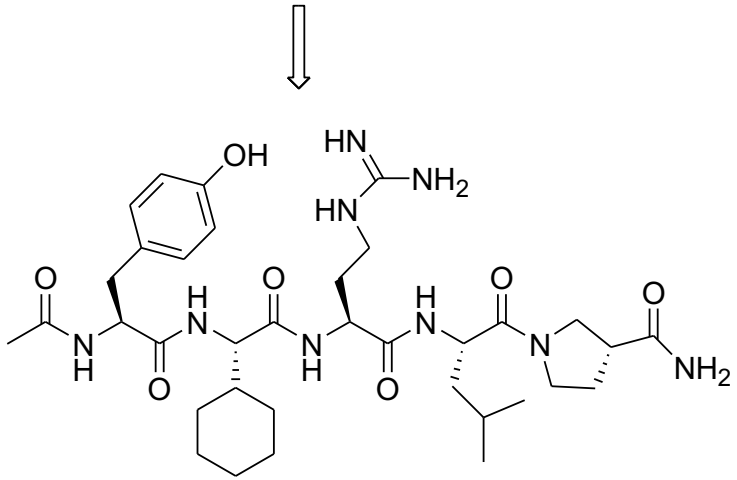
Application of the split-mixed method for discovery of *Factor Xa* inhibitors

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

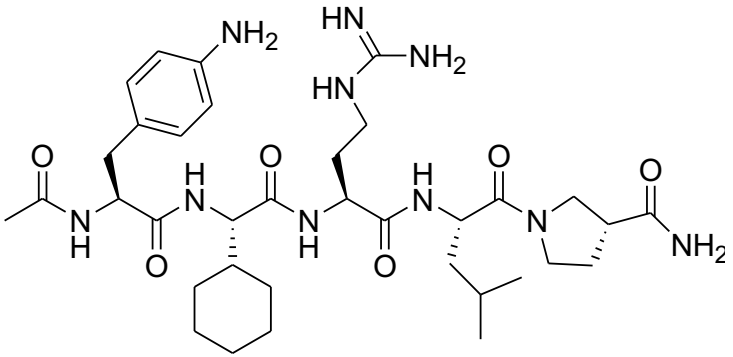
All active compounds contained **Tyr-Ile-Arg** at the N-terminus

H-Tyr-Ile-Arg-Leu-Ala-Ala-Phe-Thr-NH_2 (SEL1691; IC_{50}: 4-15μM)**)**

Drug Discovery Today **1998**, *3*, 223



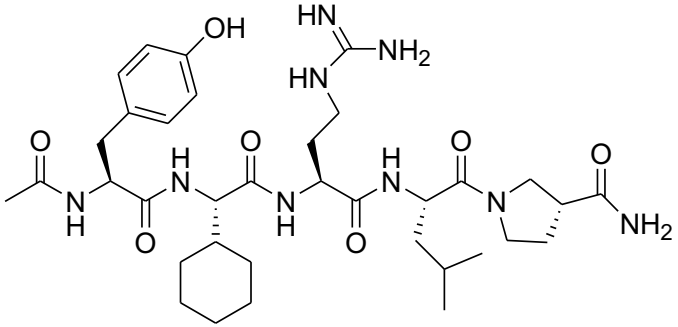
SEL2316 (IC_{50}: 80nM)



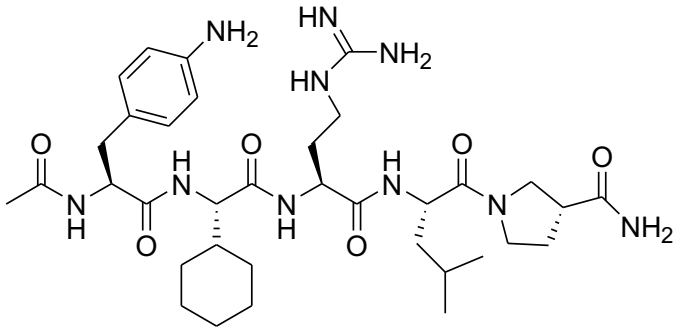
SEL2489 (IC_{50}: 25nM; half-life in rats and rabbits 8 to 10 minutes)

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

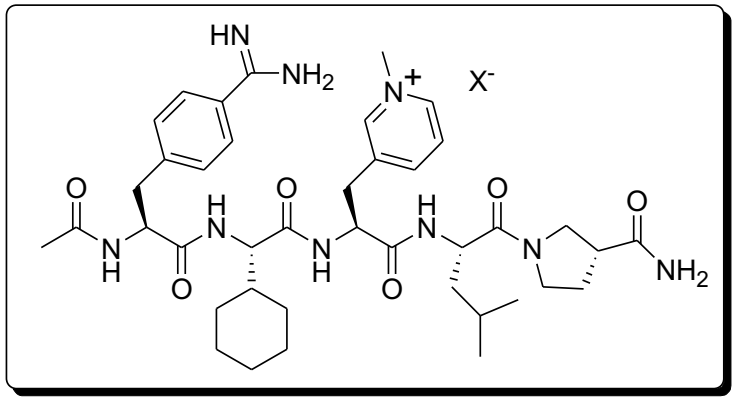
Application of the split-mixed method for discovery of *Factor Xa* inhibitors



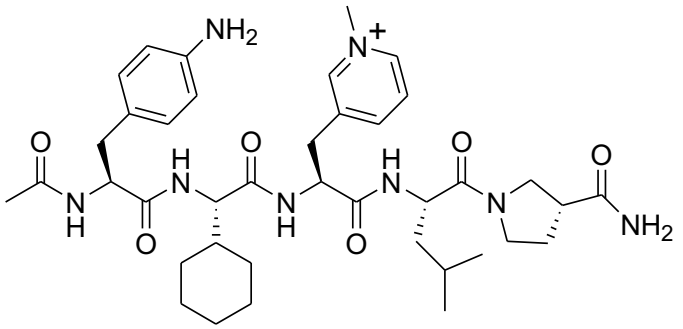
SEL2316 (IC_{50}: 80nM)



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



SEL2602 (IC_{50}: <25nM; improved half-life)



SEL2602 (IC_{50}: 285nM)

Questions

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.

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)

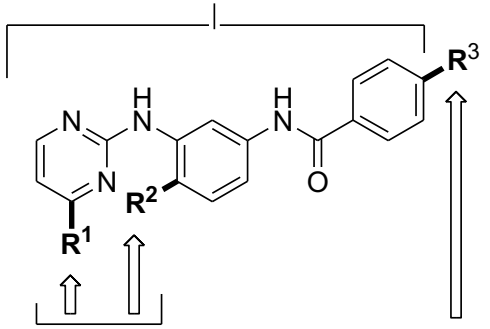
6. **Purification**: parallel flash chromatography; high-throughput HPLC coupled to MS on normal and reversed phase

7. **Analysis, stability and storage of products**

Synthesis strategies: introduction

sub-library A

scaffold



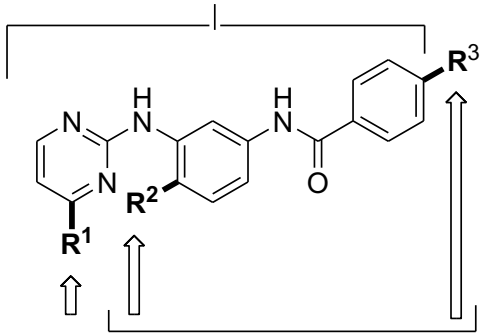
low variation substituents **high variation substituents**

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

sub-library B

scaffold



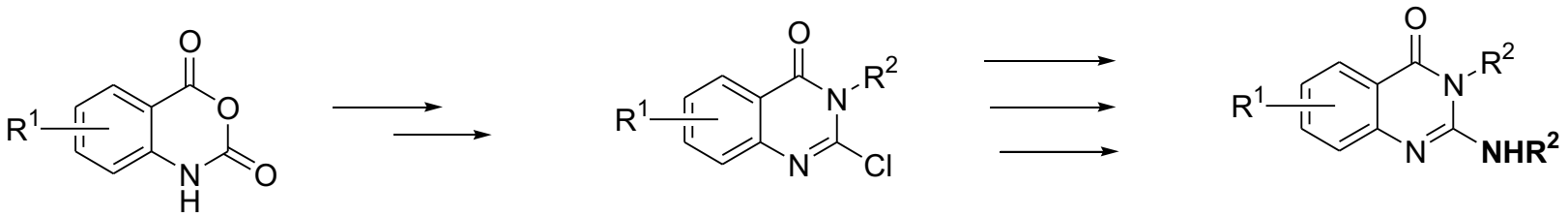
high variation substituents **low variation substituents**

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 importantfor biological activity

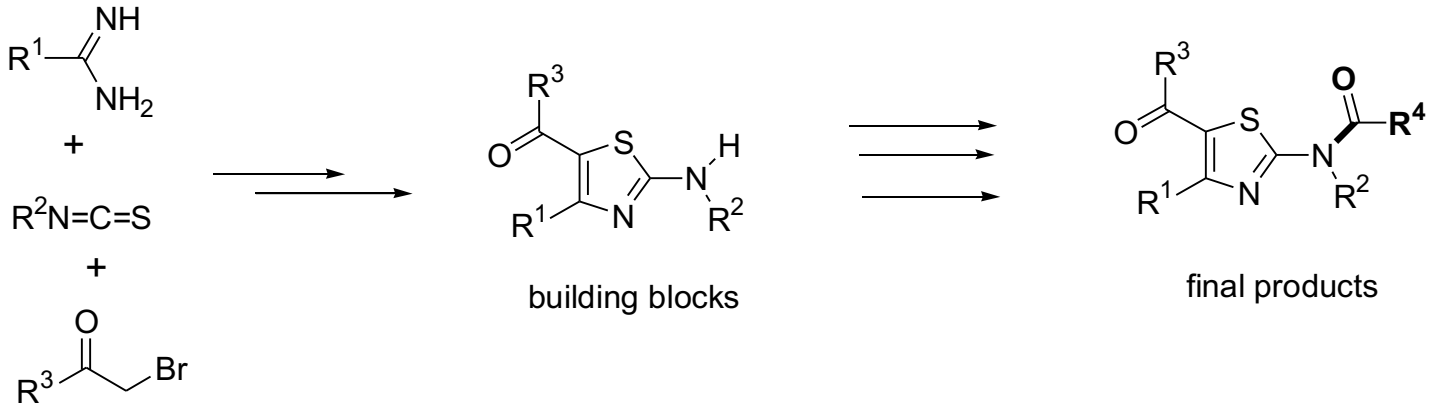
Synthesis strategies: convergent, multi-step

Multi-step synthesis of advanced building blocks (scaffold) by linear or convergent synthetic strategies and parallel conversion into final products

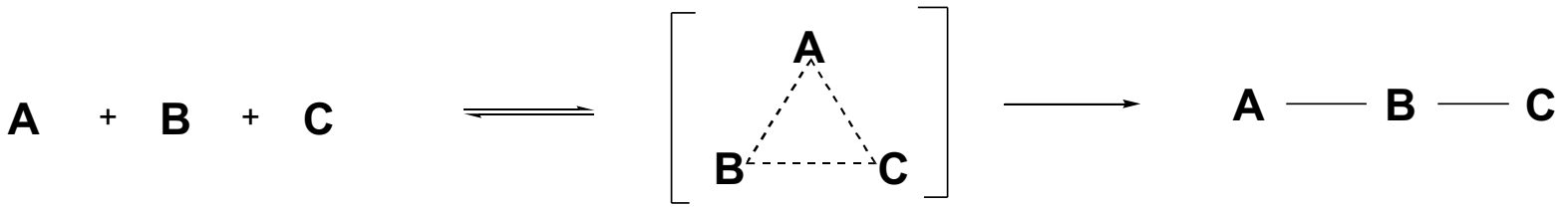


Synthesis strategies: classical multi-component approach

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



Synthesis strategies: classical multi-component approach



intermèdiaire

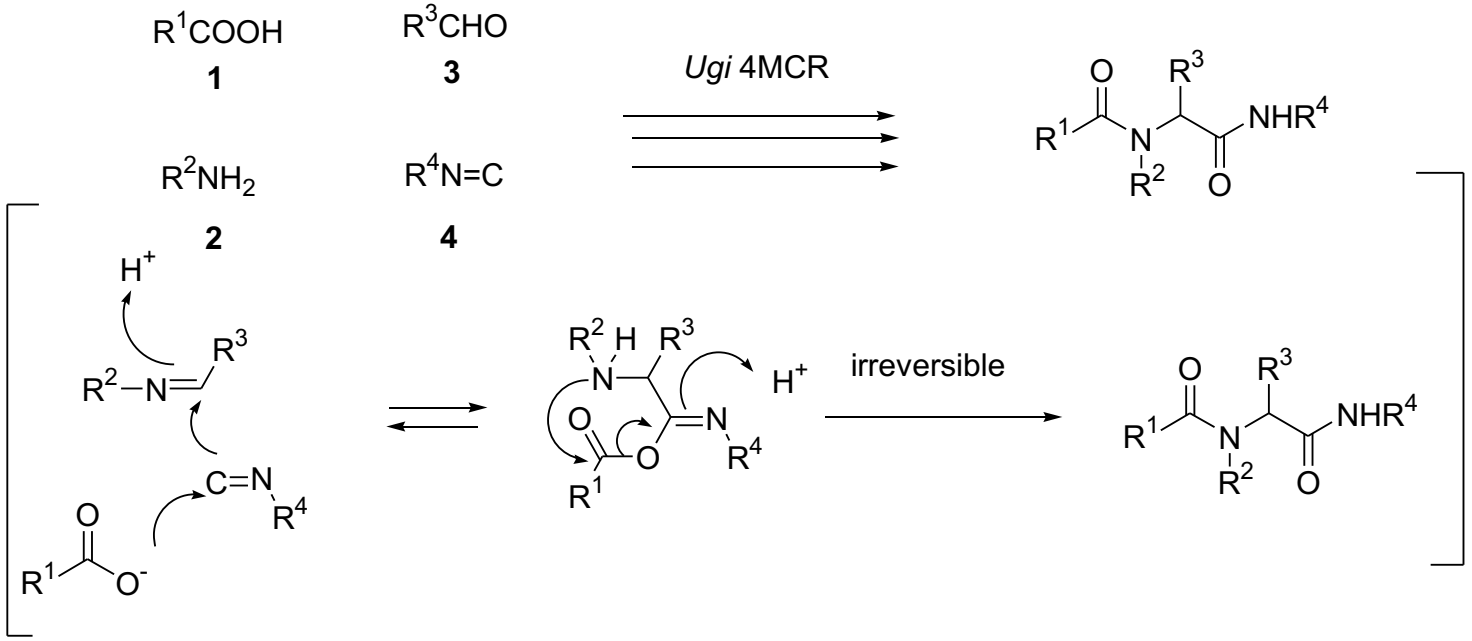
- Classical multi-component reactions (MCR's) are have in common that components (e.g. **A**, **B**, **C**) react in a reversible way to a reactive intermediate, which reacts in a irreversible way to the product. Thus, the sequence by which the components are added does nor affect product formation.

- 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**

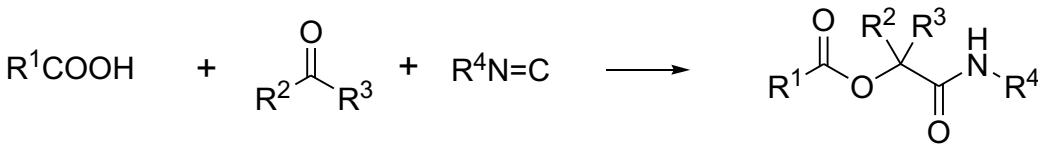
Synthesis strategies: classical multi-component approach



The classical multi-component reactions are ideally suited for parallel synthesis, however, they yield generally the same scaffold (limited scaffold diversity)

Synthesis strategies: classical multi-component approach

Passerini 3-MCR

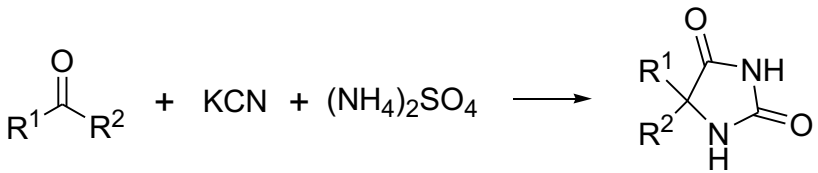


H. Passerini, *Gazz. Chim. Ital*. **1921**, *51*, 126

Strecker synthesis

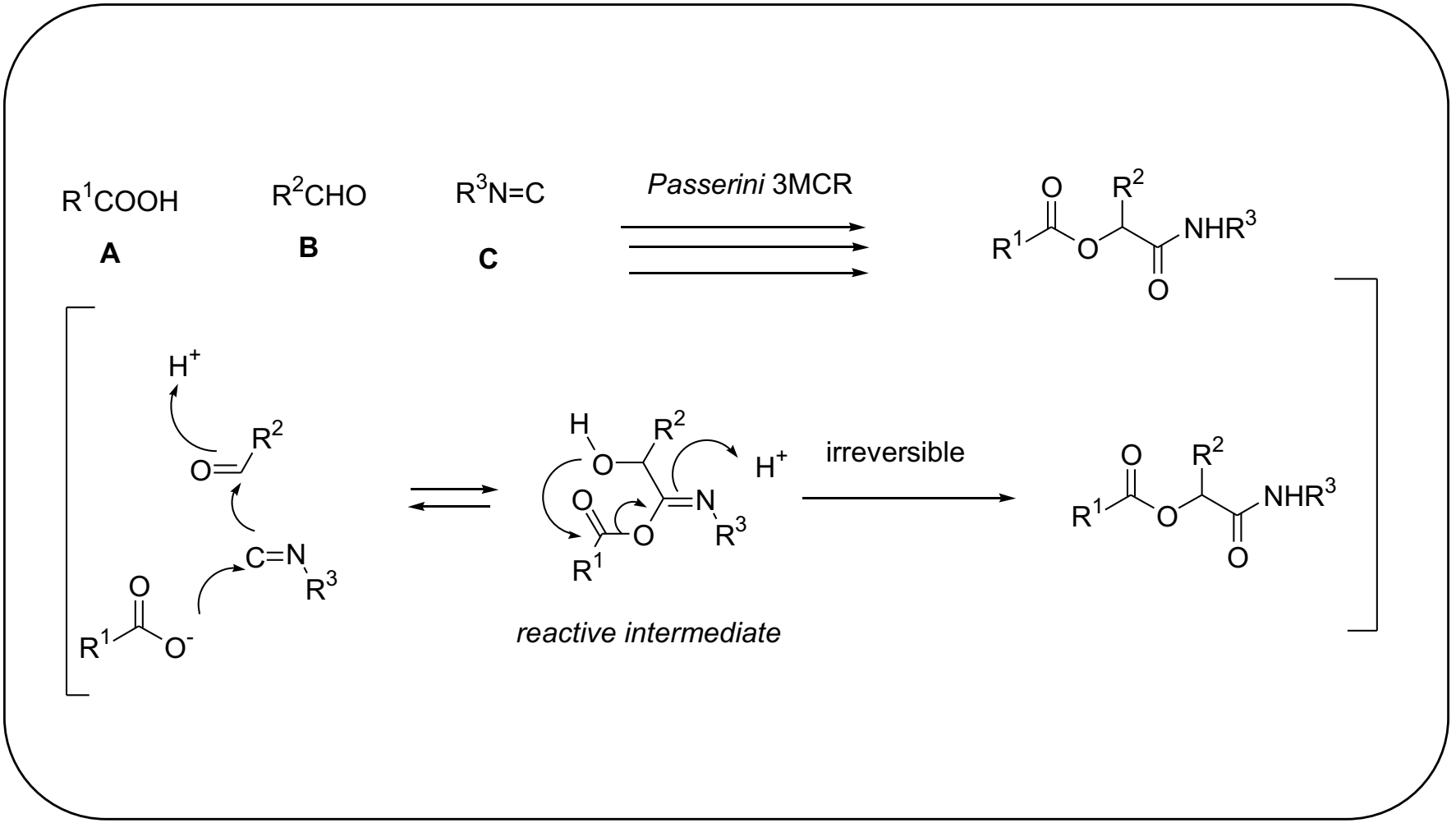
A. Strecker, *Justus Liebigs Ann. Chem.* **1854**, *91*, 345; ibid. **1890**, *23*, 1474

Bucherer-Bergs variation of the Strecker synthesis



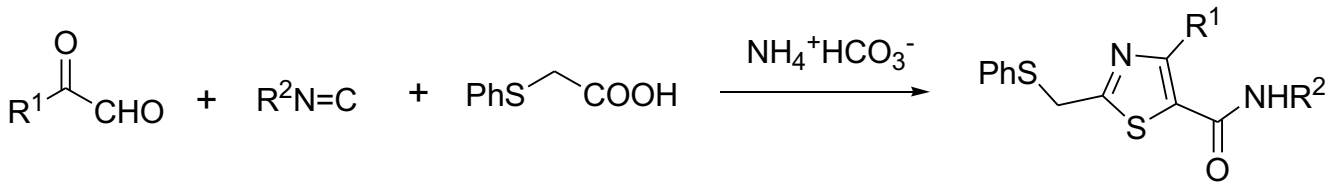
H. T. Bucherer et al. J. Prakt. Chem. 1934, 140, 69; ibid. 1934, 140, 28

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

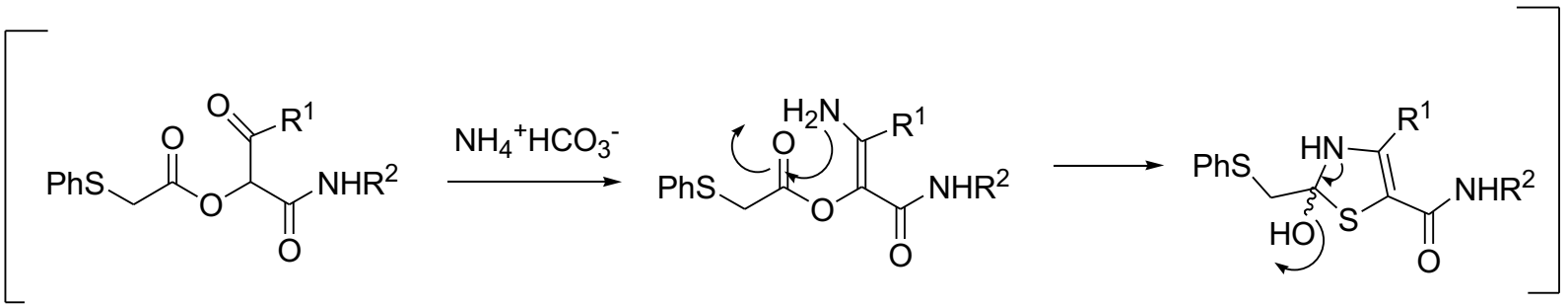


Synthesis strategies: classical MCRs

Modified Passerini 3-MCR

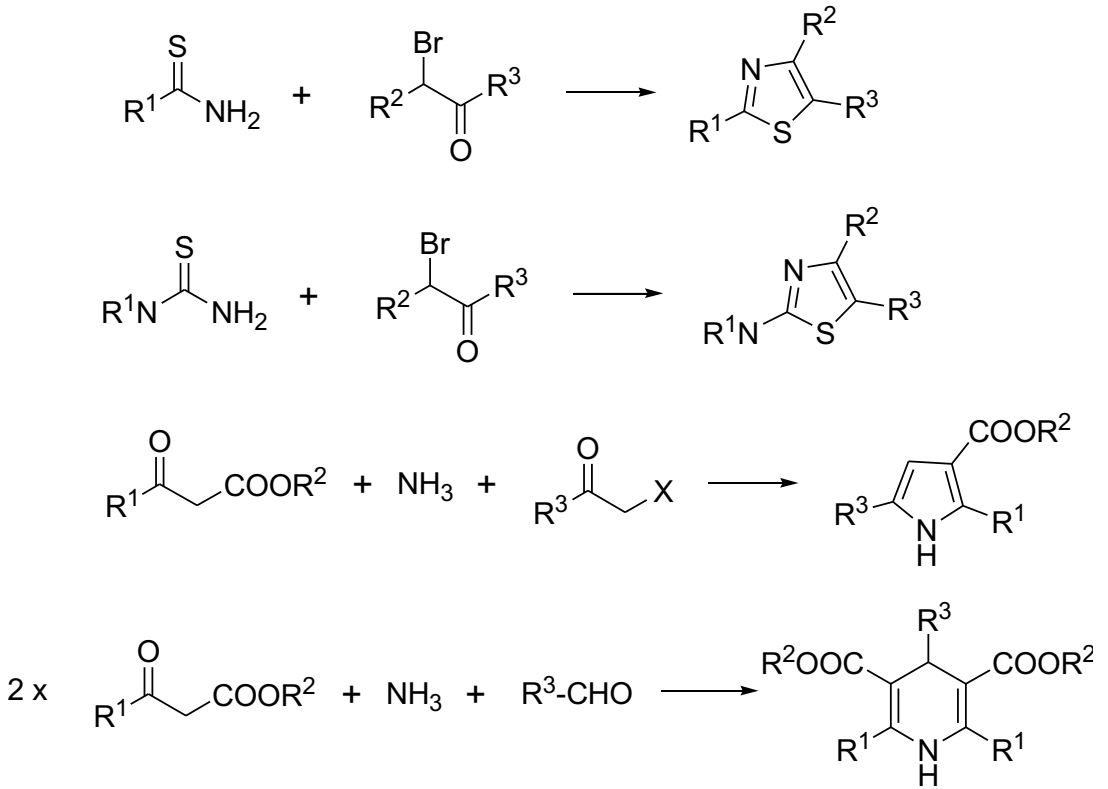


R. Bossio et al. *J. Chem. Res.* **1991**, *15*, 320



Synthesis strategies: classical MCRs

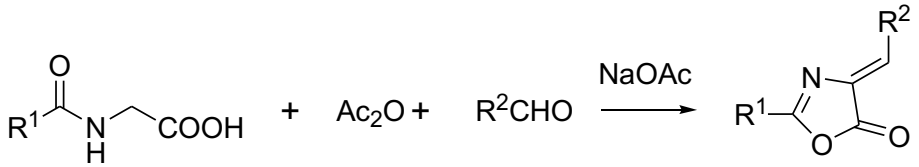
Hantzsch MCR's



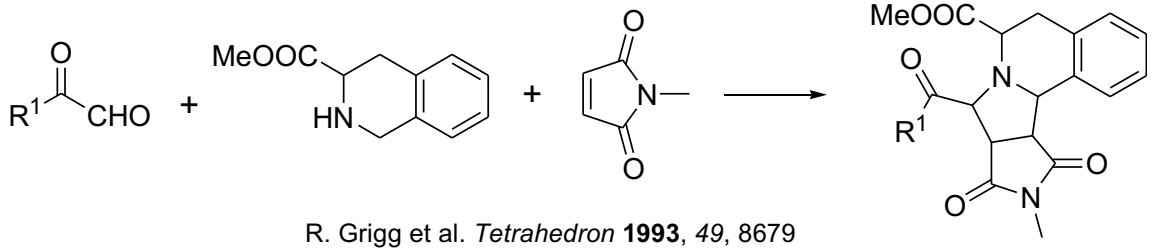
A. Hantzsch, *Ber. Deutsch. Chem. Ges*. **1890**, *23*, 1474

Synthesis strategies: Classical MCR's

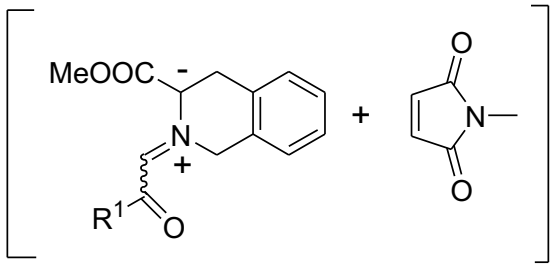
Erlemeyer azlactone synthesis



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

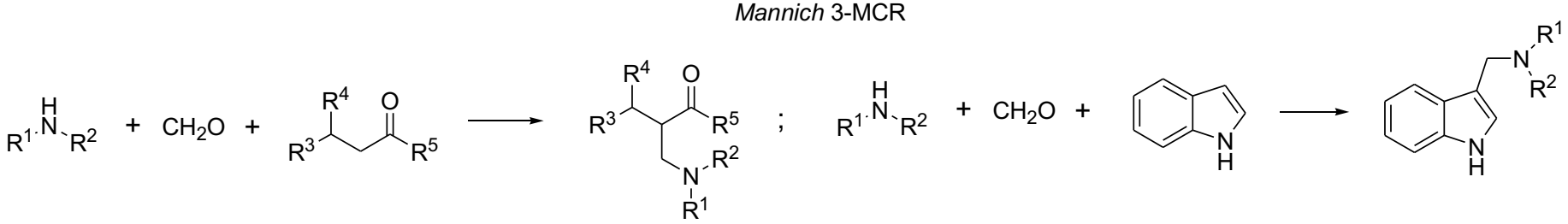


R. Grigg et al. *Tetrahedron* **1993**, *49*, 8679



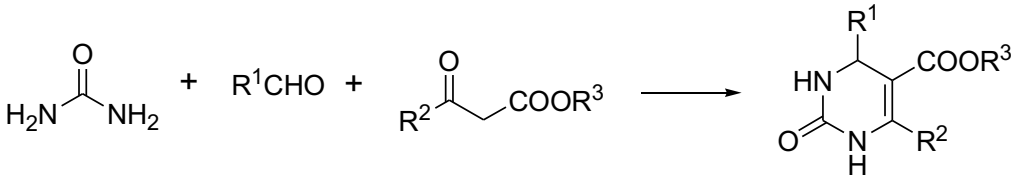
Synthesis strategies: classical MCRs

Mannich 3-MCR



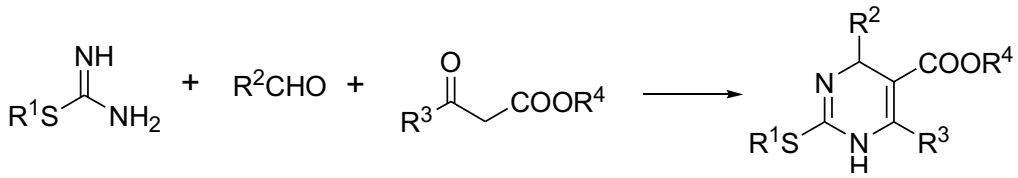
C. Mannich et al. *Arch. Pharm.* **1921**, *250*, 647

Biginelli 3-MCR



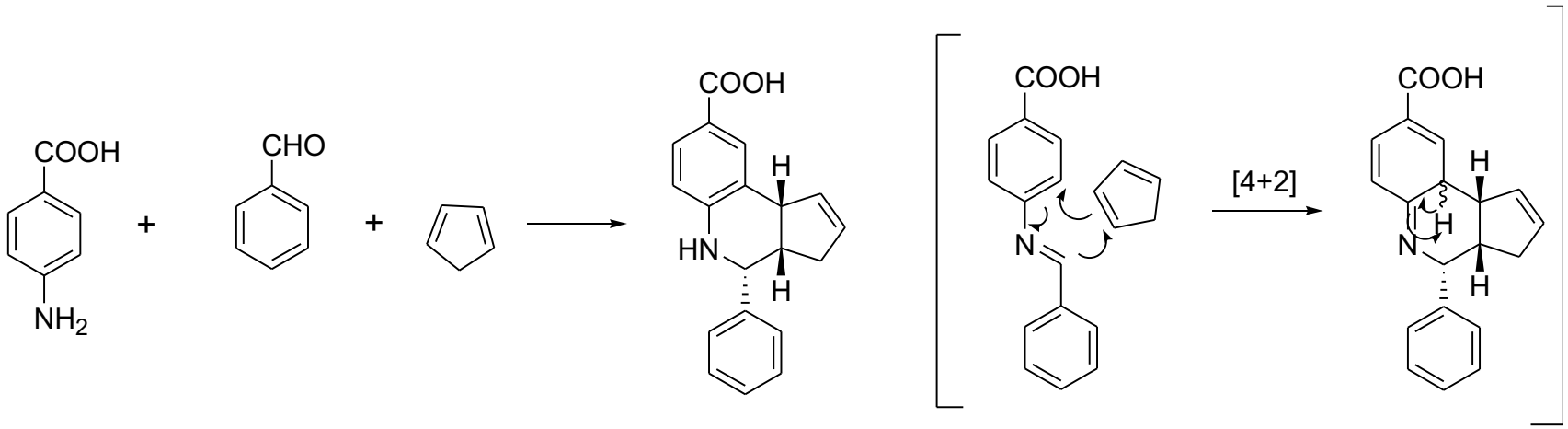
P. Biginelli, *Ber. Deutsch. Chem. Ges*. **1893**, *26*, 47; ibid. **1891**, *24*, 2962

Biginelli 3-MCR (Atwal variation)



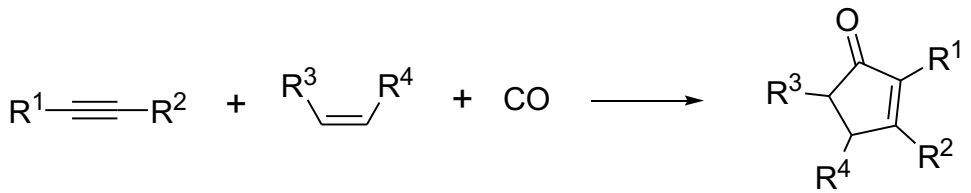
Synthesis strategies: classical MCR's

Grieco's 3-MCR



P. Grieco et al. *Tetrahedron Lett.* **1988**, *29*, 5855; R. W. Armstrong et al. *Tetrahedron Lett.* **1997**, *38*, 6163

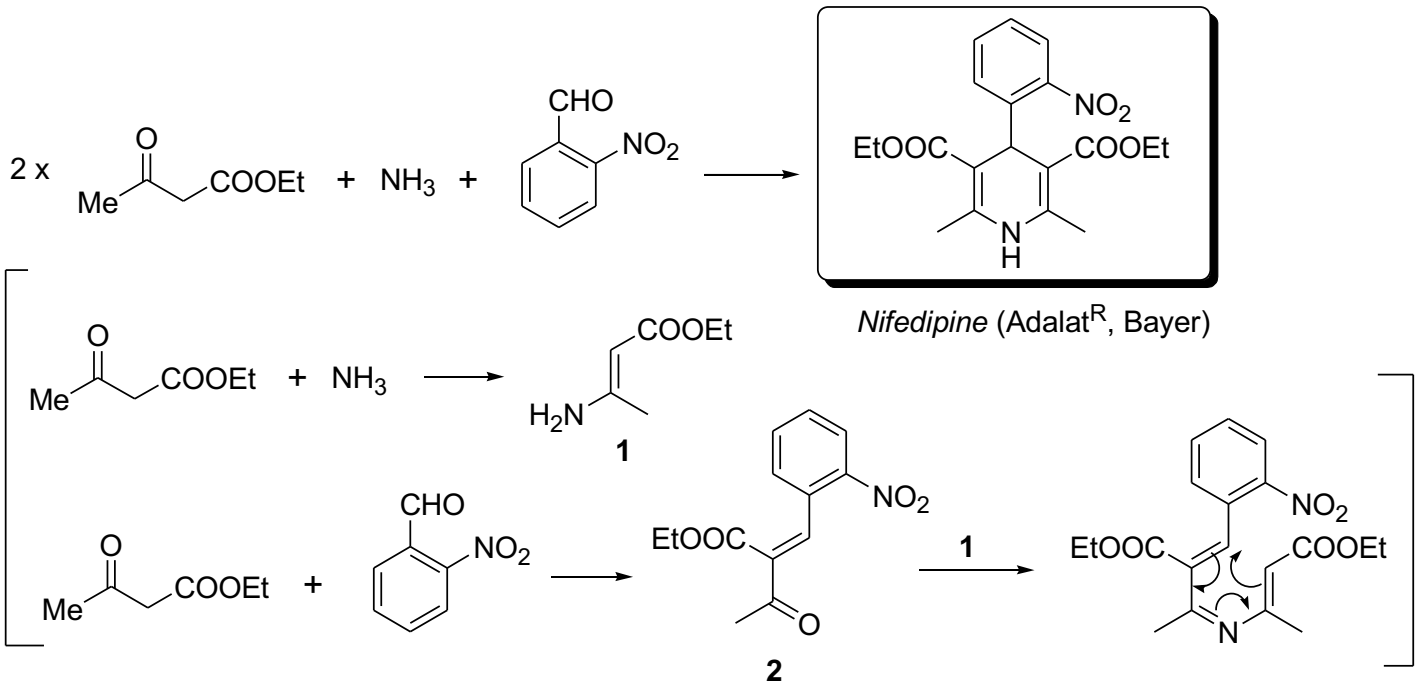
Pauson-Khand MCR



U. I. Khand et al. *J. Chem. Soc. Perkin Trans. 1* **1973,** *9*, 977

Synthesis strategies: classical MCRs: applications

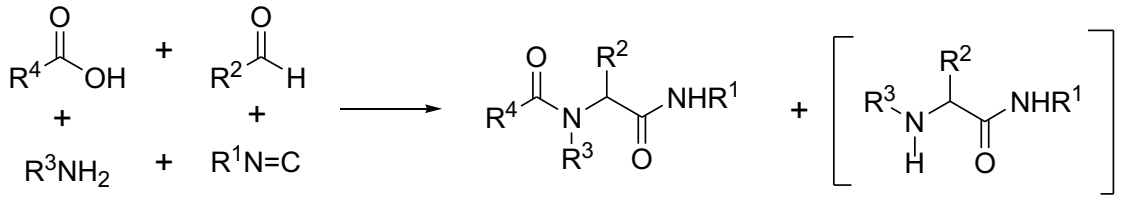
Applications of MCR's



- *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 *Hatzsch*-type 3-MCR in a very efficient and cheap way.

