Combinatorial libraries: strategies and methods for ‘lead’ discovery

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Key sources of information

- WWW:
  - Diversity information pages [http://www.5z.com/divinfo/]
  - *J.Combinatorial Chem.* [http://acsinfo.acs.org/journals/jcchff/index.html]
  - *Combi. Chem. H.T.S.* [http://www.bscipubl.demon.co.uk/cchts/index.html]
- Books
  - *Combinatorial peptide and nonpeptide libraries-a handbook*, Ed. G.Jung, VCH, Weinheim, **1996**.
  - *Combinatorial chemistry-synthesis and application*, S.R.Wilson, A.W.Czarnik, Wiley, New York, **1997**.
- Reviews
  - 'Combinatorial chemistry', *Chem. Rev*. **1997**, *97*(2), special issue.
  - 'Combinatorial chemistry', *Curr. Opin. Chem. Biol.* **1998**, *2*(3) & **1999**, *3*(3).
  - 'Combinatorial chemistry', S. Borman, *Chem & Eng. News* **1997**, *Feb24*, 43.

Format and scope of lecture

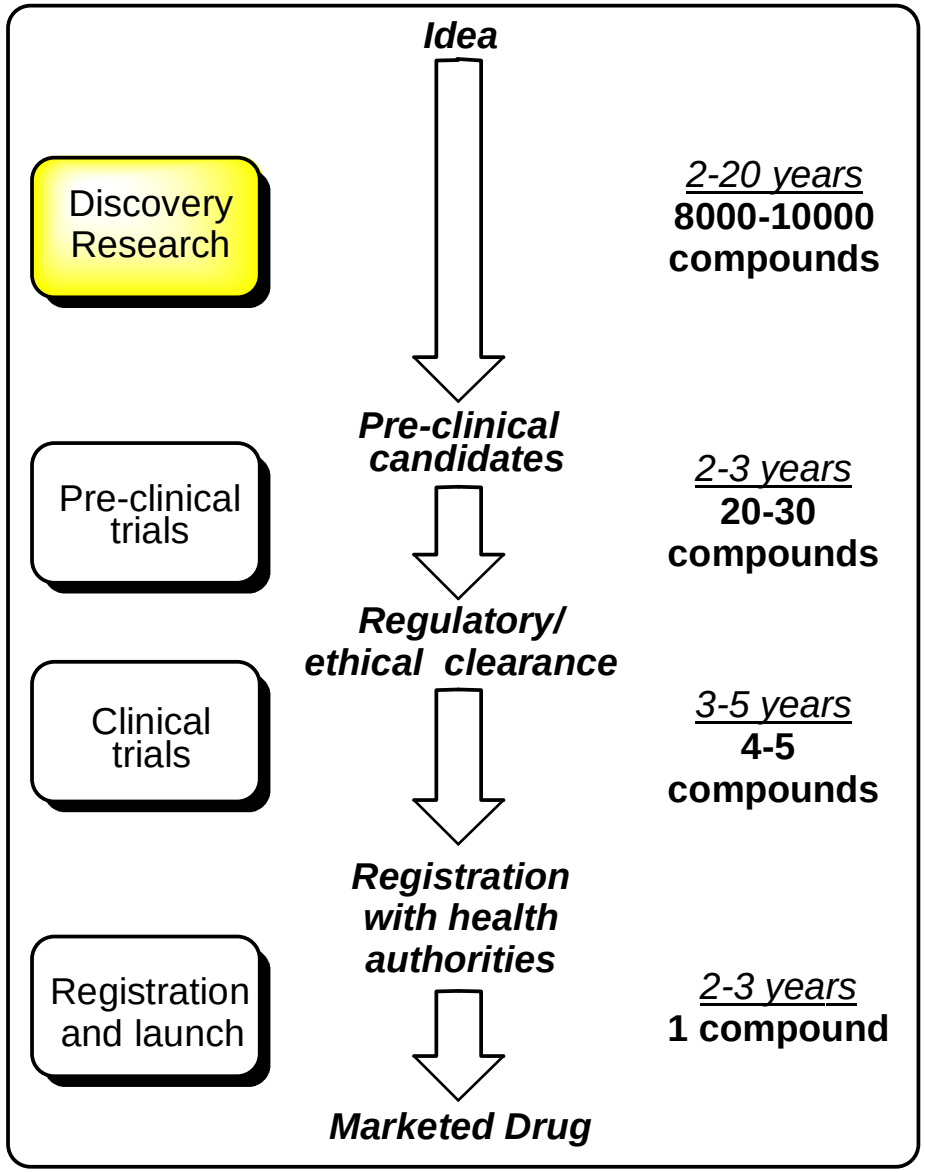
- What is combinatorial chemistry?
- The drug discovery process
- Approaches to combinatorial library synthesis:
  - mix and split synthesis
  - parallel synthesis
  - encoded tagging
- Library types:
  - oligomeric libraries
  - template based libraries
- Combinatorial drug discovery!

What is combinatorial chemistry?

Combinatorial chemistry is a useful tool for rapidly optimizing molecular properties, particularly ones that are difficult to design *a priori*…

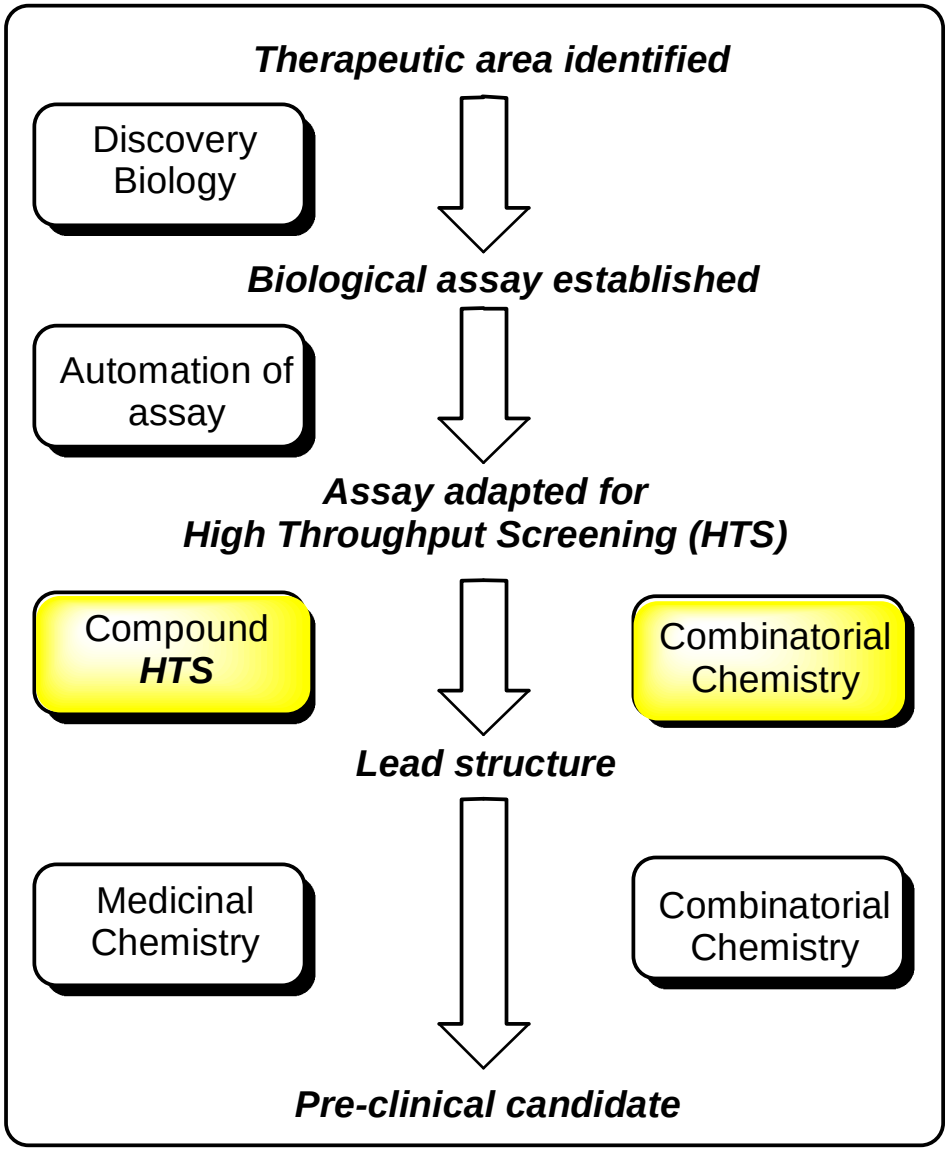
Nature uses a combinatorial approach to generate diverse functional macromolecules such as antibodies to recognize a vast array of antigens.

The drug discovery process



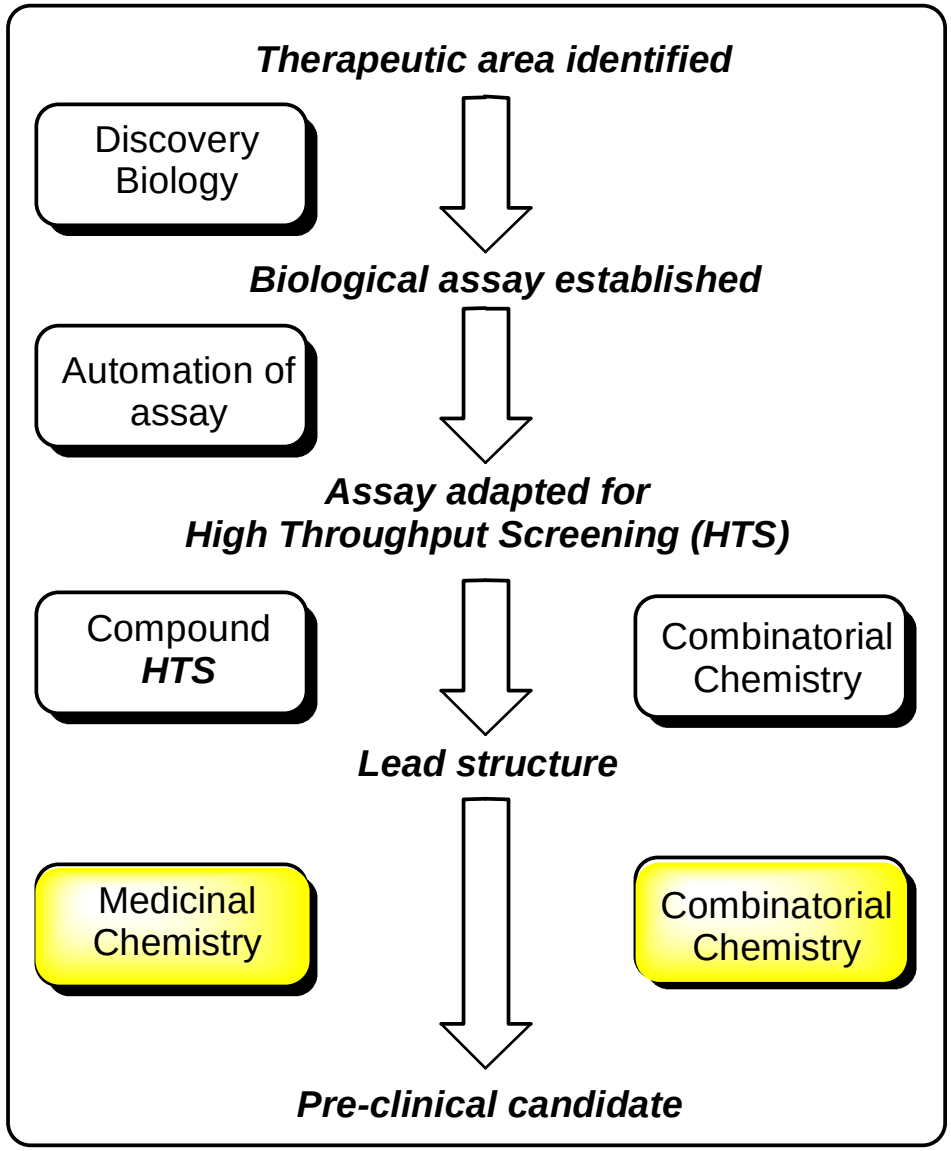
- The total cost of bringing a new drug to market is typically ~£250m (i.e. EXPENSIVE!)
- Of this, £170m is spent on DISCOVERY RESEARCH.
- This reflects the large amount of TIME involved in synthesising new compounds.
- A typical chemist can synthesise ~100 compounds a year using traditional techniques.
- ***S***OLID ***P***HASE ***O***RGANIC ***S***YNTHESIS (*SPOS*) and COMBINATORIAL CHEMISTRY are beginning to revolutionise this situation.

