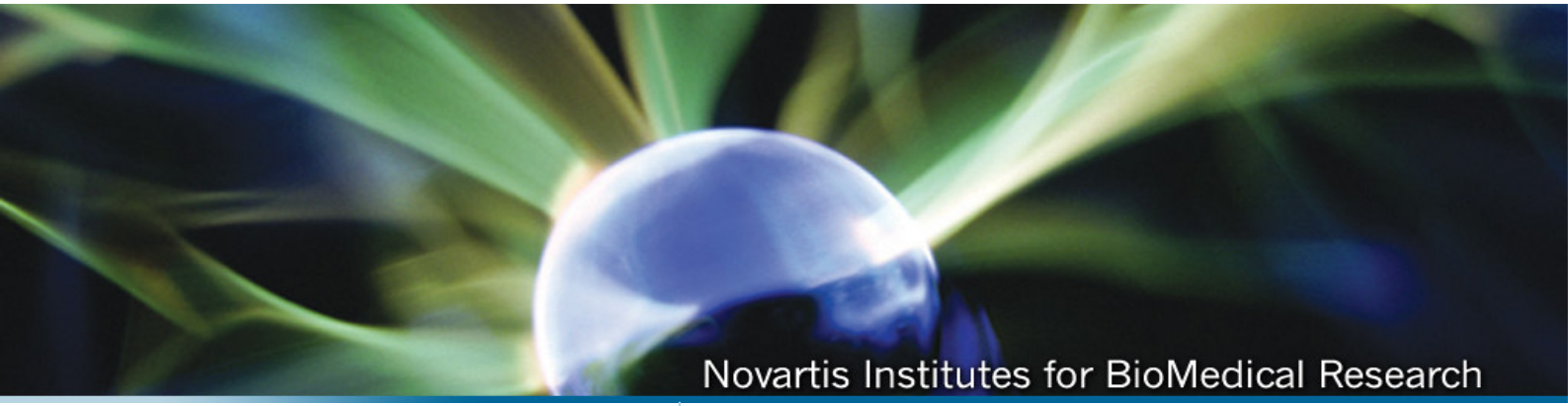
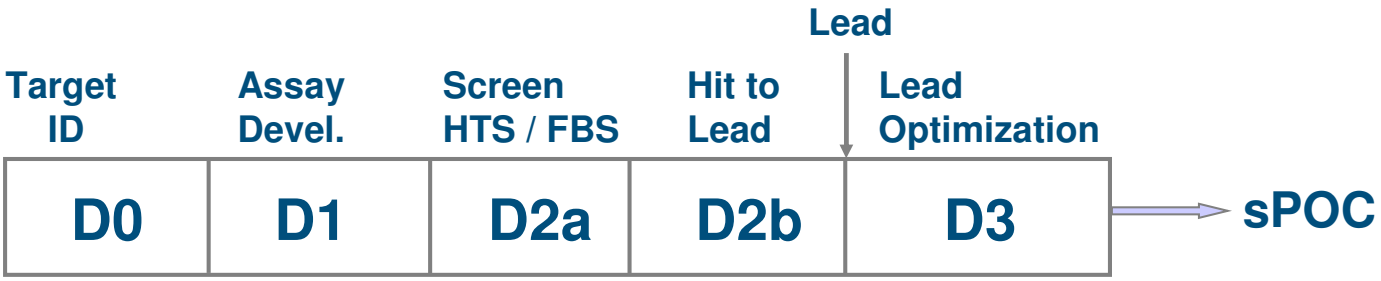


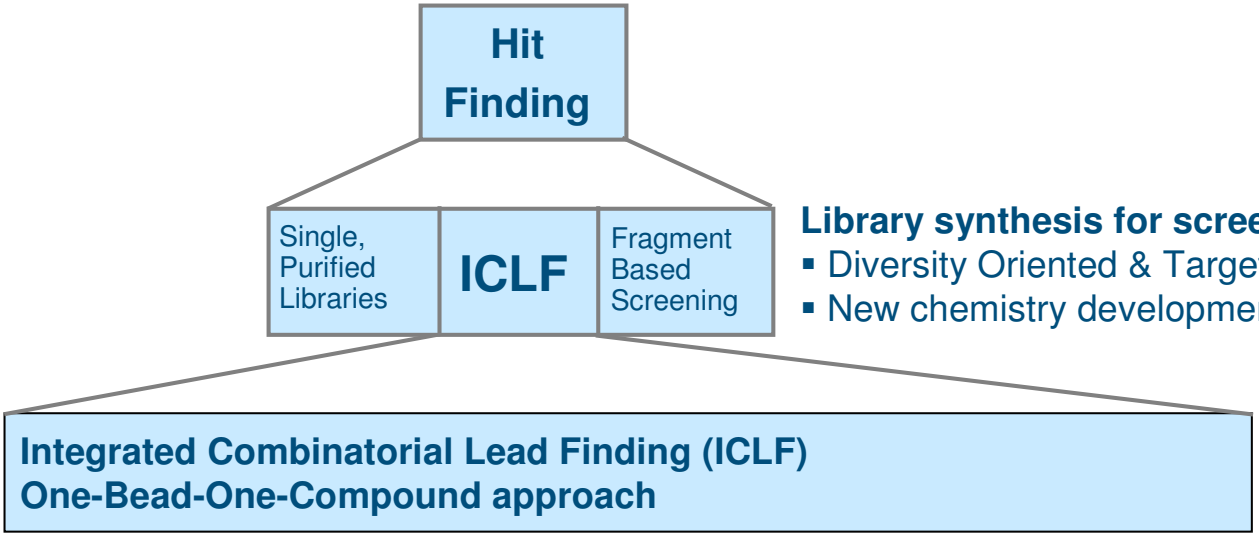
Expedite Hit Discovery: Split & Pool in the 21st Century

Dr. Andreas Marzinzik

NOVARTIS

Lead Finding in Drug Discovery

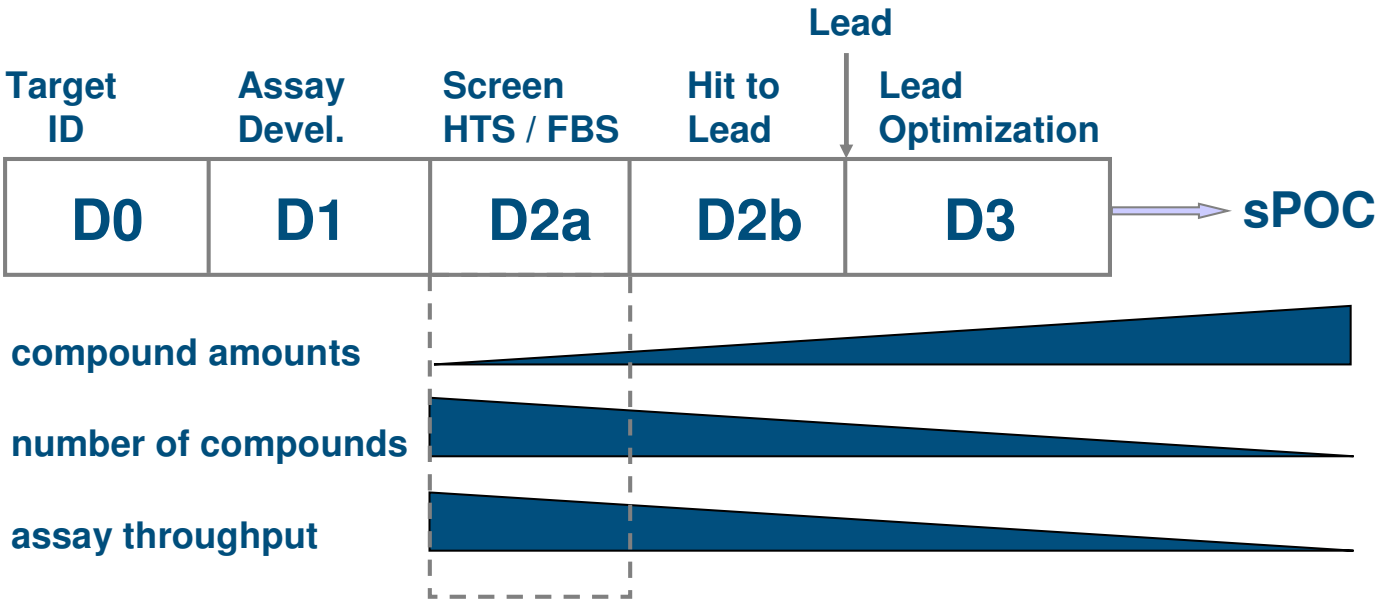




Library synthesis for screening:

- Diversity Oriented & Target Class
- New chemistry development

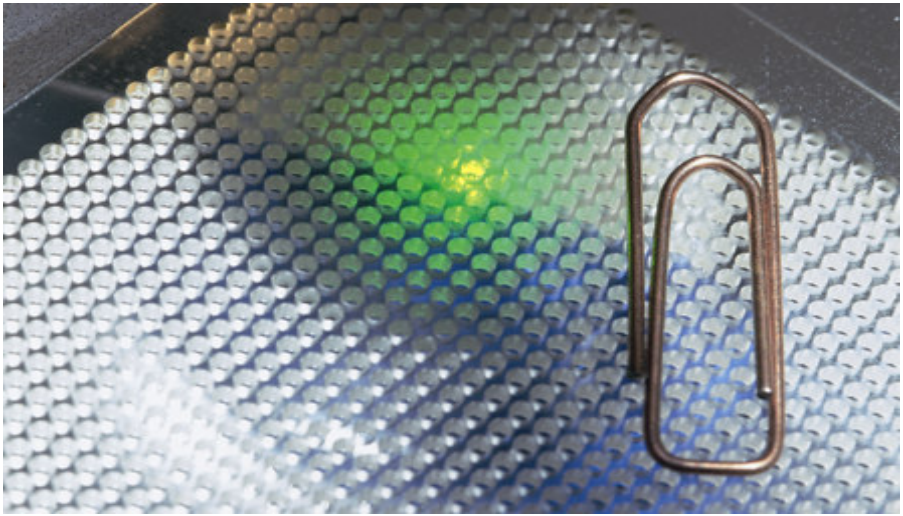
Lead Finding in Drug Discovery



Provide hits with modular structures amenable to rapid optimization by previously validated combinatorial chemistry

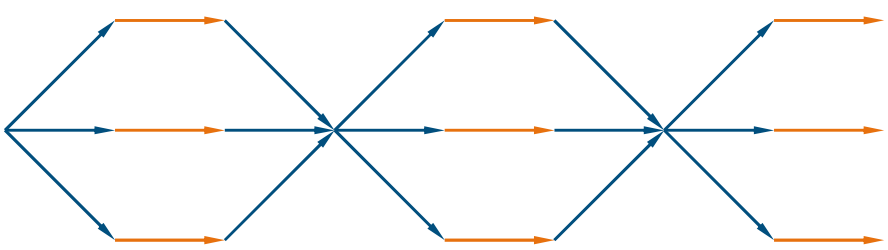
Assay Sensitivity and Throughput

UHTS Plate:	> 2000 wells / plate
Assay Volume:	**1-2 µl**
Microtiter Plate:	96 wells/ plate
Assay Volume:	200µl



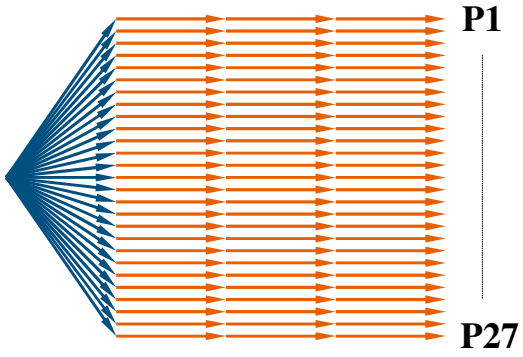
The Power of Split-Pool Synthesis

Split-and-Mix: 27 products in 9 synthesis steps



One-bead-one-cpd

Parallel Synthesis, 27 products in 81 synthesis steps

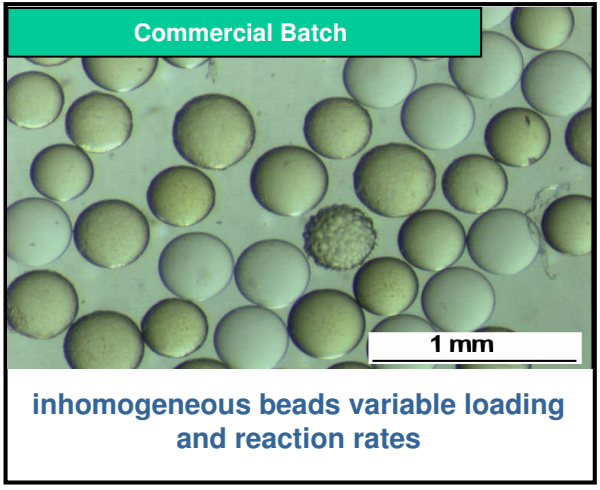


Synthesis step

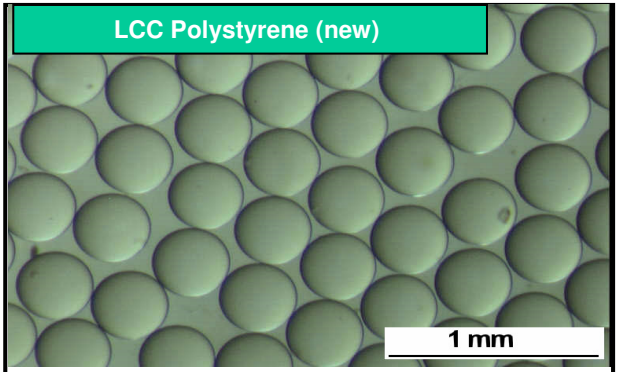
Mix/split

Monodispersed Beads

Requirements: we need a well defined reaction compartment



inhomogeneous beads variable loading and reaction rates



200 µm beads, S.D. ± 2%
1g appr. 225'000 beads

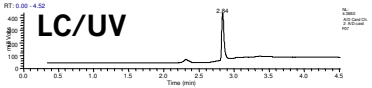
⇒ Challenge: how do we analyse reactions on beads?