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

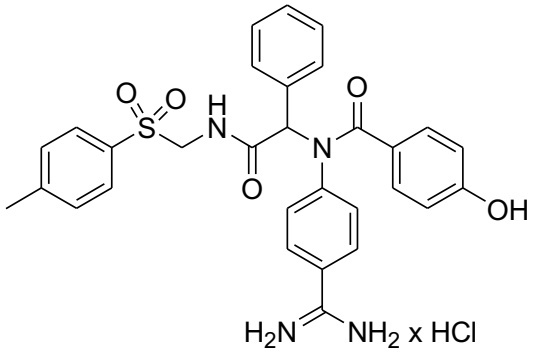


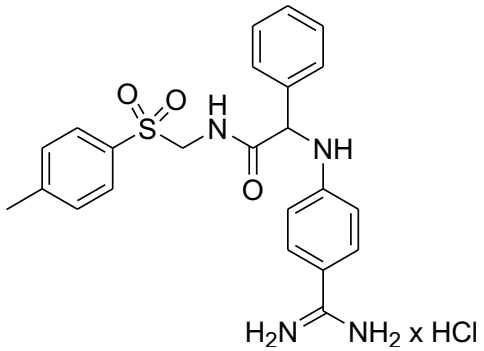
L. Weber et al. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2280

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

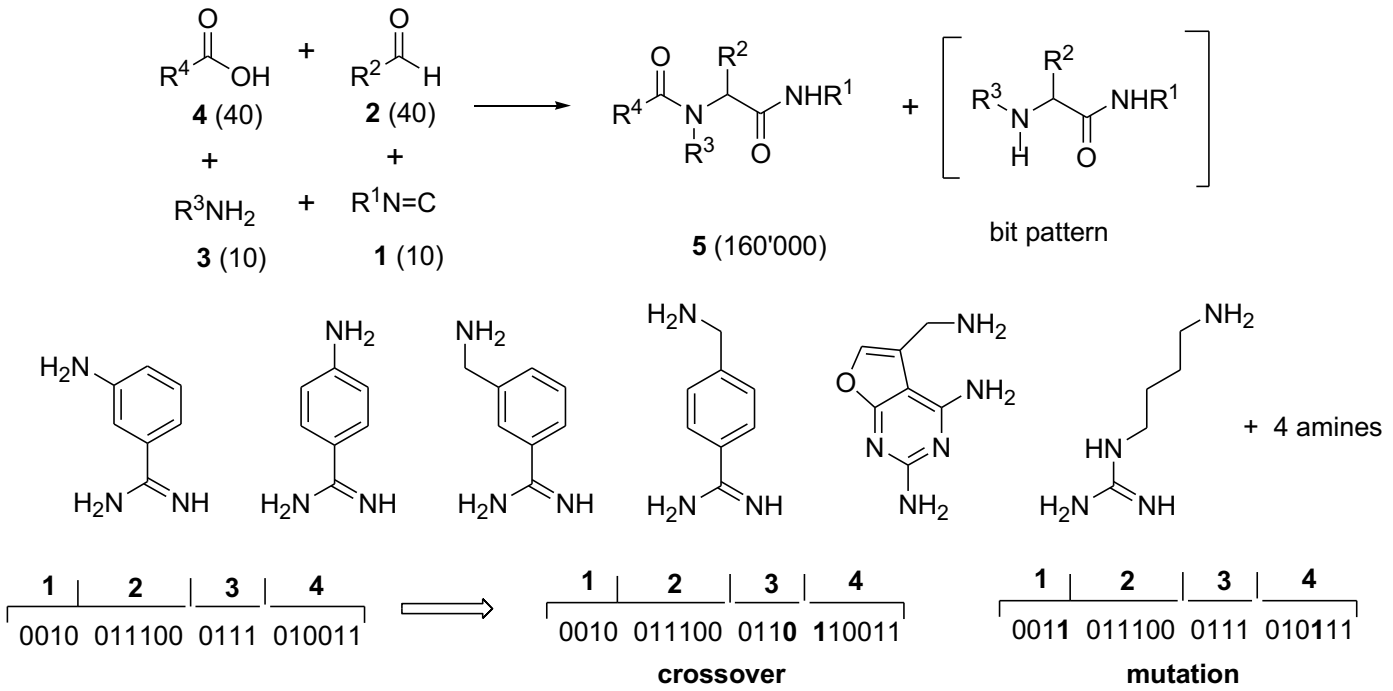
Parameters: inputs acids, isocyanates, aldehydes, amines; biological activity (inhibition of thrombin)

Genetic operations: replication, mutation and crossover





L. Weber et al. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2280



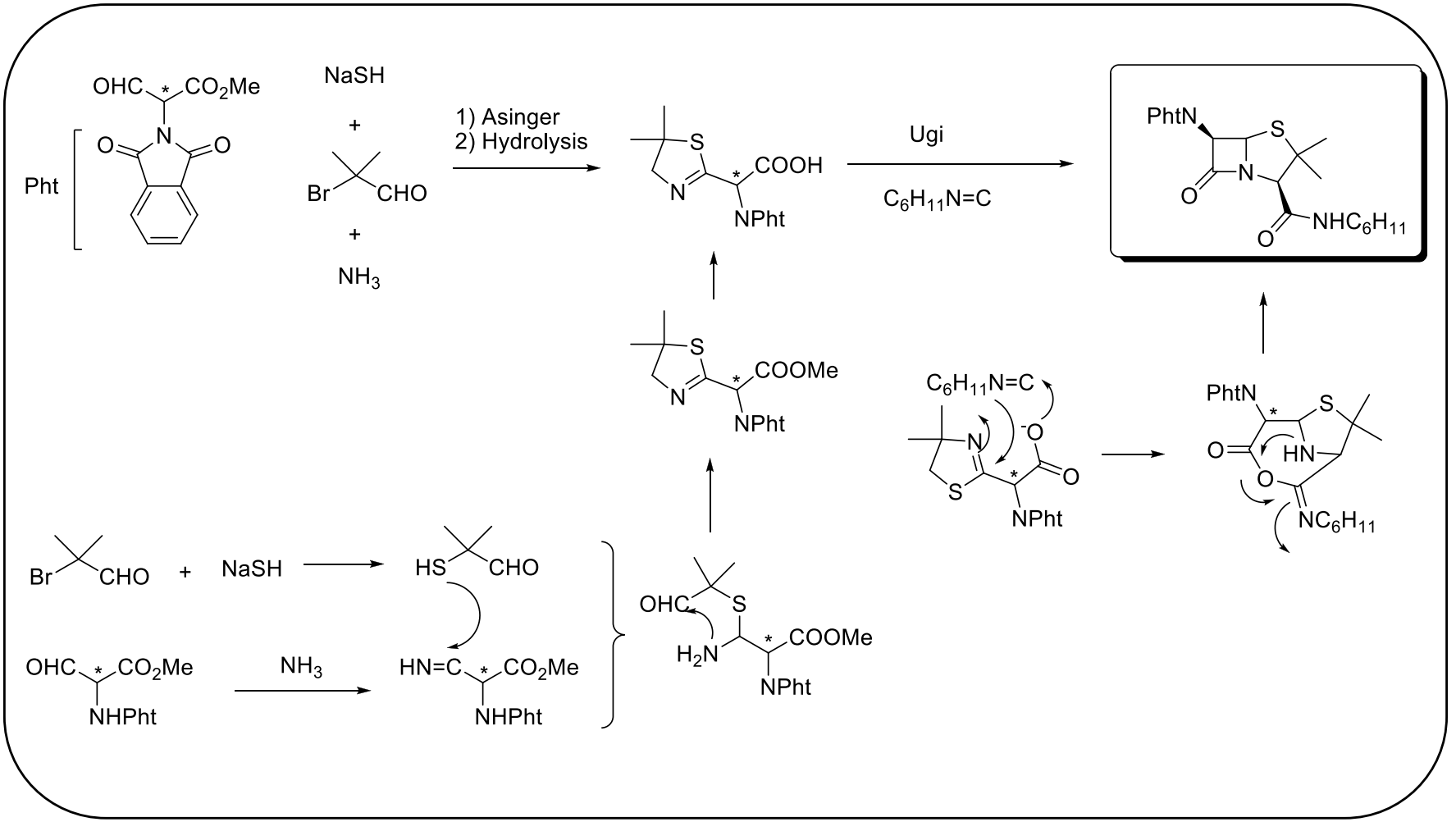
1st generation: random selection of 20 bit patterns: synthesis

2nd 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

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

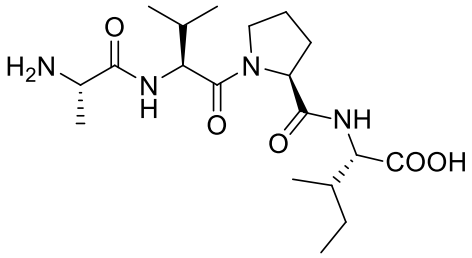
after 16 cycles, the average effective inhibitory concentration (EC_{50}) of the 20 best compounds was submicromolar

Synthesis strategies: application of the Asinger-Ugi 6-MCR: Penicillin derivatives

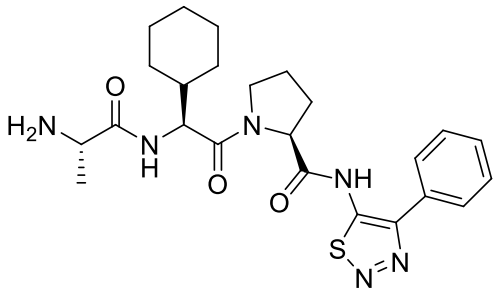


Synthesis strategies: application of the Ugi reaction: Inhibitors of IAPs

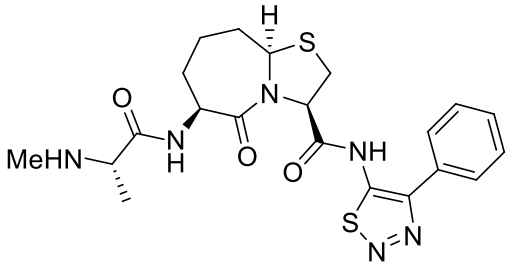
- Apoptosis (programmed cell death) is an essential part of normal homeostasis (self-regulation). Evasion of apoptosis by cells is one of the hallmarks of cancer (D. Hanahan et al. *Cell* **2000**, *100*, 57-70);
- Inhibitors of apoptosis (IAP's) are a family of proteins (8 members in human) that inhibit 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



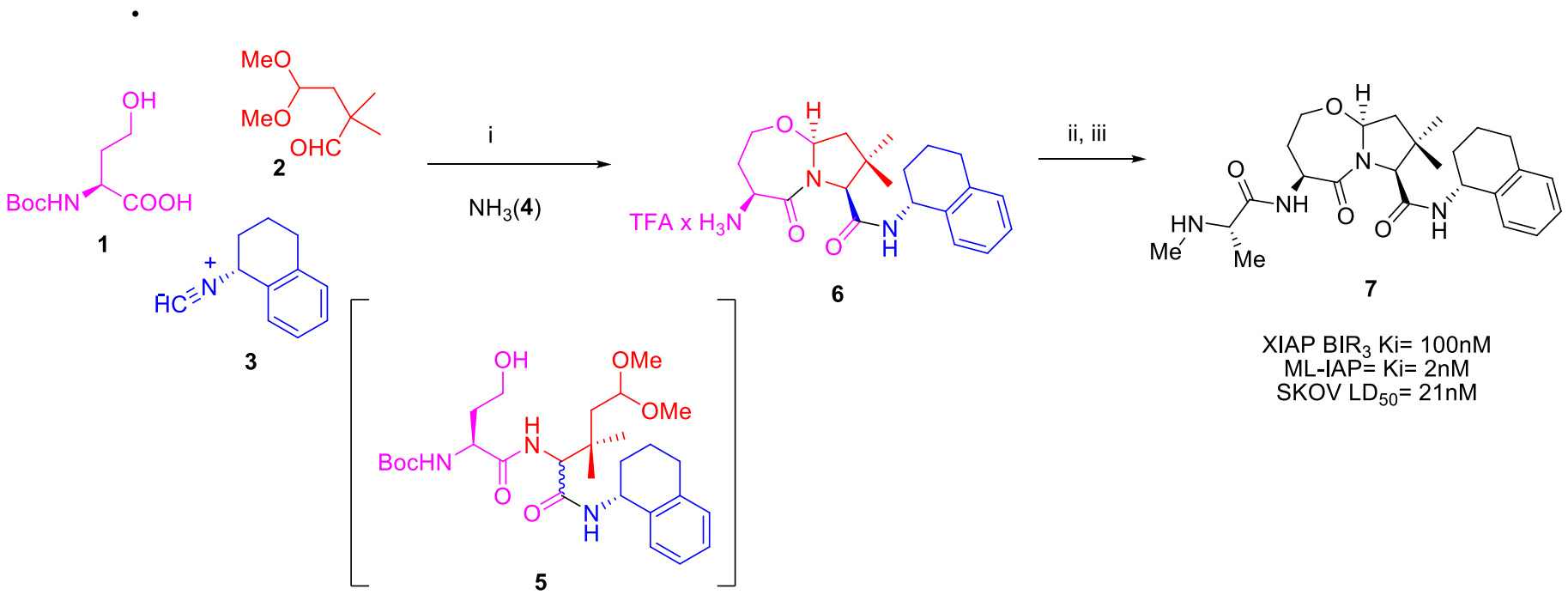
Genentech (GDC-0152)



Genentech (Smac mimetic)
K. Zobel et al. *ACS Chem. Biol.* **2006**, *1*, 525-533

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

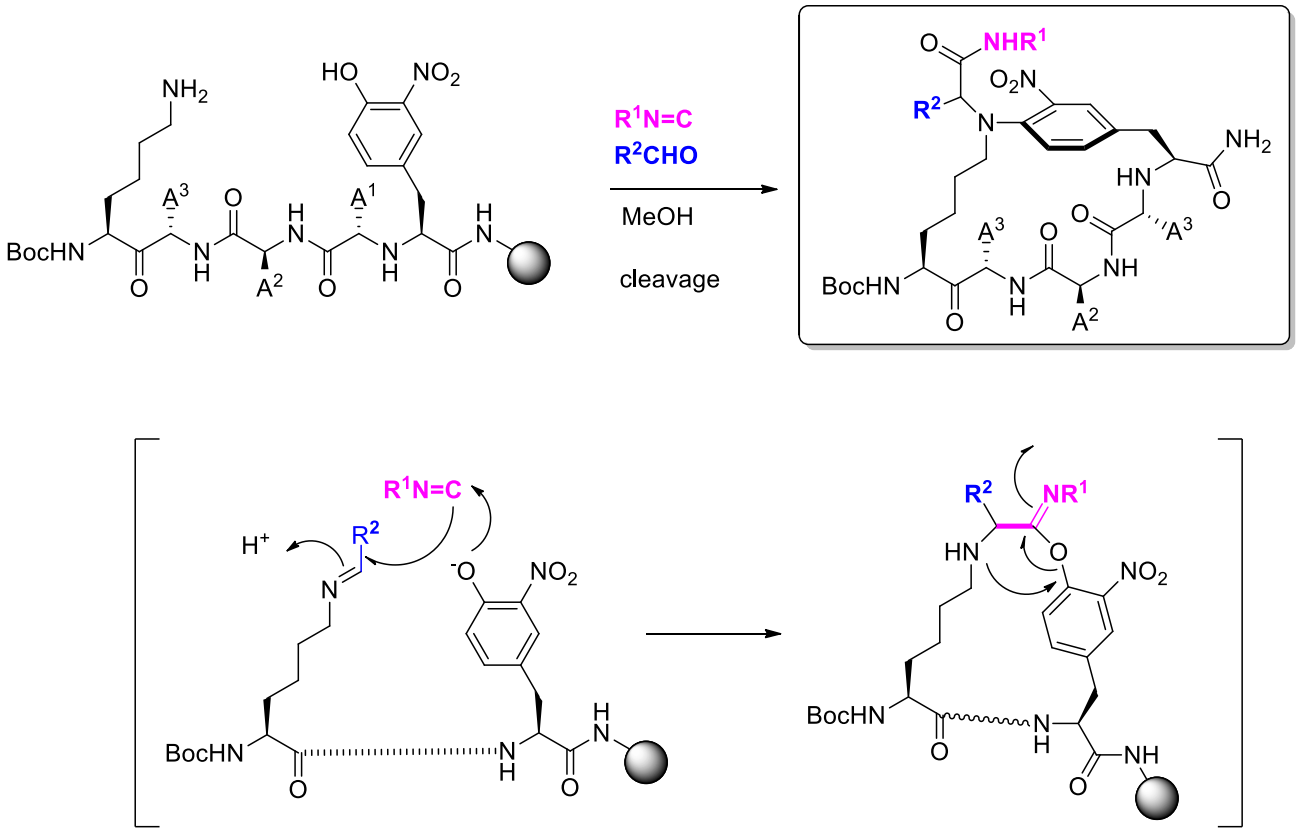
Synthesis strategies: application of the Ugi reaction: Inhibitors of IAPs



i: **1**, **2**, **3**, **4**, TFE, microwave, 80°; then TFA 8-10 equiv.), DCM, 32°; ii: Boc-N-Me-Ala, HOBT, EDC, NMM, THF; iii: TFA 8-10 equiv.), DCM, 32°

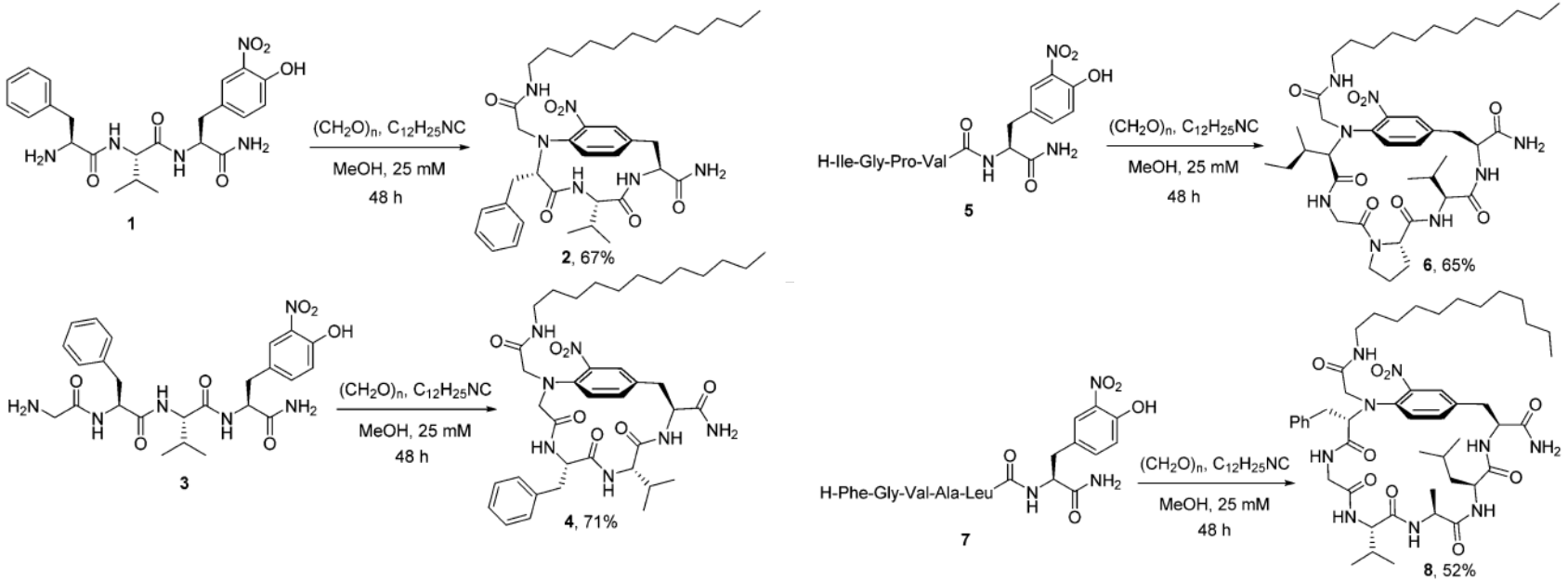
M. Vamos et al. *ACS Chem. Biol*. **2013**, *8*, 725-732

Synthesis strategies: MCRs: Ugi-Smiles macrocyclization



M. C. Morejon et al. *Org. Lett.* **2016** (DOI: 10.1021/acs.orglett.6b02001

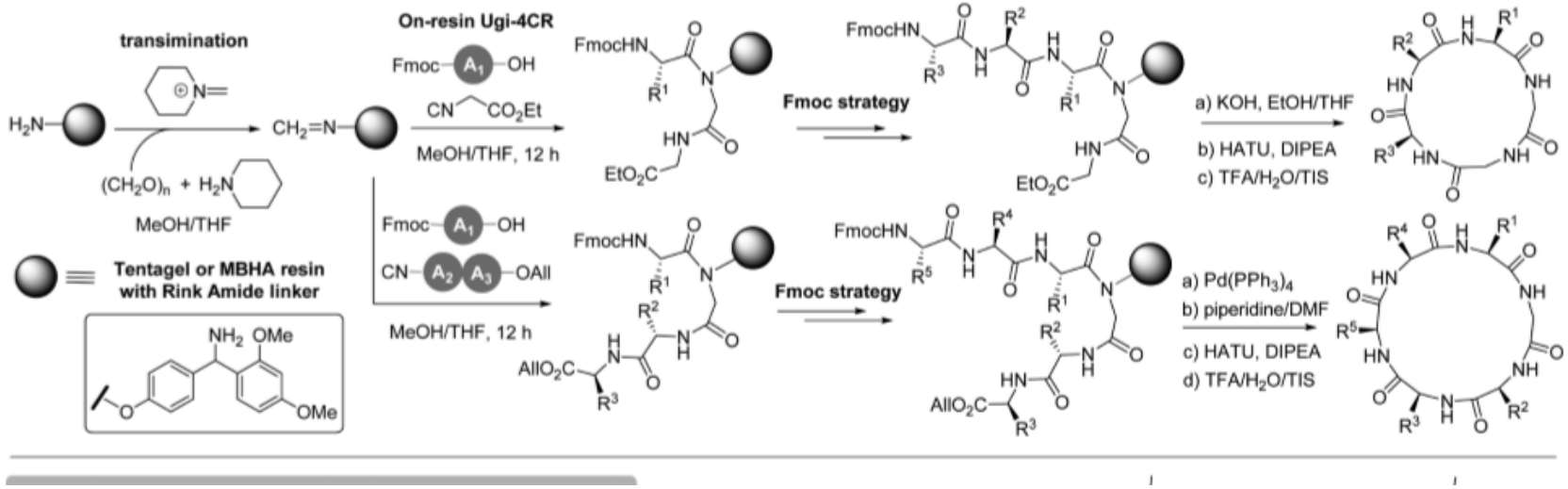
Synthesis strategies: MCRs: Ugi-Smiles macrocyclization



M. C. Morejon et *al Org. Lett*. **2016** (10.1021/acs.orglett.6b02001)

MCRs: Ugi 4MCR macrocyclization strategy on solid phase

Scheme 4. Solid-Phase Synthesis of Cyclic Peptides by a Multicomponent Backbone Amide Linker (BAL) Strategy

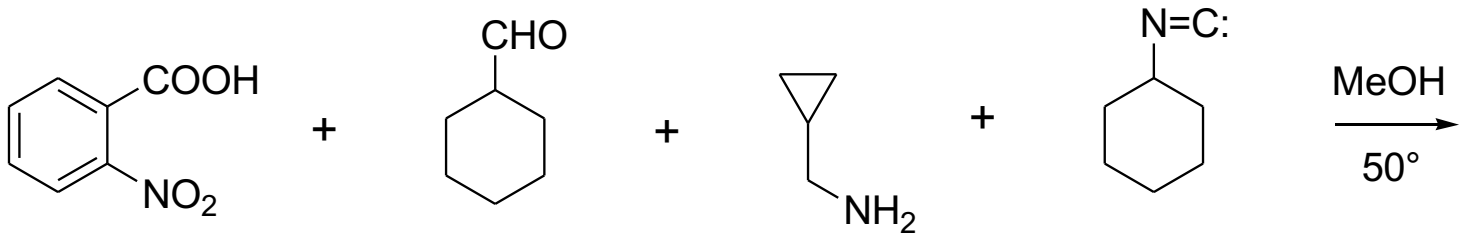


A. R. Puentes et *al Org. Lett*. **2017** (DOI: 10/1021/acs.0rglett.7b01761)

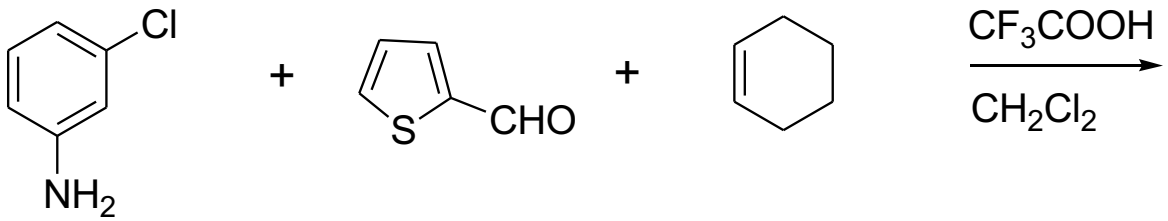
Questions

1. Please name three classical multi-component reactions (MCR‘s)?
2. Give possible products of the following MCR‘s

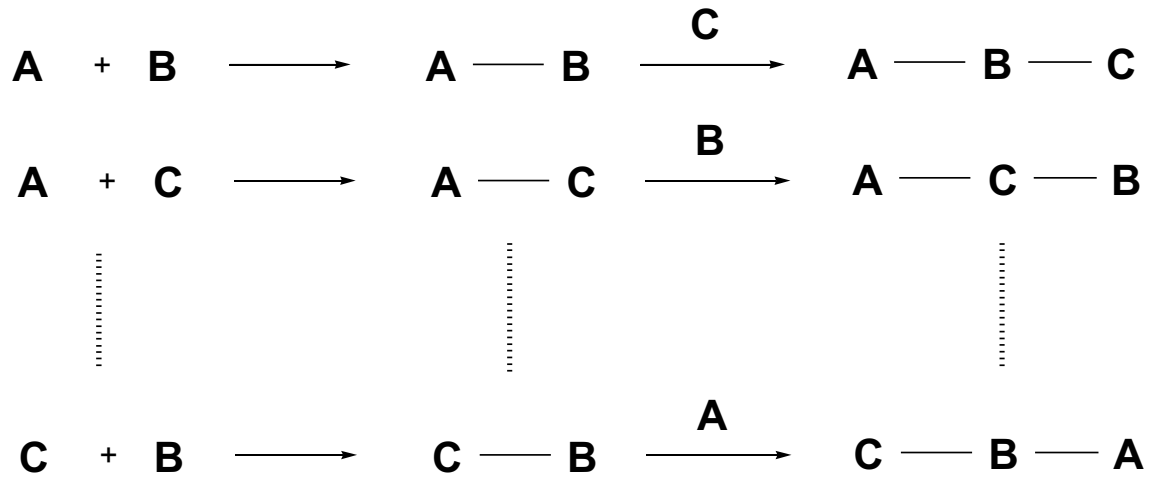
a)



b)



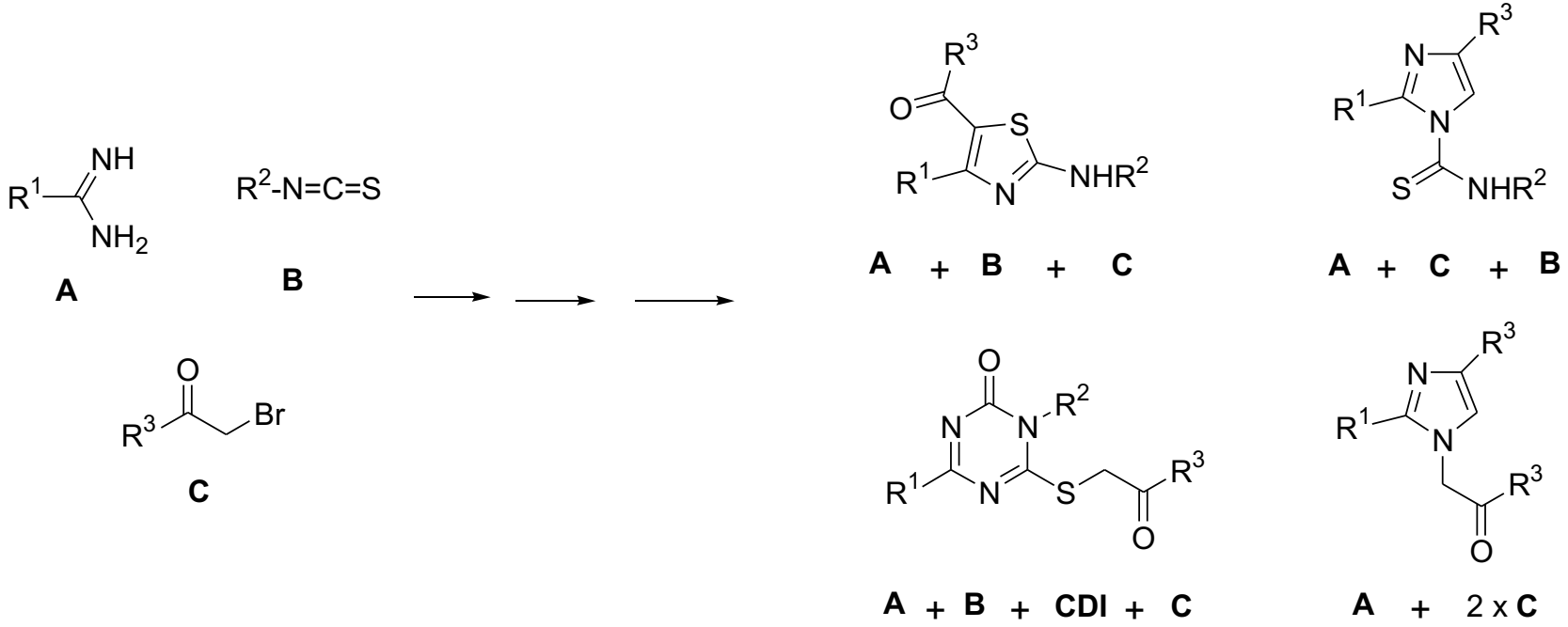
Synthesis strategies: sequential multi-component reactions (SMCRs)



- 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.

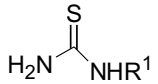
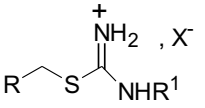
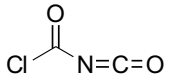
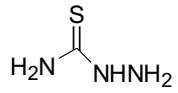
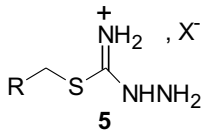
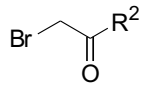
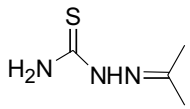
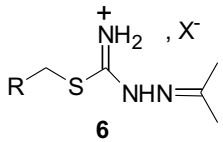
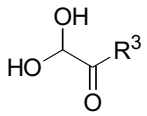
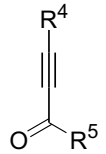
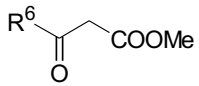
Synthesis strategies: sequential multi-component reactions



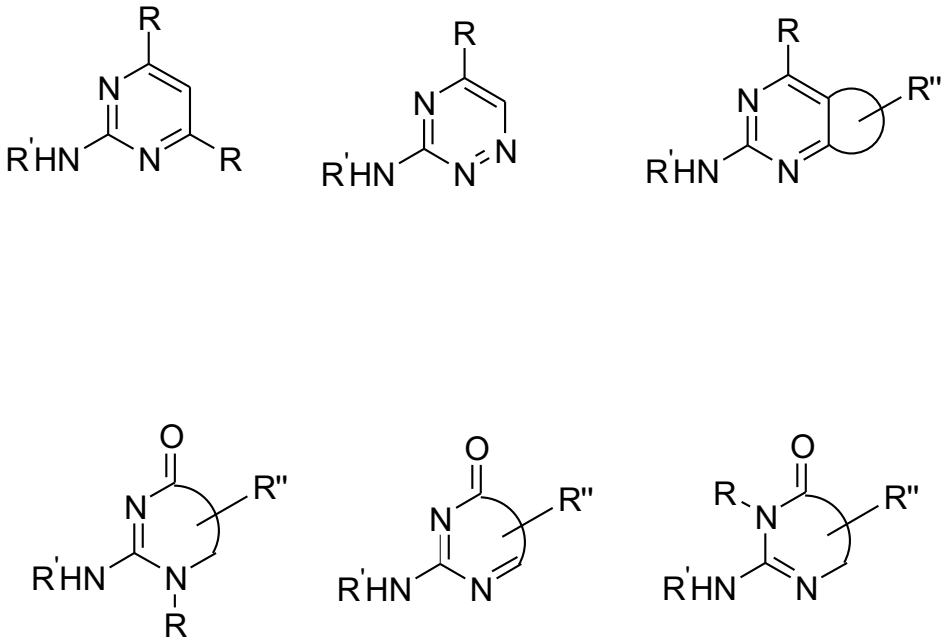
Sequential multi-component reactions (MCR's) offer the same advantages as the classical MCR's: in addition several different scaffolds can be obtained employing the same set of building blocks

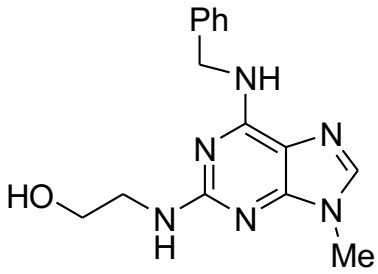
D. Obrecht, P. Ermert, 5th Ineternational conference on Synthetic Organic Chemistry (ECSOC-5); www.mdpi.org/ecsoc-5/, September 1-30, 2001, [B0005]

Synthesis strategies: sequential multi-component reactions

bis-donors	bis-acceptors	acceptor-donors	electrophiles	nucleophiles
 **1**	 **4**	 **7**	R^7—N=C=S **12**	R^9—X (X: Cl, Br, I) **14**	R^{10}—NH_2 **15**
 **2**	 **5**	 **8**	R^8—N=C=O **13**		
 **3**	 **6** (X: Cl, Br)	 **9**			
		 **10**			
		 **11**			

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

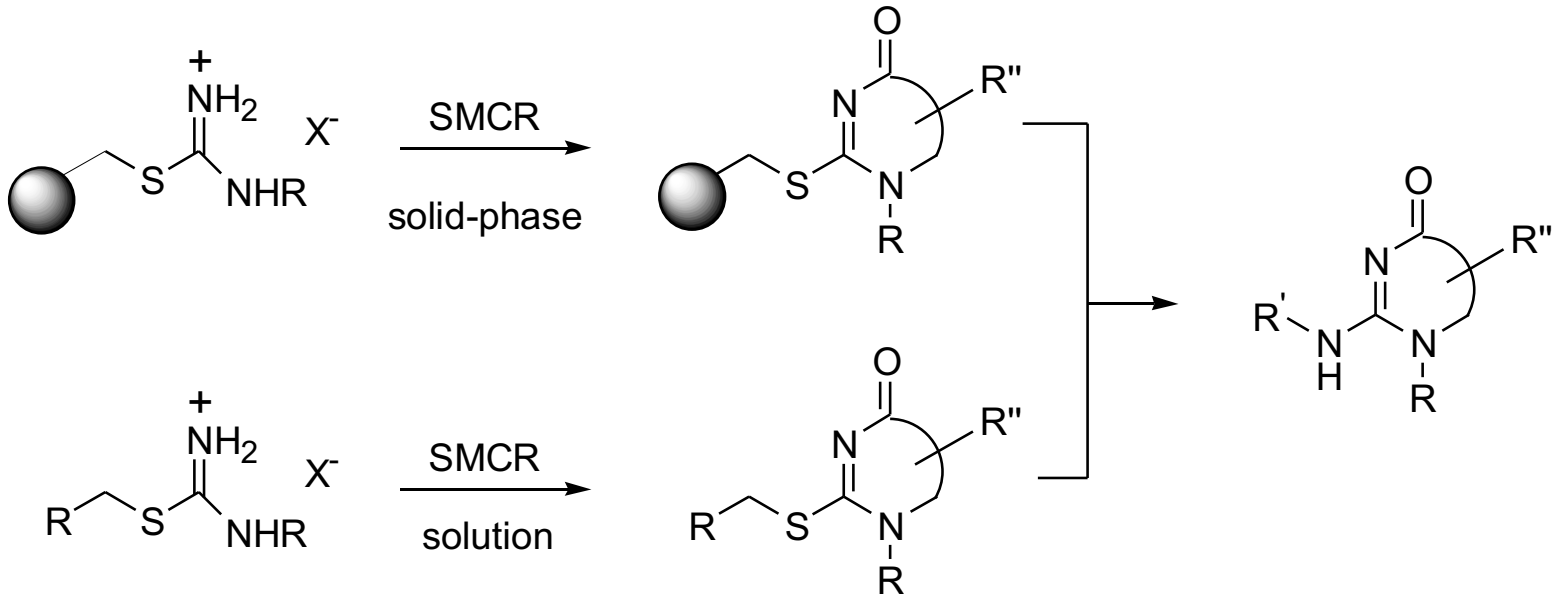




Olomoucin

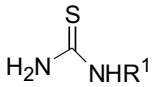
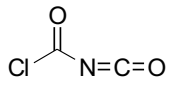
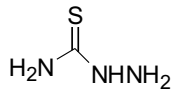
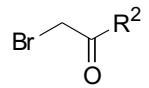
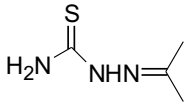
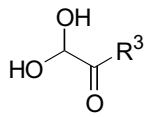
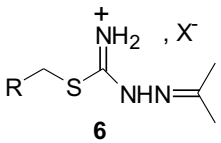
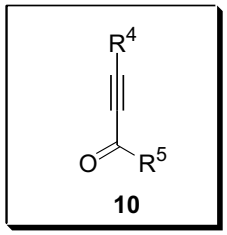
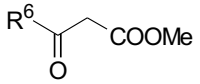
D. Obrecht, P. Ermert, 5th Ineternational conference on Synthetic Organic Chemistry (ECSOC-5); www.mdpi.org/ecsoc-5/, September 1-30, 2001, [B0005]

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

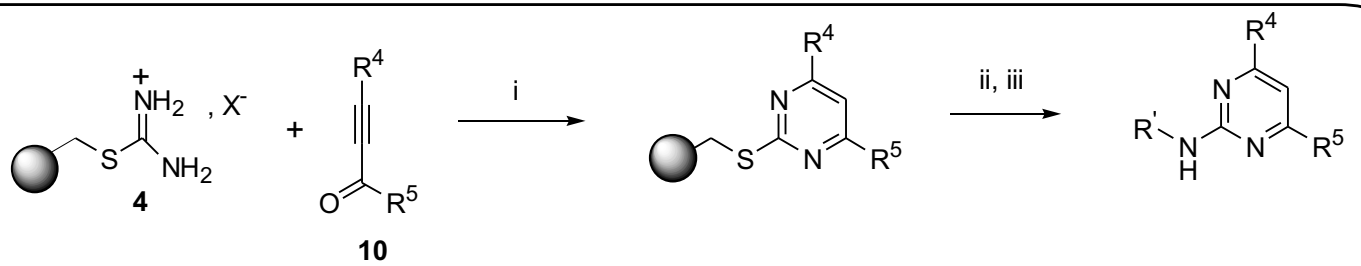


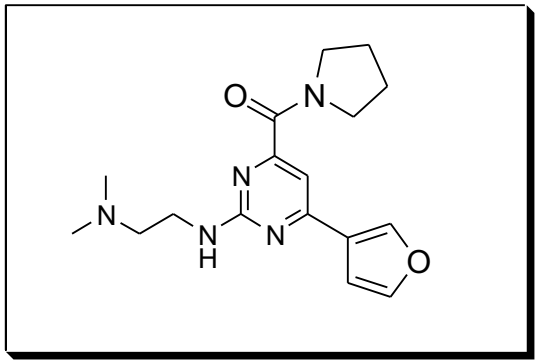
D. Obrecht, P. Ermert, 5th Ineternational conference on Synthetic Organic Chemistry (ECSOC-5); www.mdpi.org/ecsoc-5/, September 1-30, 2001, [B0005]

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

bis-donors	bis-acceptors	acceptor-donors	electrophiles	nucleophiles
 **1**	 **7**	$R^7-N=C=S$ **12**	R^9-X (X: Cl, Br, I) **14**	$R^{10}-NH_2$ **15**
 **2**	 **8**	$R^8-N=C=O$ **13**		
 **3**	 **9**			
 **6** (X: Cl, Br)	 **10**			
	 **11**			

Synthesis strategies: Sequential multi-component reactions





i: DIPEA, DMF; ii: m-CPBA, CH_2Cl_2; iii: $R'NH_2$ (**15**), dioxane, 80-100° [8].