Discovery chemistry: stage 1



- **High Throughput Screening (*HTS*):**
  - Rapid, automated screening of compounds for specific biological activity.
- **Role of combinatorial chemistry:**
  - Very large libraries.
  - Maximum diversity libraries.
  - Mix and split libraries (& parallel synthesis).
  - Mixtures of compounds (& single compounds).

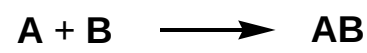
Discovery chemistry: stage 2



- **Medicinal chemistry:**
  - Systematic optimisation of molecular and physicochemical properties of lead compound
- **Role of combinatorial chemistry:**
  - Small libraries.
  - ‘Targeted/focussed’ libraries.
  - Parallel synthesis libraries.
  - Single compounds.

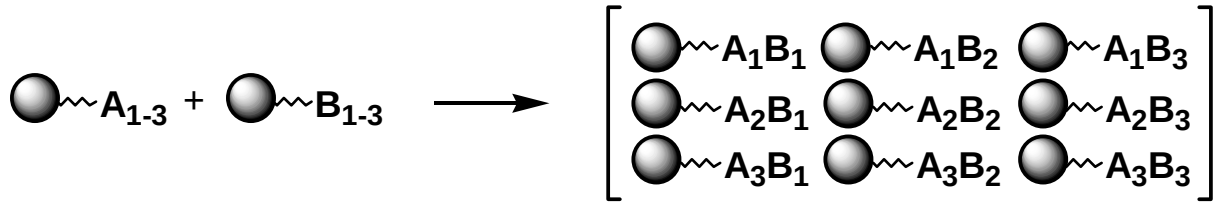
Traditional *vs.* combinatorial

- Traditional synthesis:



compounds prepared one at a time, characterised and screened

- Combinatorial synthesis:



reaction of 3 reagents $\mathbf{A_x}$ with 3 reagents $\mathbf{B_y}$ provides a library of 3^2 (i.e. 9) compounds $\mathbf{A_xB_y}$

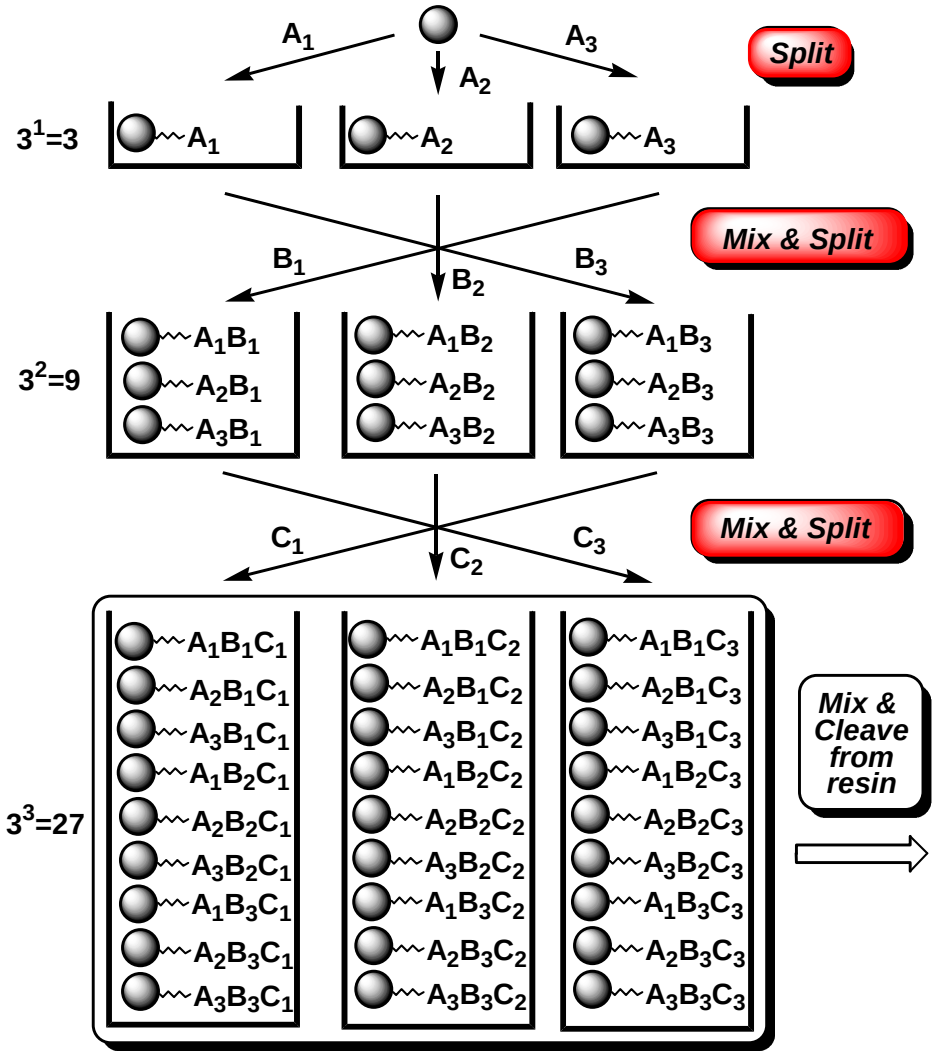
introduction of a third set of 3 reagents $\mathbf{C_z}$ increases the library size to 3^3 (i.e. 27) compounds $\mathbf{A_xB_yC_z}$

Approaches to ‘combinatorial’ library synthesis

- ***In vivo* - biological methods:**
  - **Phage display, plasmids, polysomes etc.**

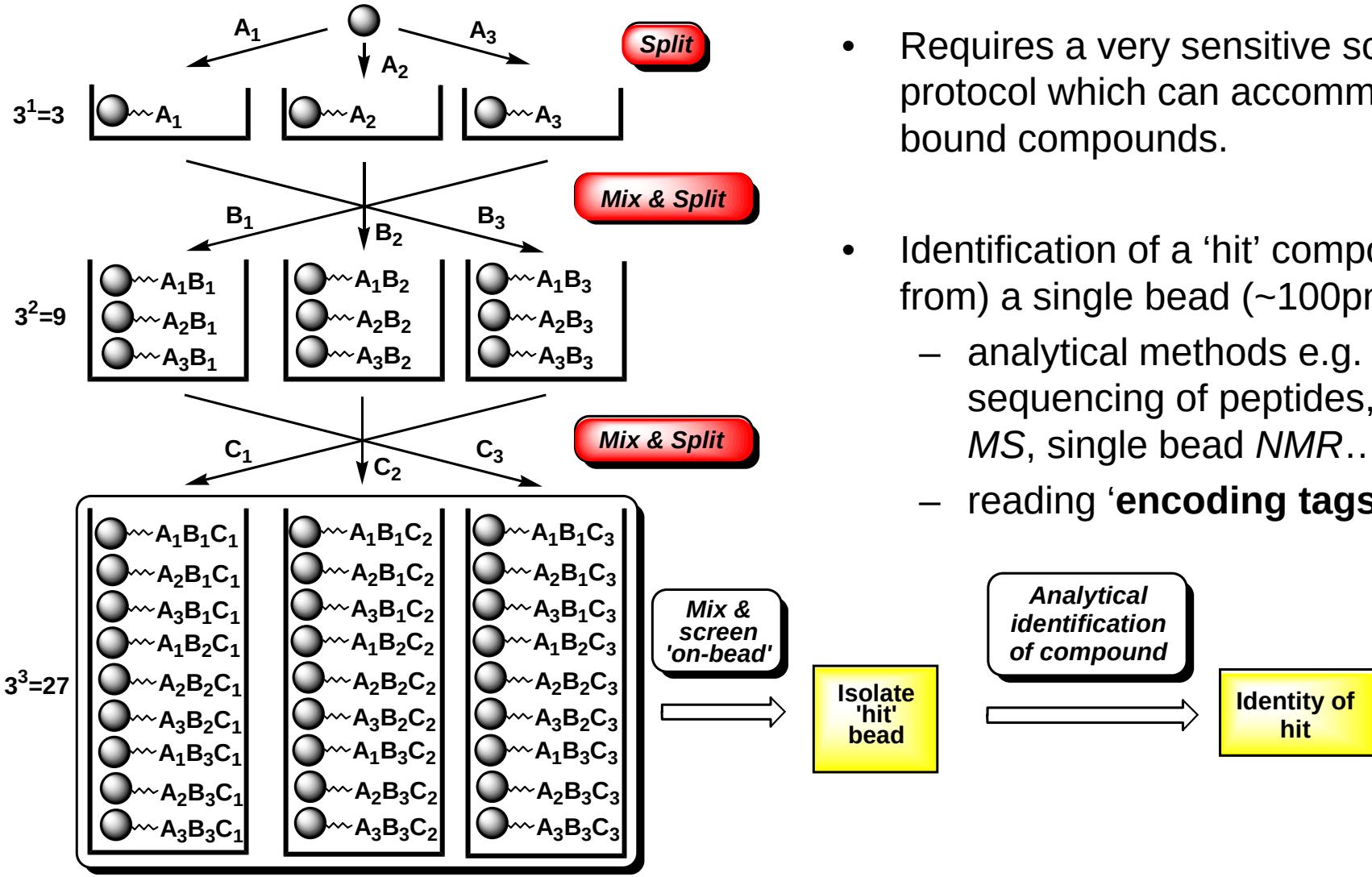
- ***In vitro* - synthetic methods:**
  - **Mix and split using *S*olid *P*hase *O*rganic *S*ynthesis (*SPOS*).**
    - Cleavage from the solid support following ‘mix and split’ results in complex mixtures (pools) of compounds. Screening of these mixtures yields ‘hits’ whose identity must be determined by ‘deconvolution’.
    - If screening can be performed ‘on-bead’ (i.e. ‘one-bead one-compound’ libraries) then deconvolution can be avoided.
  - **Parallel synthesis using *S*olid *P*hase *O*rganic *S*ynthesis (*SPOS*).**
    - Spatially separate synthesis of single compounds whose identity is uniquely defined by their location.