Single Bead Analysis

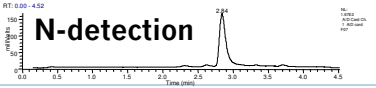
Off-bead LC/MS/N

Identity

Purity

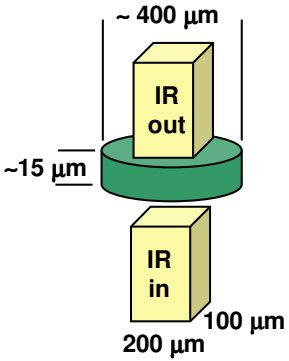


Quantity

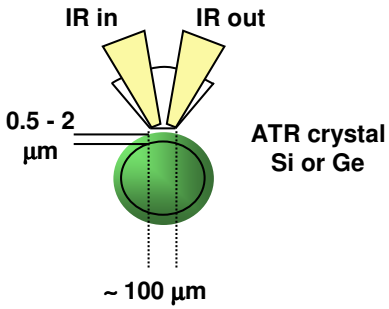


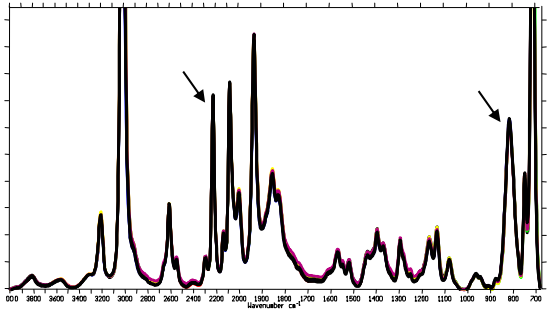
On-bead IR

Transmission



AT Reflectance

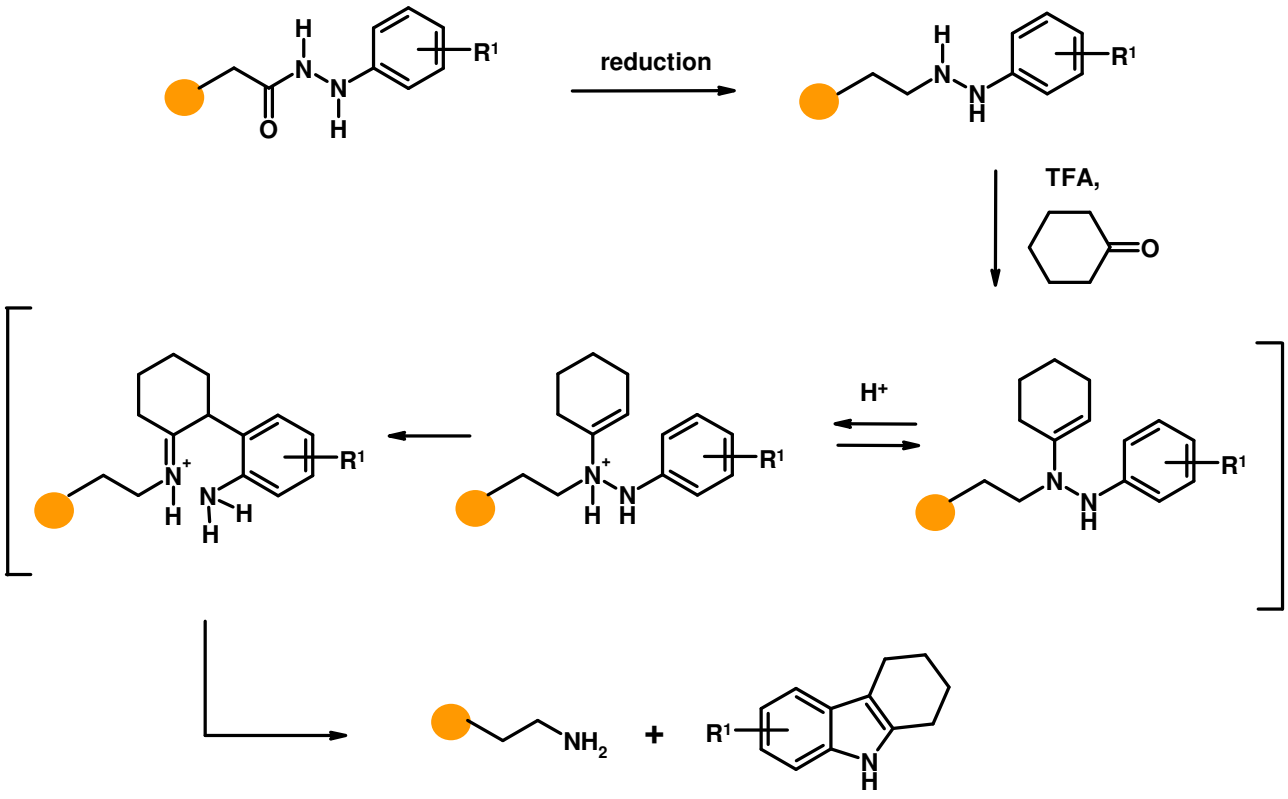




Library Design

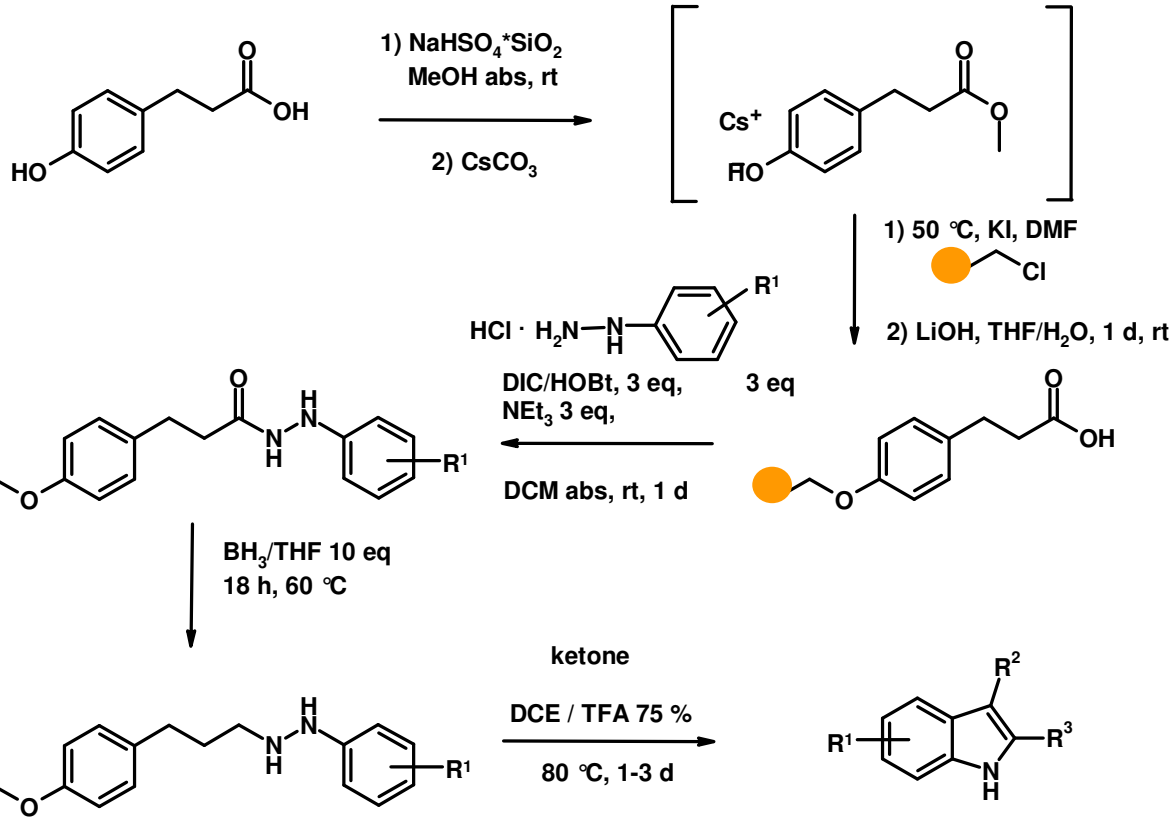
- **Medicinal chemistry aspects**
  physicochemical, potency, diversity, ADME/tox
- **Novelty**
- **Similarity to active compound classes**
  focus, complementarity
- **Modularity of the scaffold**
  BB availability, library size, feasibility…
- **Complexity of Chemistry**
  time of development, purity...

Fischer Indole Synthesis



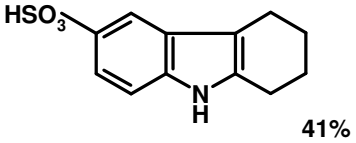
Rosenbaum C., Katzka C., Marzinzik A., Waldmann H. *Chem. Comm.* **2003**, *15*, 1822-1823.

Traceless Fischer Indole Synthesis

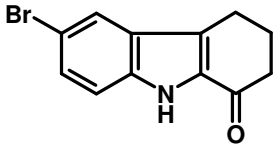


Rosenbaum C., Katzka C., Marzinzik A., Waldmann H. *Chem. Comm.* **2003**, *15*, 1822-1823.