D. Obrecht, P. Ermert, 5th Ineternational conference on Synthetic Organic Chemistry (ECSOC-5); www.mdpi.org/ecsoc-5/, September 1-30, 2001, [B0005]

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

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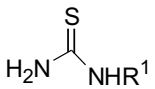
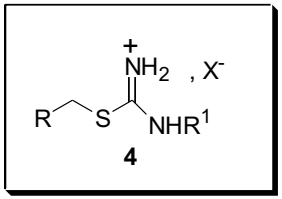
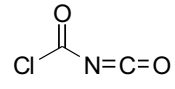
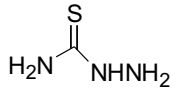
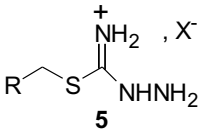
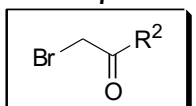
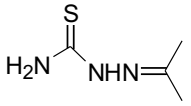
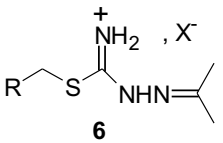
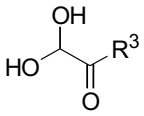
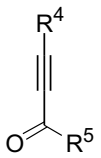
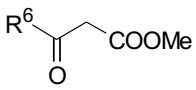
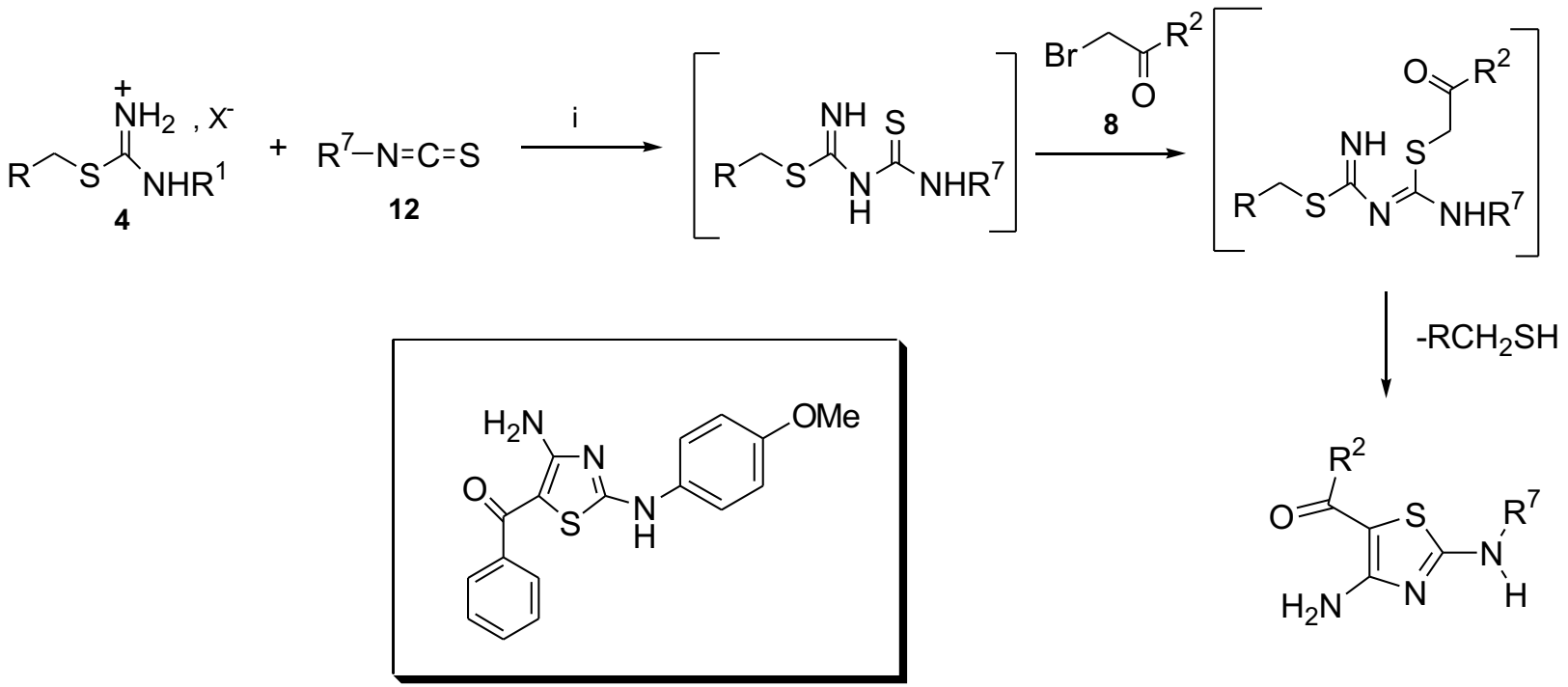
 **1**	 **4**	 **7**	$R^7-N=C=S$ **12**	R^9-X (X: Cl, Br, I) **14**	$R^{10}-NH_2$ **15**
 **2**	 **5**	 **8**	$R^8-N=C=O$ **13**		
 **3**	 **6** (X: Cl, Br)	 **9**			
		 **10**			
		 **11**			

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



i: DBU, DMF, 0°; then DBU and **8**, r.t.

D. Obrecht, P. Ermert, 5th Ineternational conference on Synthetic Organic Chemistry (ECSOC-5); www.mdpi.org/ecsoc-5/, September 1-30, 2001, [B0005]

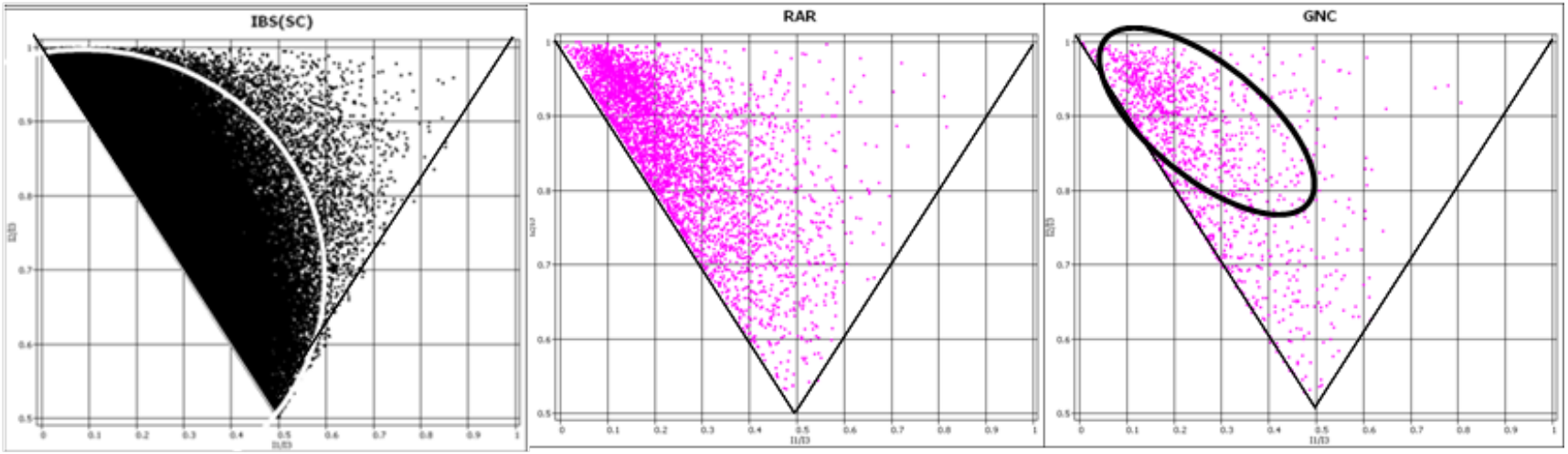
Synthesis strategies: Diversity-oriented synthesis (DOS)

- During 1995-2005 large small molecule libraries were synthesized exhibiting limited 3D-diversity
- Large combinatorial libraries have many linear (cigare-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 sperical-shape

(A. K. Gosh, *J. Org. Chem*. **2010**, *75*, 7967-7989; D. J. Newman et al., *J. Nat. Prod*. **2007**, *70*, 461-477; E. M. Driggers et al. *Nature Rev. Drug Discov*. **2008**, *7*, 608-624)

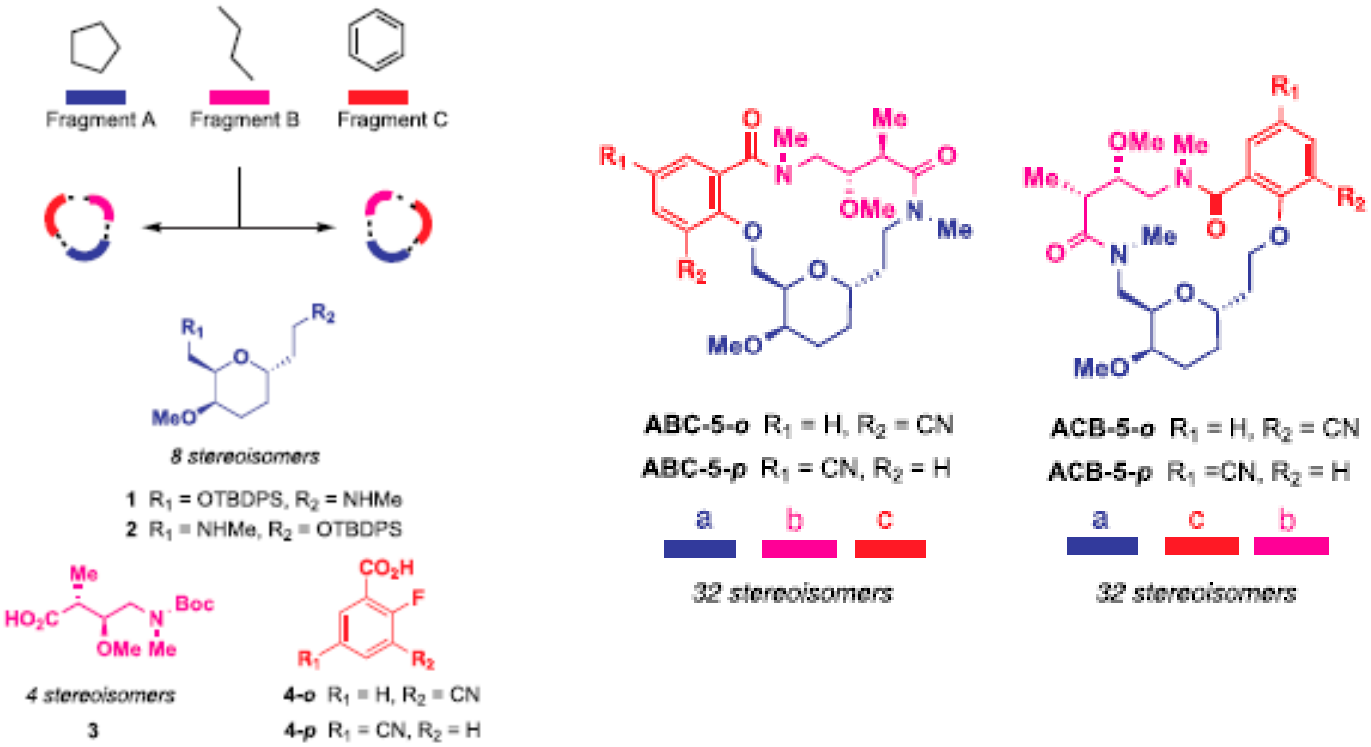
- Natural products often require a large and complex multistep synthesis effort. Diversity-oriented synthesis aimes at synthesizing natural product-like libraries via common synthetic precursors

(S. L. Schreiber, *Nature* **2009**, *457*, 153-154)



Synthesis strategies: Diversity-oriented synthesis

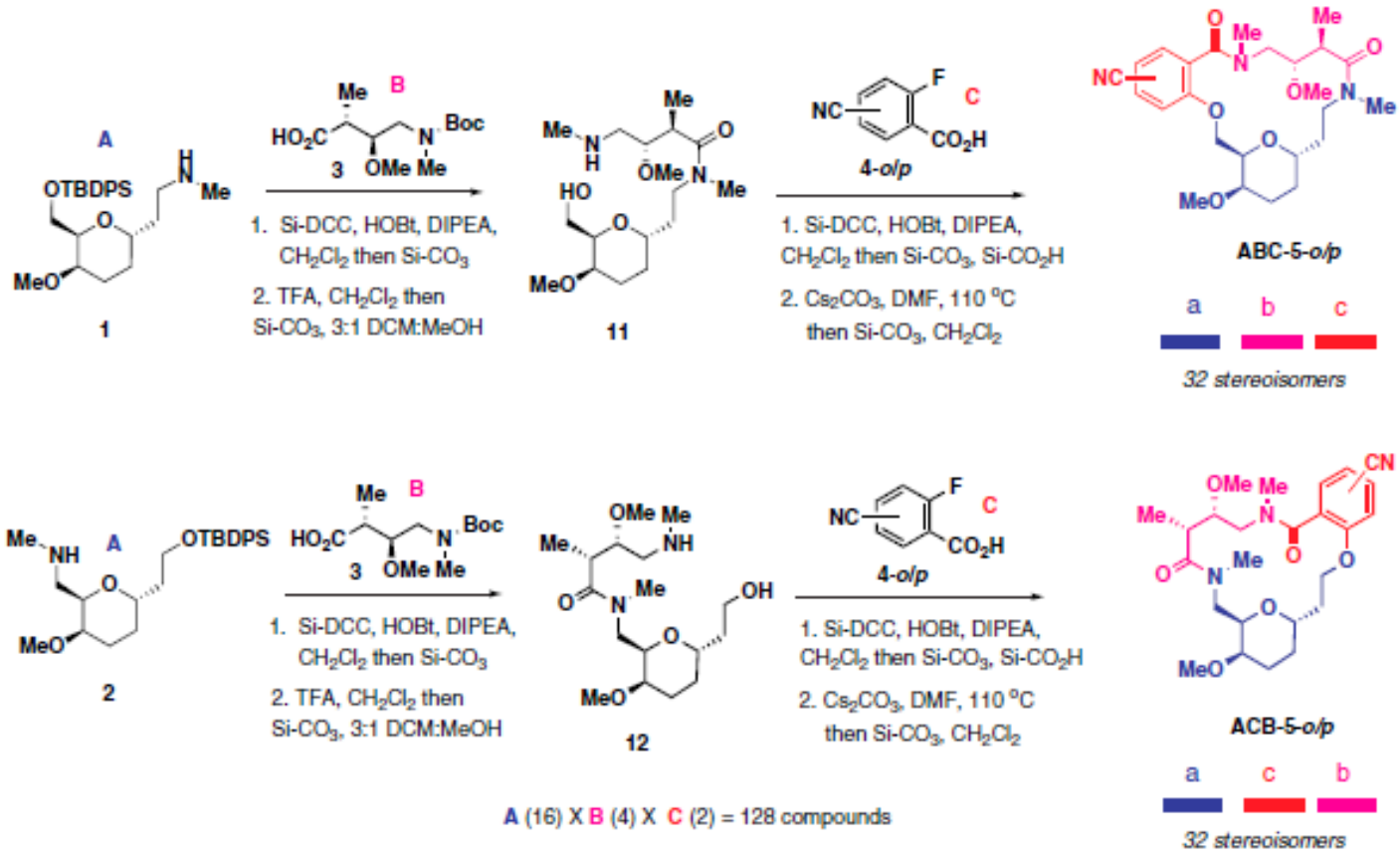
Fragment-based domain shuffling approach



E. Comer et al. *PNAS* **2011**, *108*, 6751-6756

Synthesis strategies: Diversity-oriented synthesis

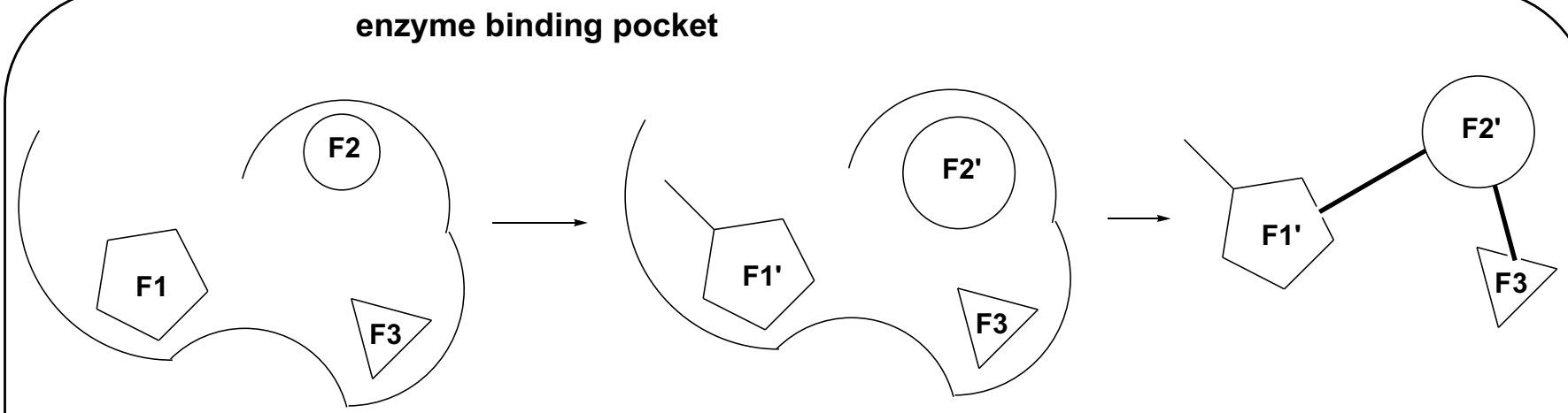
Fragment-based domain shuffling approach



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:

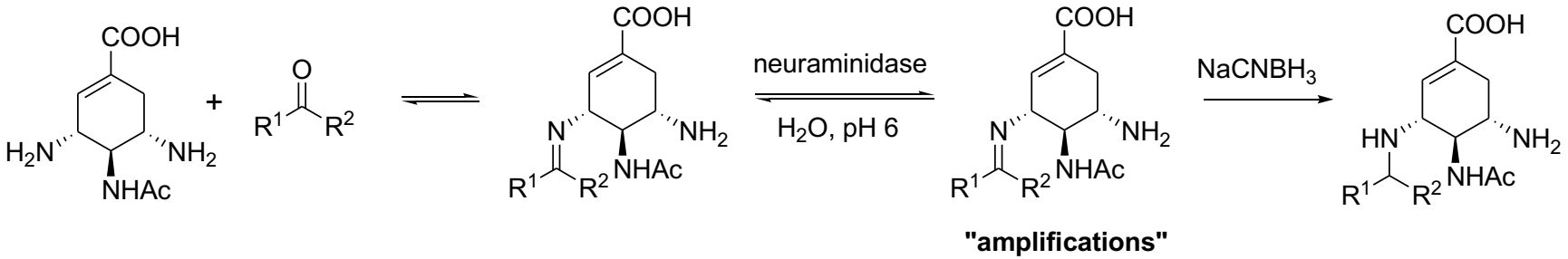
-An initial fragment is optimized (e.g. **F1** to **F1'** and **F2** to **F2'**)

C. Fragment linking:

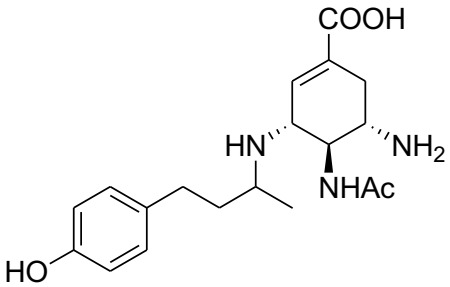
-Two ore more fragments, which bind to proximal parts of the active site, are joined together
-very challenging

-D. C. Rees et al. *Nature Rev. Drug Disc*. **2004**, *3*, 660-672
-P. Hayduk et al. *Nat. Rev. Drug Disc*. **2007**, *6*, 211-219

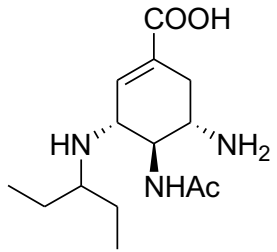
Synthesis strategies: Dynamic Combinatorial Synthesis



"amplifications"



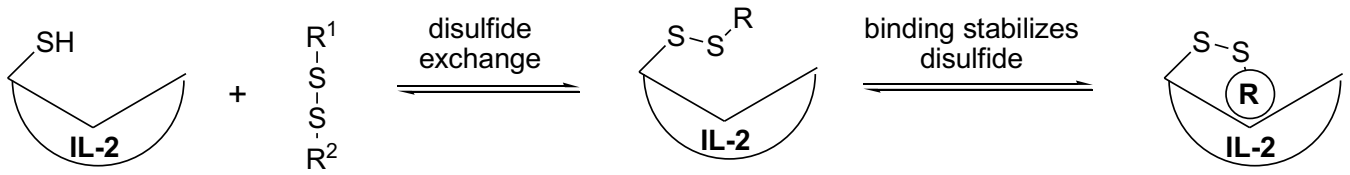
amplification= 84
Ki = 700nM



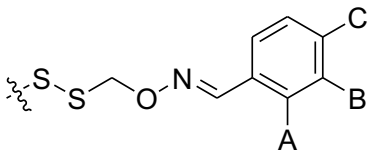
amplification> 30
Ki = 85nM (strongest binder)

M. Hochgürtel et al. *J. Med. Chem.* **2003**, *46*, 356-8

Synthesis strategies: Dynamic Combinatorial Synthesis: disulfide thethering

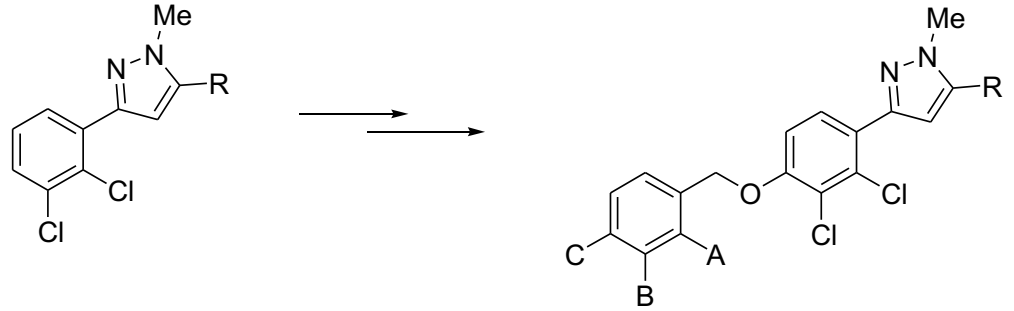


best R series:



A, B, C: H, CO_2H, CO_2Me or MeO

improve design of a known inhibitor with tethering "hit"



existing inhibitor
IC_{50} = 3 μM

improved inhibitors
IC_{50} = 0.2 μM

J. A. Wells et al. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 9367-72; A. C. Brainsted et al. *J. Am. Chem. Soc.* **2003**, *125*, 3714-15

Synthesis strategies: Click chemistry

Click Chemistry: Diverse chemical function from a few good reactions

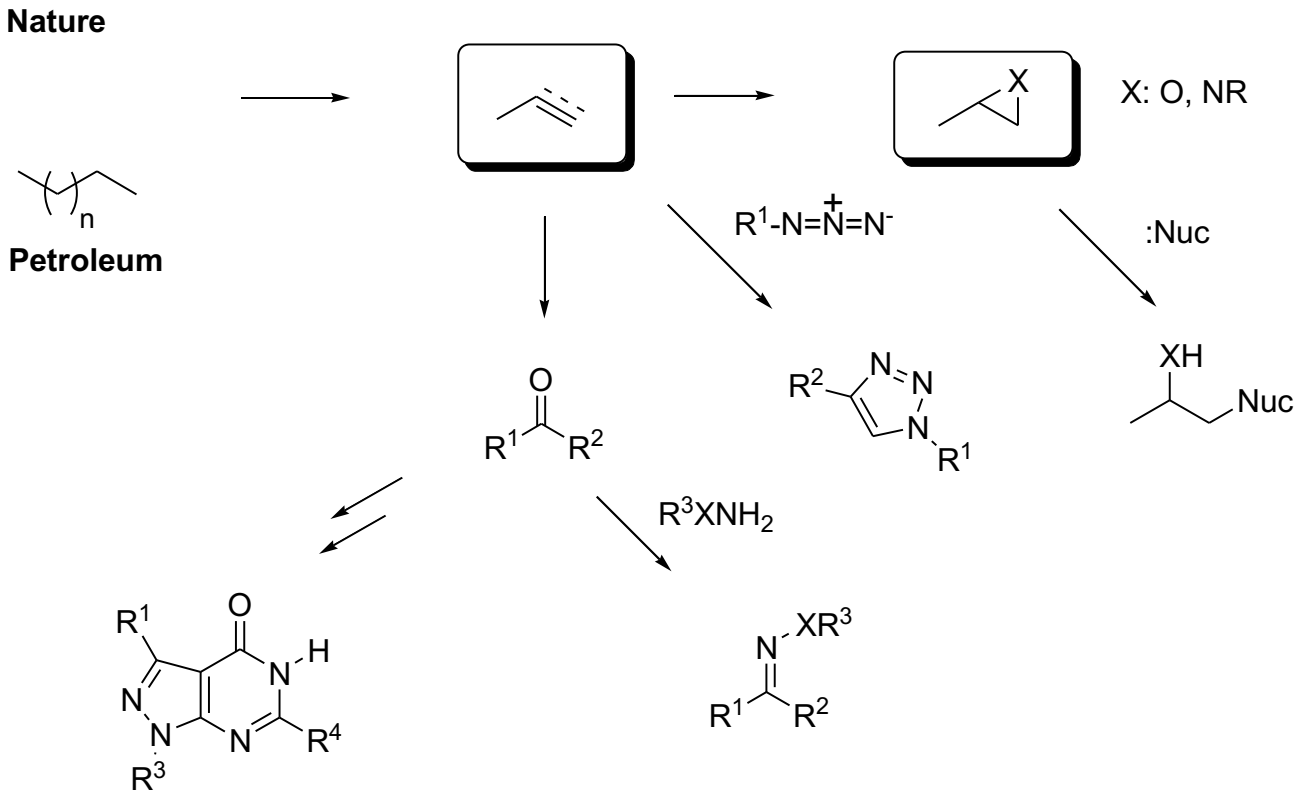
H. C. Kolb, K. B. Sharpless, *Angew. Chem. Int. Ed*. **2001**, *40*, 2004

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

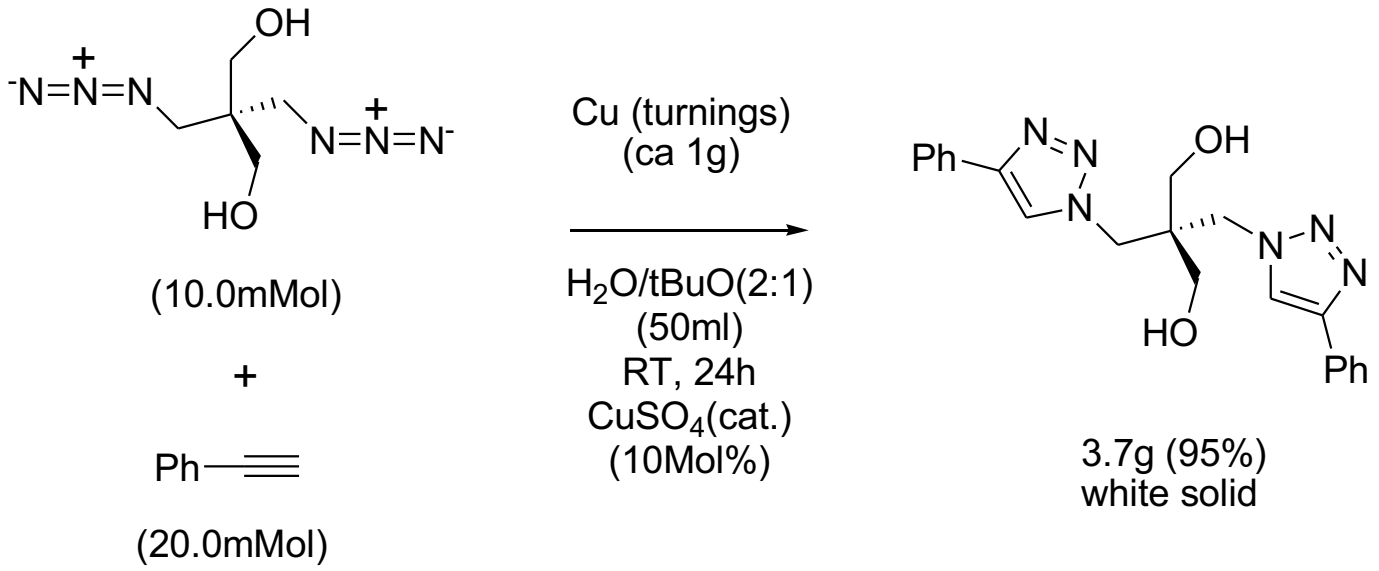
Synthesis strategies: Click chemistry



H. C. Kolb, K. B. Sharpless, *Drug Discovery Today* **2003**, *8*, 1128-37

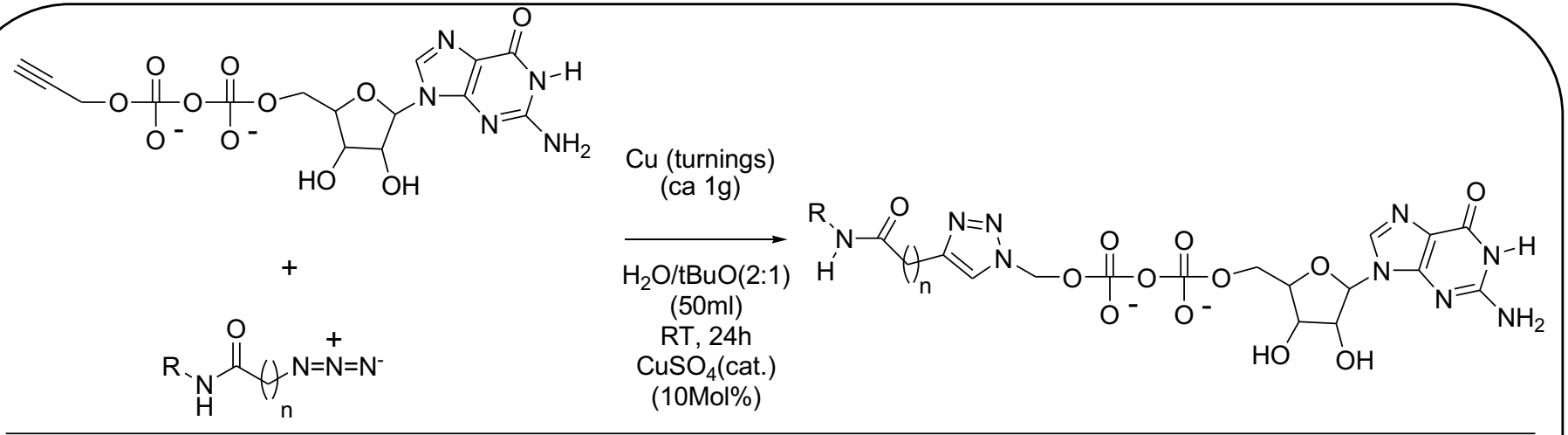
Synthesis strategies: Click chemistry

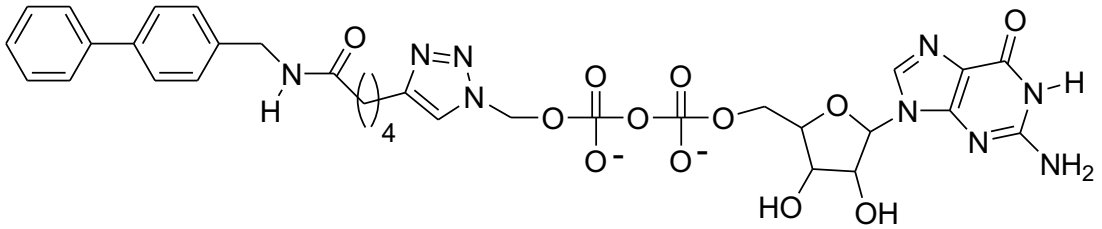
[1,3]-Dipolar additions of acetylenes and azides



V. V. Rostovtsev et al. *Angew. Chem. Int. Ed*. **2002**, *41*, 2596

Synthesis strategies: application of Click chemistry





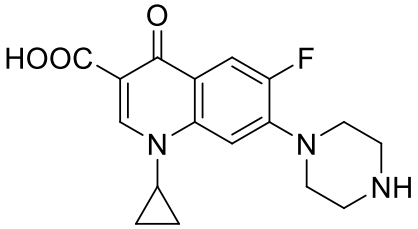
Ki: 62nM; inhibition of fucosyl transferase

cancer metastasis; lymphocyte trafficking

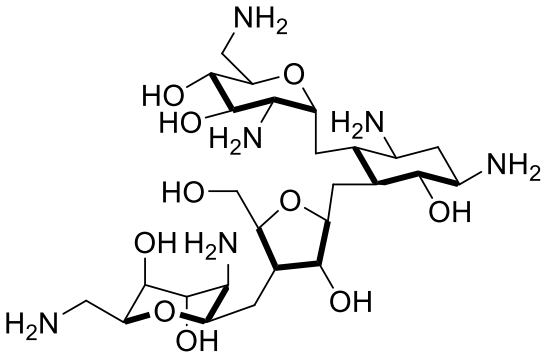
Lee et al. *J. Am. Chem. Soc*. **2003**, *125*, 9588-89