Mix & split synthesis: libraries of mixtures of compounds in solution



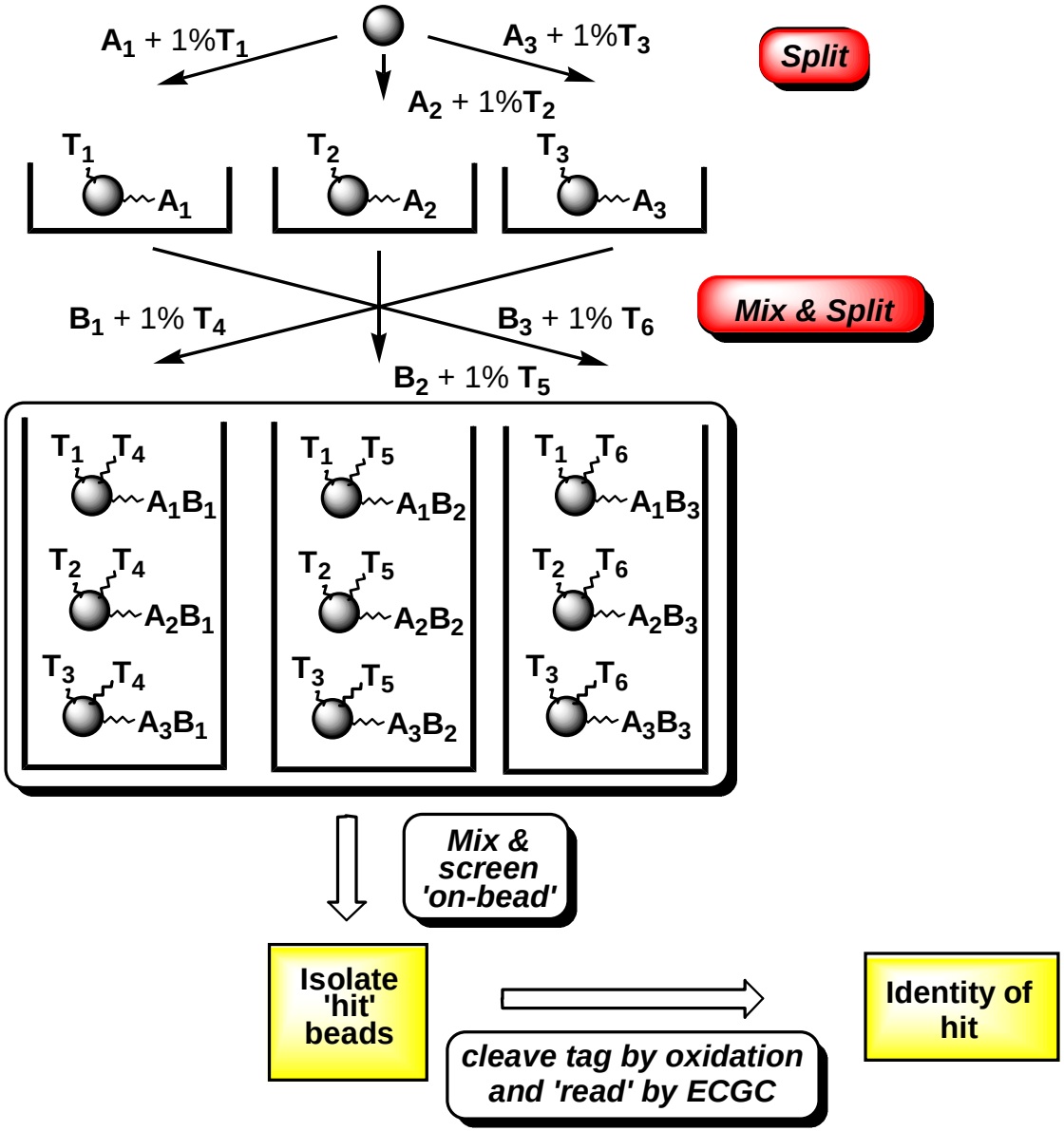
- Screening complex mixtures of compounds in solution can give false ‘hits’ due to synergistic effects.
- Identification of a compound within the mixture responsible for the ‘hit’ requires iterative **deconvolution**.
- Houghton *Nature*, **1991**, *354*, 84.

Mix & split synthesis: ‘one-bead one-compound’ libraries



- Requires a very sensitive screening protocol which can accommodate resin bound compounds.
- Identification of a ‘hit’ compound on (or from) a single bead (~100pm) by:
  - analytical methods e.g. Edman sequencing of peptides, *MALDI-TOF MS*, single bead *NMR*…
  - reading ‘**encoding tags**’ on beads.

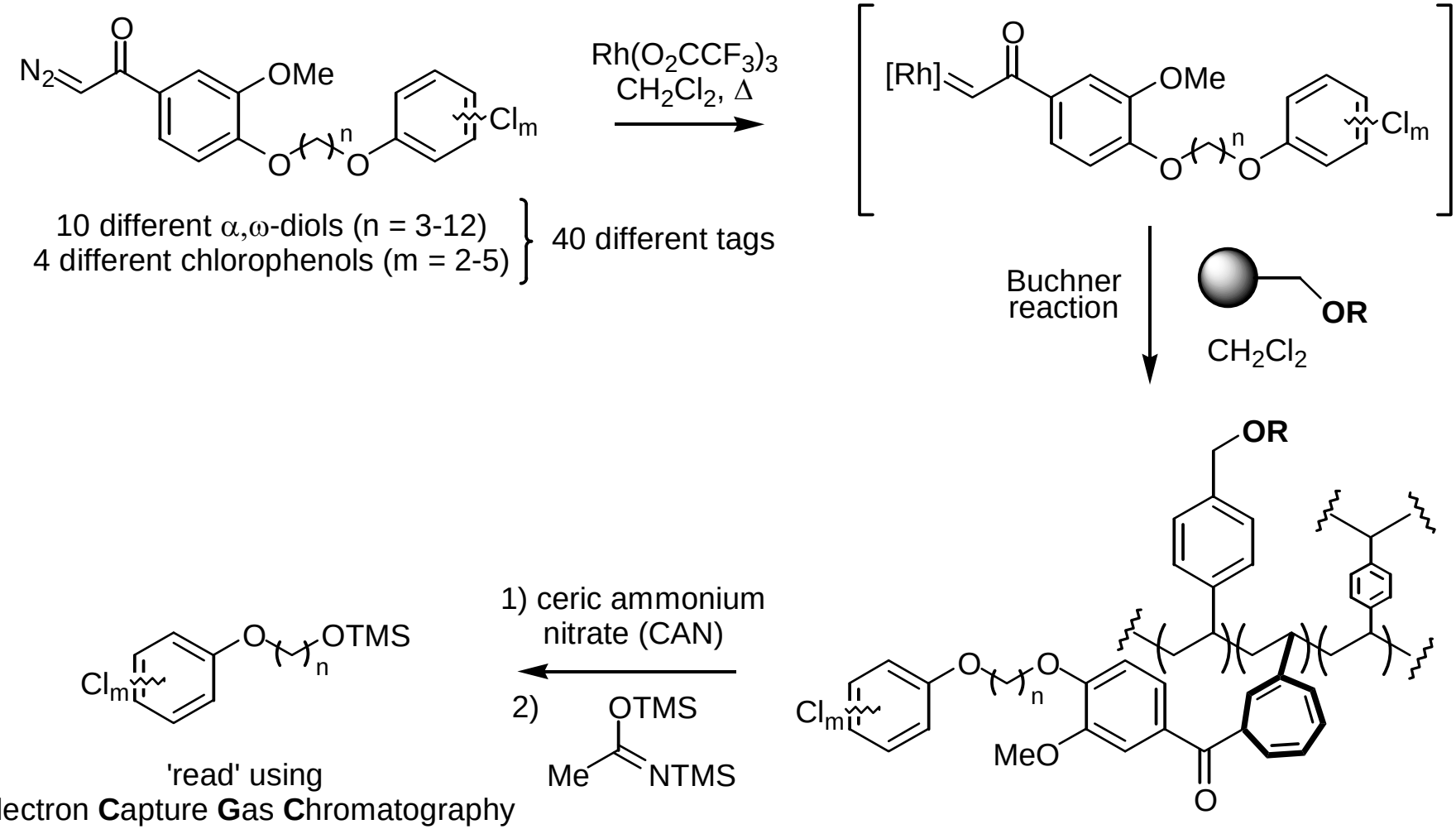
Clark Still's encoded tagging protocol



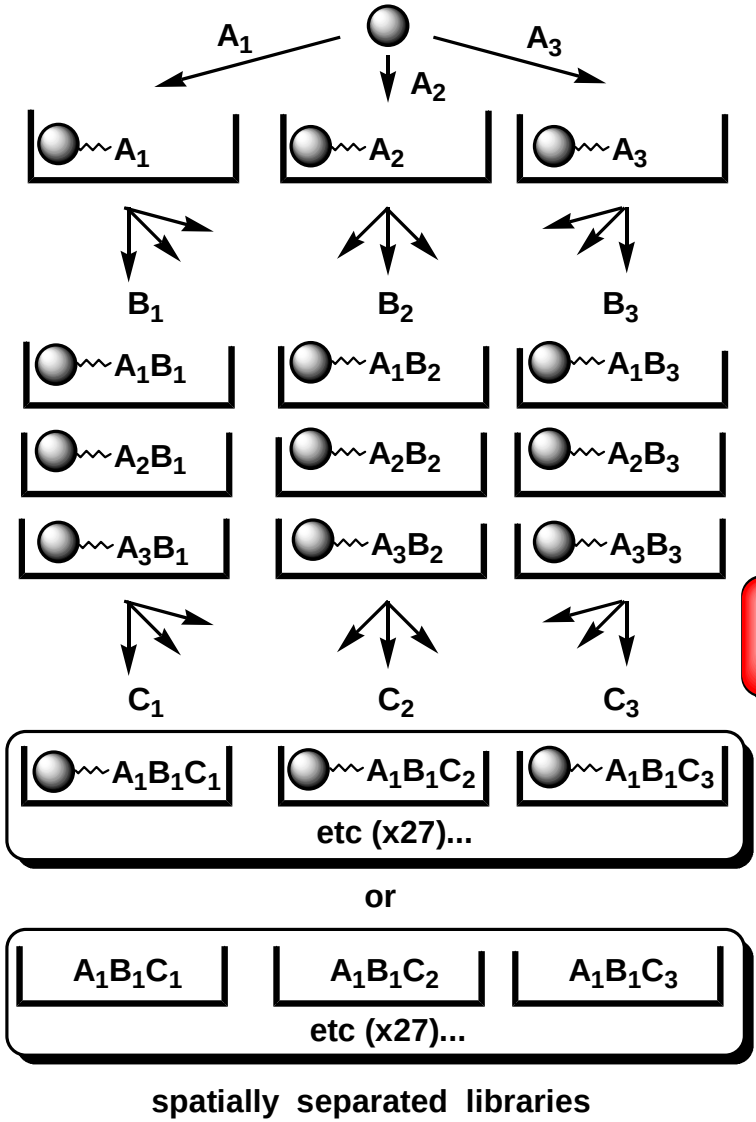
- Still *Acc. Chem. Res.* **1996**, *29*, 155.
- Each 'monomer' used in the library synthesis has an associated encoded tag.
- The tags are chlorinated aromatic compound which can be analysed at sub-picomolar levels by ***E***lectron ***C***apture ***G***as ***C***hromatography (*ECGC*).
- Allows for hit identification at one-bead fidelity for any type of library