Results of Fischer Indole Synthesis



41%



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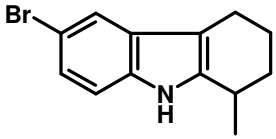

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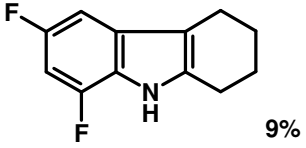
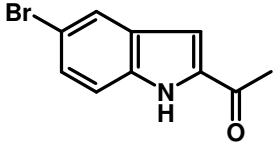
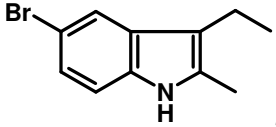


9%



39%



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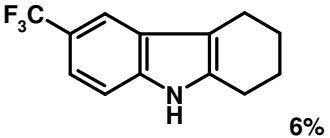

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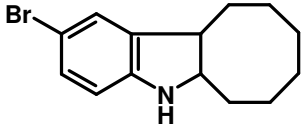

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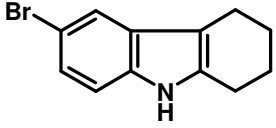

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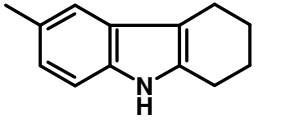

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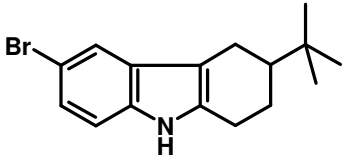
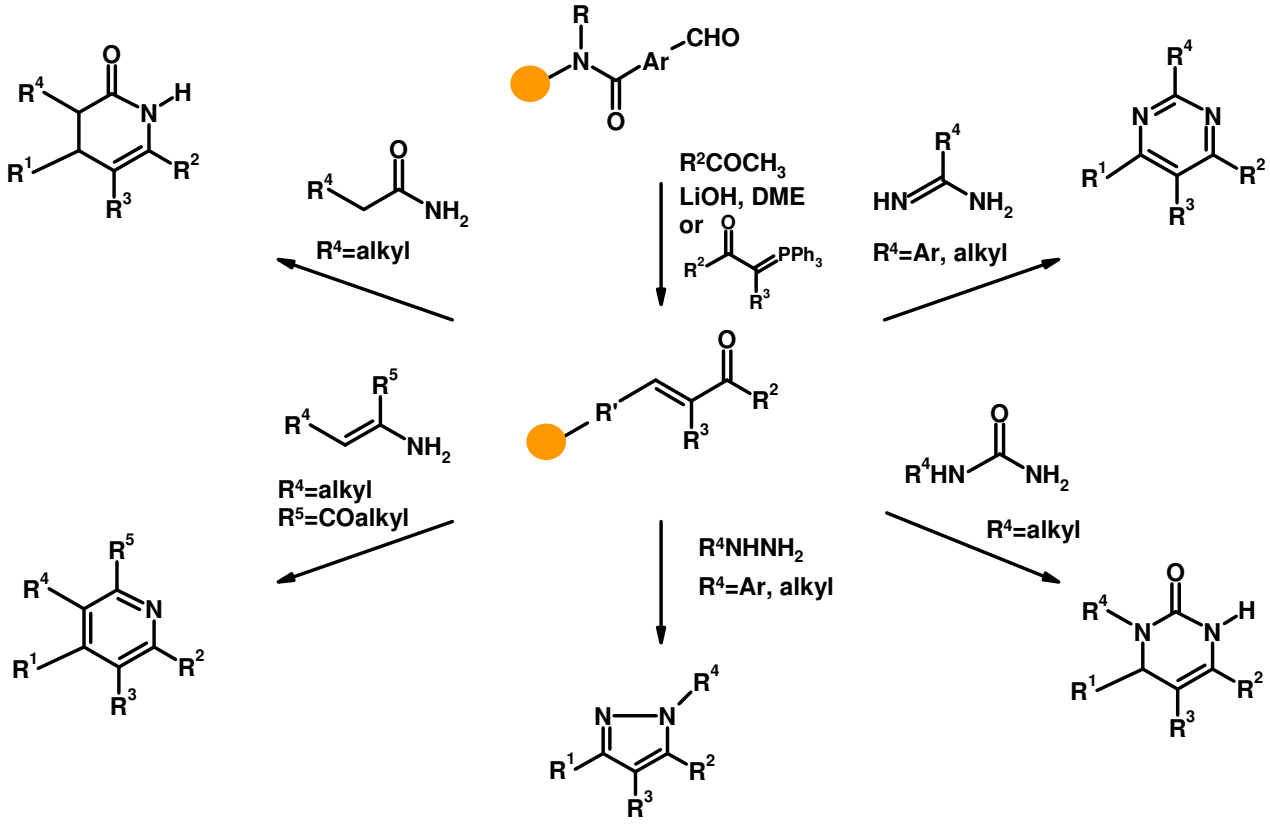


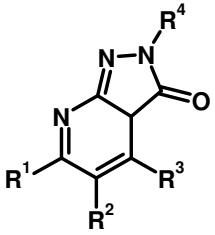
24%

Rosenbaum C., Katzka C., Marzinzik A., Waldmann H. *Chem. Comm.* **2003**, *15*, 1822-1823.

Diversity Platform

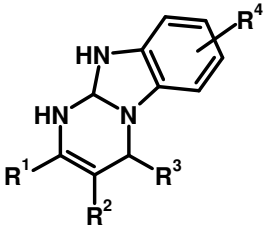


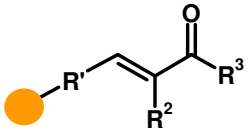
Annelated Heterocycles



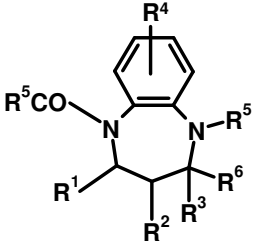
3-amino-5-hydroxypyrazole
(R^5 = H), NaOEt
DMSO, rt, 16 h