Dramatic rate acceleration of the azide-alkyne cycloaddition by sequestering the two components inside the host structure (enzyme or receptor)

Synthesis strategies: application of Click chemistry



Ciprofloxacin (Cipro; Bayer)

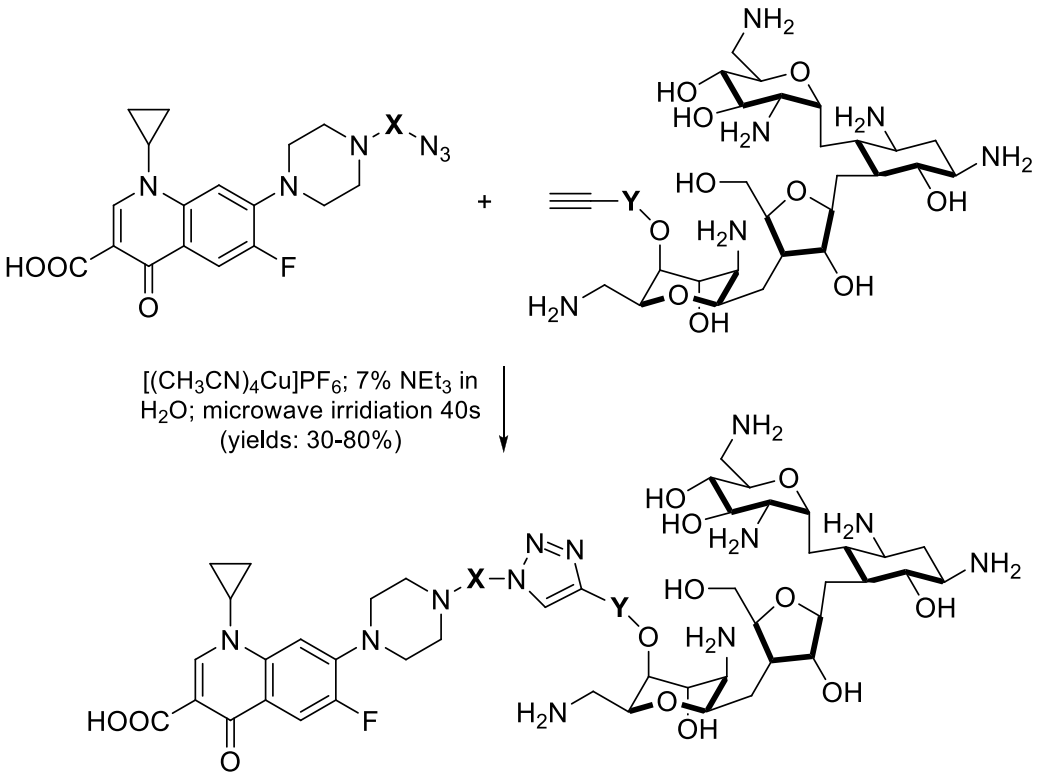


Neomycin B

V. Pokrovskaya et al. *J. Med. Chem.* **2009,** *52*, 2243-2254

-Emerging resistance in clinical isolates of bacteria render existing antibiotics such as Neomycin and Ciprofloxaxin inactive
-Enzymes such as aminoglycoside 3‘-phsphotransferases 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

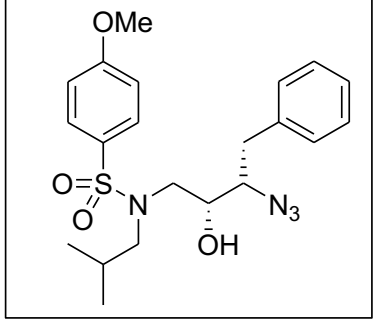


Cipro-NeoB hybrids

-biological activities (MICs) depended significantly on the variable spacer groups X and Y
-best combinations were X= -$(CH_2)_2$- and Y= -CH_2OCH_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

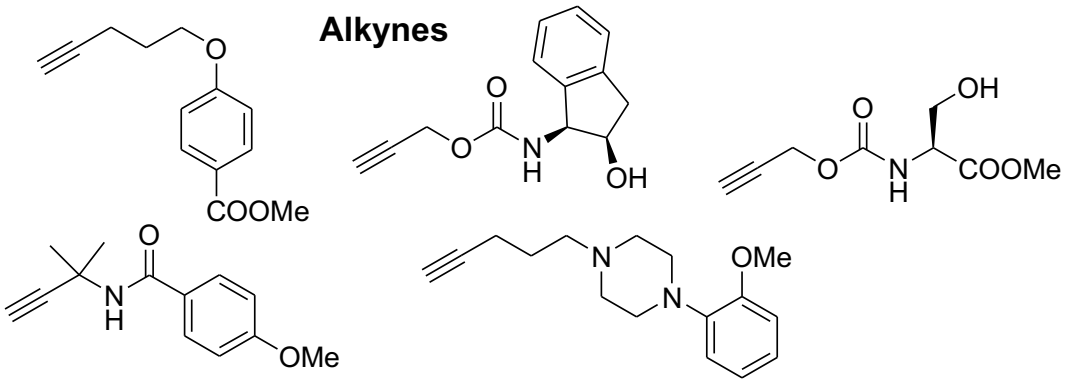
Synthesis strategies: application of Click chemistry

Azide 1

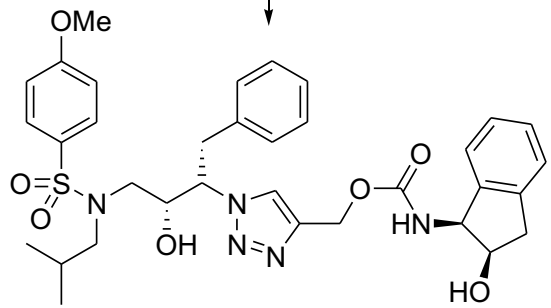


x

Alkynes



HIV-protease (SF-2), buffer, 23°, 24h



Ki = 1.7 nM

M. Whiting et al. *Angew. Chem. Int. Ed*. **2006**, *45*, 1435-39; K. B. Sharpless, R. Manetsch, *Exp. Opin.Drug. Disc*. **2006**, *1*(6), 525-38

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

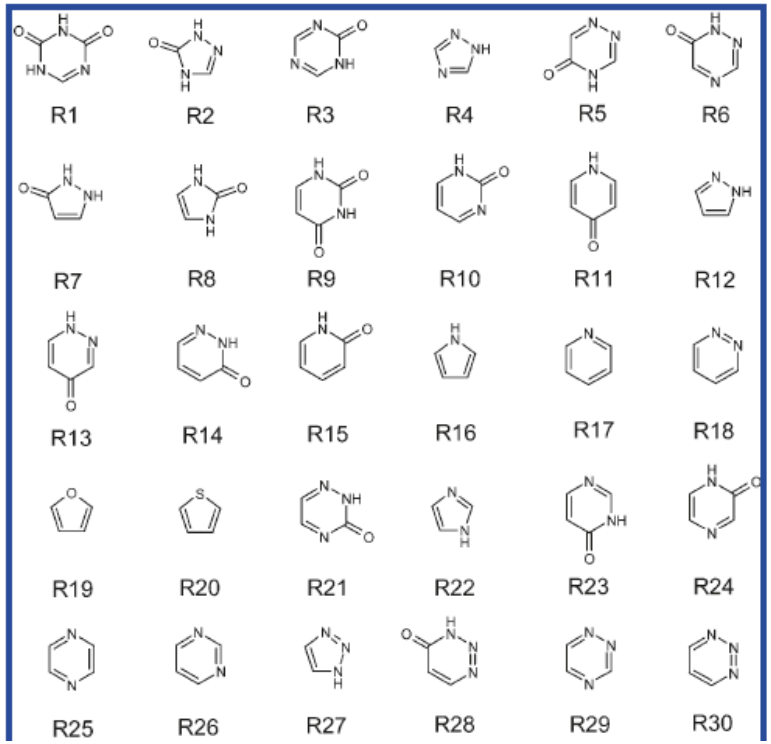
-fragments can be assembled in a thermodynamically or kinetically controlled fashion: *dynamic combinatorial synthesis*

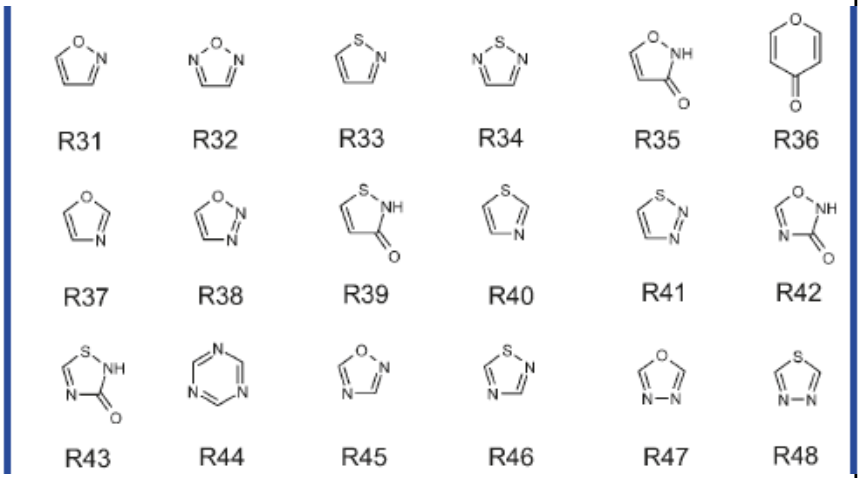
-fragments can be assembled using *click chemistry*

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

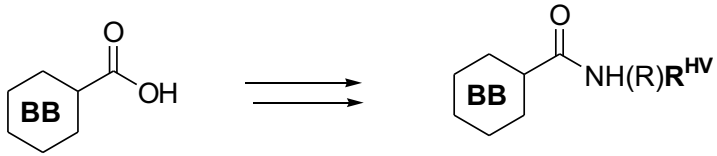


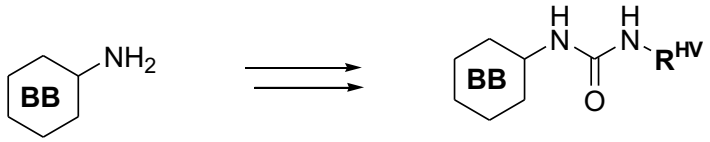


R. Ward et al. *J. Męd. Chem*. **2011**, *54*, 4670-4677; S. D. Roughley et al. *J. Med. Chem.* **2011**, *54*, 3451-3479

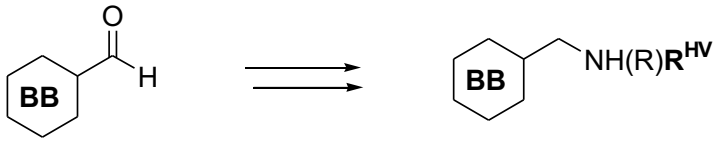
Most important reactions used in parallel and combinatorial synthesis

Formation d`amides et d`urées:

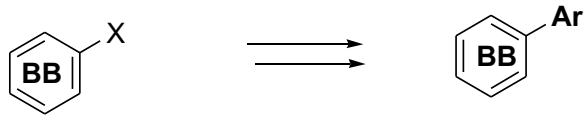




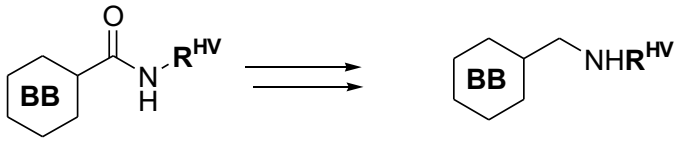
Amination réductrice:



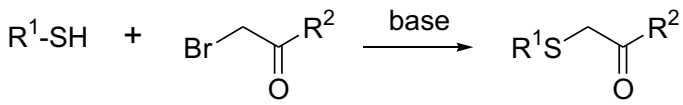
Couplage Suzuki:



Réduction au diborane:

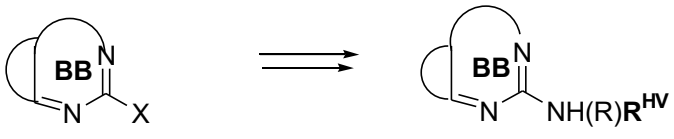


Alkylation du groupe thiol:

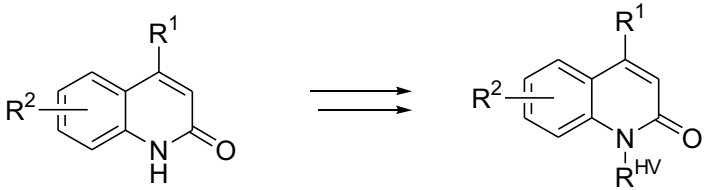


Most important reactions used in parallel and combinatorial synthesis

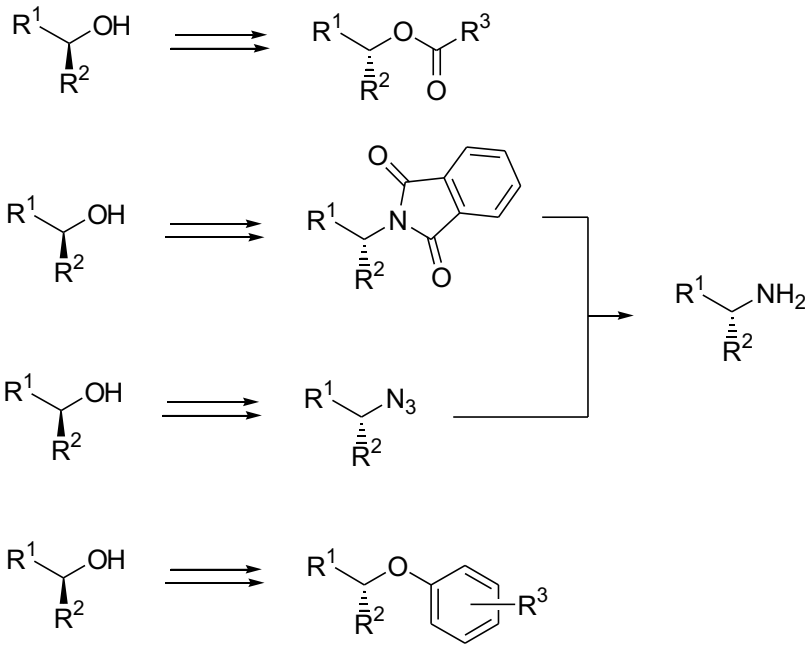
Substitution nucleophillique:



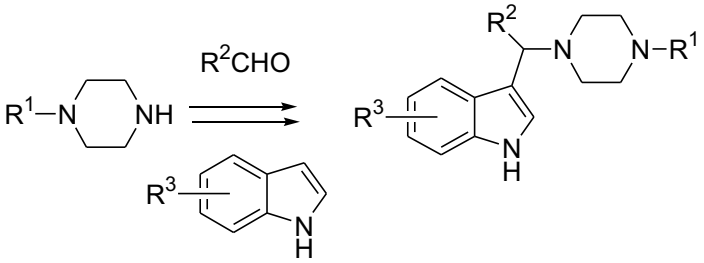
Alkylation de NH activés:



Réaction de Mitsunobu:



Réaction de Mannich:



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 libaries?

3. What is the rule of 3?

Parallel work-up procedures

Extractions : principle

Liquid-liquid extractions

Solid-phase extractions

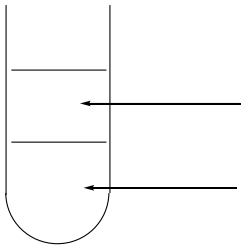
Solid-supported scavengers

Ion-exchange resins

Fluorous phase extractions

Parallel work-up procedures: principle

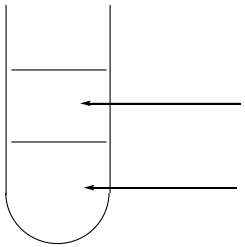
1. Two phase extractions: manuel extraction



Upper phase: **contains product** (EtOAc or fluorous phase): separated manually

Lower phase: contains impurities (aqueous phase)

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

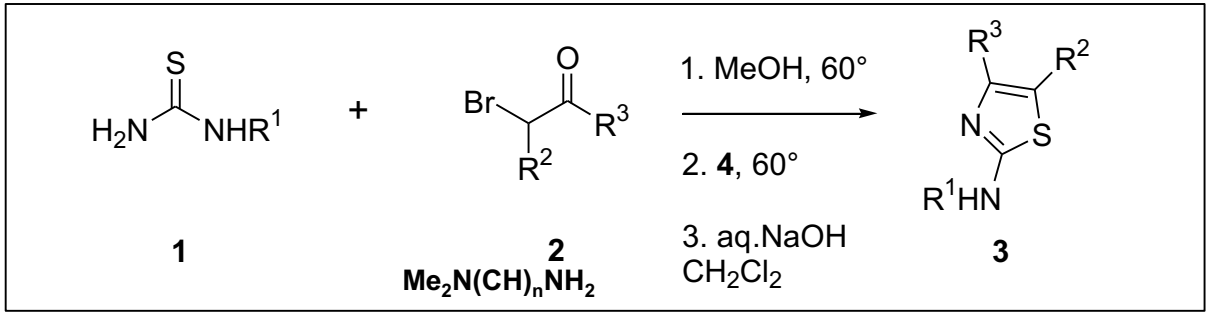


Upper phase: contains impurities (aqueous phase): separated by robot

Lower phase: **contains product** ($CHCl_3$ or CH_2Cl_2): dried and evaporated

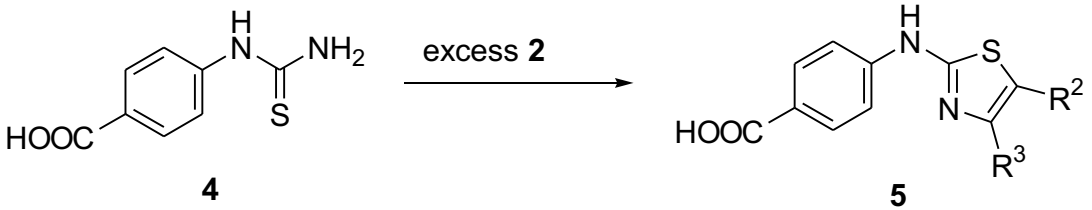
Parallel work-up strategies: liquid-liquid extractions

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



1 **2** **3**

$Me_2N(CH)_nNH_2$



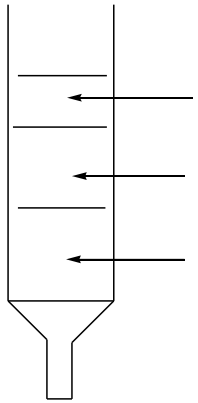
4 **5**

Products of type **5** are soluble in the *basic aqueous phase*

A. Chuchulowski, T. Masquelin, D. Obrecht, J. Stadlwieser, J.-M. Villalgordo, *Chimia* **1996**, *50*, 530

Parallel work-up strategies: solid-phase extractions

Solid phase extractions/filtrations

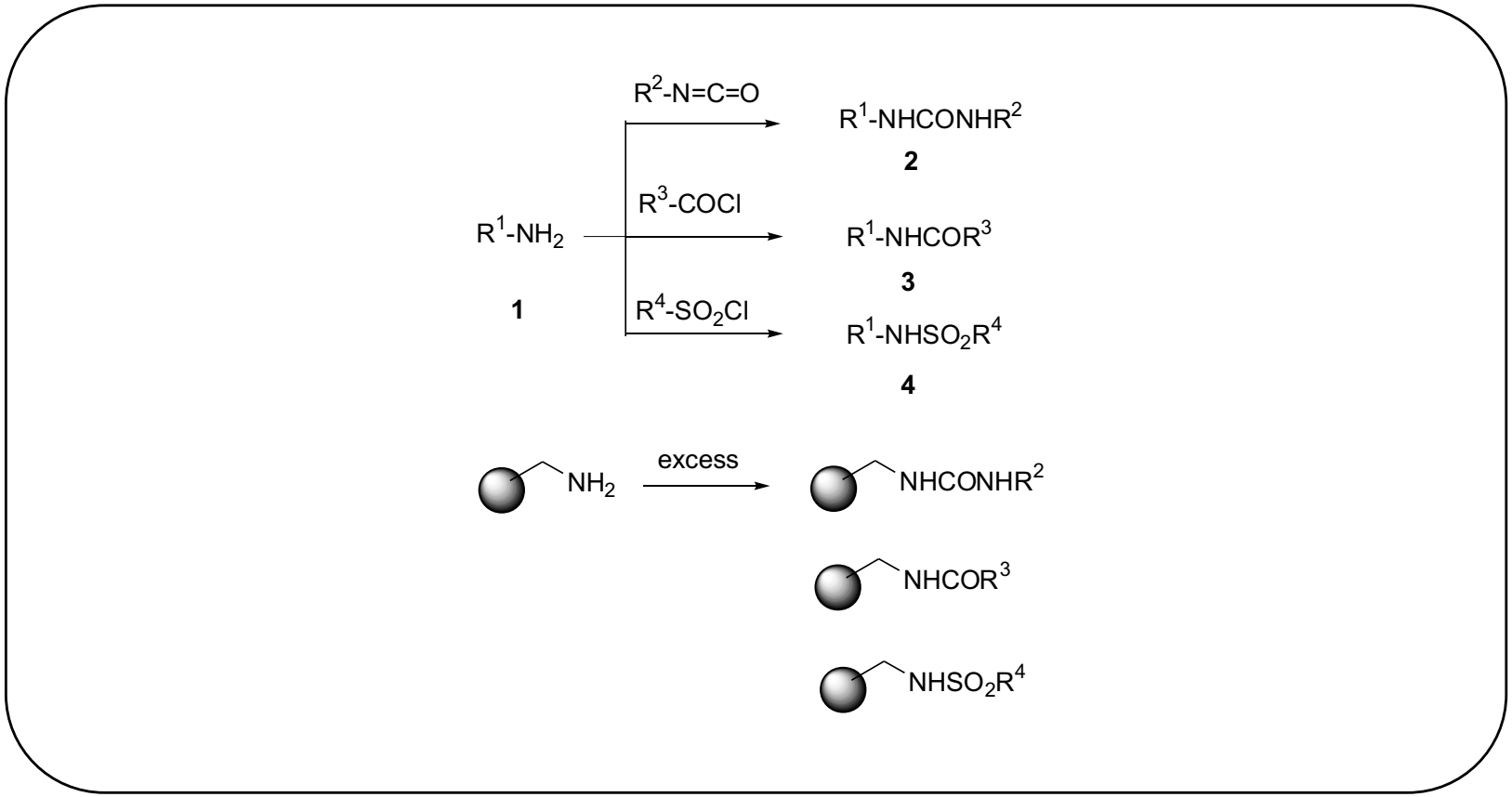


Solid phase: one or several solid pahses are filled into a polypropylene syringe or cartridge

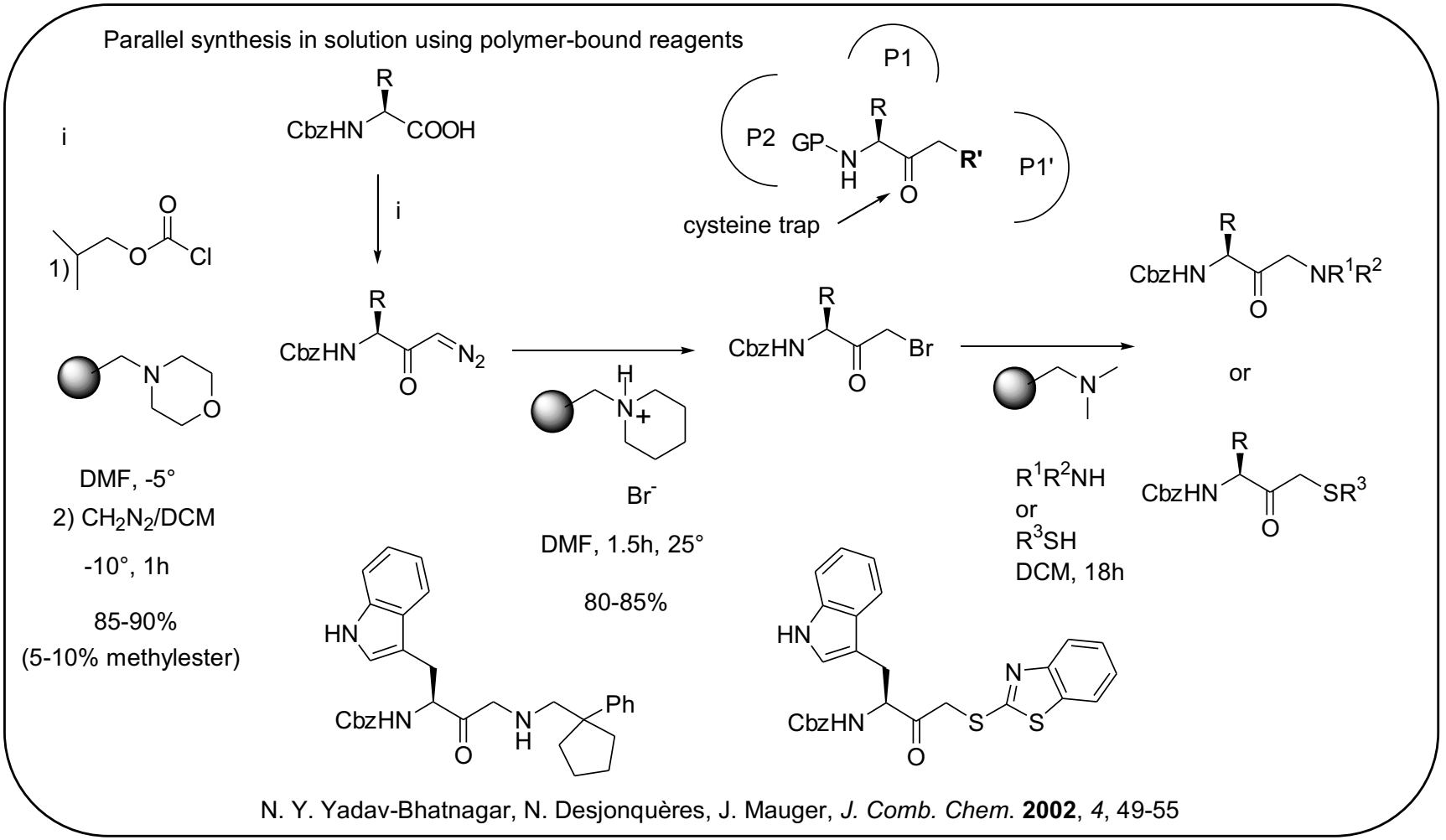
Solid phases: SiO_2; Al_2O_3; ``ion exchange resins (basic, acidic and mixed bed)``; Kieselguhr; $MgSO_4$; polymère functionalisé: -NH_2, -SH, -PPh_2, COOH, CHO, CH_2OH, isothiourée, N_3...;

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



Parallel work-up strategies: solid-supported scavengers

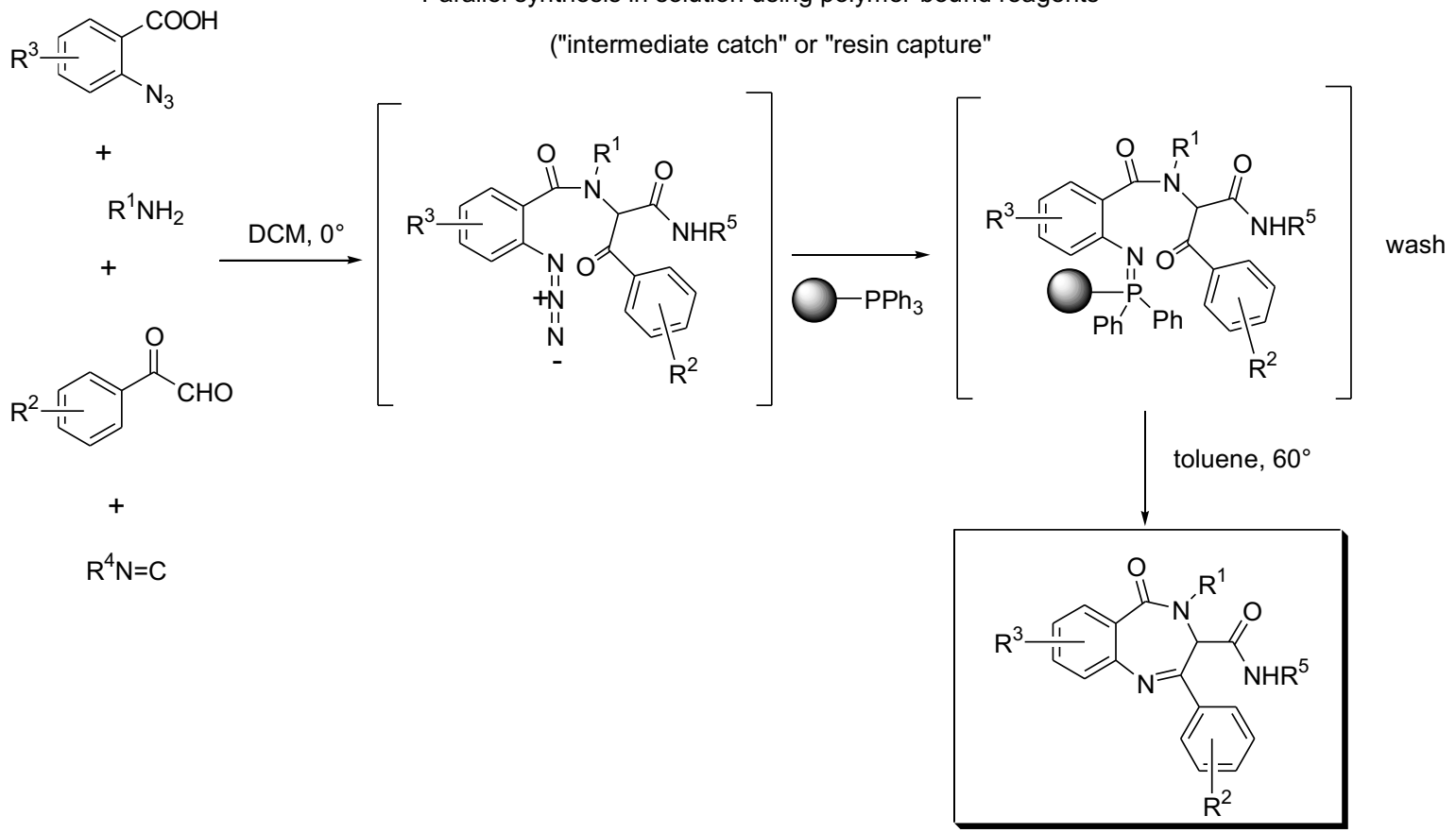


N. Y. Yadav-Bhatnagar, N. Desjonquères, J. Mauger, *J. Comb. Chem.* **2002**, *4*, 49-55

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

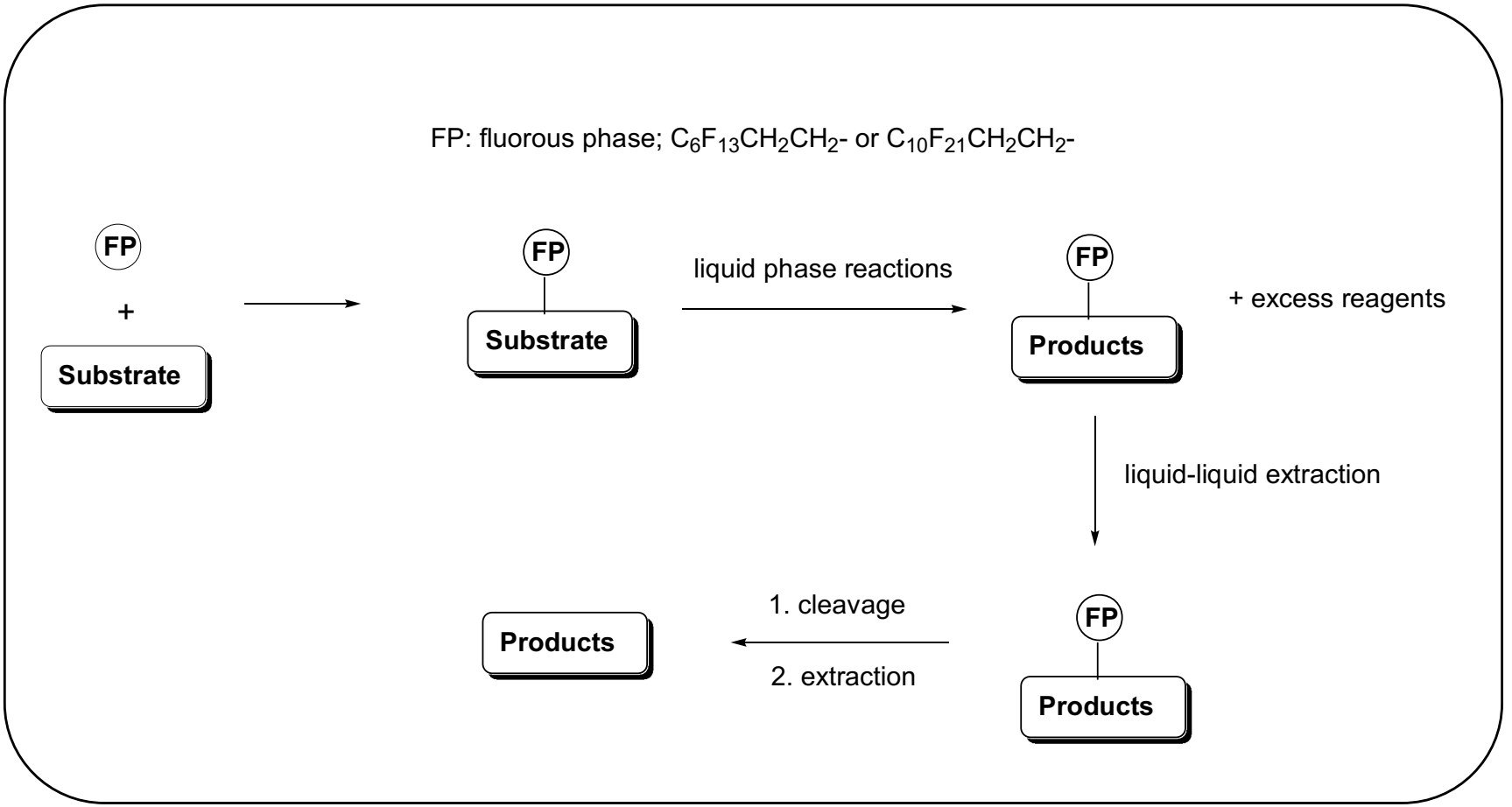
Parallel synthesis in solution using polymer-bound reagents

("intermediate catch" or "resin capture"



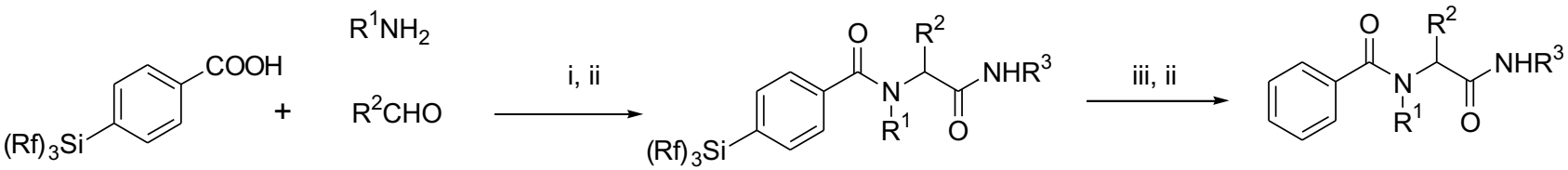
A. Chucholowski, D. Heinrich, B. Mathis, C. Müller, *Generation of benzodiazepin and benzodiazocin libraries through resin capture of Ugi-4CC*, conference: 214th ACS national meeting, Las Vegas, **1997**