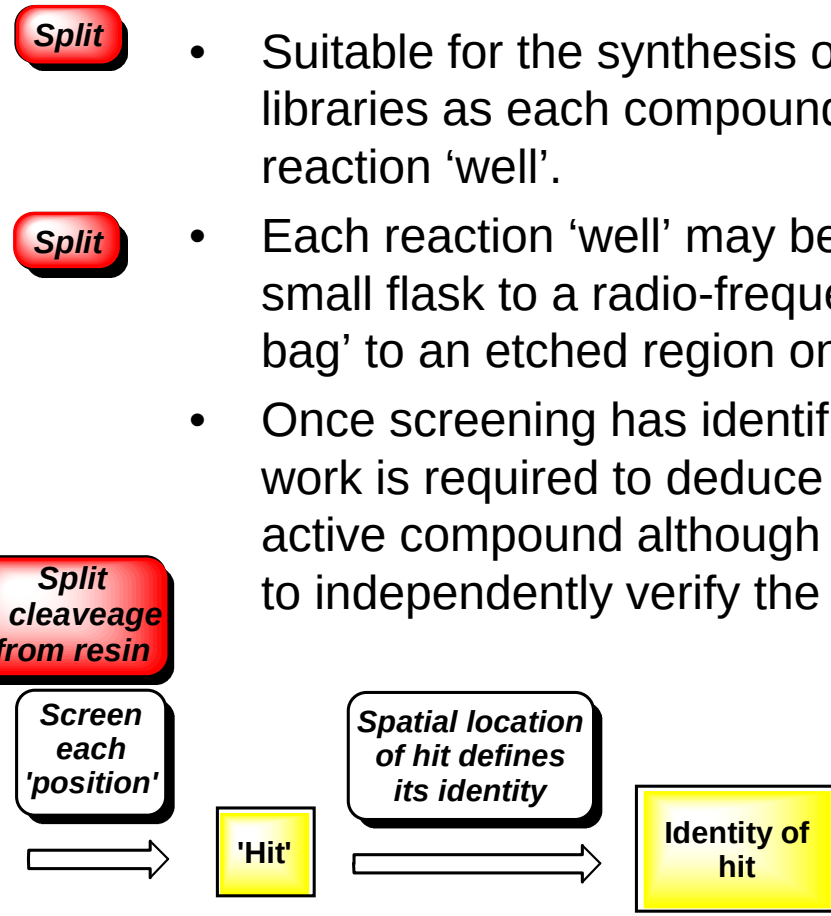
Mechanism of Clark Still encoded tags

- Still *J. Org. Chem.* **1994**, *59*, 4723.



Parallel synthesis: spatial separation gives single compounds

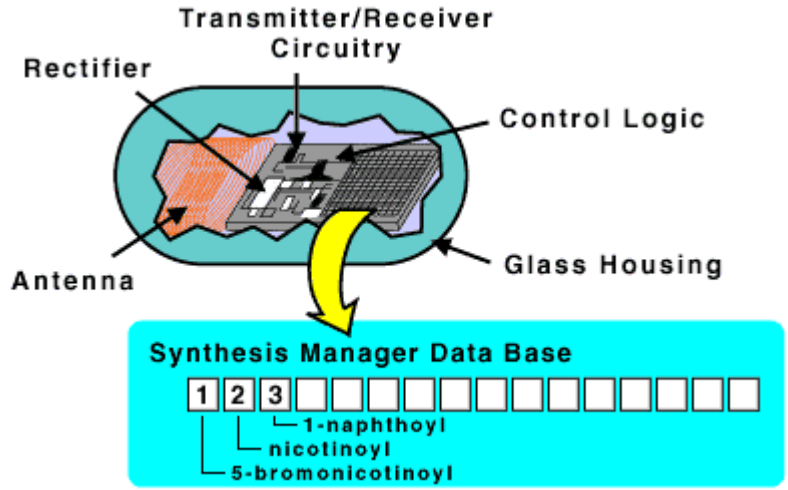




- Suitable for the synthesis of relatively small libraries as each compound requires its own reaction ‘well’.
- Each reaction ‘well’ may be anything from a small flask to a radio-frequency tagged ‘tea bag’ to an etched region on a silicon chip!
- Once screening has identified a hit no further work is required to deduce the identity of the active compound although it is routine practice to independently verify the structure.

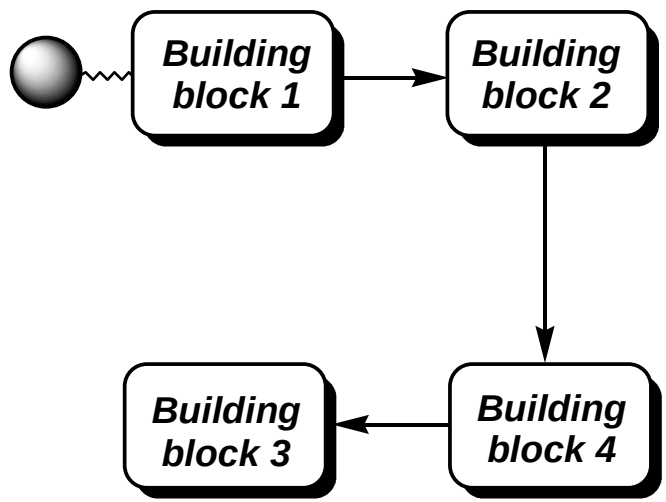
Keeping track of ‘tea-bag’ parallel synthesis: Irori radio-frequency tagging

- http://www.irori.com/



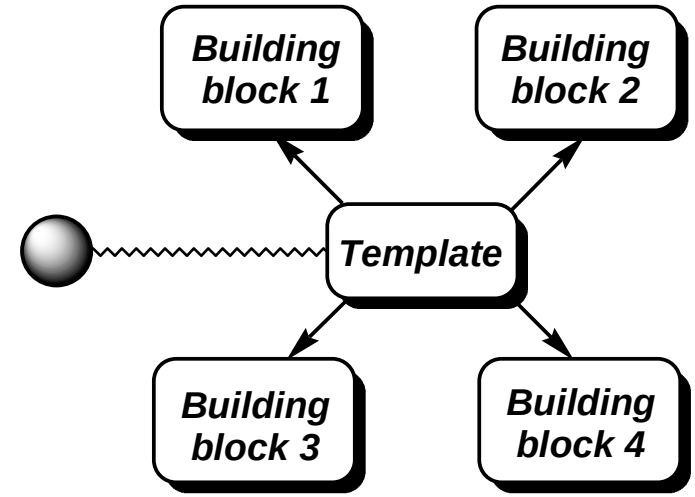
Library types

- **There are essentially two strategically distinct types of library at the molecular level:**



Oligomeric

peptides/peptoids
oligonucleotides
oligosaccharides
unnatural oligomers
polyaromatics

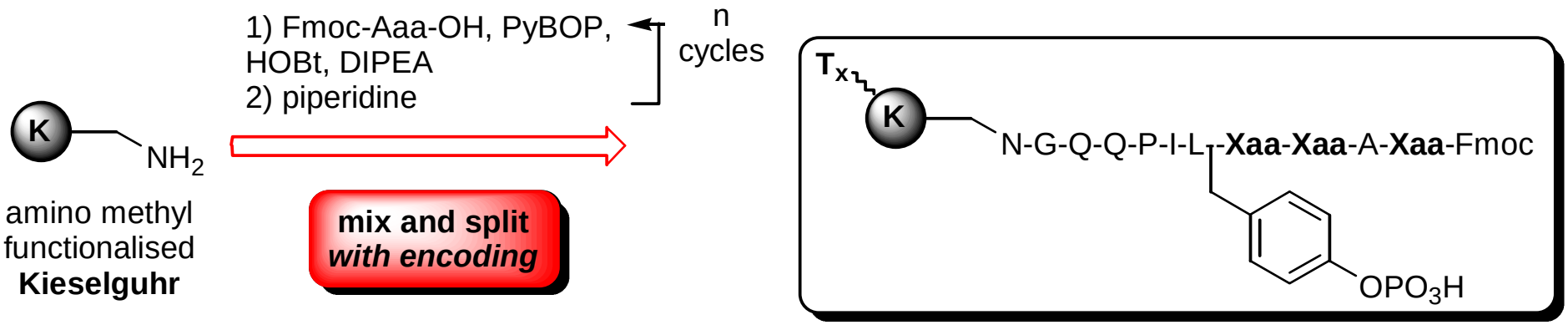


Template based

drug-like molecules
natural product-like molecules
heterocycle based molecules

Balasubramanian's peptide library

- Protein tyrosine phosphatase substrate library (oligomeric).
- Balasubramanian *J. Am. Chem. Soc.* **1997**, *119*, 9568.
- **Library synthesis:**



encoding via dummy peptide sequence incorporating glycine in place of phosphotyrosine

one-bead one-peptide encoded library

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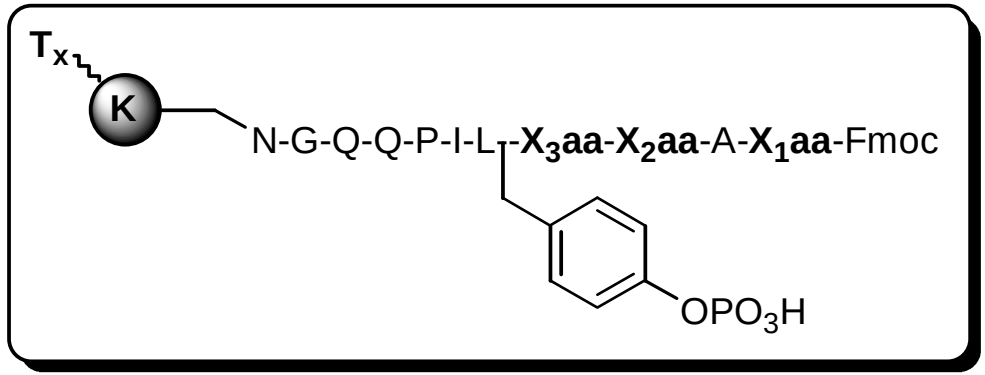


one-bead one-peptide encoded library

1) leukocyte antigen receptor protein tyrosine phosphatase (*PTP*)
2) α-chymotrypsin
3) fluorescent labelling of *N*-terminus with carboxyfluorescein: **F**