2-aminobenzimidazole
NaOEt, DMSO, rt, 16 h

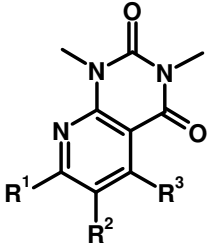




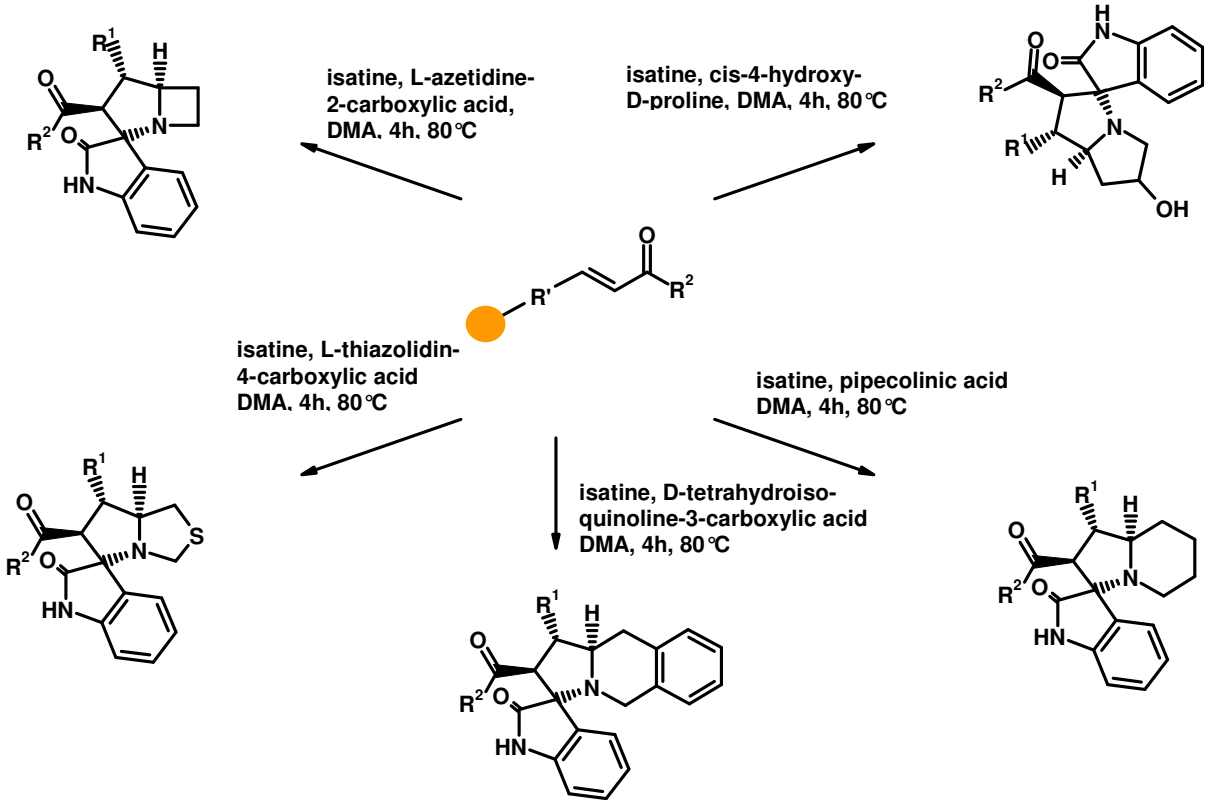
several
steps



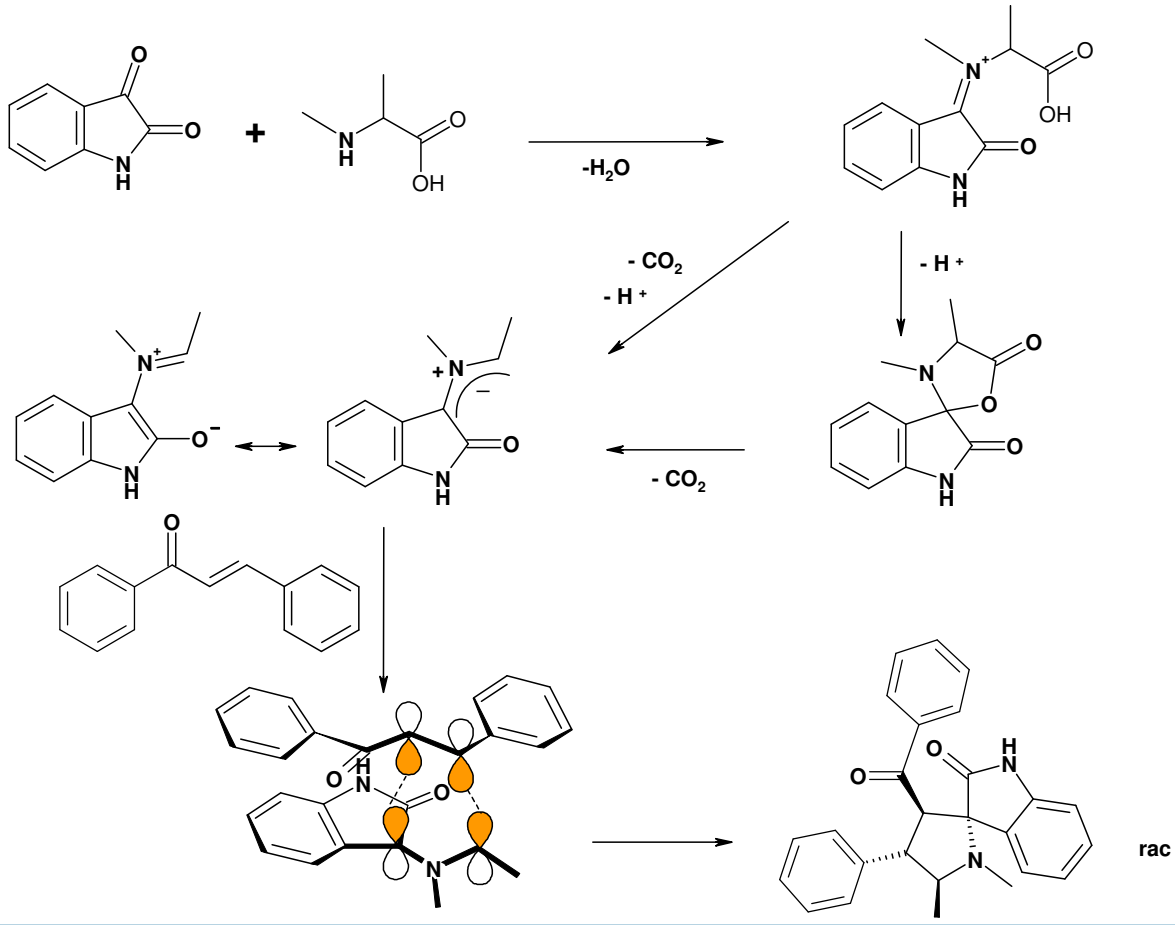
6-amino-1,3-dimethyl-
uracil , O_2, DMA, 16h, rt