Parallel work-up strategies: fluorous phases



Parallel work-up strategies: fluorous phases

Multicomponent reactions: fluorous phase extraction



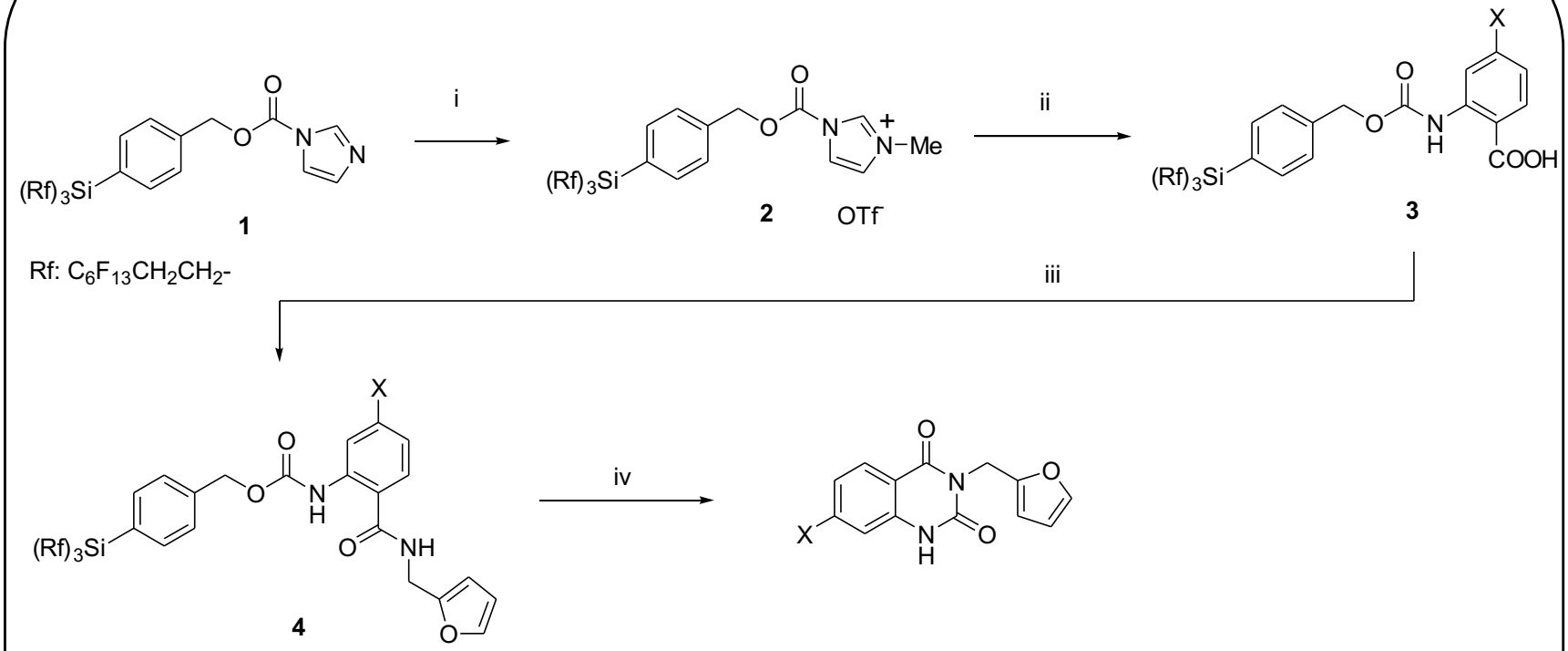
Rf: $C_{10}F_{21}CH_2CH_2$-

i: TFE, 90°, 48h; ii: liquid-liquid extraction; iii: Bu_4NF, THF, rt

A. Studer, S. Hadida, R. Ferritto, S.-Y. Kim, P. Jeger, P. Wipf, D. Curran, *Science* **1997**, *275*, 823

Parallel work-up strategies: fluorous phases

Fluorous phase extraction: cleavage by cyclization



i: MeOTf, CH_2Cl_2, 1,1,1-(trifluoromethyl)benzene(BTF); ii: anthranilic acid, DMAP, BTF, CH_2Cl_2; iii: TBTU, furfuryl amine, THF;iv: Et_3N

D. Schwinn, W. Bannwarth, *Helv. Chim. Acta* **2002**, *85*, 255

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*, *CPCR'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 adressed 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

P. Imming et al. *Nature Rev. Drug Disc.* **2006**, *5*, 821-34

1. Number of drug targets :

1997 : Drews et al. *Nature Biotechnol*. **1997**, *15*, 1318-19

-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

2002: A. Hopkins et al. *Nature Rev. Drug Disc*. **2002**, *1*, 727

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

bottom line: 300-500 targets hit by current drugs; 3'000-8'000 drugable targets

Kinase inhibitors

-Recent reviews: A. J. Bridges, *Chem. Rev*. **2001**, *101*, 2541-2571; G. Scapin, *Drug Disc.Today* **2002**, *77*, 601-611; S. Orchard, *Curr. Opin. Drug Disc. & Dev*. **2002**, *5*, 713-717; D. Fabbro, C. Garcia-Echeverria, *Curr. Opin. Drug Disc. & Dev*. **2002**, *5*, 701-712; S. K. Hanks, *The FASEB J*. **1995**, *9*, 576-596 (sequences of kinases); M. E. M. Noble, J. A. Endicott, L. N. Johnson *Science* **2004**, *303*, 1800-5; J. Zhang; P. L. Yang; N. S. Gray, *Nat. Rev. Drug Discov*. **2009**, *9*, 28-39 (Targeting cancer with small molecule kinase inhibitors);

-Three families of kinases:

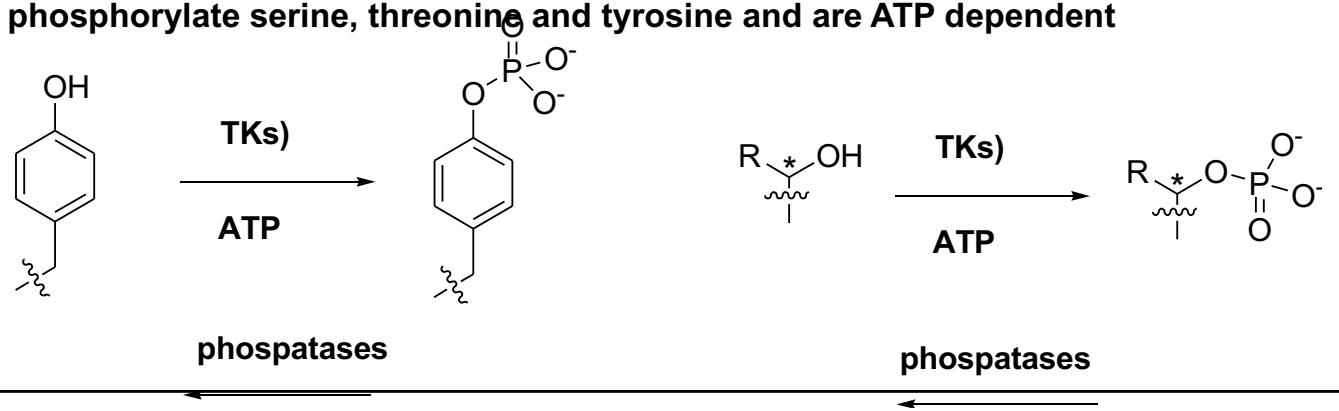
-Serine-threonine kinases (S/TKs)

-Tyrosine kinases (TKs)

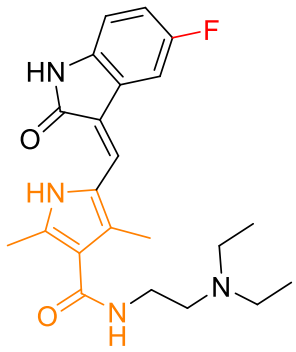
-Dual function kinases (DFKs)

-Roughly 2000 kinases known in the human genome

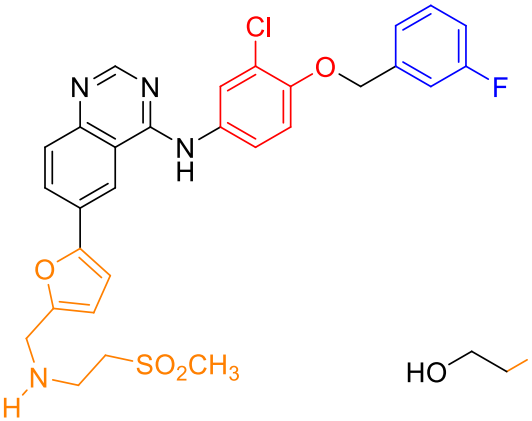
-Kinases phosphorylate serine, threonine and tyrosine and are ATP dependent



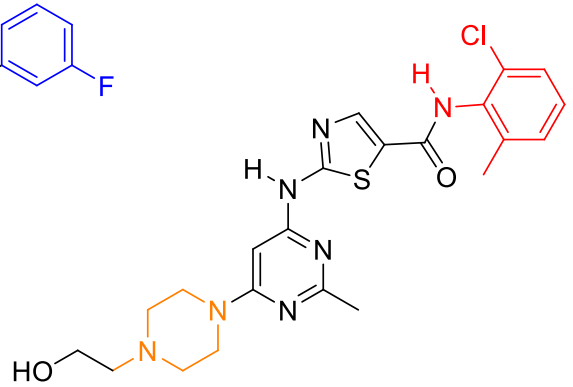
Kinase inhibitors on the market



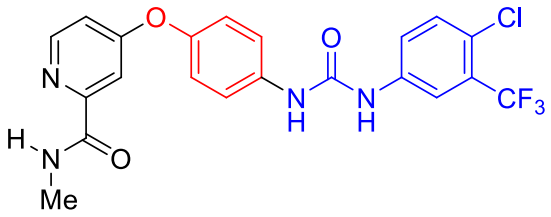
Sutent
KIT; PDGF and VEGR



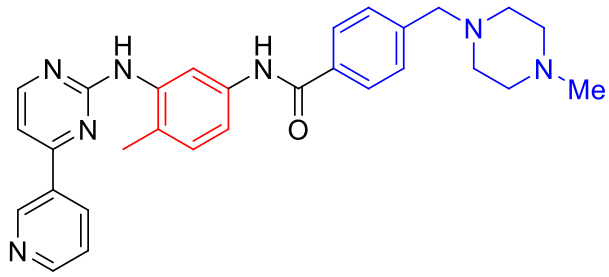
Lapatinib
EGFR; ERBB2



Dasatinib
Src family; ABL1; KIT; PDGFR; Eph



Sorafenib
KIT; PDGFR; B-Raf; VEGFR2



Imatinib (Gleevec)
Bcr-Abl; KIT; PDGFR;

GPCR's: introduction

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

G-protein: guanin nucleotide-binding protein

-240 receptors with known ligands from which only ~30 are currently investigated by pharma 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

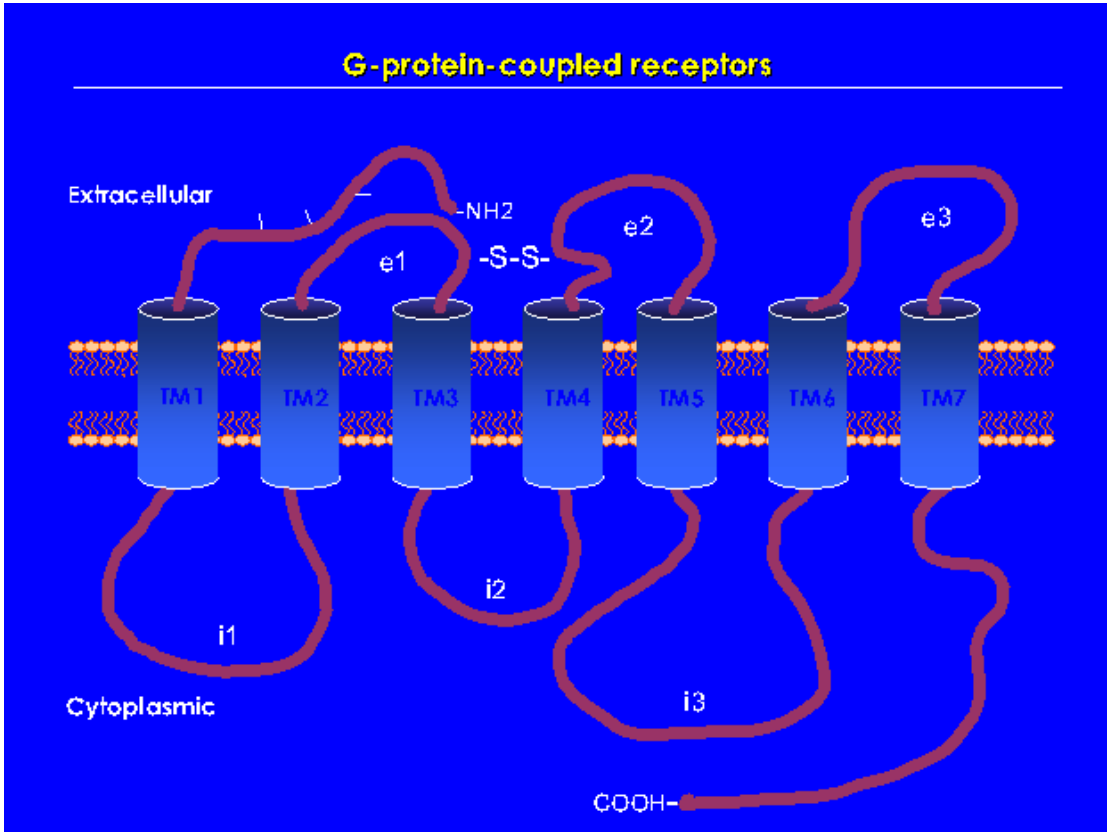
Family 2: glucagon receptor-like or secretin receptor-like GPCR's

Family 3: metabotropic glutamate receptors

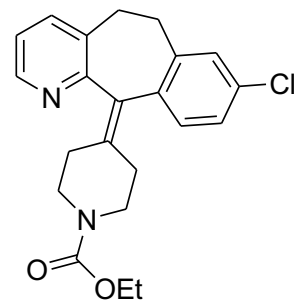
Drug design strategies for targeting G-protein-coupled-receptors: Th. Klabunde, G. Hessler, *ChemBioChem* **2002**,*3*, 928-44.

3D-structure of bovine rhodopsin: *Science*, **2000**, *289*, 739-45; *Biochemistry,* **2001**, *40*, 7761-72.

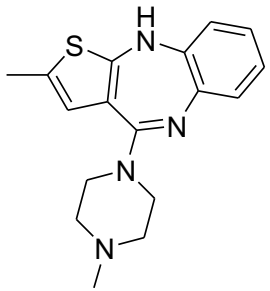
GPCR's: introduction



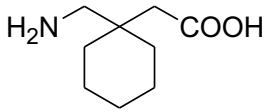
GPCR's: some best-selling drugs



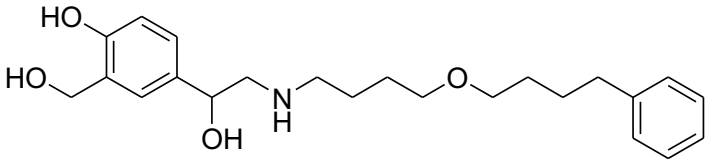
Claritin (Schering-Plough, H_1 antagonist allergies, 3.1billion USD, 2001)



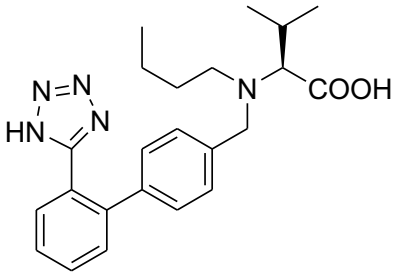
Zyprexa (Ely Lilly, D_2/D_1/5-HT_2 allergies, 2.35 billion USD, 2001)



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



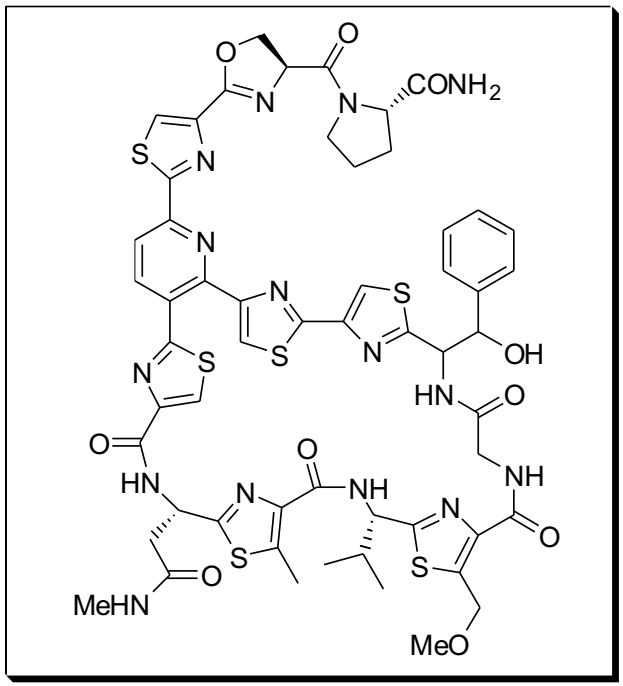
Serevent (Glaxo, β_1 agonist asthma, 0.91 billion USD, 2001)



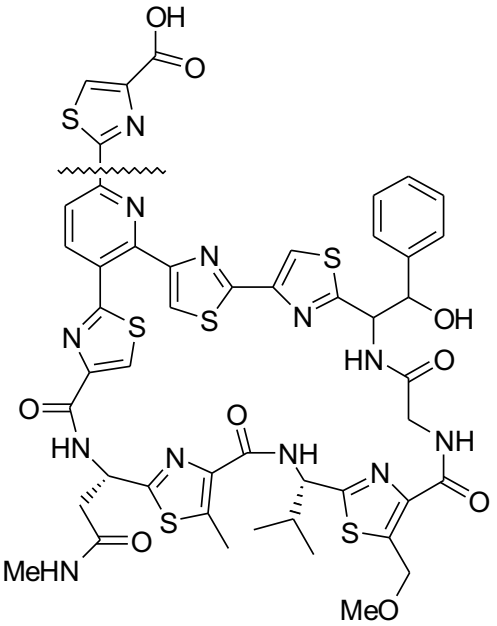
Diovan (Novartis, AT1 antaginist hypertension, 0.8 billion USD, 2001)

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

Parallel synthesis starting from a natural product-derived building block



GE2270 A

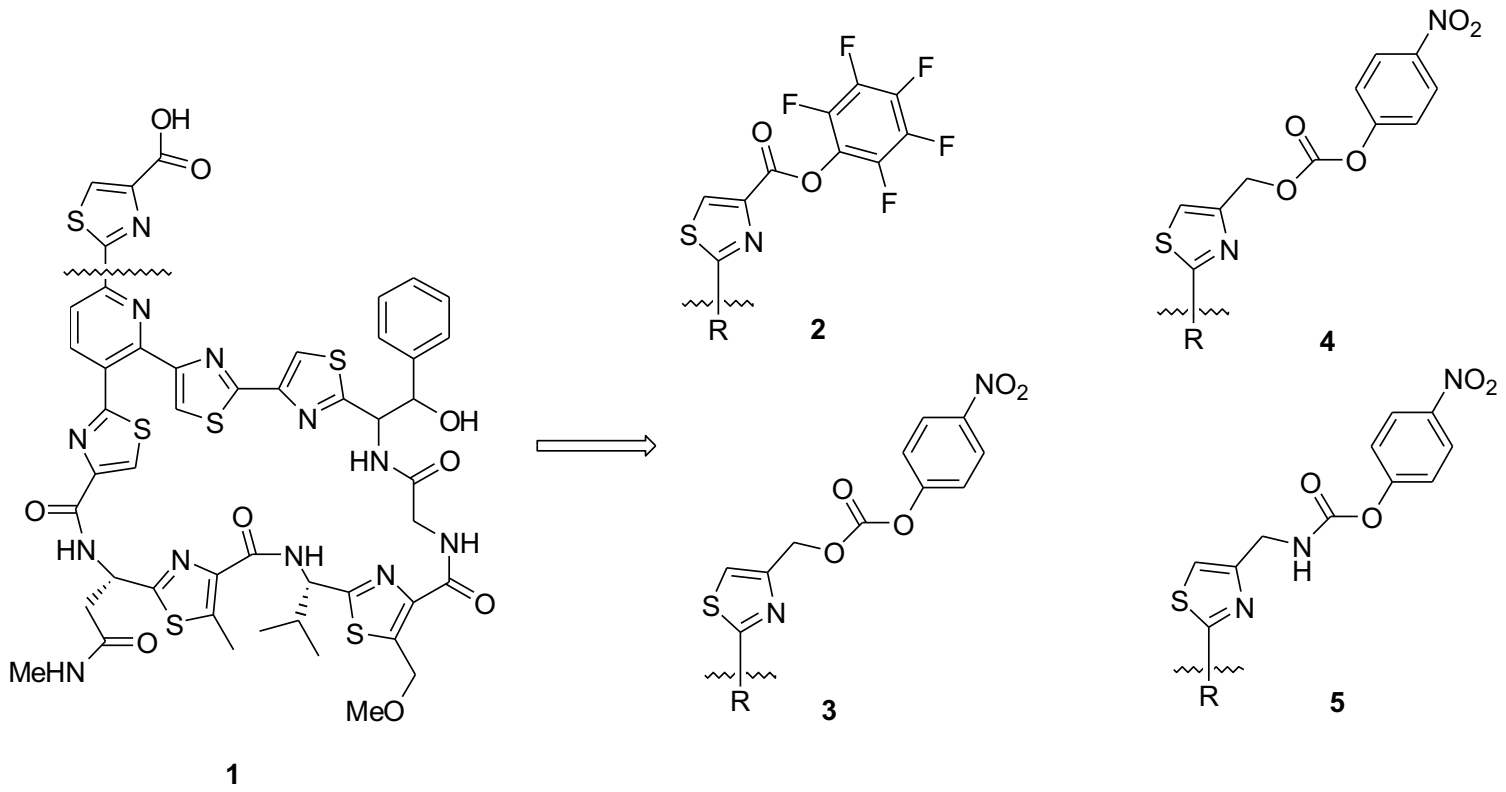


1
inhibitor of elongation factor EF-TU

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

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

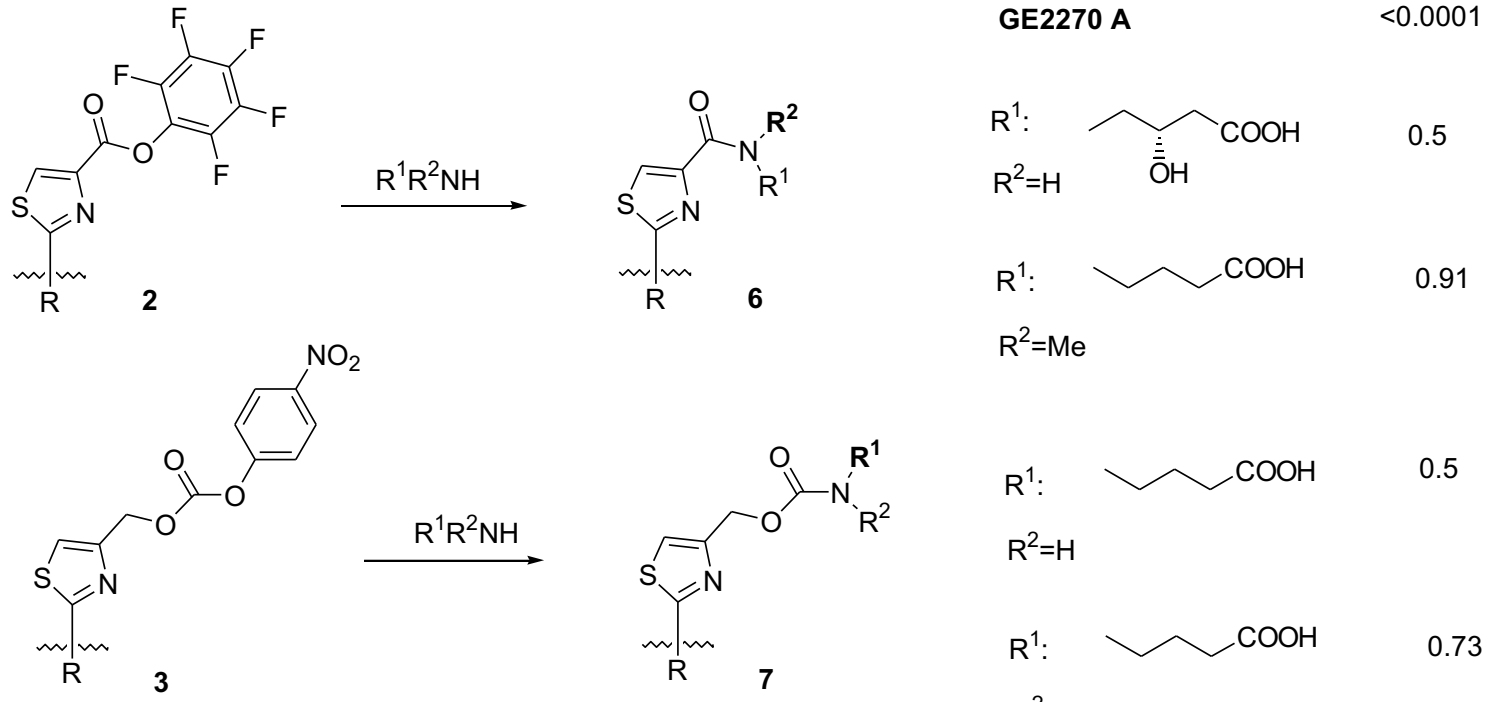
Parallel synthesis starting from a natural product-derived building block



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

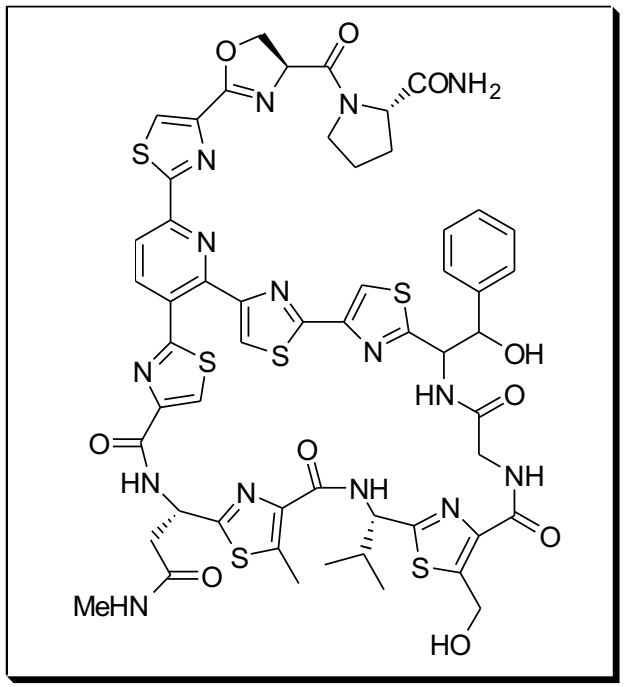


	Solubility (mg/ml)
GE2270 A	<0.0001
R^1: R^2=H	0.5
R^1: R^2=Me	0.91
R^1: R^2=H	0.5
R^1: R^2=Me	0.73

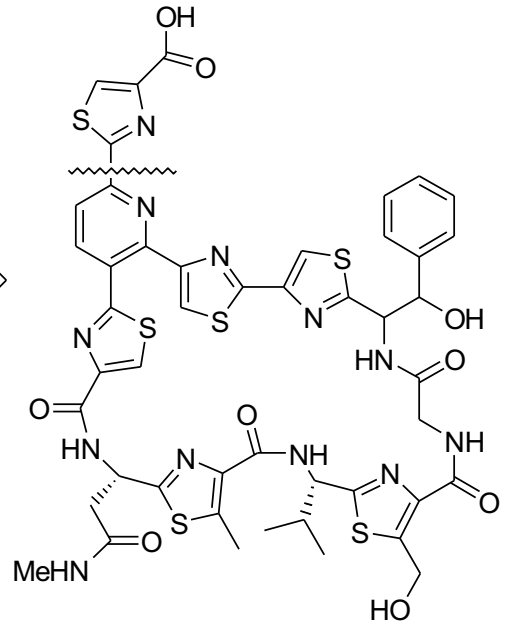
J. W. Jacobs et al. (Versicor), *40th annual ICAAC conference*, Toronto, Canada, september 17-20th, **2000**, Poster 2193 and 2194

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

Parallel synthesis starting from a natural product-derived building block



GE2270 D2

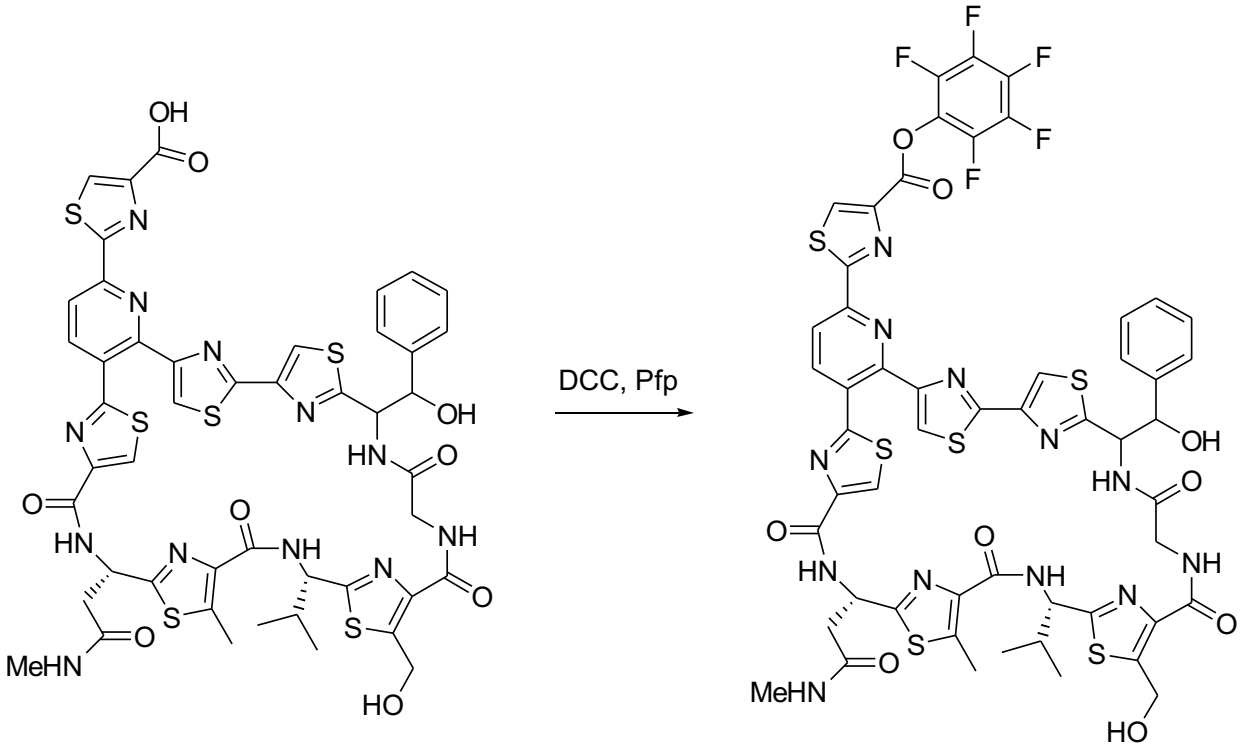


8

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

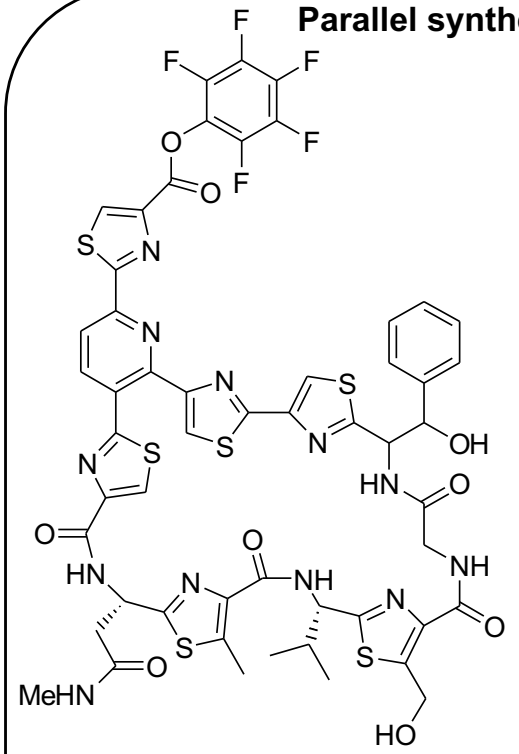


8 **9**

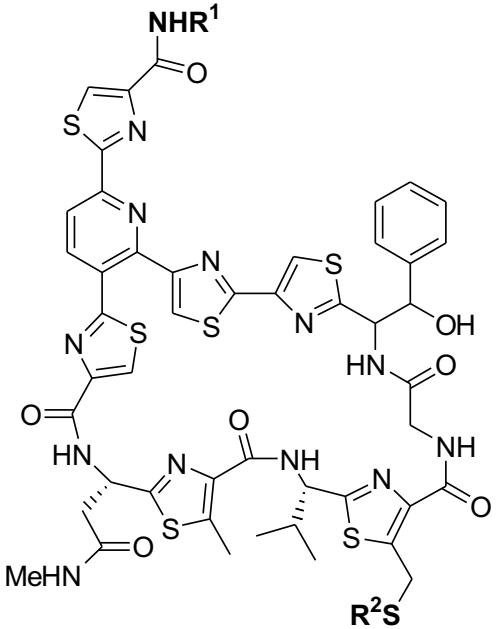
J. W. Jacobs et al. (Versicor), *40th annual ICAAC conference*, Toronto, Canada, september 17-20th, **2000**, Poster 2193 and 2194

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

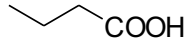
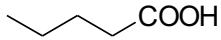
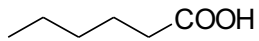
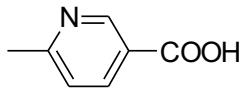
Parallel synthesis starting from a natural product-derived building block



9



10

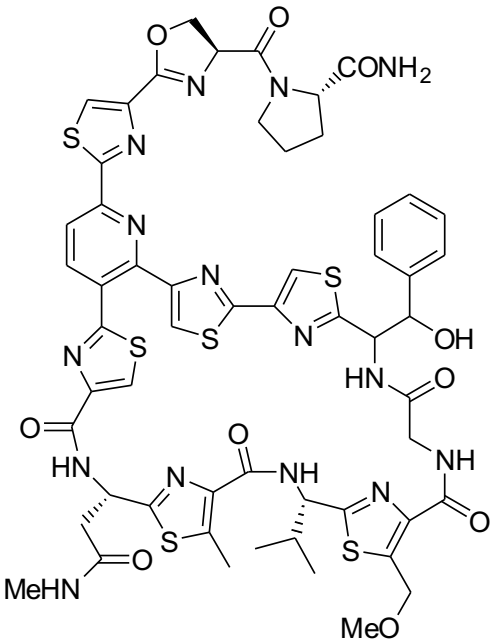
	Solubility (mg/ml)
GE2270 A	<0.0001
R^1:  R^2= SCH_3	0.44
R^1:  R^2= SCH_3	0.41
R^1:  R^2= 	>2.0

i:R^1NH_2, DMF, DIEA ; ii: H_2O; then precipitation + wash; iii: Ts_2O; CH_2Cl_2; DIEA ; iv: R2SH, DMF/aq. K_2CO_3; then precipitation + wash; v: TFA/CH_2Cl_2 (1:1); Et_2O; then precipitation + wash; then dry

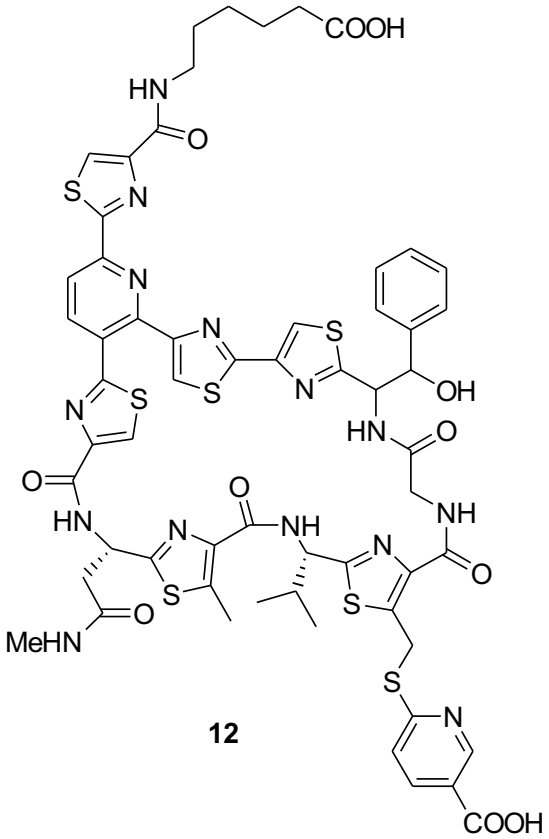
J. W. Jacobs et al. (Versicor), *40th annual ICAAC conference*, Toronto, Canada, september 17-20th, **2000**, Poster 2193 and 2194

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

Parallel synthesis starting from a natural product-derived building block



GE2270 A



12

	Solubility (mg/ml)	MIC(MRSA) (μg/ml)
GE2270 A	<0.0001	0.125
12	>2.0	0.5

J. W. Jacobs et al. (Versicor), *40th annual ICAAC conference*, Toronto, Canada, september 17-20th, **2000**, Poster 2193 and 2194

7. Appendix:

-Additional slides
-Useful definitions
-Reviews; Literature

Clinical Trials Phases

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

Clinical Trials Phases...