Hits = Tx K N-G-Q-Q-P-I-L **F**

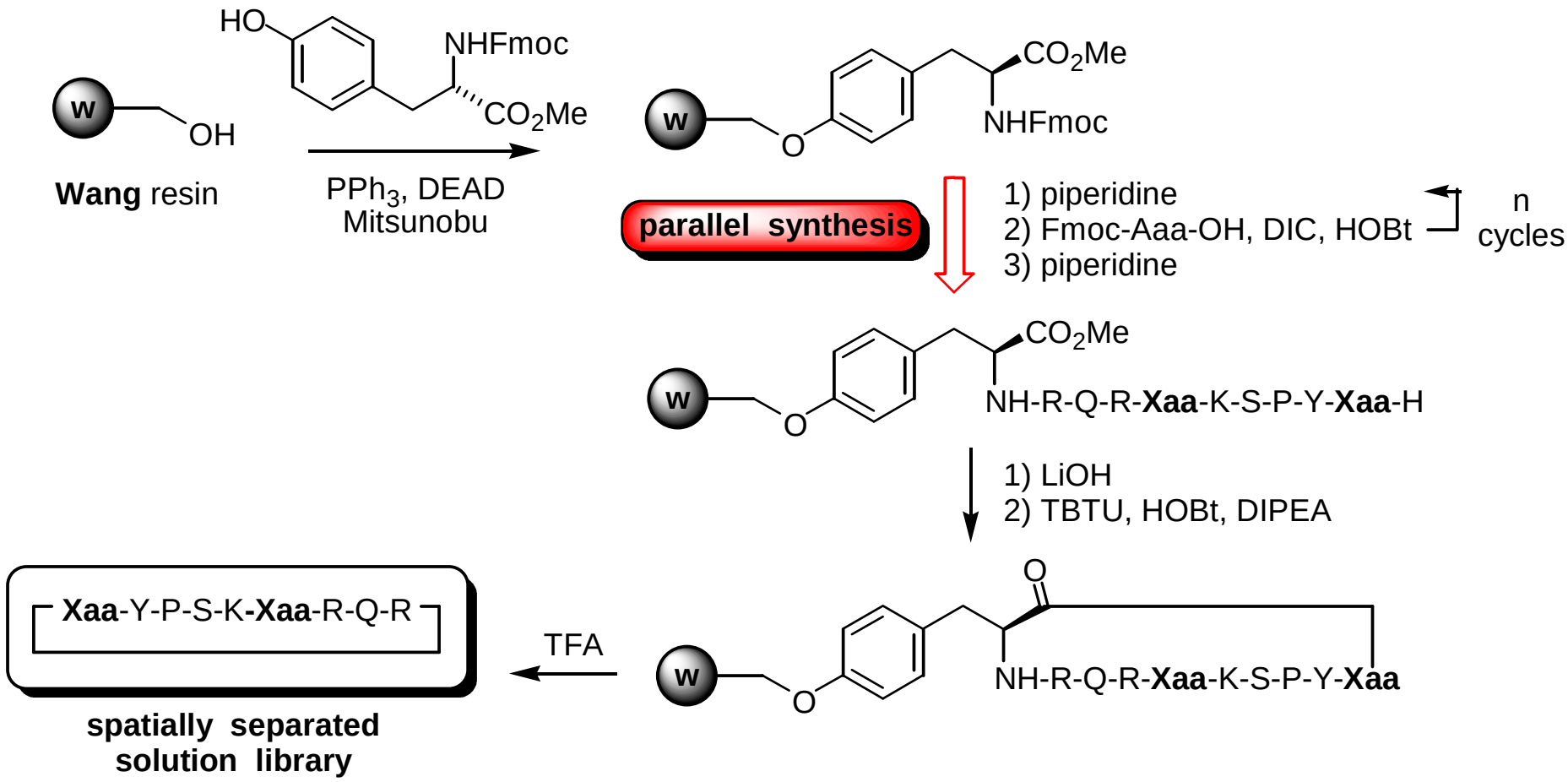
sequence encoding tag peptide

Identity of PTP substrate sequences

"preference for at least two acidic residues in variable positions and a glutamic acid residue at X_{1aa}"

Beck-Sickinger's cyclic peptide library

- Neuropeptide Y analogue library (oligomeric).
- Beck-Sickinger *J. Org. Chem.* **1999**, *64*, 4353.
- **Library synthesis:**



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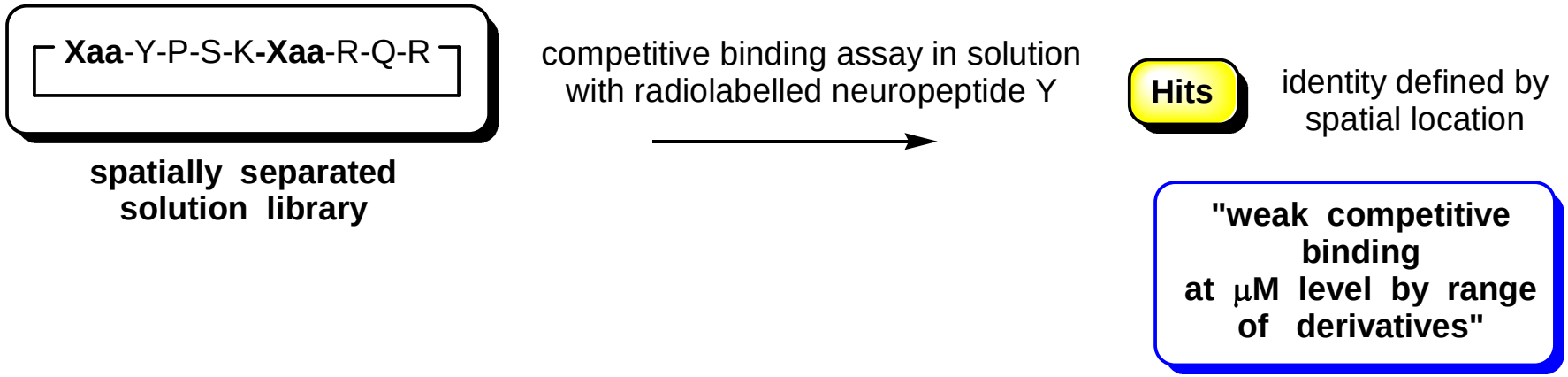


Oligonucleotide libraries

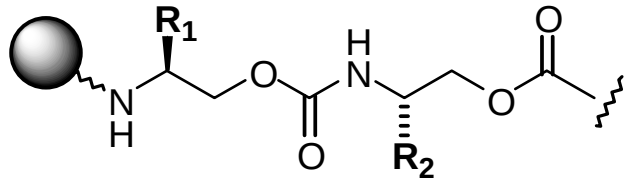
- These oligomeric libraries are generally prepared using *in-vivo* 'biological' methods and screened using ***S***ystematic ***E***volution of ***L***igands by ***Ex***ponential enrichment (SELEX) procedures.

- e.g. The discovery of very high-affinity RNA and DNA ligands to human IgE which inhibit binding to the Fcε receptor I.

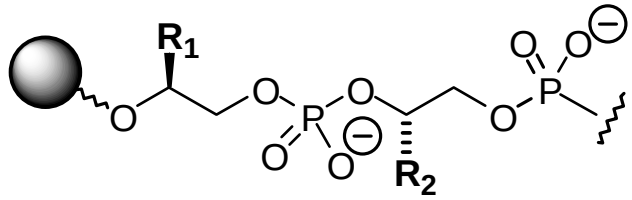
- Wiegand *J. Immunology* **1996**, *157*, 221 (and references therein).

Unnatural backbone oligomer libraries

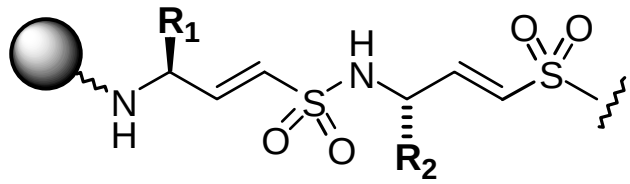
Backbone



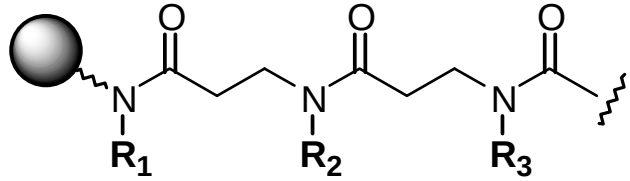
oligocarbamate



oligo-phosphodiester

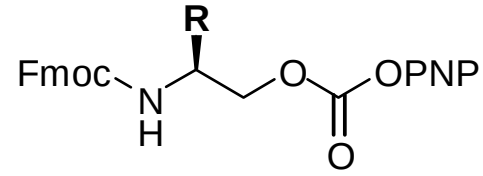


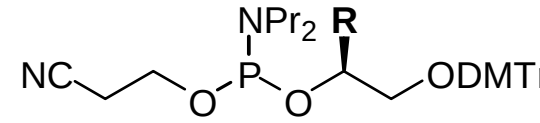
vinylogous sulfonamidopeptide

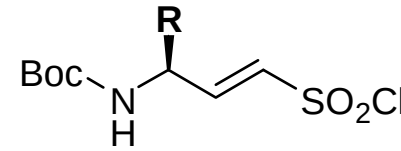


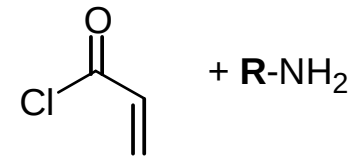
vinylogous sulfonamidopeptide

Monomers



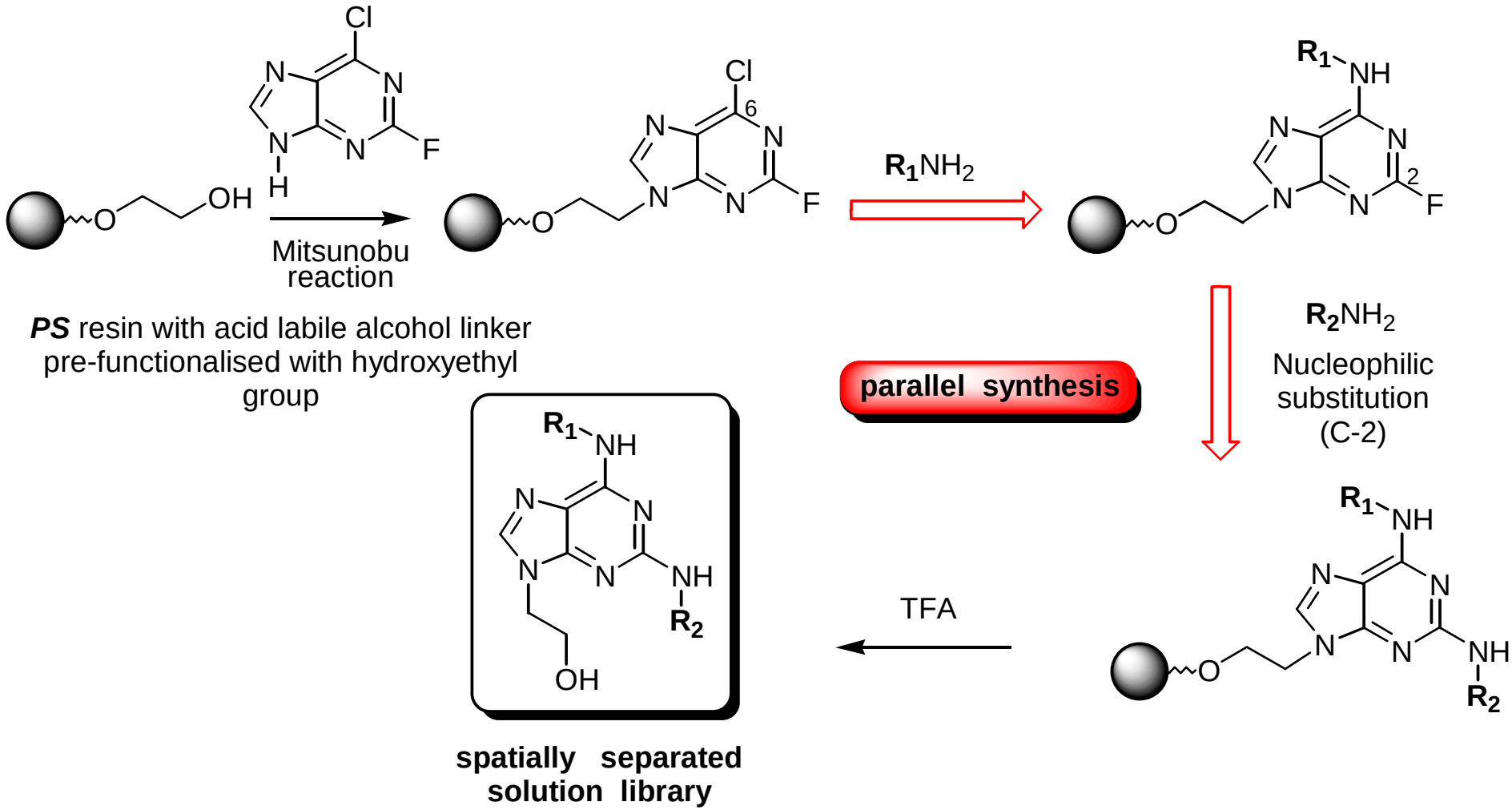






Schultz's purine library

- Kinase inhibitor library (template based).
- Schultz *Science* **1998**, *281*, 533.
- **Library synthesis:**