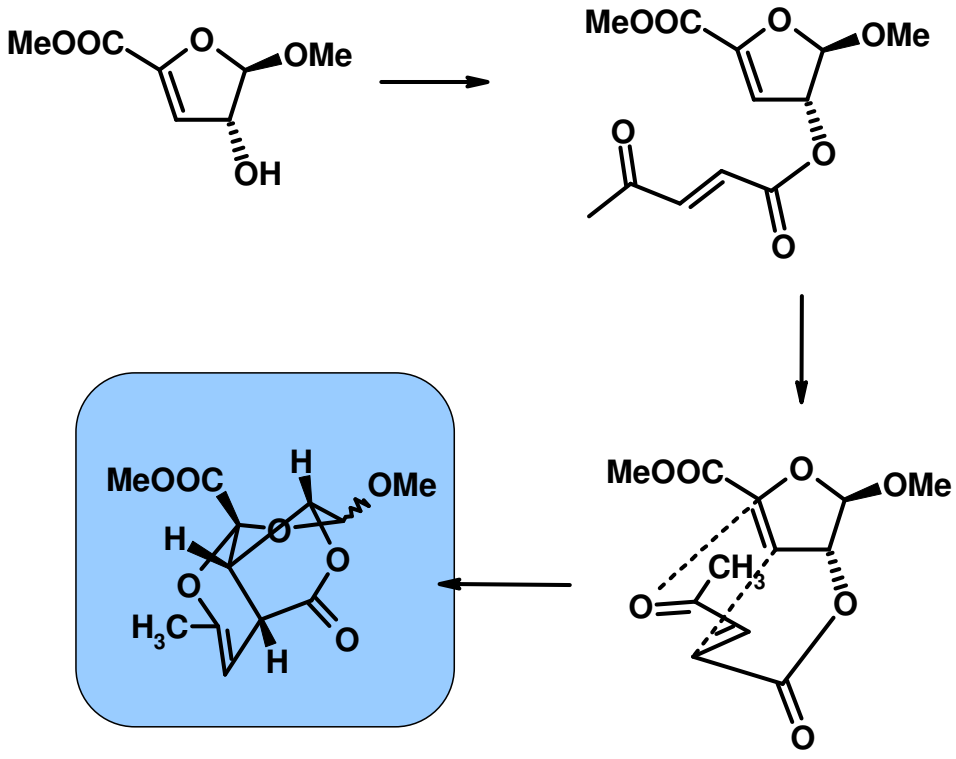
Diversity Oriented Synthesis



1,3 Dipolar Cycladdition - Mechanism

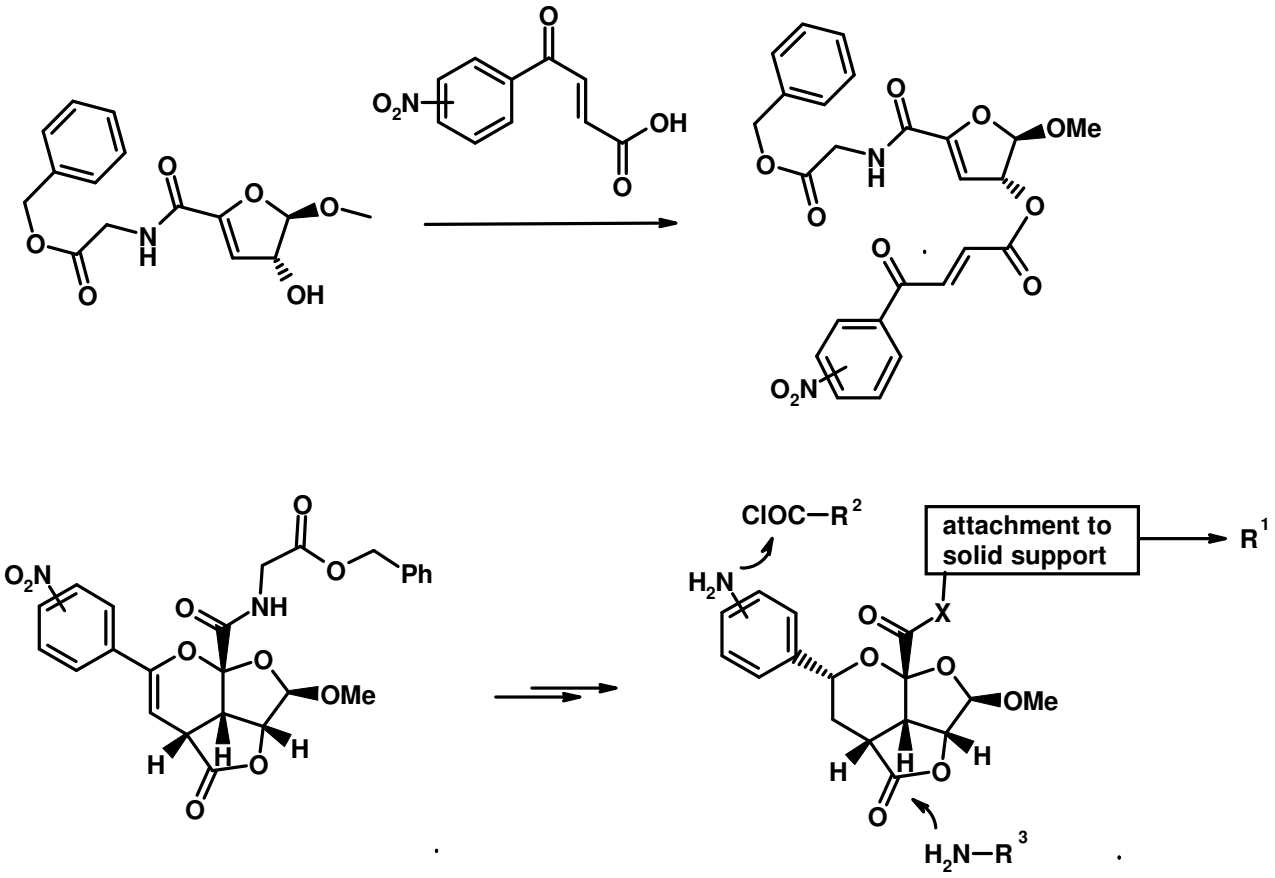


Natural Product-like Scaffold



Hetero-Diels-Alder

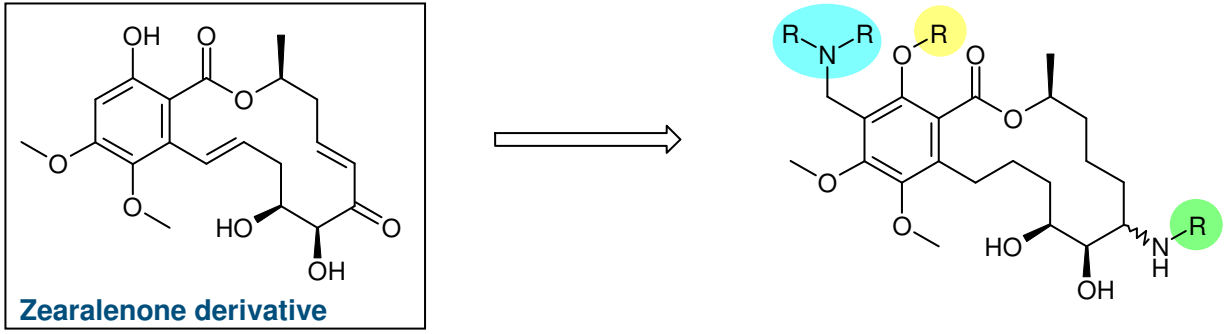
Synthesis of the Scaffold



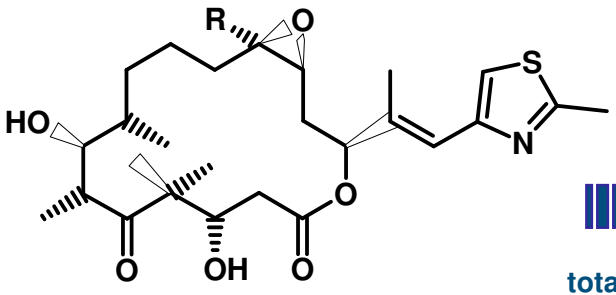
Messer R., Pelle X. , Marzinzik A. L., Lehmann H., Zimmermann J., Häner R. *Synlett* **2005**, in press.

Libraries from Natural Products

- NP have played a considerable part in the exploration and development of new drugs
- The hit-rate of pure natural compounds is higher than that of synthetic compounds
- Natural products provide unique chemical diversity, distinct from that found in the majority of synthetic libraries
- Biologically active natural products were evolutionarily selected and validated for binding to particular protein domains. Therefore such natural products are interesting starting points for library development