Phases	Goals	Subjects	Duration
Phase III	• Effectiveness (therapeutic confirmatory) • Monitor Side-effects • Compare to Commonly Used Treatments • Collect information that will allow the drug or treatment to be used safely • Only about 25 % of the experimental drug pass Phase III Trial	1000 – 5000	1-5 years years
Phase IV	• Post – Marketing (therapeutic use) • Effectiveness in General Population • Optimizing Drug Use	Patient population Sample	Ongoing Process

3. Combinatorial and Parallel Synthesis in Medicinal Chemistry

Historical background-objective

1963: Seminal paper by *R. B. Merryfield* describing for the first time the successful synthesis of a short peptide on a polystyrene resin (*J. Am. Chem. Soc*. **1963**, *85*, 2149)

1965: *Letsinger* and *Khorana* applied solid supports for the synthesis of oligonucleotides (*J. Am. Chem. Soc*. **1965**, *87*, 2149); *J. Am. Chem. Soc*. **1966**, *88*, 3181)

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

1967: Wilkinson et al. Described polymer-bound tris-(triphenylphoshine)chlororhodium as a hydrogenation catalyst (*J. Am. Chem. Soc*. **1967**, *89*, 1574)

1969: Solid-phase synthesis of Ribonuclease (*J. Am. Chem. Soc*. **1969**, *91*, 501)

1970: *H. Rapoport* introduced the term *hyperentropic efficacy* (effect of high dilution) on solid supports (*J. Am. Chem. Soc*. **1970**, *92*, 6363)

1971: *Fréchet et al*. pioneerd solid-phase synthesis in the field of carbohydrate research (*J. Am. Chem. Soc*. **1971**, *93*, 492)

1973: Application of intramolecular *Dieckmann*-condensation for the solid-phase synthesis of lactones by *Rapoport* et al. (*J. Macromol. Sci. Chem*. **1973**, 1117)

3. Combinatorial and Parallel Synthesis in Medicinal Chemistry

Historical background-objective

1973: *Leznoff et al*. described the use of polymer-supports for the mono-protection of symmetrical dialdehydes, oxime-formation, *Wittig* reaction, crossed aldol formation, benzoin-condensation and *Grignard* reaction (*Can. J. Chem.* **1973***, 51,* 3756*)*

1974: *F. Camps* describes the first synthesis of benzodiazepines on solid support (*Ann. Chim*. **1974**, *70*, 1117)

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 snthesis: 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*)*

1976-1978: *Leznoff et al*. published a series of papers dealind with the synthesis of insect sex attractants (*Can. J. Chem.*. **1977**, *55*, 1143)

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)

3. Combinatorial and Parallel Synthesis in Medicinal Chemistry

Historical background-objective

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

1974: *F. Camps* describes the first synthesis of benzodiazepines on solid support (*Ann. Chim*. **1974**, *70*, 1117)

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

1985: *Houghten et al.* described the tea-bag method for 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 couplind rates (*Mol. Immunol*. **1986**, *23*, 709)

1991: *Fodor et al.* described the VLSIPS method (very large scale immobilised polymer synthesis; photolitographic parallel synthesis (*Science* **1991**, *251*, 767)

Historical background-objective

1991: Almost simultaneously *Furka et al.* described the `portioning-mixing' method (*Int. J. Pept. Prot. Res*. **1991**, *37*, 487); *Hruby et al.* the `split synthesis' (*Nature* **1991**, *354*, 82); and *Houghten et al*. the `divide, couple and recombine`process (*Nature* **1991**, *354*, 84)

1992: Oligonucleotide-encoded chemical synthesis by *Lerner* and *Brenner* (*Proc. Natl. Acad. Sci*. *USA*, **1992**, *89*, 5181)

1992: Synthesis od 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: Binary encoded synthesis using gas chromatographically detectable chemically inert tags by *W. C. Still et al.* (*Proc. Natl. Acad. Sci*. *USA*, **1992**, *89*, 5181)

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)

1994: Use of the `safety-catch` linker principle developed by *Kenner et al.* (*J. Chem. Soc. Chem. Commun*. **1973**, 636) by *J. A. Ellman* for multidiretional cleavage from the resin (*J. Am. Chem. Soc*. **1994**, *116*, 11171)

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

Historical background-objective

1995: Use of a genetic algorythm for the selection of the products of an *Ugi* four component reaction (*Angew. Chem. Int. Ed. Engl*. **1995**, *34*, 2280)

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

1997: Combination of a cyclo-condensation reaction, multicomponent diversification and multidirectional resin cleavage using a novel `safety-catch`- and traceless linker yielding highly diverse pyrimidines by *D. Obrecht et al.* (*Chimia* **1996**, *11*, 530; *Helv. Chim. Acta* **1997**, *80*, 65) and *L. M. Gayo et al.* (*Tetrahedron Lett*. **1997**, *38*, 211)

1997: Synthesis of a taxoid library using radiofrequency-encoding (*J. Org. Chem.* **1997**, *62*, 6092)

2001: *Click Chemistry*: Diverse Chemical Function from a few good reactions: H. C. Kolb, K. B. Sharpless, *Angew. Chem. Int. Ed*. **2001**, *40*, 2004-21; ibid *Drug Discovery Today* **2003**, *8*, 1128-37.

2001: *Dynamic Combinatorial Chemistry*: J. M. Lehn et al. *Science* **2001**, *291*, 2331-32.

2001: *Using an enzyme‘s active site to template inhibitors*: R. Nguyen, I. Huc, *Angew. Chem. Int. Ed*. **2001**, *40*, 1774

2005: *Receptor-assisted Combinatorial Chemistry: Thermodynamics and Kinetics in Drug Discovery:* J. D. Cheeseman et al. *Chem. Eur. J.* **2005**, *11*, 1708-16

2006: *In situ* click chemistry: a powerful means for lead discovery: B. K. Sharpless et al. *Expert Opin. Drug Discov*. **2006**, *1*(6), 525-38

Historical background-objective

2004: **Fragment-based drug discovery**: D. A. Erlanson, R. S. McDowell, T. O'Brien, J. Med. Chem. **2004**, *47*, 3463-3482; D. C. Rees, M. Congreve, R. Carr, Nat. Rev. Drug Discov. **2004**, *3*, 660-672

2008: **„Build-couple-pair"** strategy as a basis for diversity-oriented synthesis (DOS): D. Morton et al. Angew. *Chem. Int. Edn* **2008**, *48*, 104-109

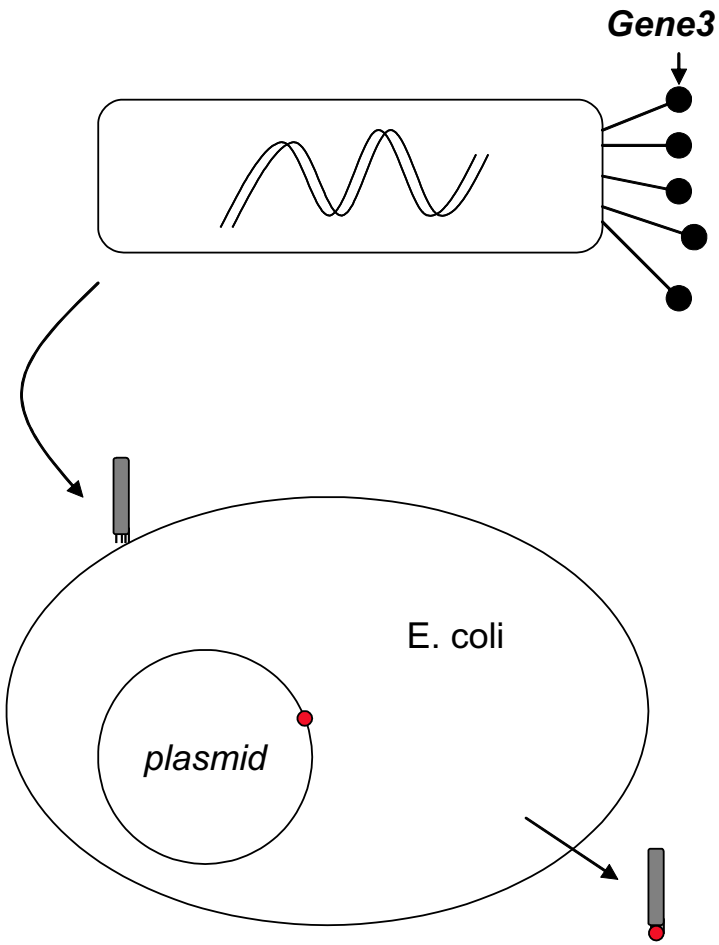
2009: Diversity-oriented Synthesis (**DOS**): S. Schreiber, *Nature* **2009**, *457*, 153-154

2011: Collective synthesis of natural products: S. Jones et al. *Nature* **2011**, *475*, 183-188

2011: High-throughput discovery of new chemical reactions: D. W. Robbins et al. *Science* **2011**, *333*, 1423-1427

2011: A radical approach to disversity: D. A. Nagib et al. *Nature* **2011**, *480*, 224-227

Examples for libraries synthesized on solid-phase: peptides: phage display



The native phage contains a DNA genome surrounded by a protein coat. At one end of the phage are 5 copies of the ***Gene3*** gene product expressed from the phage genome.

The phage infects a host bacterial cell (e.g. *E. Coli*) and uses the bacterium to replicate itself, leading to secretion of progeny phage.

In phage display, the E. Coli host contains a DNA plasmid encoding *Gene3* fused to either a protein of interest, or a library of random peptides. As the phage replicates, Gene3 fusion proteins (expressed from the plasmid) are incorporated into the phage coat. Libraries of phages can be produced, with each bacterium producing phages with a unique peptide displayed at its surface determined by the plasmid (the phage also contains the at this point) of the host cell.

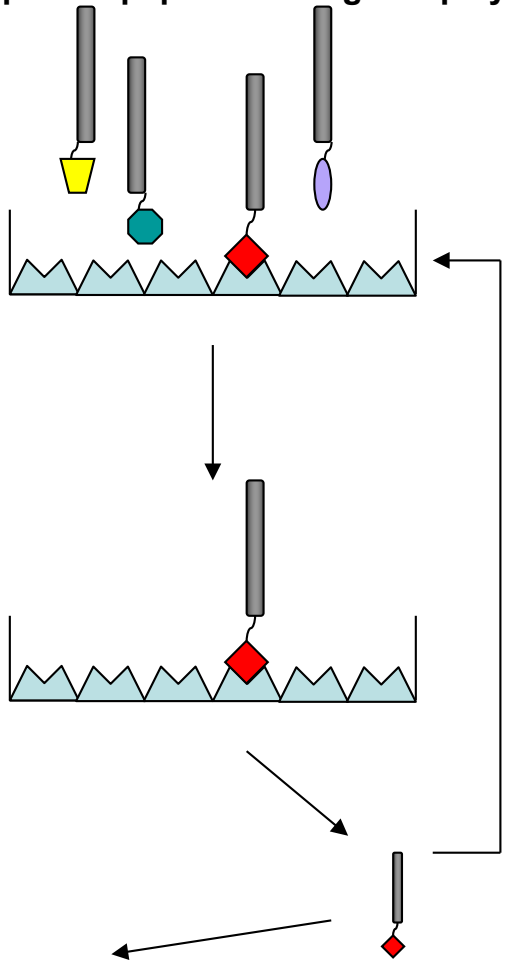
G. P. Smith et al. Meth. Enzymol. 1993, 217, 228; J. K. Scott et al. Curr. Opin. Biotechnol. 1994, 5, 4)

Examples for libraries synthesized on solid-phase: peptides: Phage display panning techniques

A library of phages, each displaying a unique peptide sequence, is allowed to bind to a plate coated with the target molecule (e.g. protein).

Unbound phages are washed away.

Specifically bound phages are eluted.



The eluted phages are amplified and panning process is repeated several times.

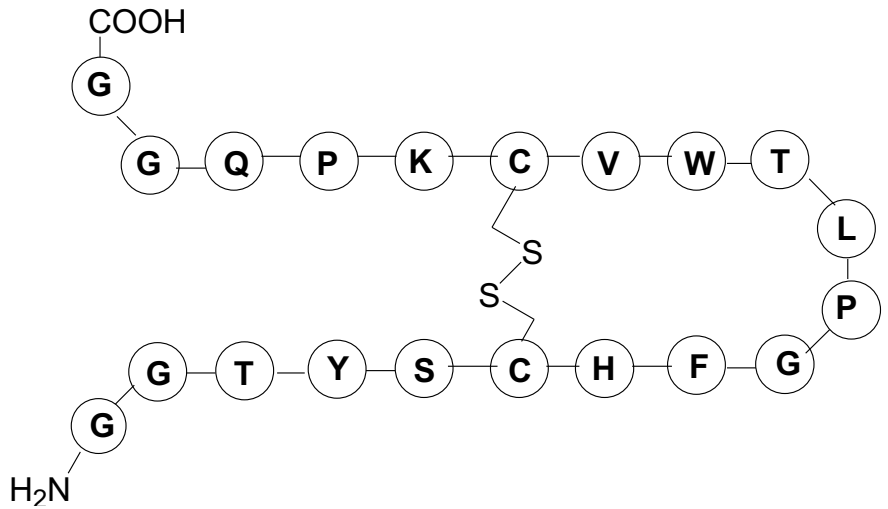
After 3-4 rounds of panning, individual phage clones are isolated and sequenced to determine the sequence of the displayed peptide.

Examples for libraries synthesized on solid-phase: phage display

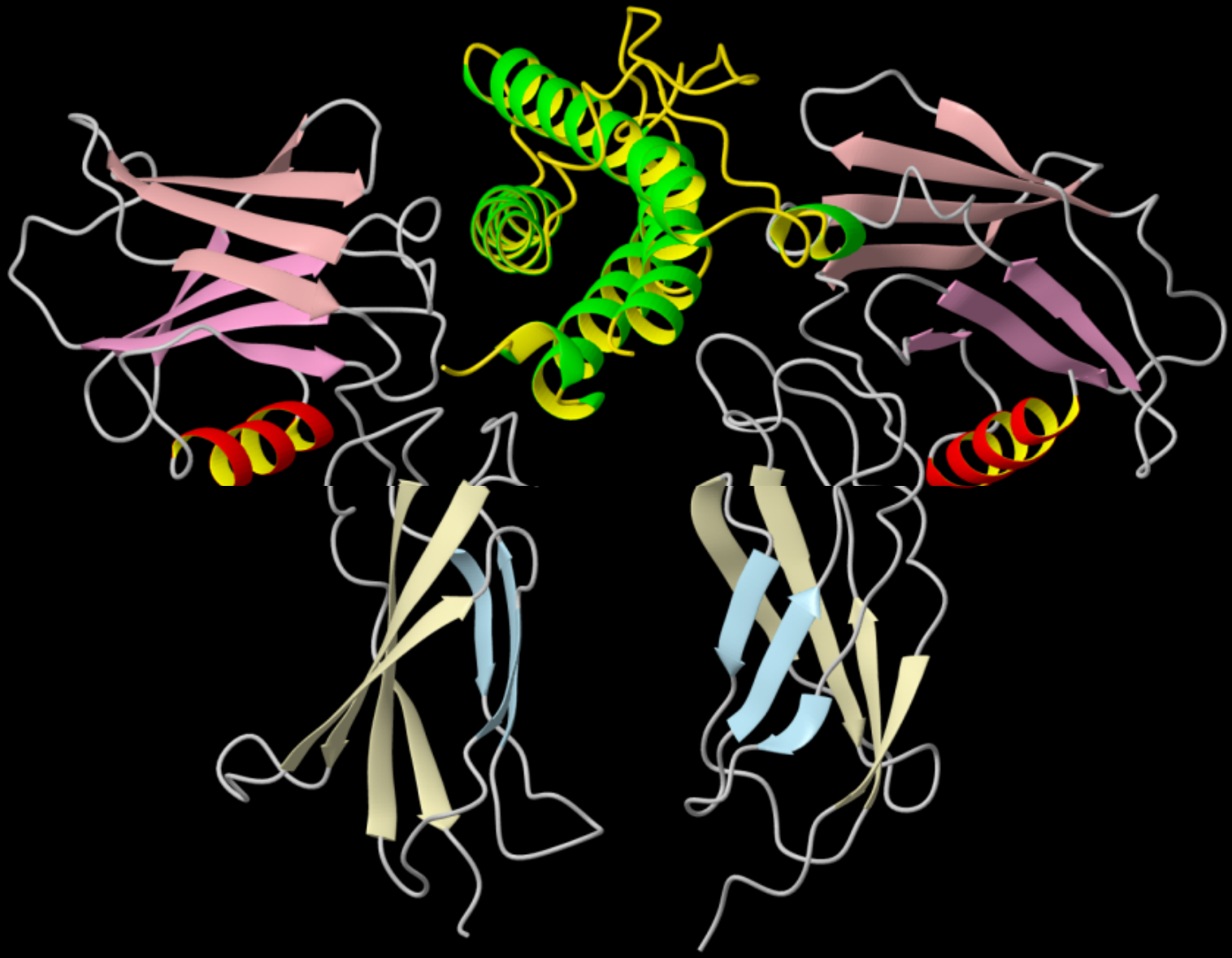
Functional mimicry of a protein hormone by a peptide agonist: **EPO receptor complex**; *Science* **1996**, *273*, 464-471

Erythropoetin (EPO) is the primary hormone that regulates the proliferation and differentiation of immature erythroid cells. Recombinant human EPO is widely used in the treatment of patients with anemia due to renal failure, cancer chemotherapy, and AZT treatment. The *EPO receptor* belongs to the cytokine receptor superfamily, which includes receptors for other hematopoetic growth factors, such as *interleukins* (IL) and *colony-stimulating factors* (CSF), as well as *growth hormone* (GH), *prolactin*, and *ciliary neurotrophic factor* (CNTF).

Screening of a phage libray (*Annu. Rev. Microbiol*. **1993**, *47*, 535) against immobilized EPOR gave an active consens sequence, and a very potent member of the family with agonistic activity *in vitro* and *vivo* was identified (see Figure).



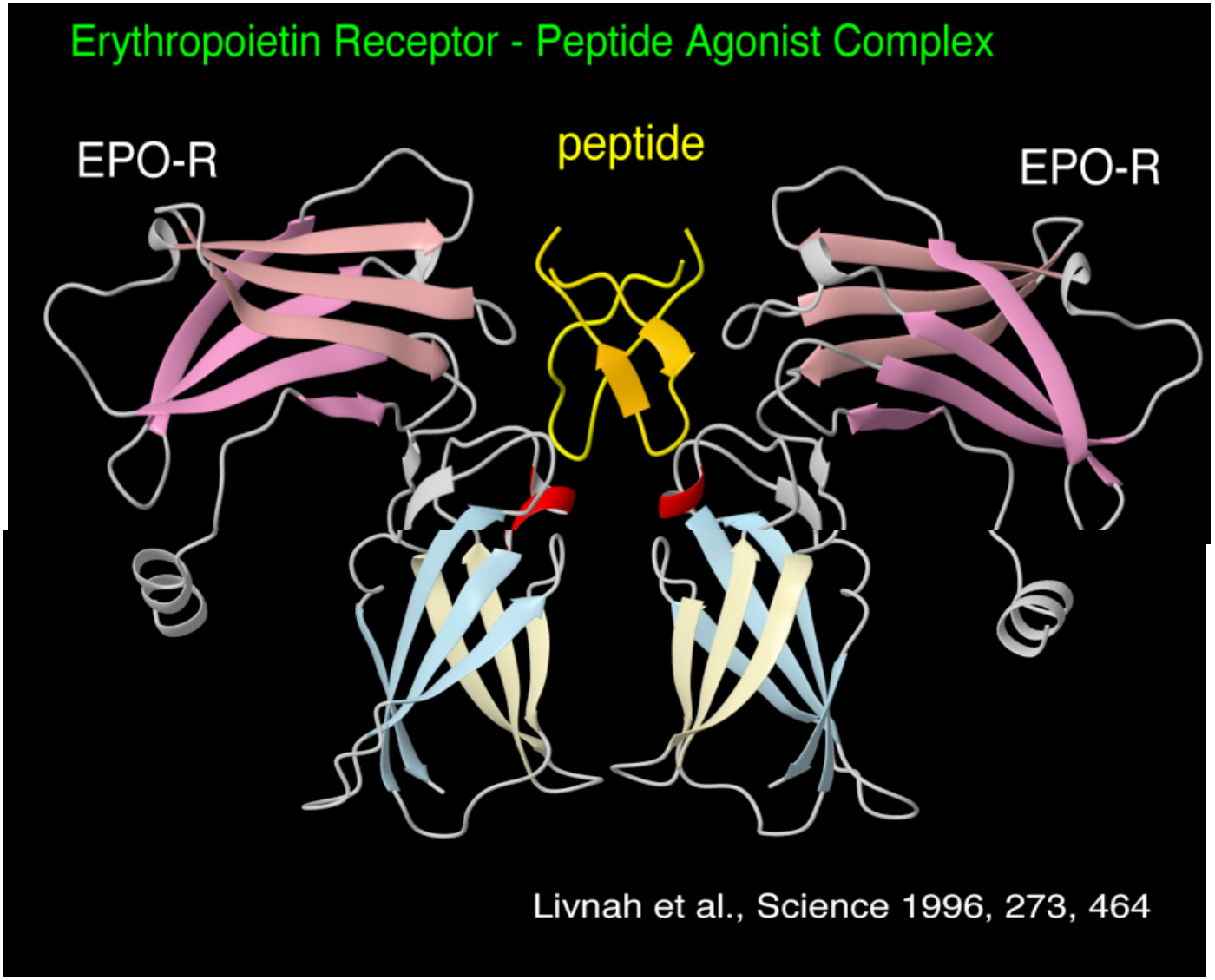
EPO plus receptor



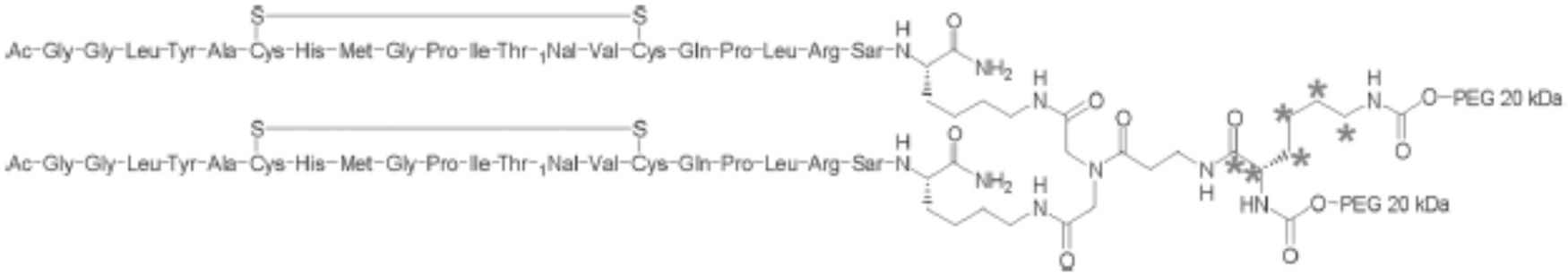
H. Zhan et al. Nature, (1998), 395, 511.

Medicinal Chemistry : Combinatorial Chemistry-Parallel Synthesis

4. Combinatorial Synthesis of Biopolymers



- Covalently linked dimeric analogues of EMP1 were subsequently developed at Affimax as EPO mimetics;
- A pegylated version (peginesatide, hematide) with long half life was selected for clinical development for treatment of patients with chronic kidney disease (CKD)-associated anemia (patients with inadequate production of EPO by the damaged kidney)



Peginesatide (Hematide); Phase III

The development of peginesatide is a most impressive example for a functional mimicry of a protein by a much smaller peptide derivative

K. W. Woodburn et al. *Xenobiotica* **2012**, *42*, 660-670

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

Characteristic of large surface protein-protein interactions

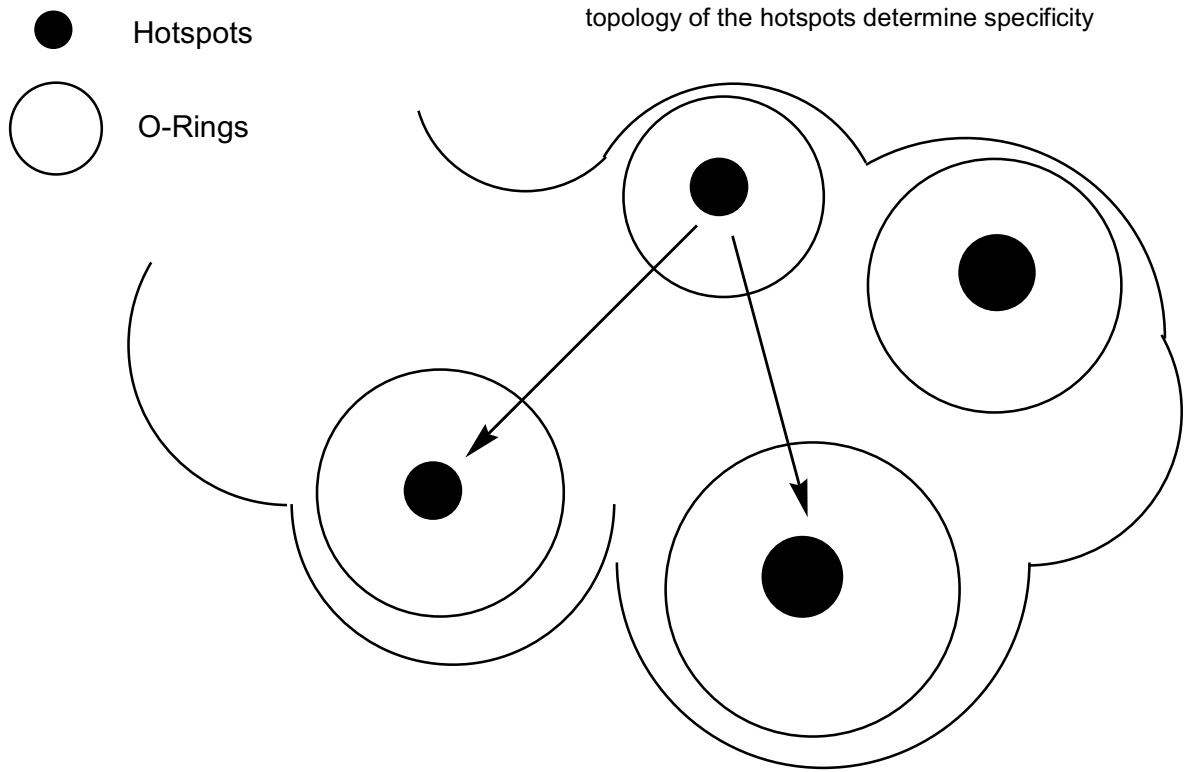
- Fundamental to the functioning of biological systems
  - –many proteins function as part of complexes
  - –cell to cell signalling
  - –cell adhesion
  - –long distance communication (hormones)
- Specific inhibition offers important therapeutic potential:

- Generally form across a large area of interacting surfaces: 700-1300 A^2 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

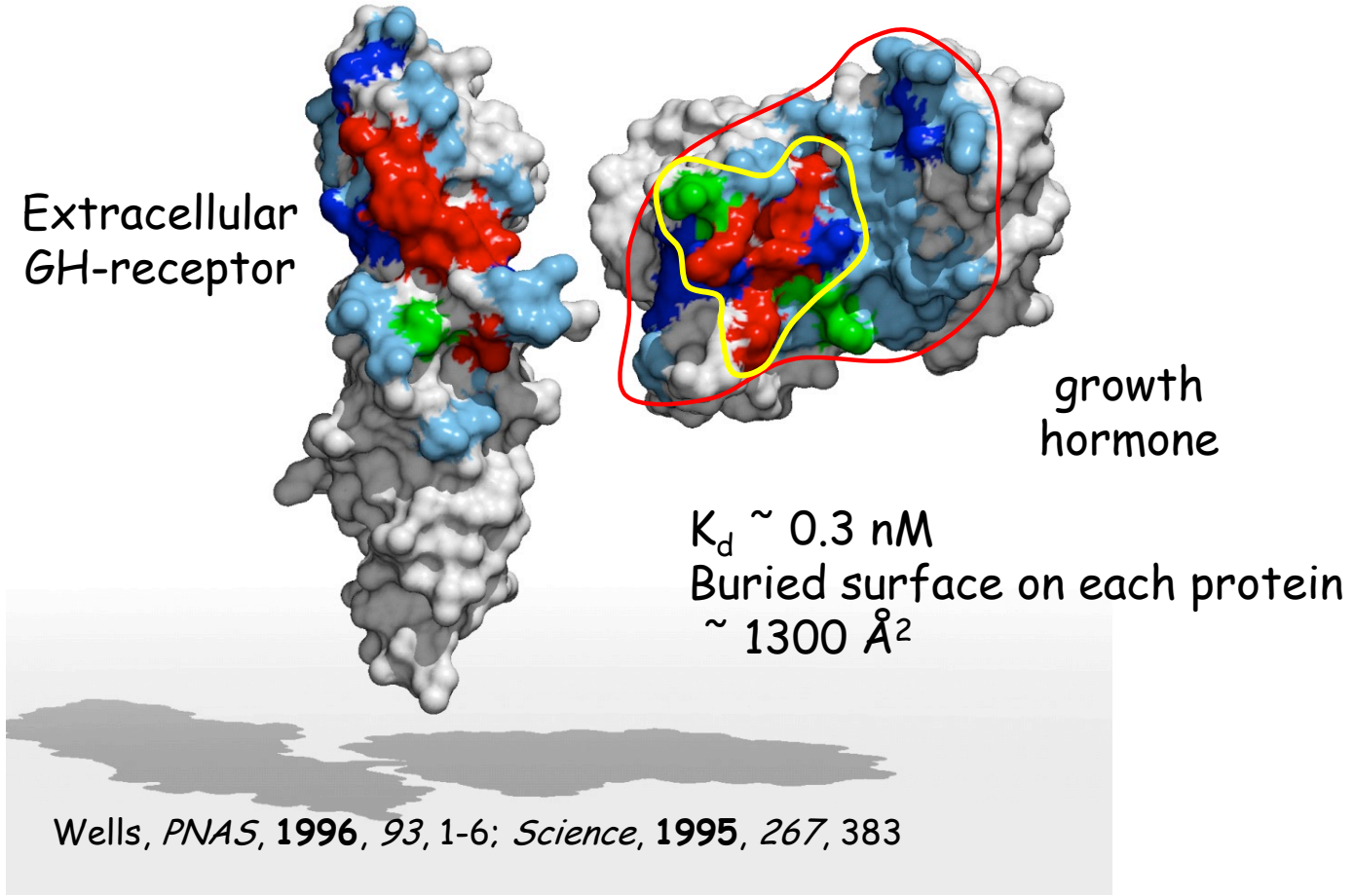
6.5. Examples for libraries synthesized on solid-phase: inhibitors of protein-protein interaction

average contact surface area in protein-protein interactions: 600-900 $Å^2$

Bogan, A. A.; Thorn, K. *J. Mol. Biol.* **1998**, *280*, 1-9



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



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

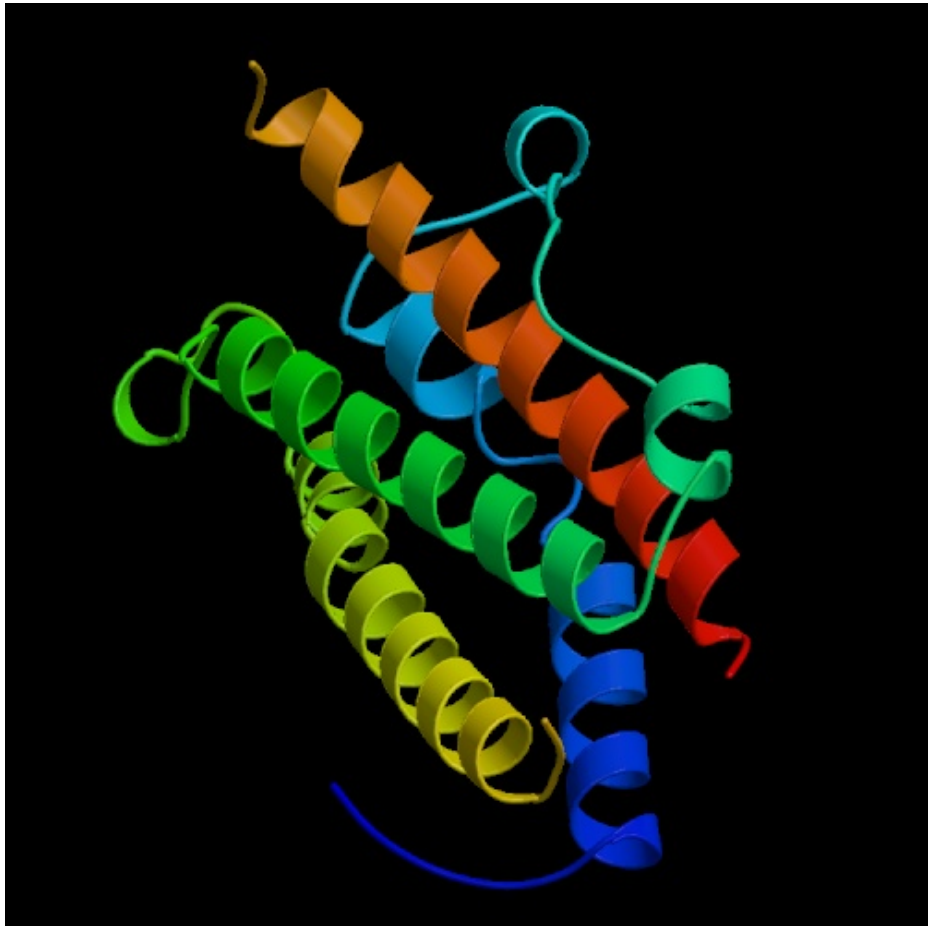
Petidic α-helix mimetics as inhibitors of protein-protein interactions

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

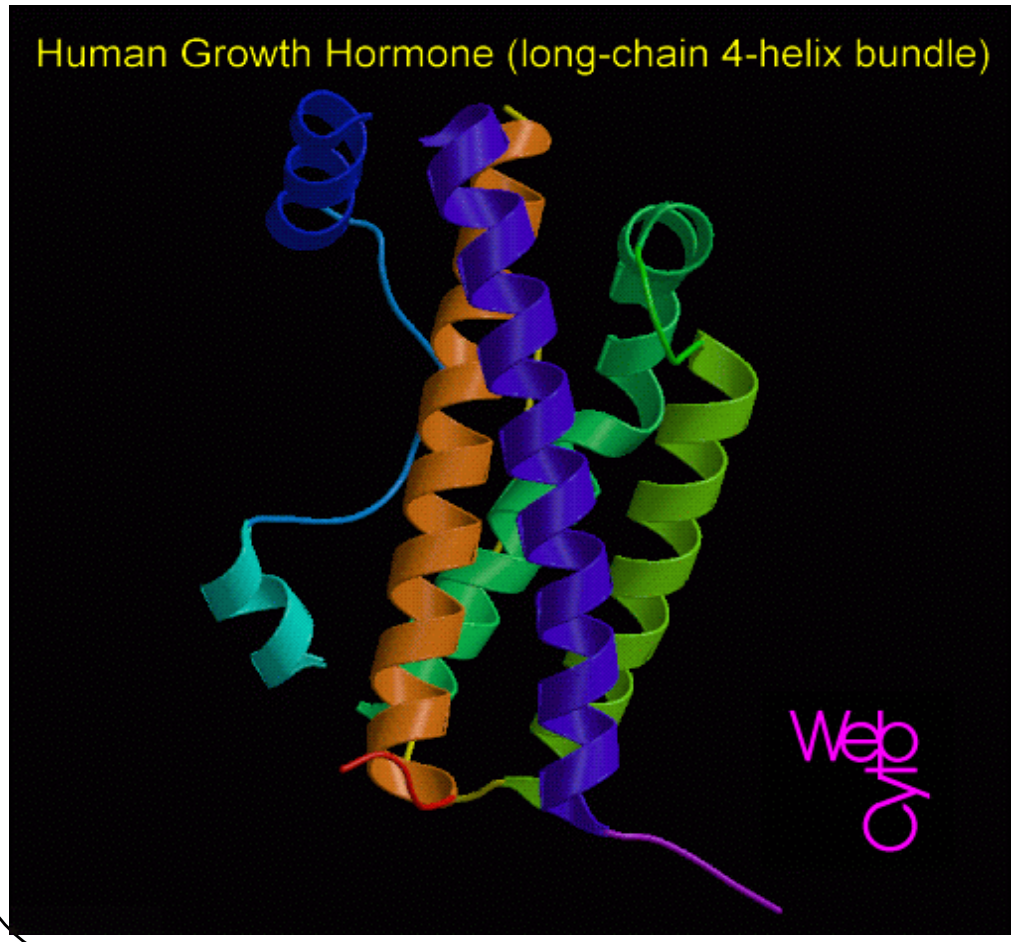
-Part of a large family of important proteins