PS resin with acid labile alcohol linker pre-functionalised with hydroxyethyl group

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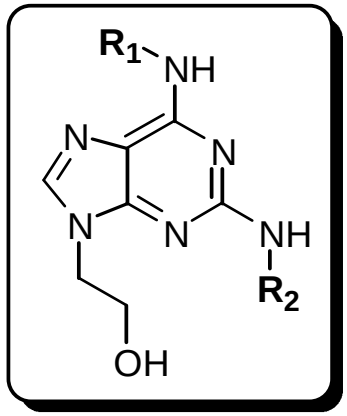
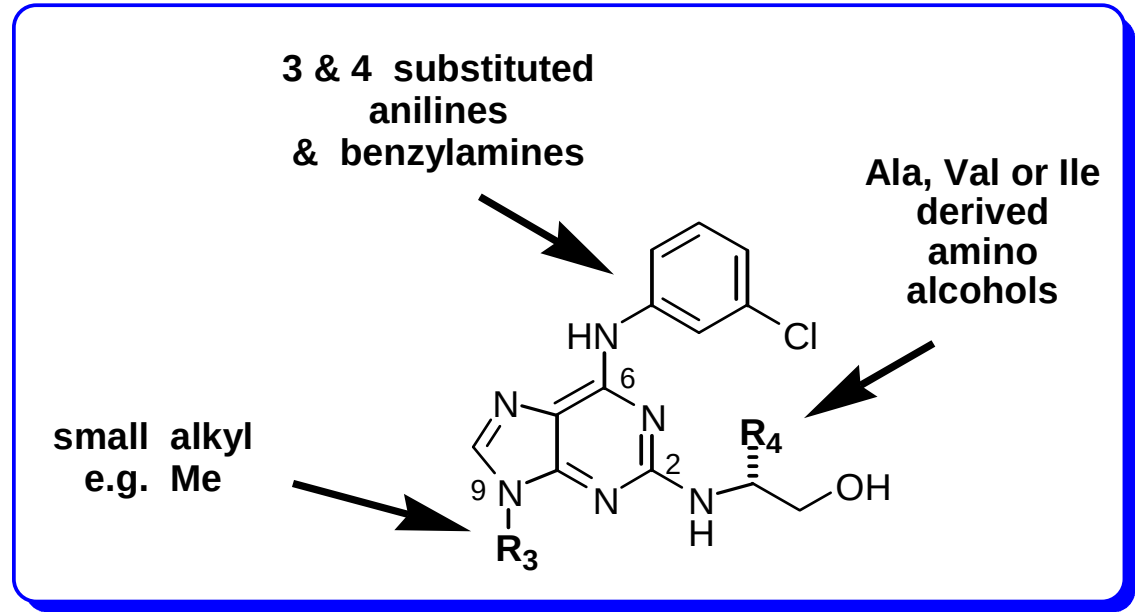


spatially separated solution library

Screen each position for inhibition of h-CDK2-cyclin A kinase complex

Hits

identity defined by spatial location



Schreiber's 'natural product' library

- Protein epitope binding library (template based).
- Schreiber *J. Am. Chem. Soc.* **1998**, *120*, 8565.
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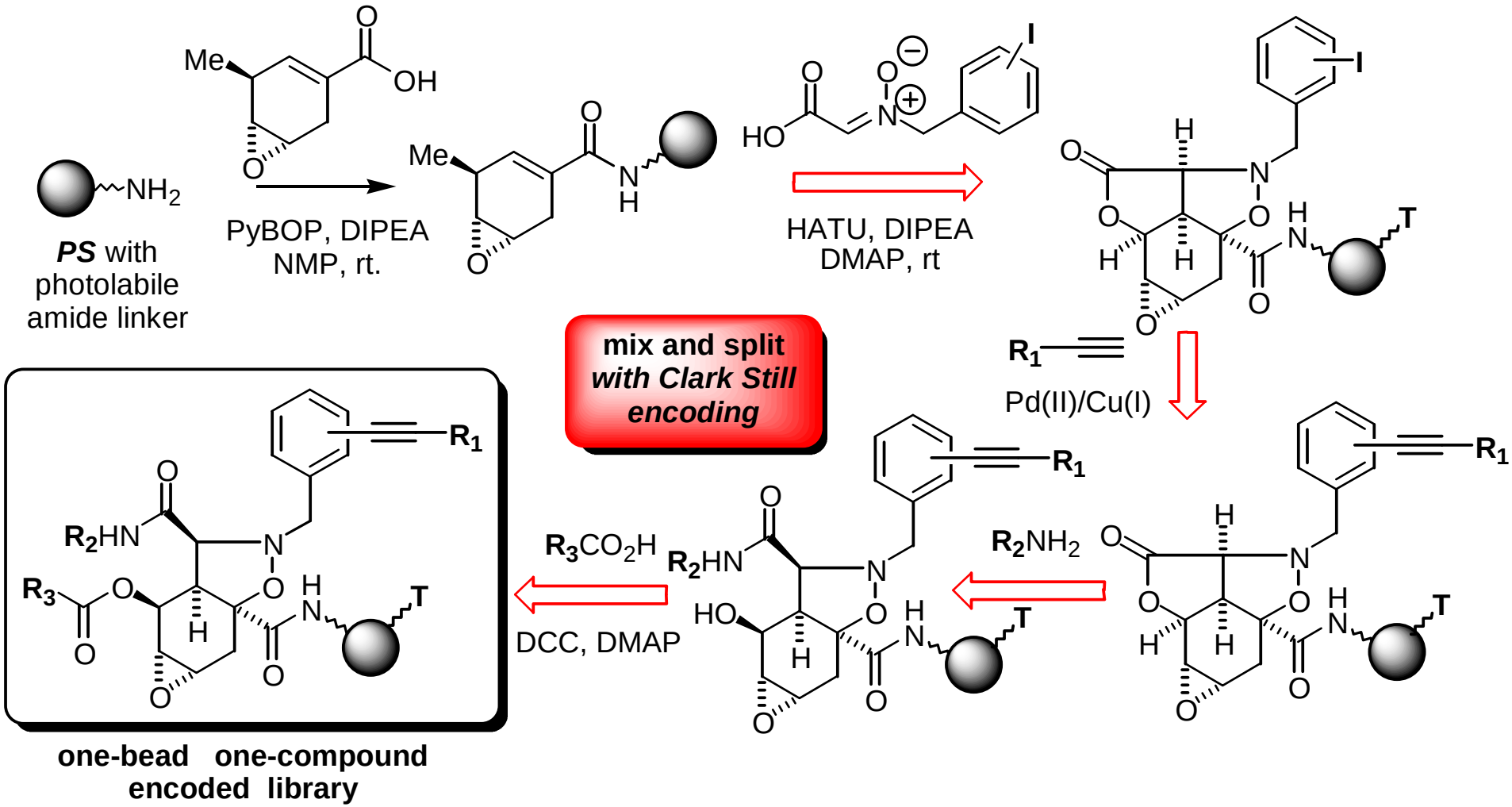

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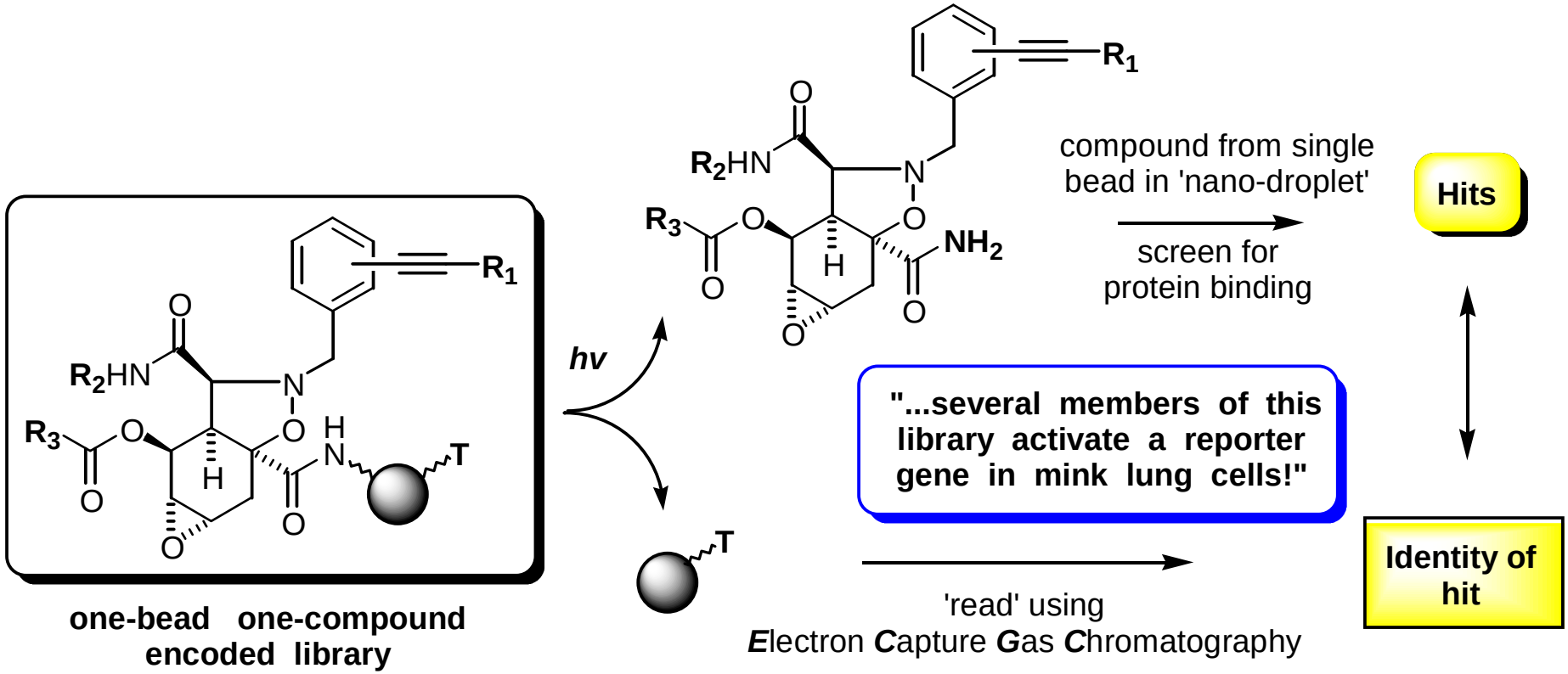