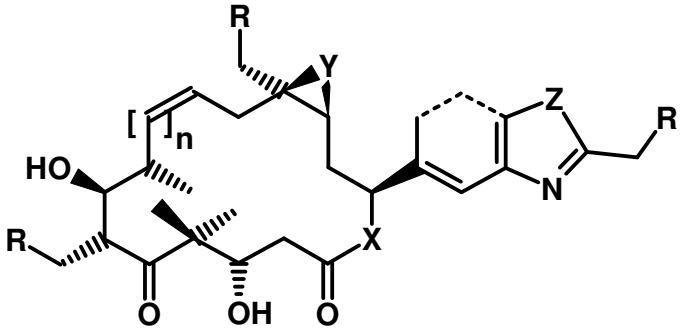
Libraries from Natural Products

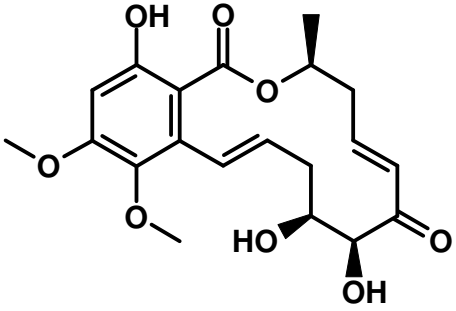


Epothilone B

LO

total synthesis,
semi-synthesis

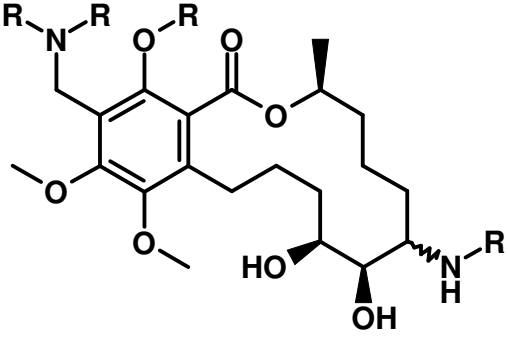




Zearalenone derivative

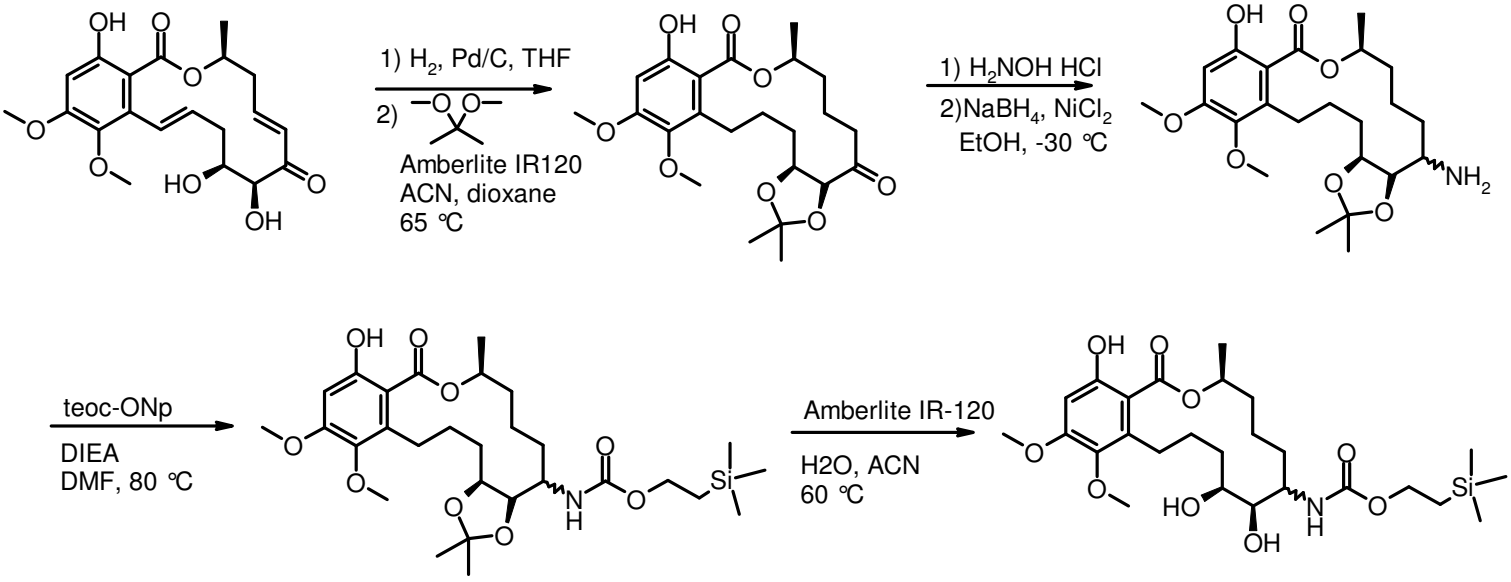
LF

semi-synthesis



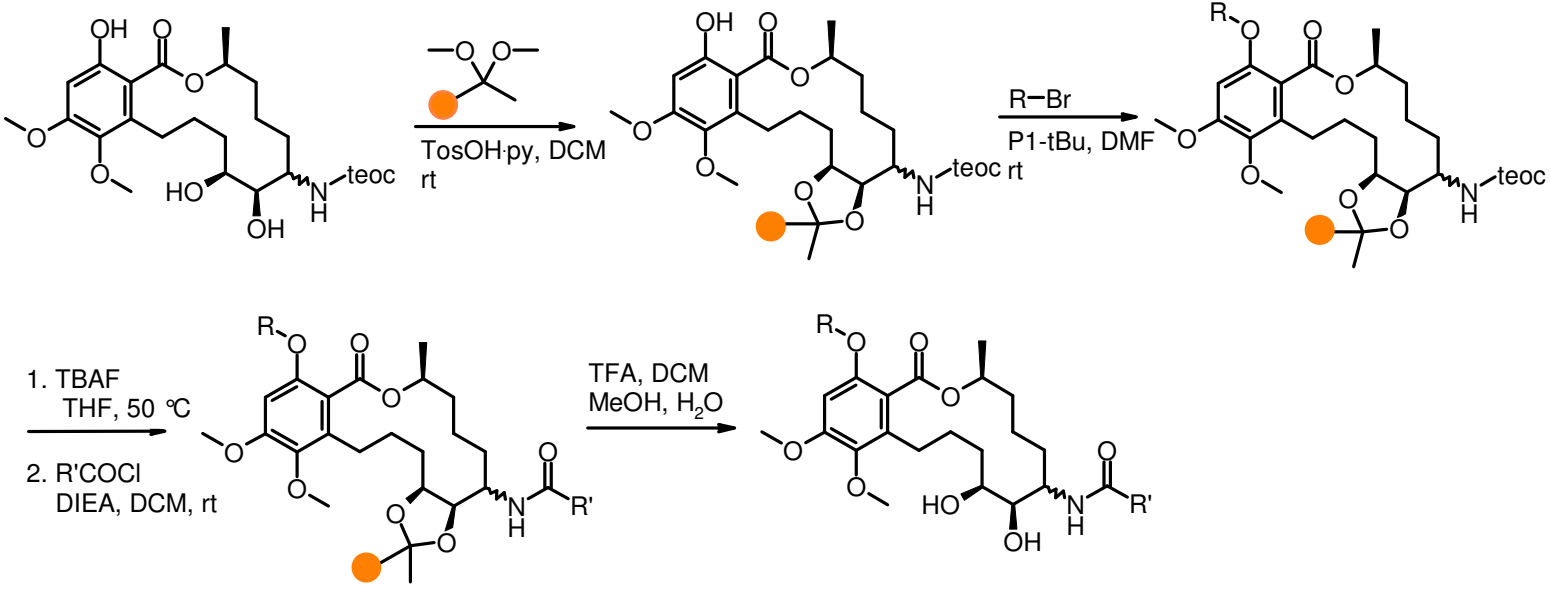
Cachoux F., Isarno T., Wartmann M., Altmann K.-H. *Angew. Chem., Int. Ed.* **2005**, *44*, 7469

Template Generation



Grosche, P; Akyel, K. G.; Marzinzik, A. L. *Synthesis* **2005**, *12*, 2015

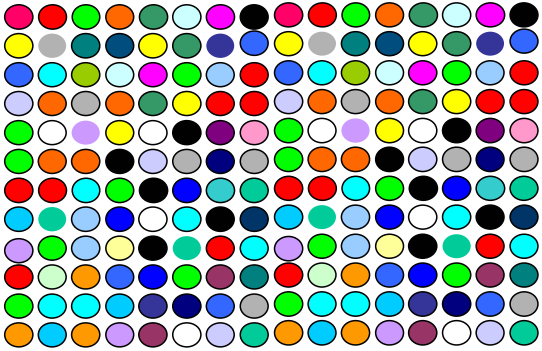
Zearalenone-derived Library



⇒ Challenge: how do we identify active cpds from split-pool synthesis?

Split & Pool Synthesis

Split & pool synthesis:



Sizeable amounts / well

One bead / well

Mixture

- Amount: as much as you want
- Screening of mixtures

\+ save of screening cost

\- no SAR, “mixtures problems”

Single Compound

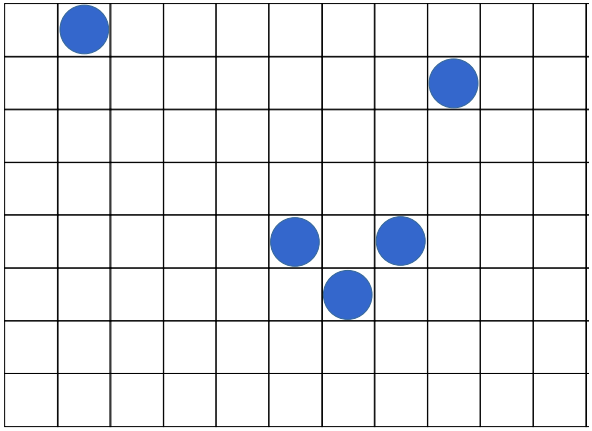
- Amount 1-30 nMol
- Deconvolution
- Screening with HT formats