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

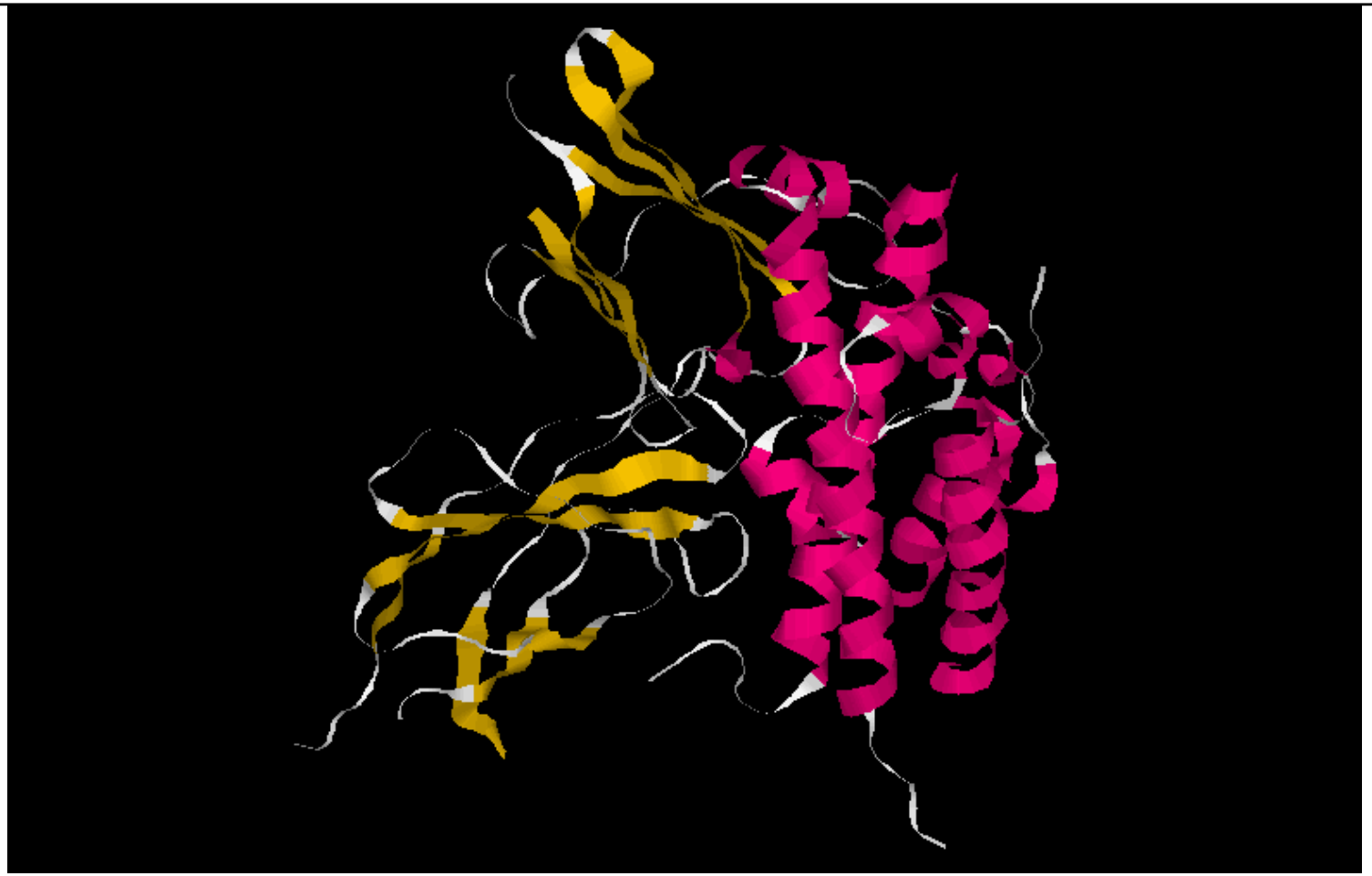
Family

Long Chain 4-helix bundle	Short Chain 4-helix bundle	Dimeric-dimeric 4-helix bundle
Growth Hormone	IL-2	IL-10
Prolactin	IL-4	IFN-G
IL-6	IL-13	IFN-B
IL-3	IFN-a	
IL-7	IL-5	
LIF	GM-CSF	
OSM	M-CSF	
CNTF		
CDF		

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

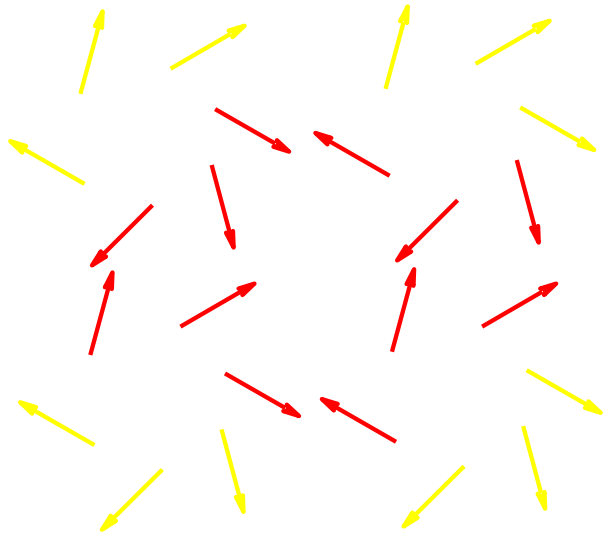


Examples for libraries synthesized on solid-phase: four helix bundle proteins



Note side-on interactions of α-helices

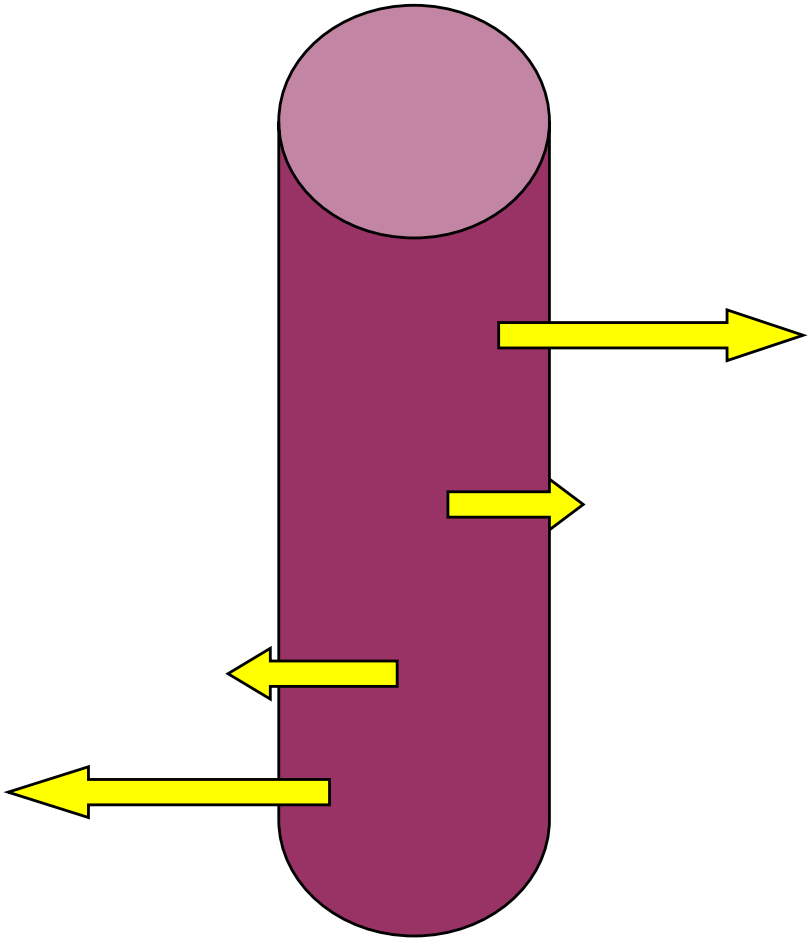
Examples for libraries synthesized on solid-phase: four helix bundle proteins



-α-helices cluster with hydrophobic residues poiting 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 momomers

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” α-helix interactions through 3 side-chains

-Large - 100k compounds

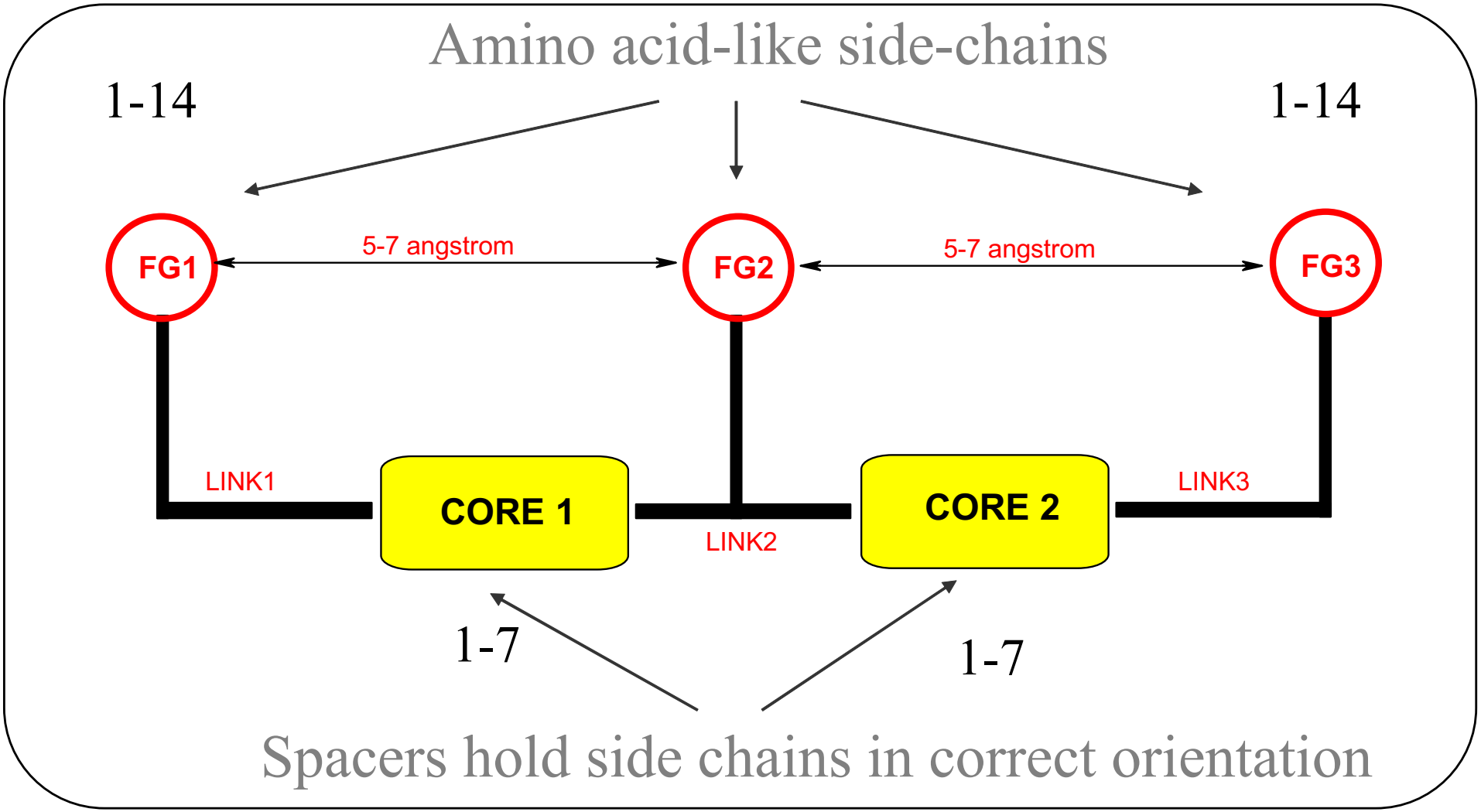
-Non-peptidic

-Split - mix synthesis on solid phase

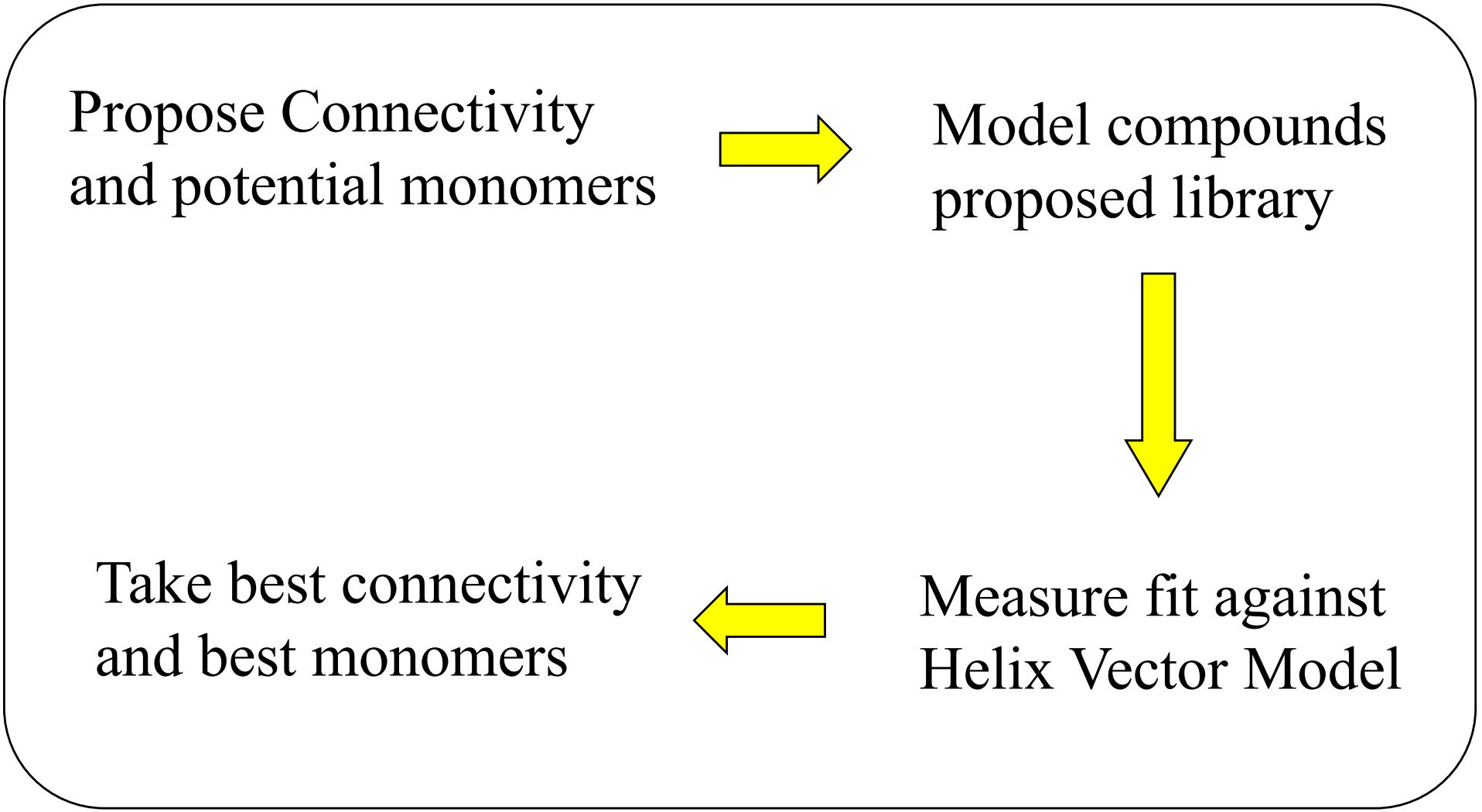
-Fully Encoded / Partial Release Technology

-384 screening format

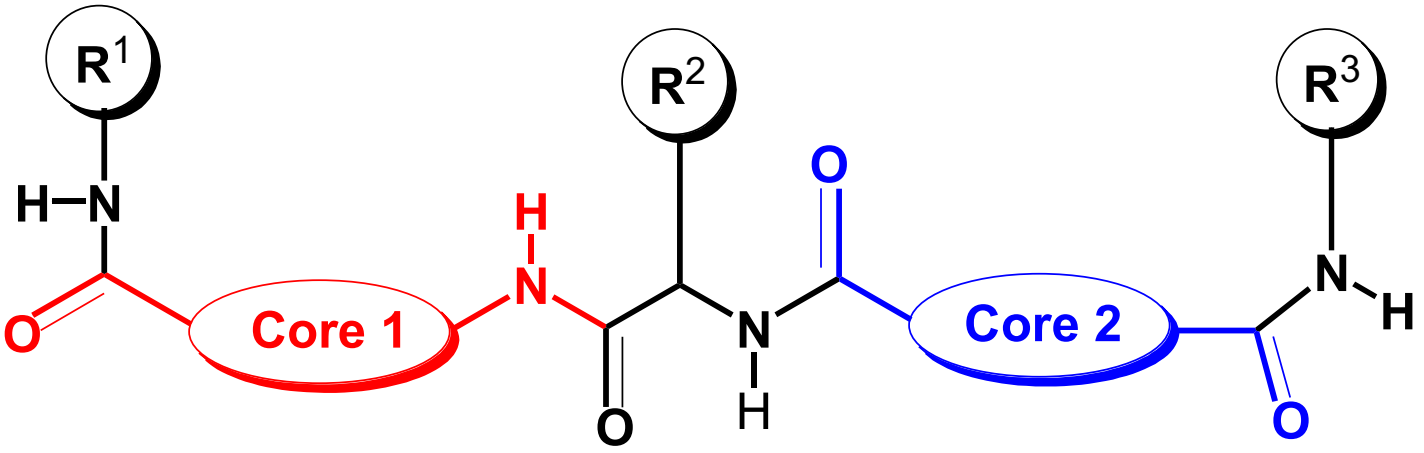
Examples for libraries synthesized on solid-phase: four helix bundle proteins



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



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



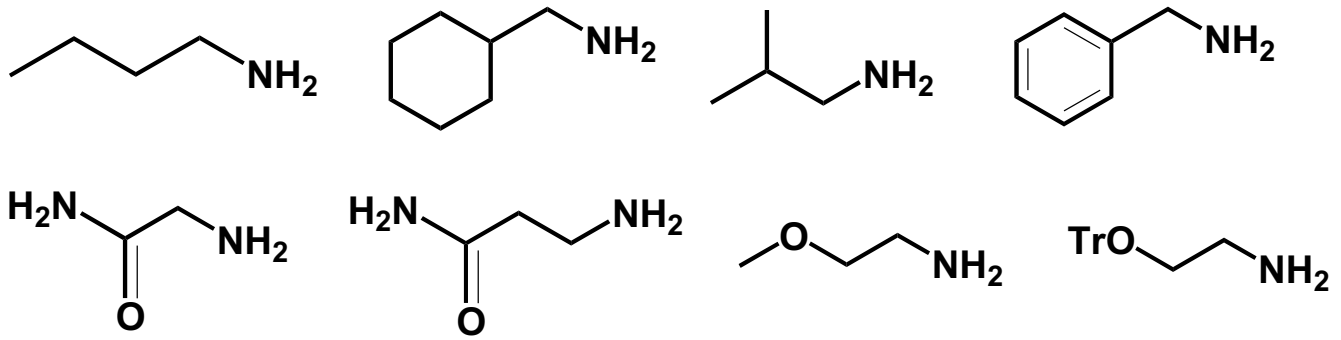
	Amines	Amino acids	α-Amino acids	Amino acids	Amines
	14	7	14	7	14
Tags required	4	3	4	3	3

Total number of compounds: 134'456

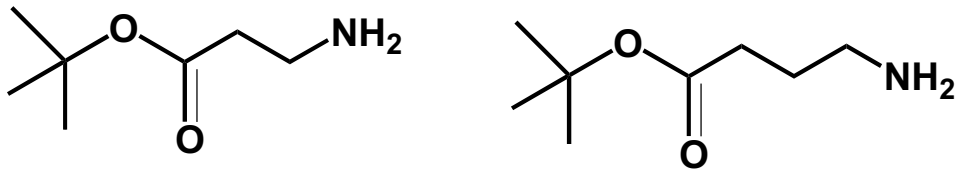
Examples for libraries synthesized on solid-phase: four helix bundle proteins

amines

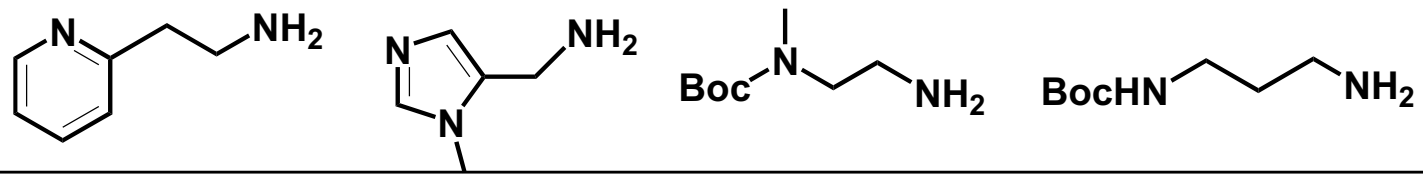
neutral



acidic

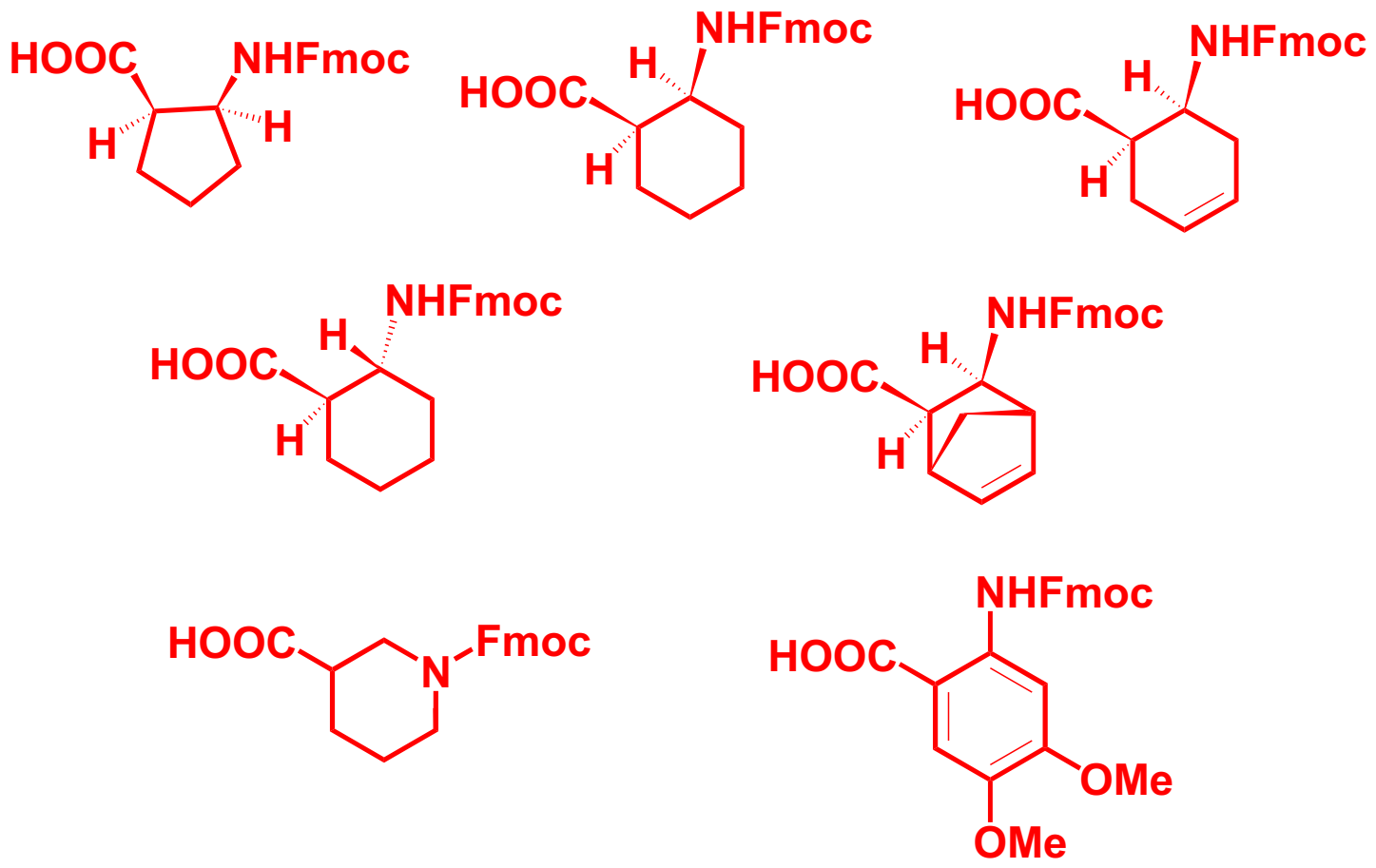


basic

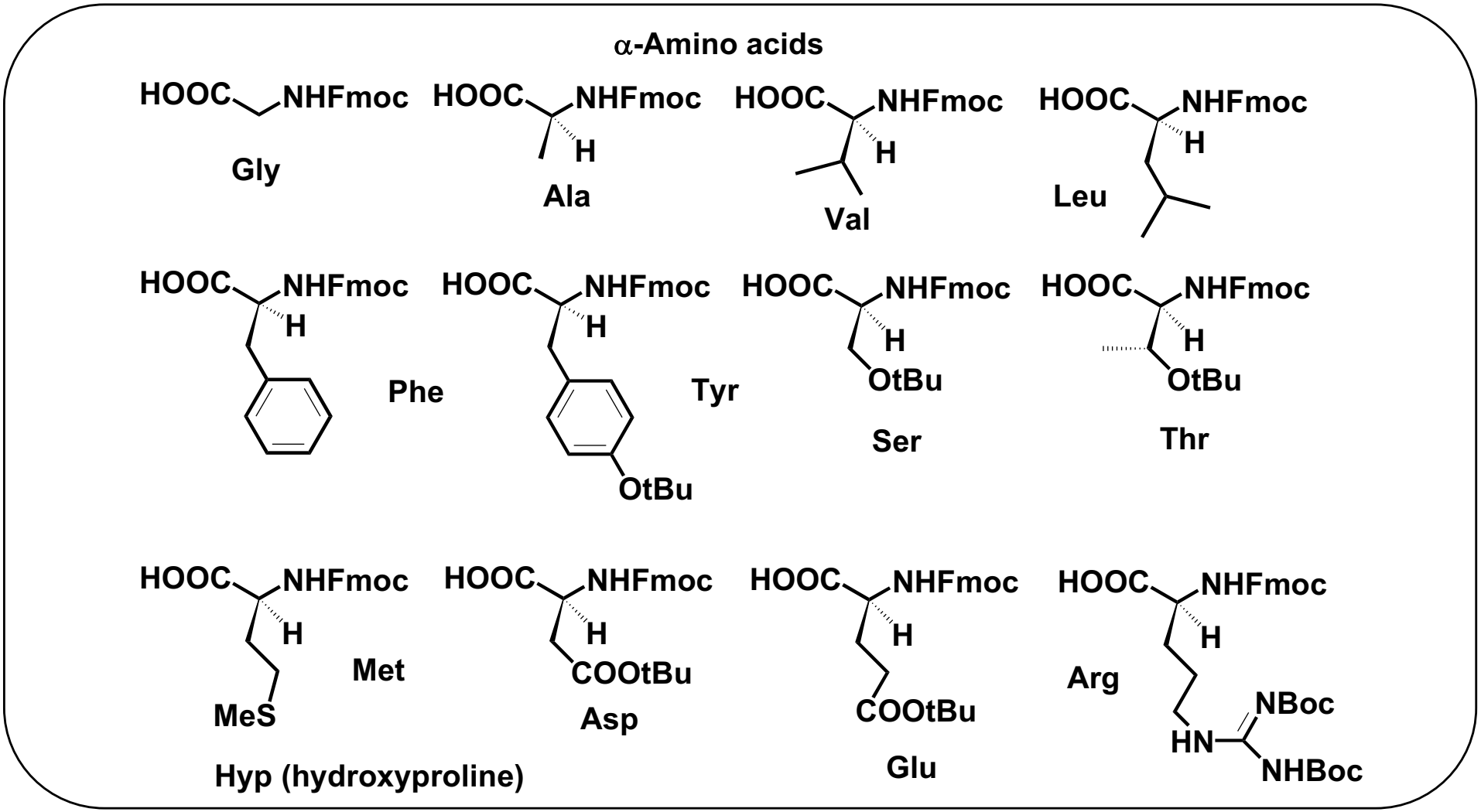


6.5. Examples for libraries synthesized on solid-phase: four helix bundle proteins

Core 1 amino acids

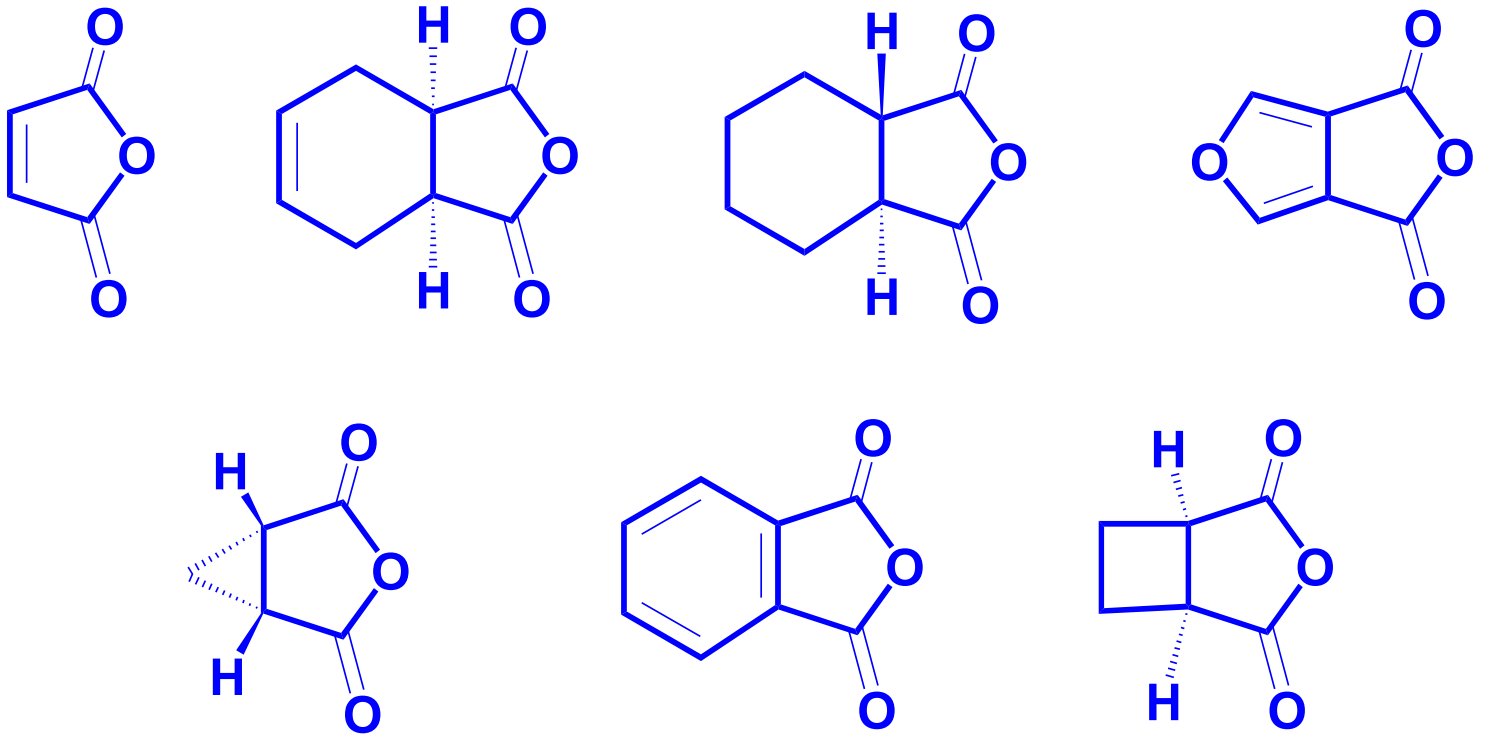


Examples for libraries synthesized on solid-phase: four helix bundle proteins



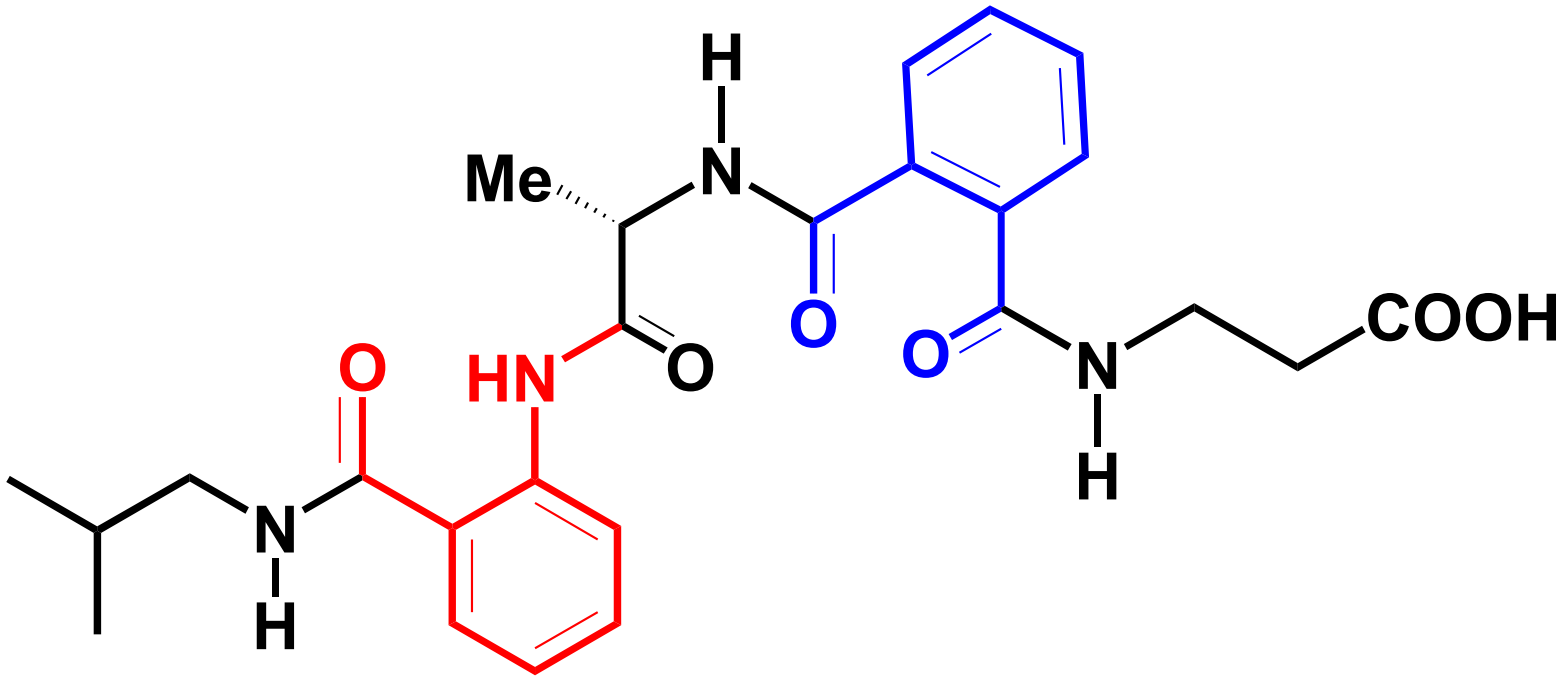
Examples for libraries synthesized on solid-phase: four helix bundle proteins

Core 2: Diacids (Anhydrides)



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

Major conformers closely match α-helix in side-chain display vectors



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

Concepts

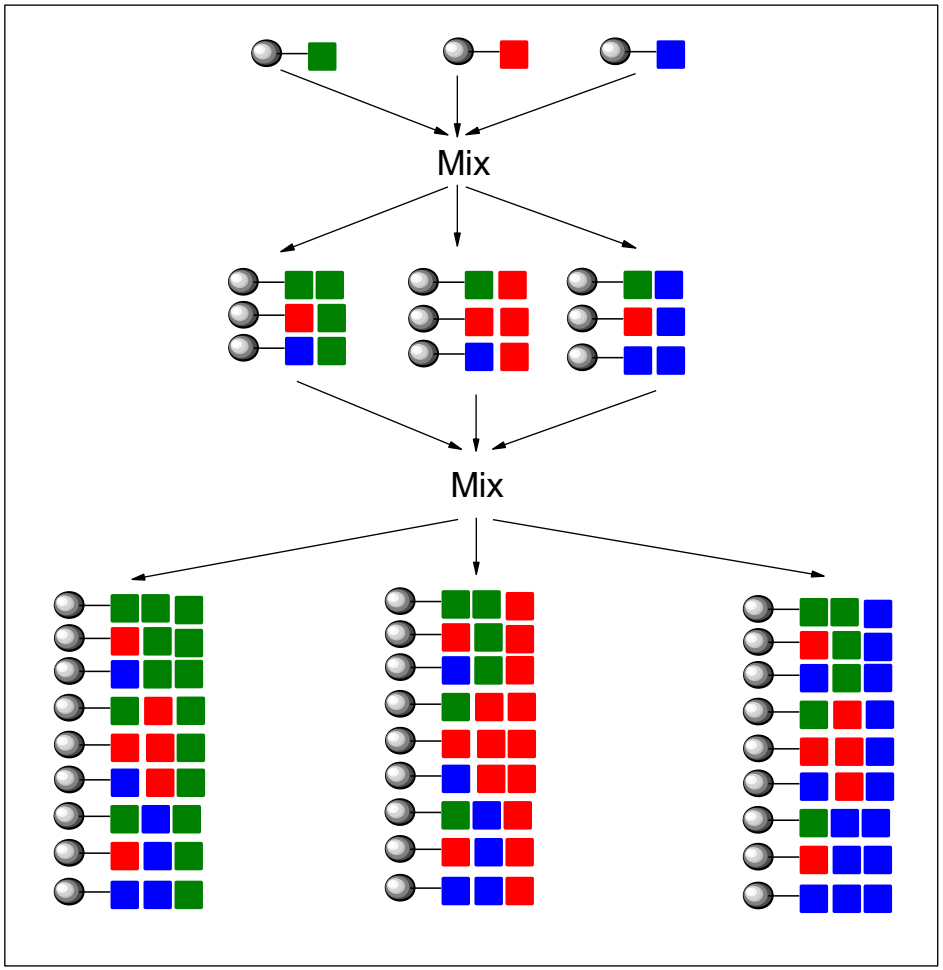
- -Split Mix synthesis
- -Library encoding
- -Differential release
- -Single Bead screening