Nicolaou's sarcodictyin library

- Tubulin-microtubule disruptant library (template based).
- Nicolaou *J. Am. Chem. Soc.* **1998**, *120*, 10814.
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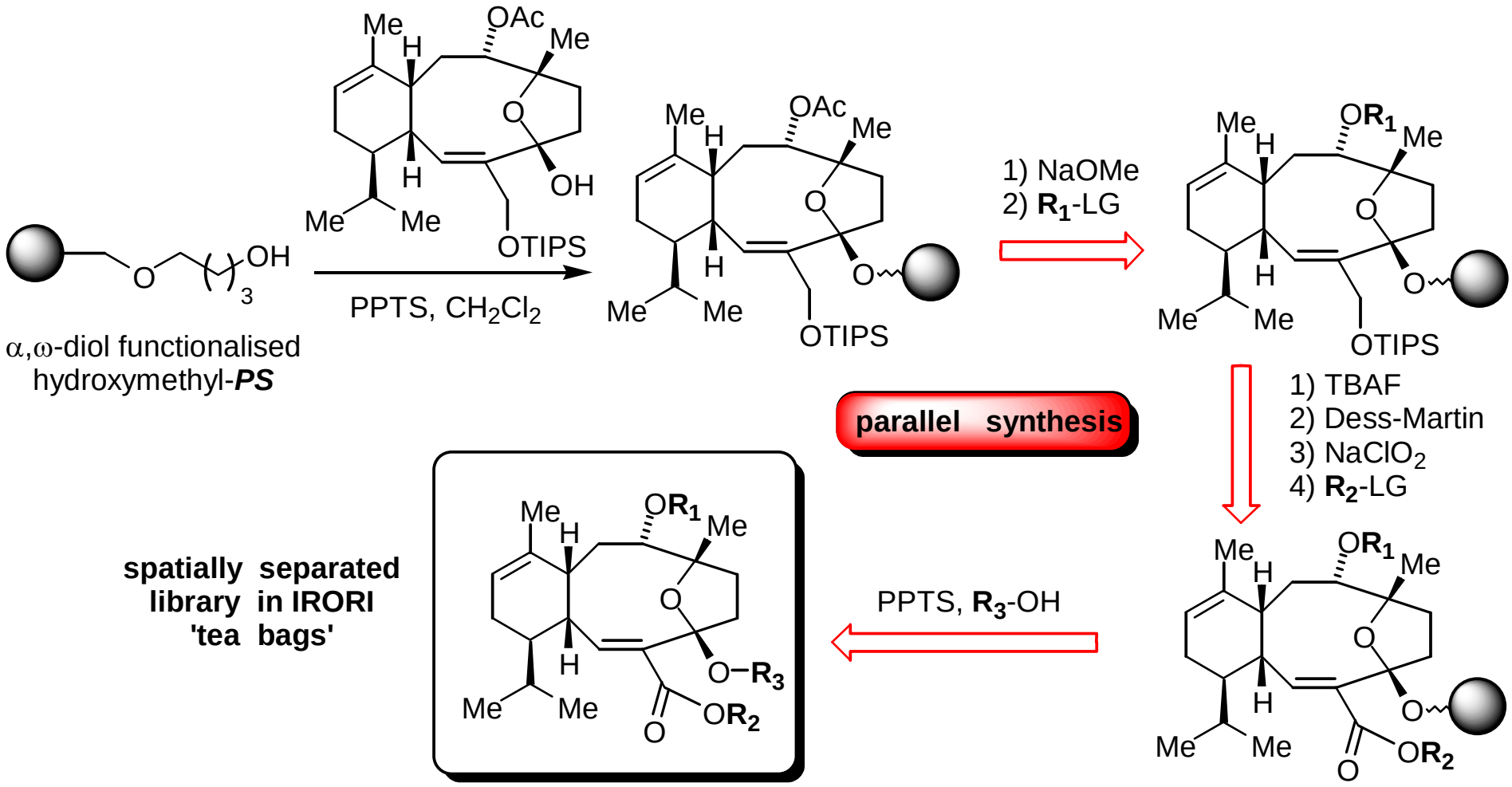

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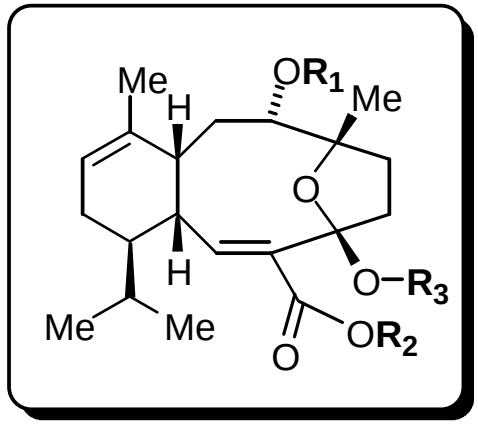
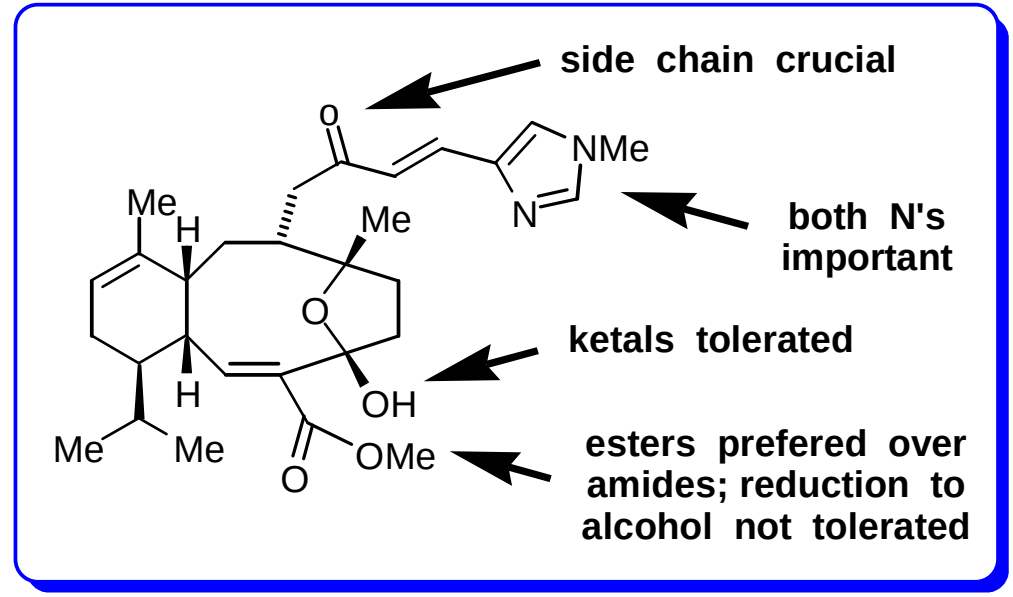


spatially separated library in IRORI 'tea bags'

screen for induction of tubulin polymerisation
and
cytotoxicity with ovarian cancer cells

Hits

identity defined by spatial location



DeWitt's quinolone library

- Ciprofloxazin analogue library (template based).
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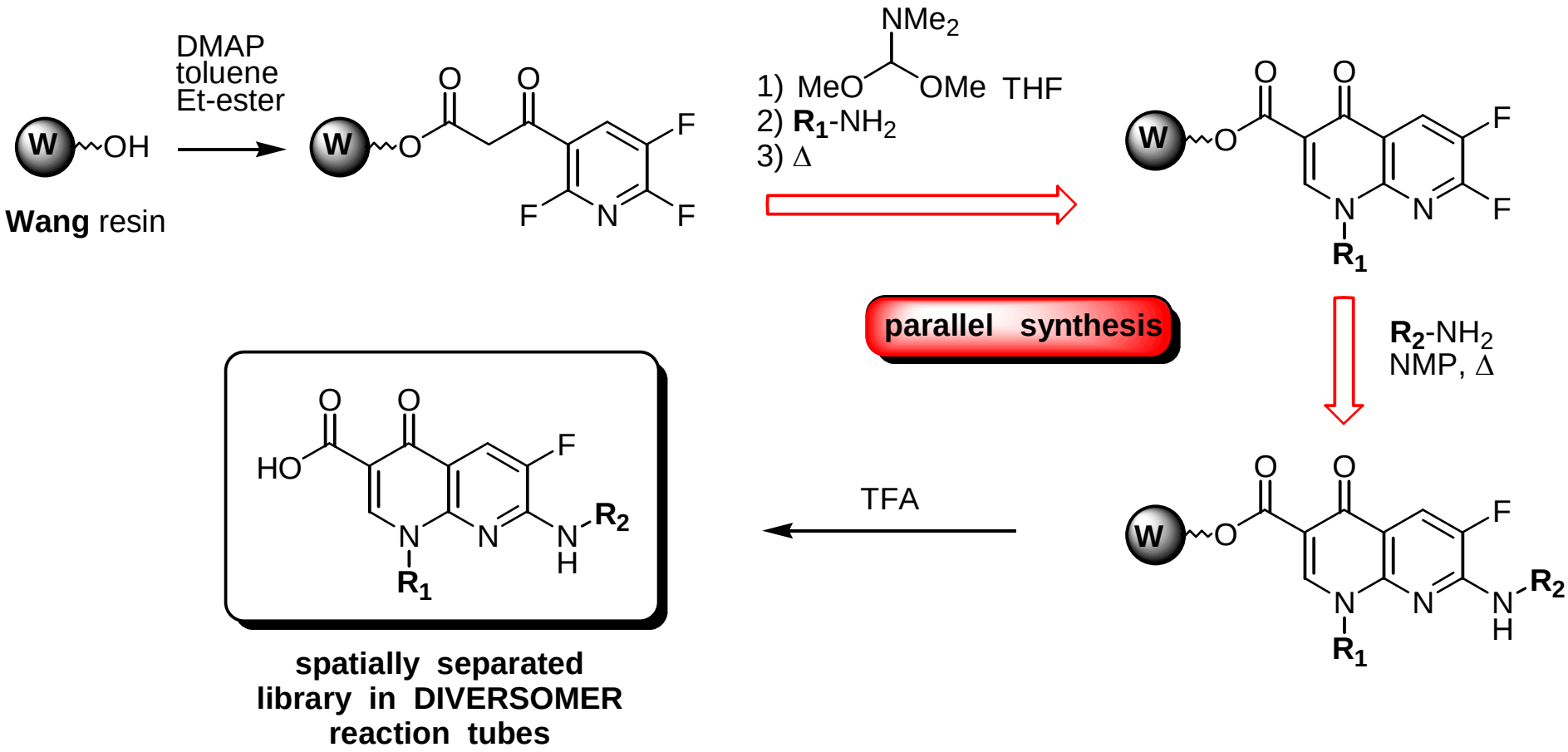