\+ no false positives, SAR possible

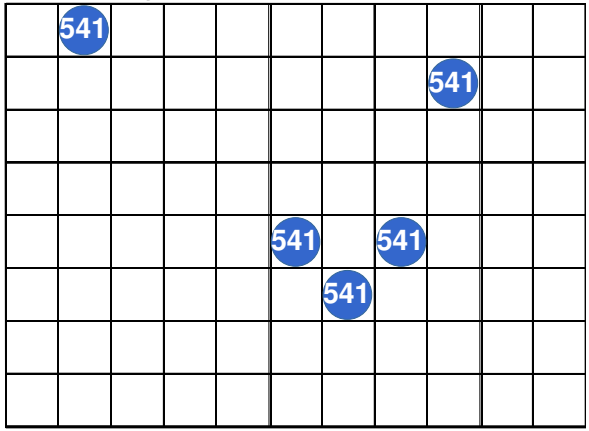
\- redundancy, amount

Success in HTS Assay

Activity profile



MS analysis



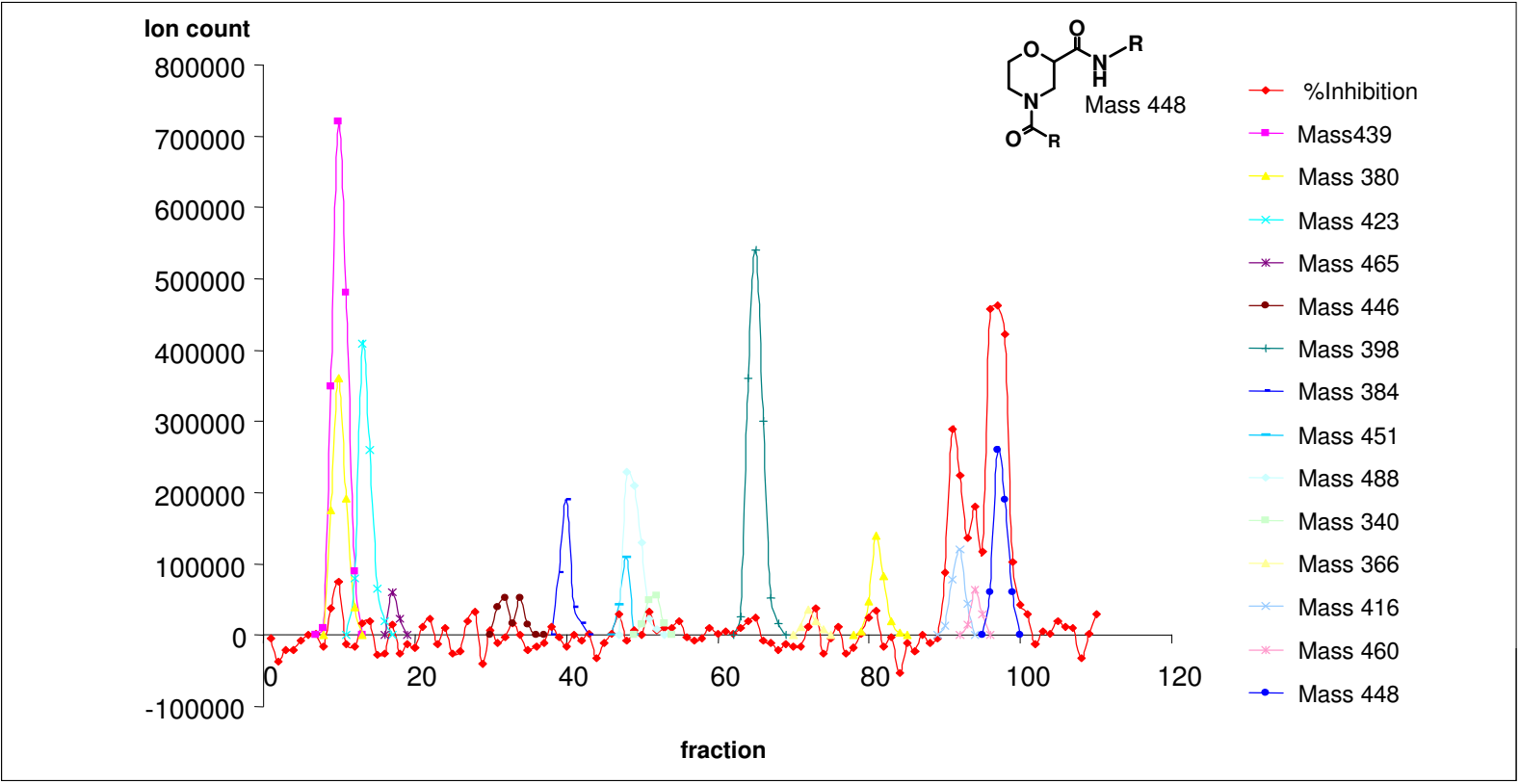
Circle diameters correspond to inhibition

● **>80% inhibition**

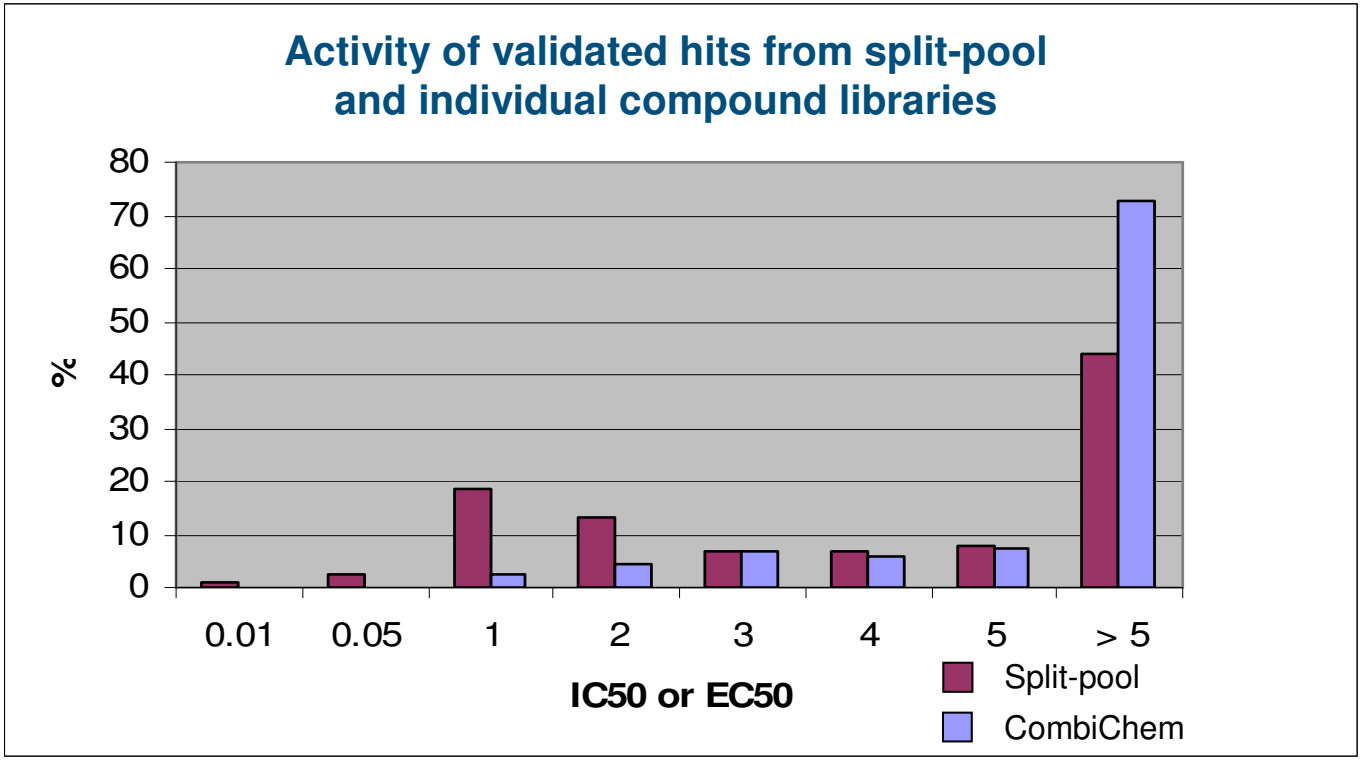
Numbers correspond to M^+

541	448	458	461	477
510	517	530	531	537

FLIPR-Screen: Fractionation Data



Activity of Hits from Single Beads



Analysis by F. Stoll, CADD

The Result

- Proprietary compounds
- Complex chemical structures
- High diversity
- High quality
- Better success-rate than “single” compounds from CC
- Fast follow-up
- Which compound will give the next success story?

Acknowledgements

Chemistry/Technology

Fred Berst
Werner Breitenstein
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Markus Vögtle
Jürg Zimmermann

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Faouria Nassur
Xavier Pelle
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Erich Spieser