3 building blocks
3 products in pools of 1

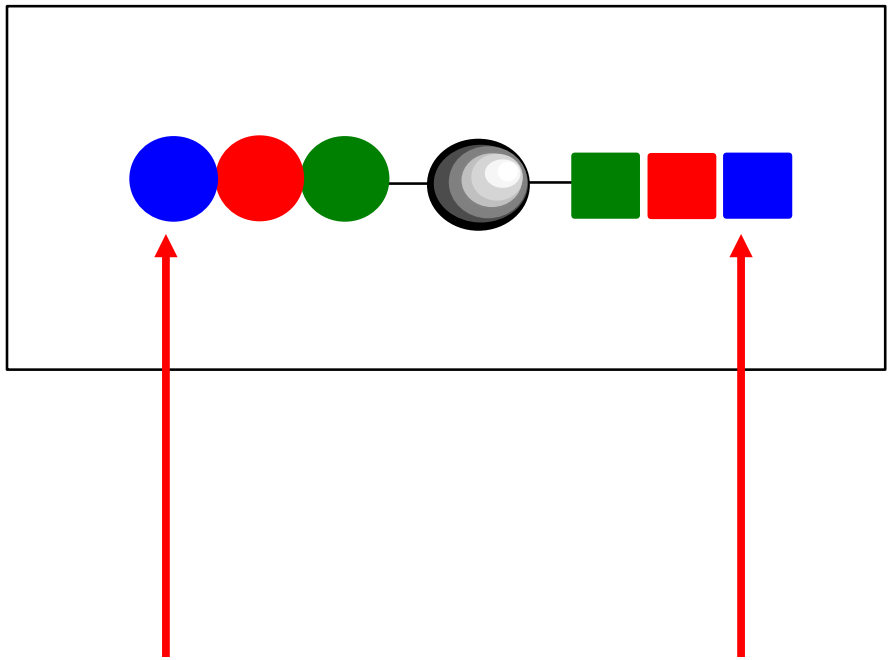
9 products in pools of 3

27 products in pools of 9

1 bead = 1 compound



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

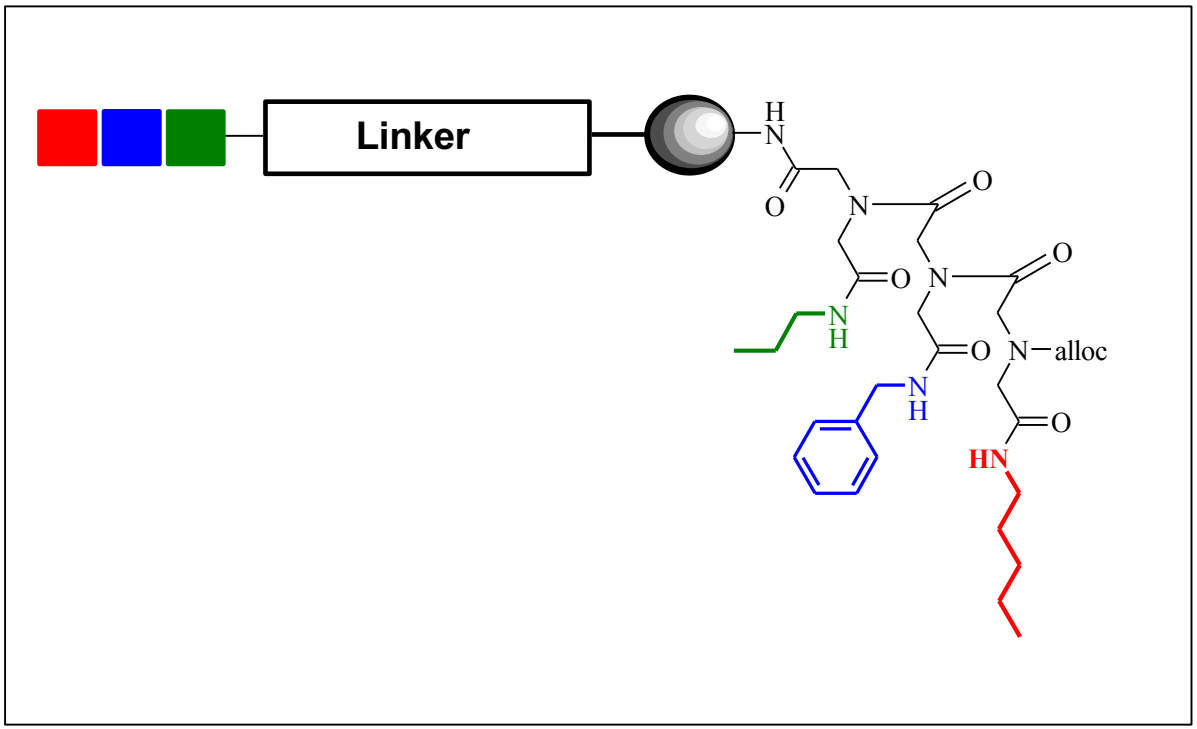


Codes for each building block

Building Blocks

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

Affimax encoding strategy

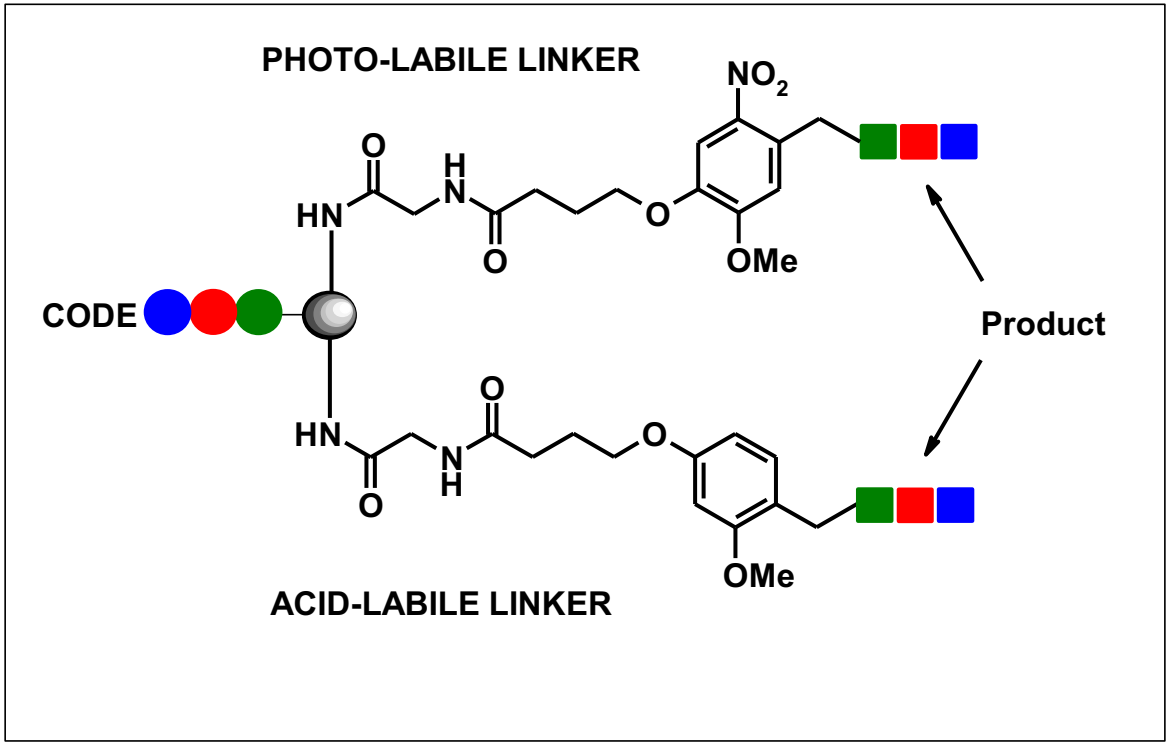


Product on acid-
or photolabile linker

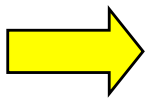
-Codes are different amines
-Cleaved with cHCl
-Dansylate and analyse by hplc

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

Differential release



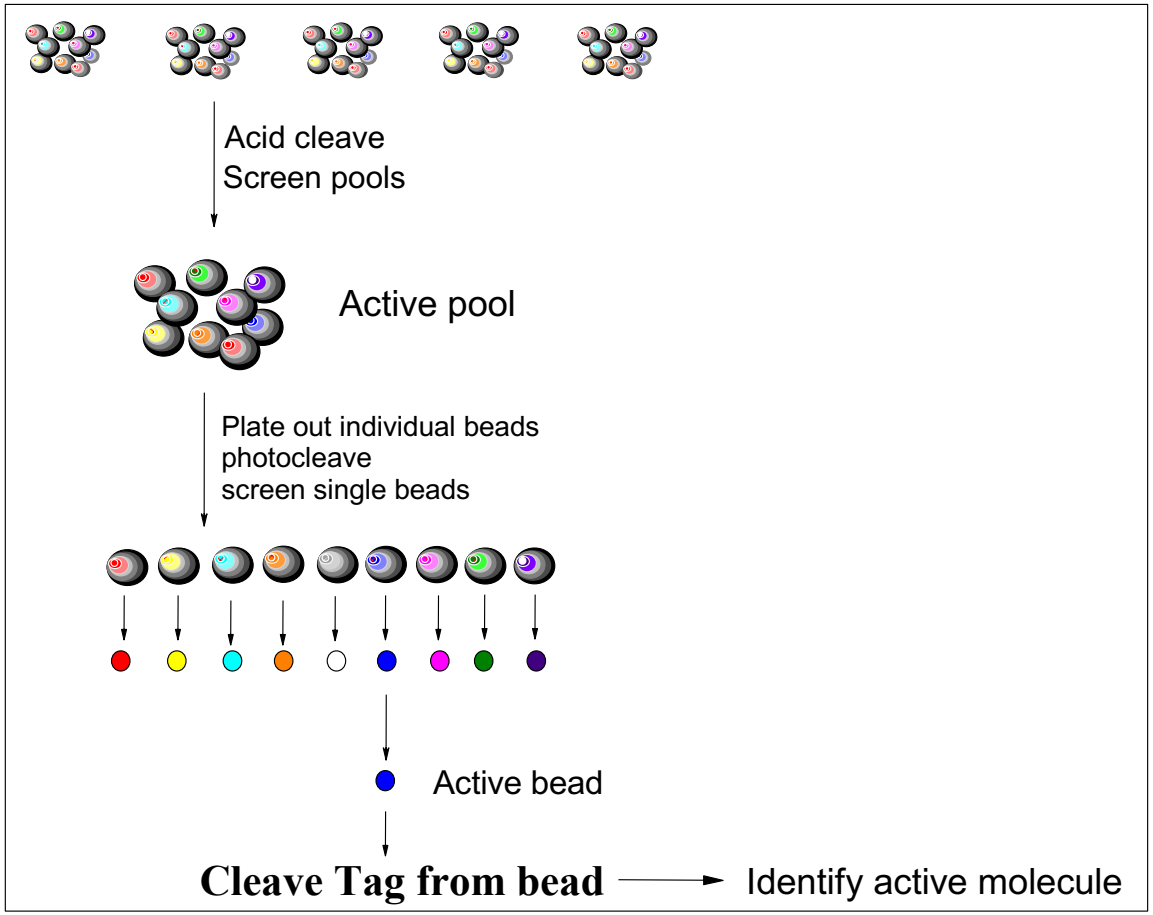
50% on acid labile linker
50% on photolabile linker



Product can be released
twice at different times

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

Single bead screening



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”

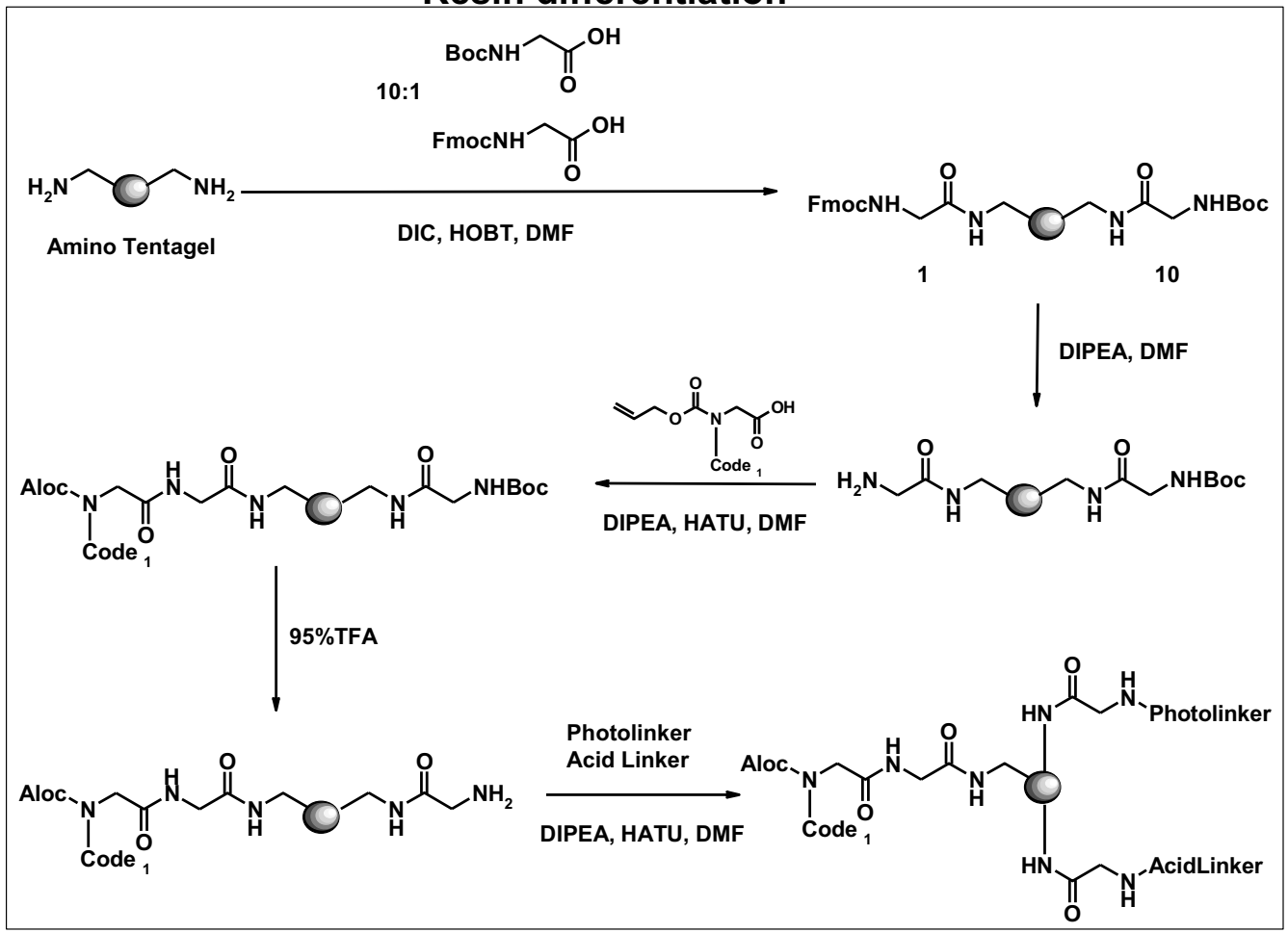
-Analysis throughout

- -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

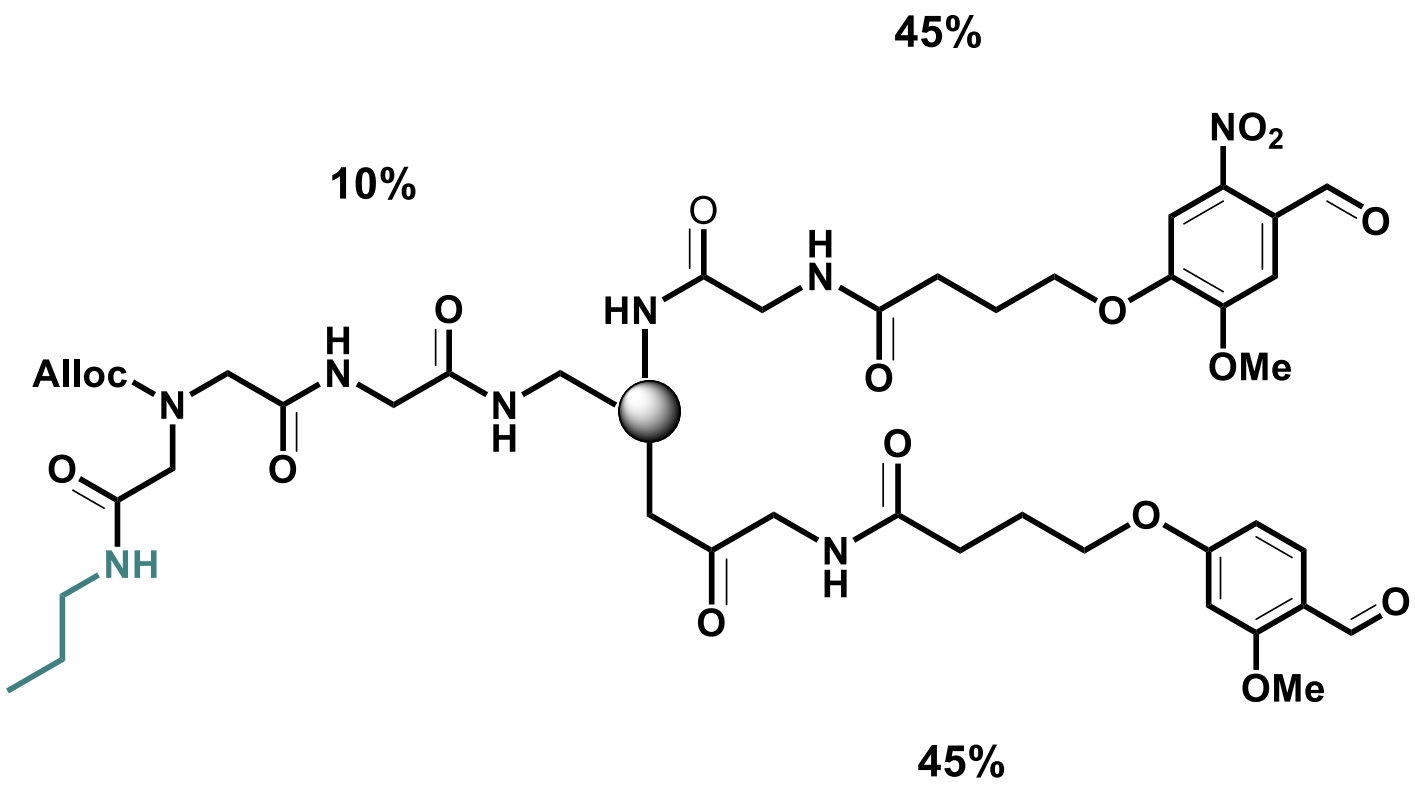
Examples for libraries synthesized on solid-phase: four helix bundle proteins

Resin differentiation



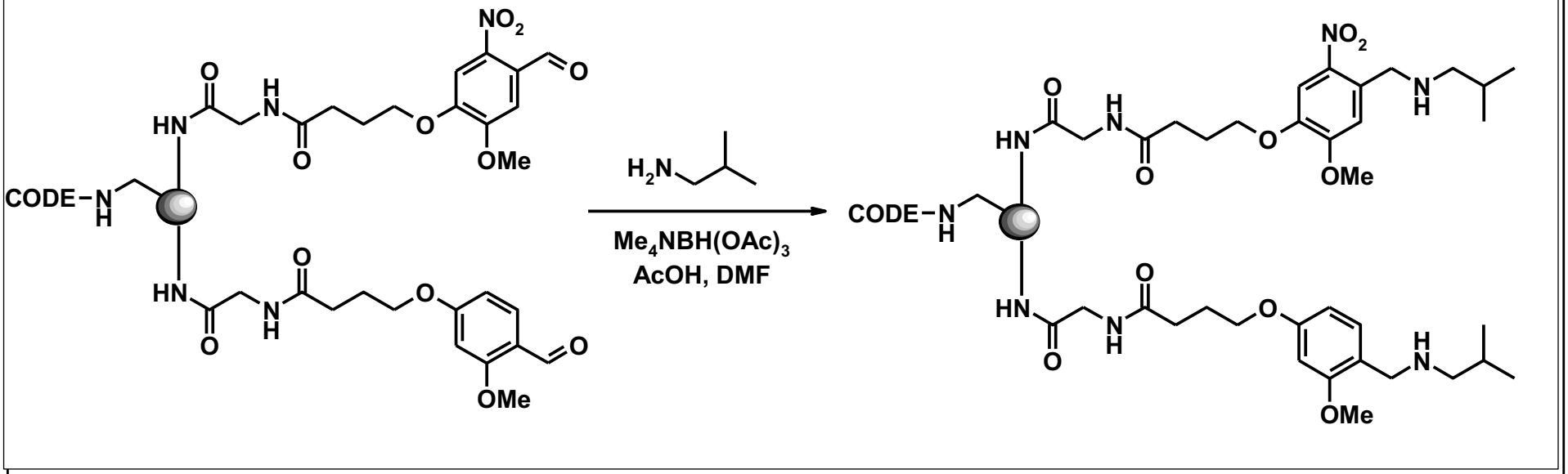
Examples for libraries synthesized on solid-phase: four helix bundle proteins

Prepared resin

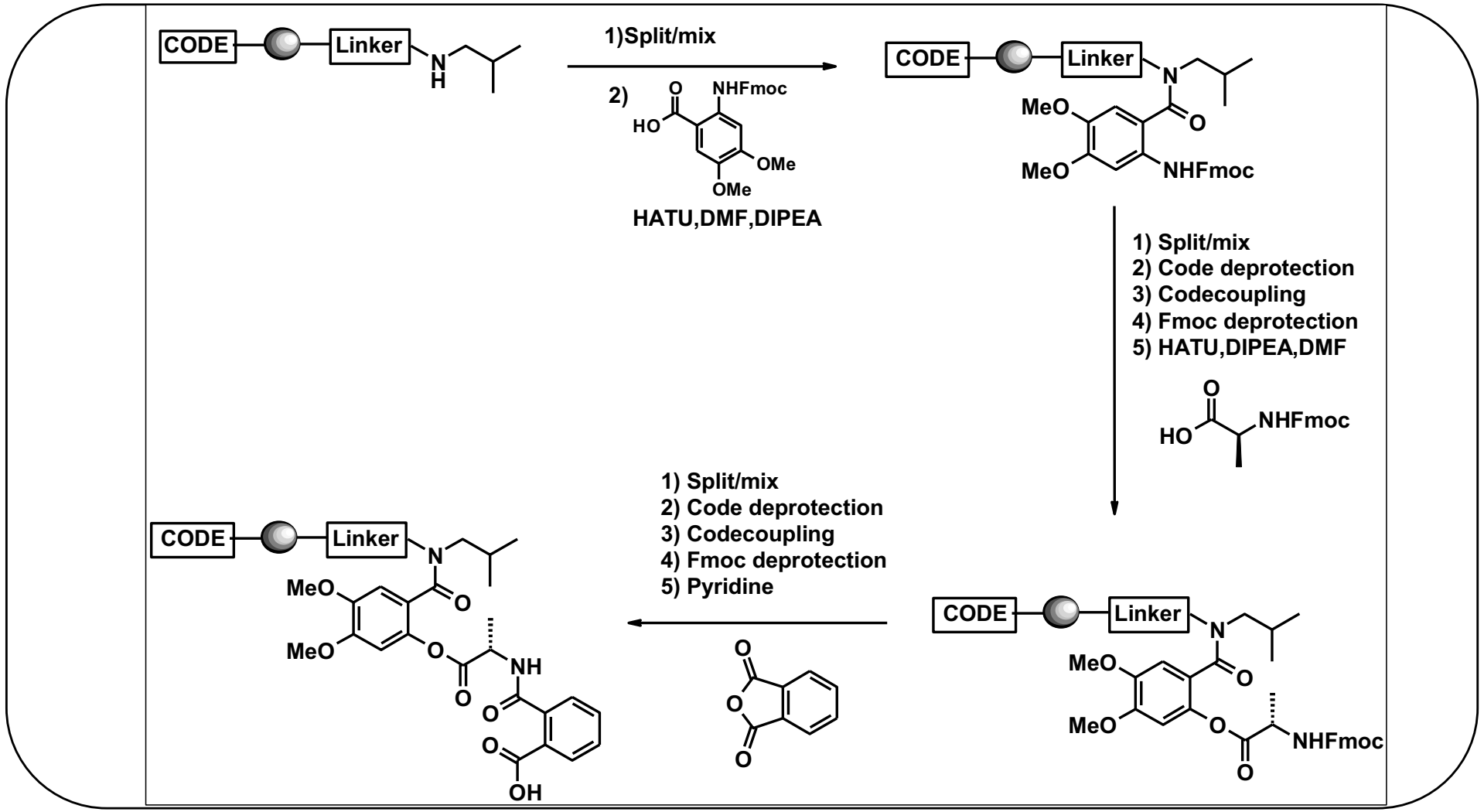


Examples for libraries synthesized on solid-phase: four helix bundle proteins

Reductive amination

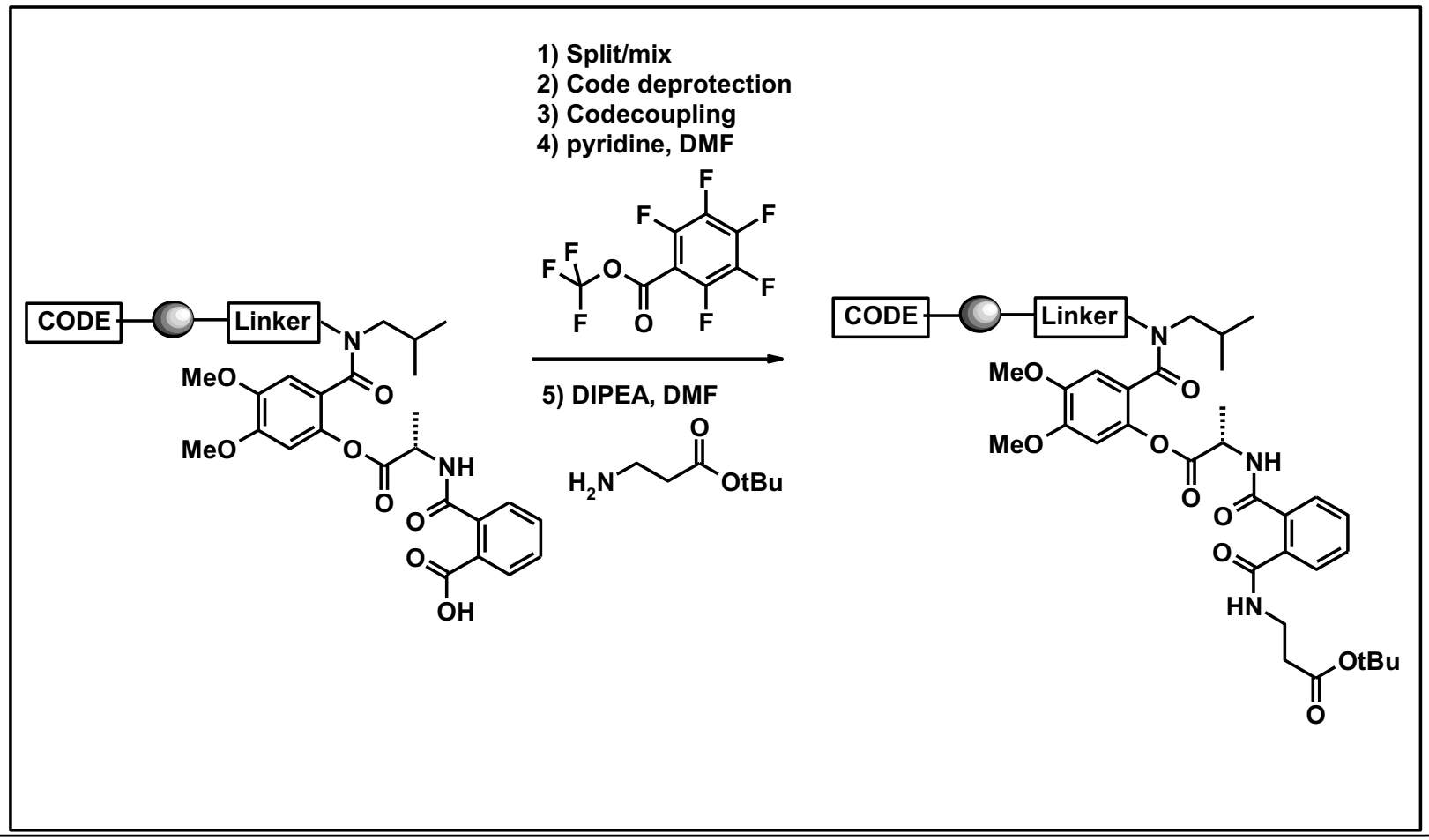


Examples for libraries synthesized on solid-phase: four helix bundle proteins



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

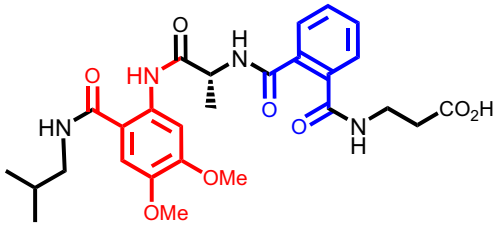
Library synthesis 2



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

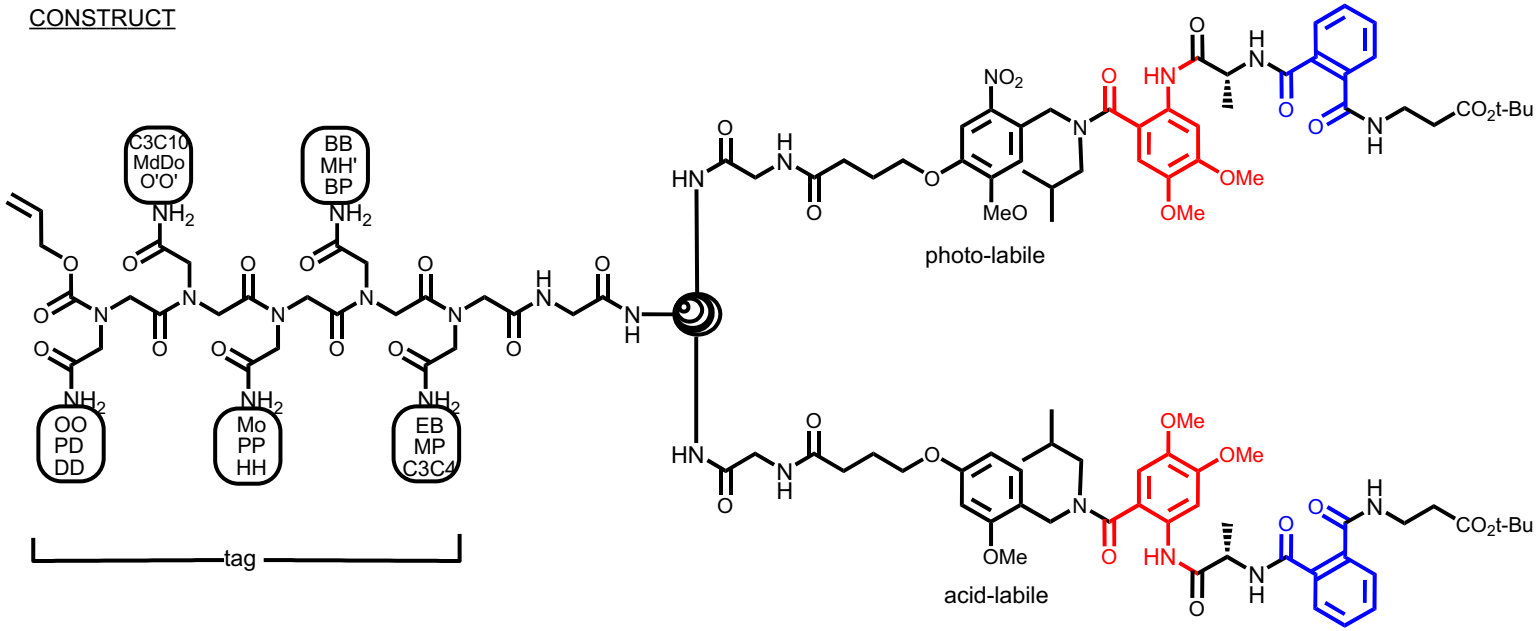
Final construct

TARGET



$C_{27}H_{34}N_4O_8$
[542.6]

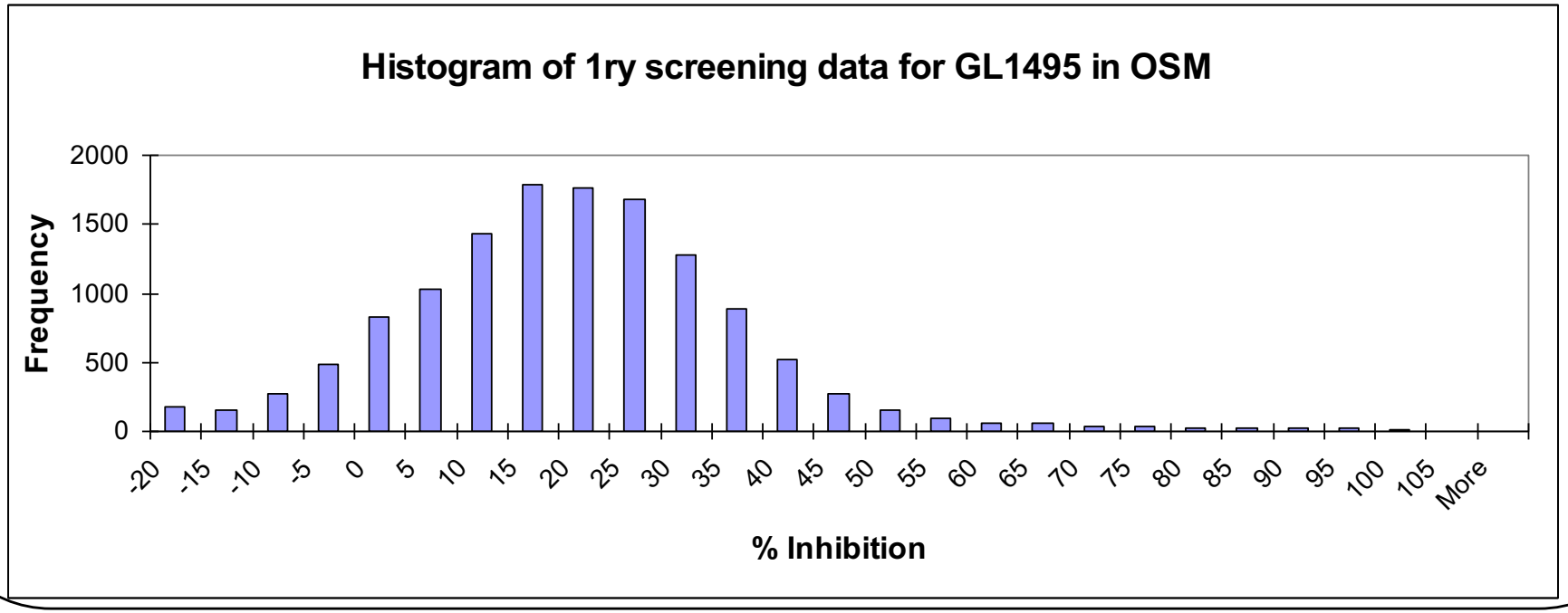
CONSTRUCT



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



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?

Source	Number	Hits	Leads
GSK compound collection	250.000	3134	0
Natural product extracts	70.000	18	0
Aptamers	2000.000	78	13
Apha helix library GL1495	134.456	21	5

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

Some useful definitions in medicinal chemistry

EC50: effective dose for a 50% of maximal response

Dose: 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.

SAR: structure activity relashionship. Correlation between chemical structure and biological activity.

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.

Some useful definitions in medicinal chemistry

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, Elimination and Toxicity

ADMET: **A**dsorption, **D**istribution, **M**etabolism, **E**xcretion(**E**limination) and **T**oxicity

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

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 polymera
- -*Hydrolases* (serine proteases, metalloproteases etc.)
- -*Lyases* (DOPA decarboxylase, carbonic anhydrase et
- -*Isomerases* ((DNA gyrases, topoisomerases etc.)
- -*Ligases* (dehydropteroate synthase, mTOR etc.)

Targets hit by current drugs

2.3. Receptors:
-*Direct ligand-gated ion channels* (GABAA, acetylcholi
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*
-*Noradrenalin/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. bevazizumab; Avastin)
-*Lymphocyte function-associated recepto* (LFA-1; efalizumab)
-*Epidermal growth factor receptor* (EGFR (e.g. cetuximab)
-*h-EGFR-2* (e.g. trastzumab; Herceptin)
-*Immunoglobulin E* (IgE; e.g. omalizumab Xolair)
-*CD-3*
-*CD-20* (Rituximab; Mabthera)
-*CD-33* (Gemtuzumab))
-*CD-52* (Alemtuzumab)
-*TNF*α (Adalimumab; infliximab; Enbrel)

Targets hit by current drugs

G-Protein Coupled Receptors (GPCR's):

- *Acetylcholin receptors* (muscarinic rece
  MCR 1-4)
- *Adenosin receptors*
- *Adrenoreceptors* (α1, α2, β1)
- *Angiotensin receptors*
- *Calcium-sensing receptors*
- *Cannabinoid receptors* (CB1, CB2)
- *Cysteinyl-leukotriene receptors*
- *Dopamine receptors*
- *Endothelin receptors*
- *$GABA_B$ recptors*
- *Glucagon receptors*
- *Glucagon-like peptide-1 receptor* (GLP-
- *Histamin receptors* (H1, H2)
- *Opioid receptors* (μ, κ, δ)
- *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*
- *Erythropoetin 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*

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