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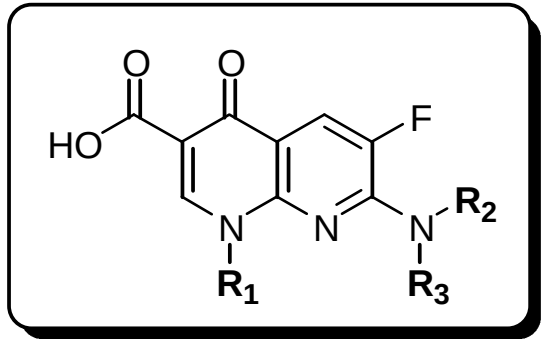

spatially separated library in DIVERSOMER reaction tubes

Screen for gyrase inhibition in solution

Hits

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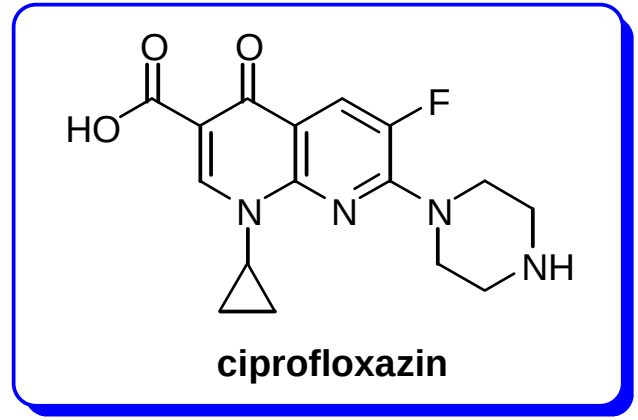

ciprofloxazin

Gallop's mercaptoacyl proline library

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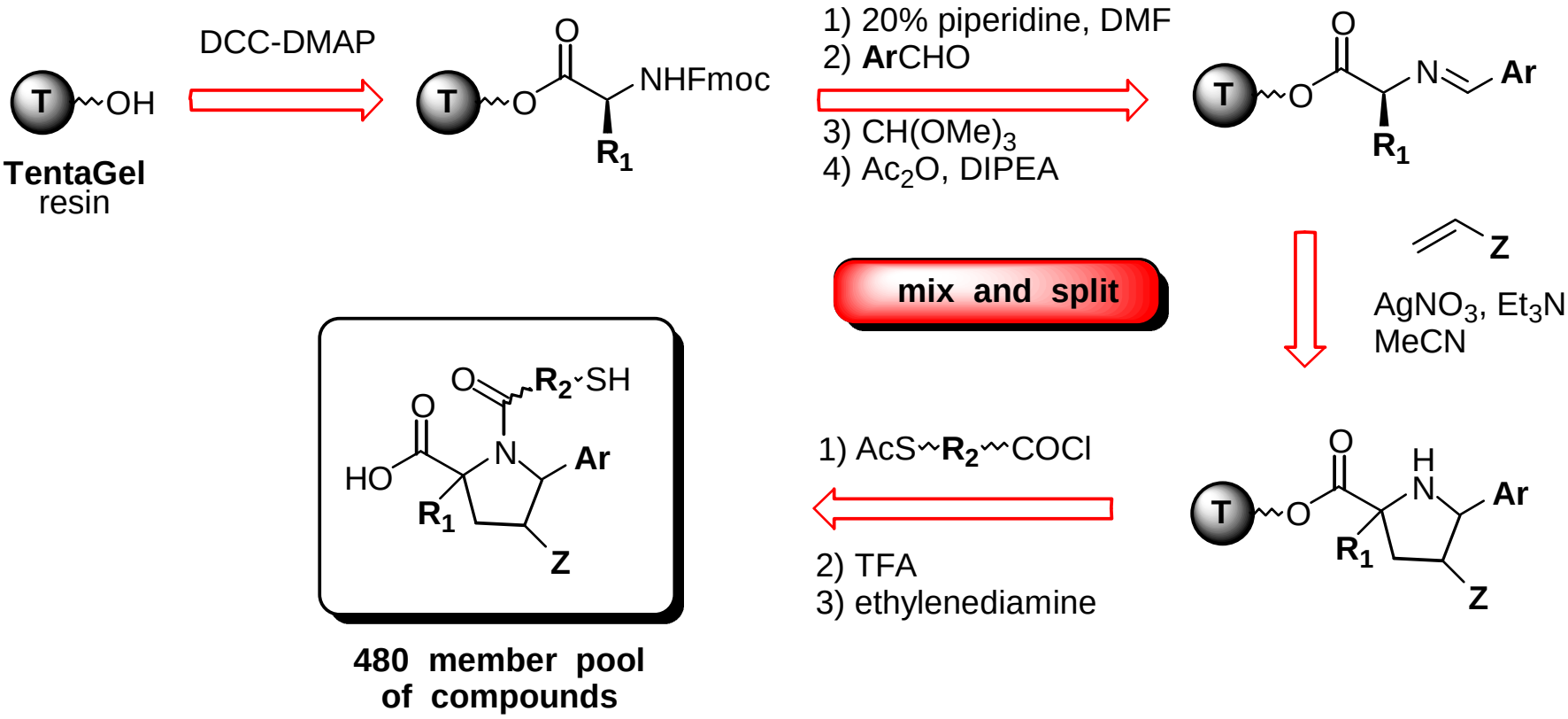

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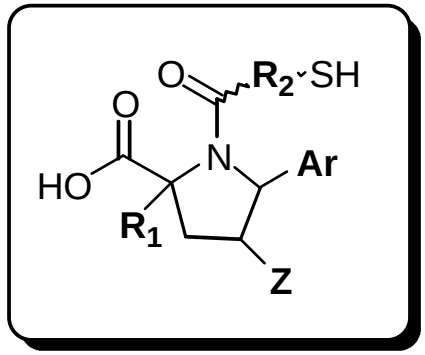
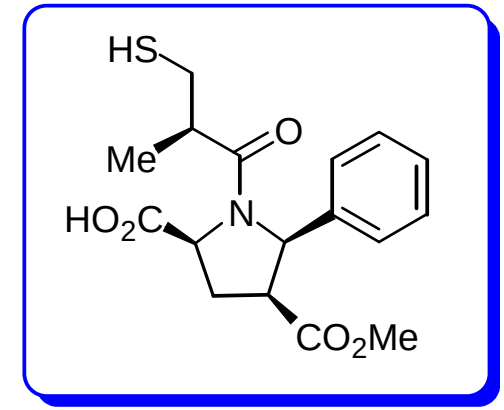


480 member pool of compounds

Screened for ACE inhibition

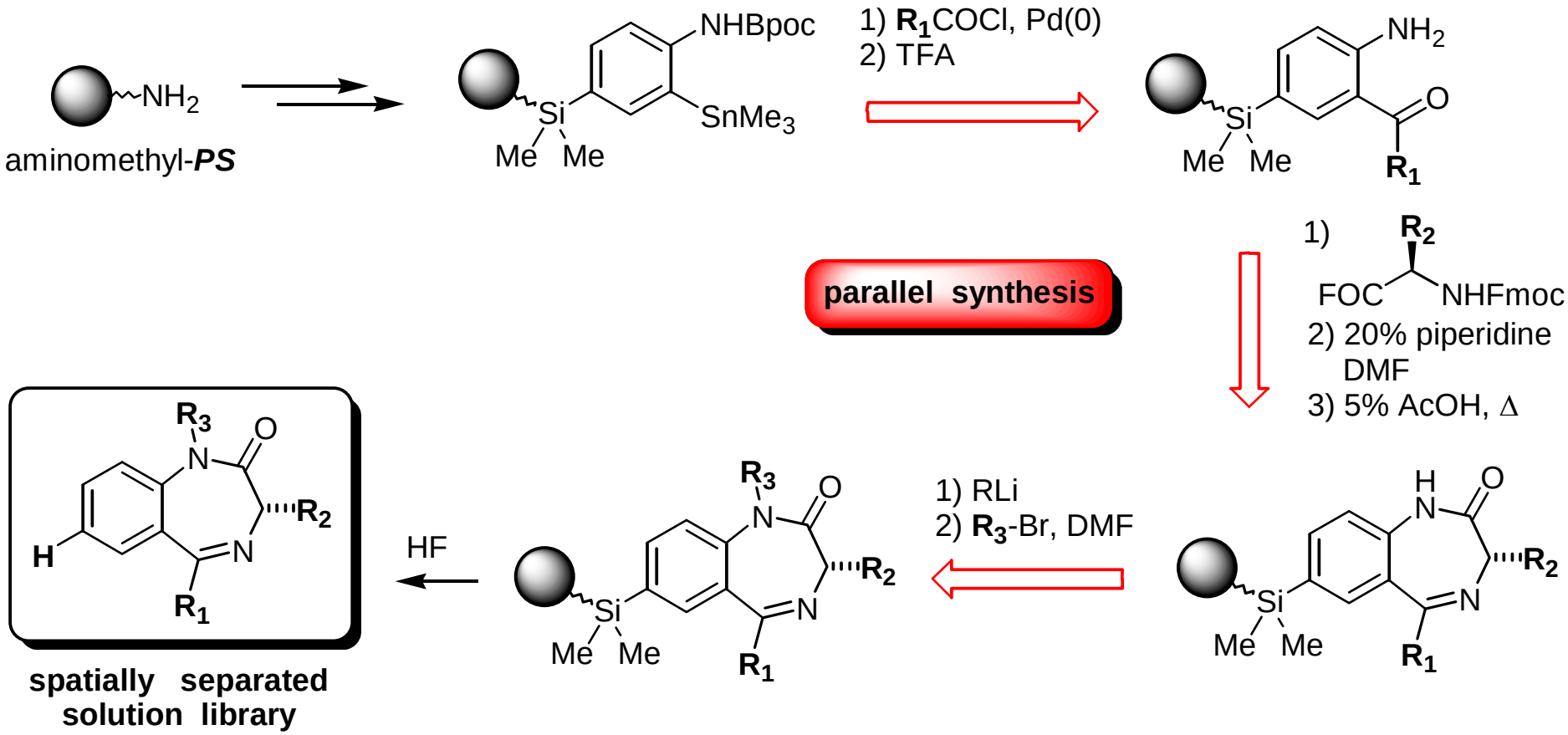
DECONVOLUTION
VIA FOUR ITERATIONS OF SUB-LIBRARY RE-SYNTHESIS AND SCREENING



K_i ~ 160pM
3 x more potent than captopril

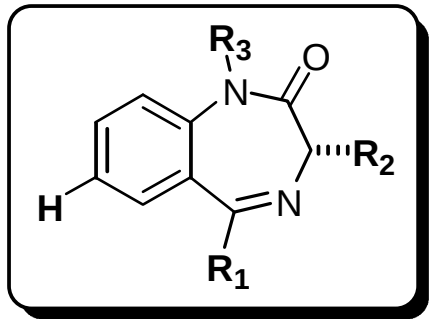
Ellman's benzodiazepine library

- Benzodiazepine library (template based).
- Ellman *J. Org. Chem.* **1997**, *62*, 2885.
- **Library synthesis:**



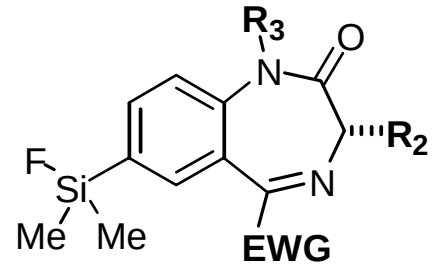
Ellman’s benzodiazepine library

- Benzodiaxepine library (template based).
- Ellman *J. Org. Chem.* **1997**, *62*, 2885.
- **Library screening:**



spatially separated solution library

screening of this library was not reported because it pertained that some of the compounds in the library still contained silicon due to an anomolous cleavage mechanism which was particularly troublesome when $\mathbf{R_1}$ was an electron withdrawing substituent



Summary

- What is combinatorial chemistry?
- The drug discovery process
- Approaches to combinatorial library synthesis:
  - mix and split synthesis
  - parallel synthesis
  - encoded tagging
- Library types:
  - oligomeric libraries
  - template based libraries
- Combinatorial drug discovery.