



Harvard Institute of Chemistry and Cell Biology (ICCB)
The Initiative For Chemical Genomics (ICG)

*Diversity-Oriented Synthesis (DOS):
A Platform for Discovery in Chemical Genetics*

Jared Shaw – Institute Fellow (ICCB)

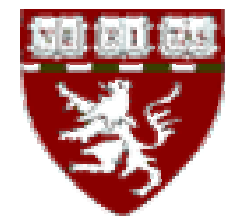
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<http://chembank.med.harvard.edu>

http://iccb.med.harvard.edu/chemistry/shaw_group/index.htm



Harvard Institute of Chemistry and Cell Biology

The Initiative For Chemical Genomics

- **Diverse Goals of the ICCB**
 - Chemistry - To bring the power of modern synthetic chemistry to bear on problems in Cell Biology.
 - *Diversity-oriented, split-pool synthesis*
 - Biology - To increase our understanding of fundamental mechanism in cell biology.
 - *Via Screening broadly for activities/phenotypes.*

Information Analysis enables both of the above.
(this powers our iterative approach)

Key Enabling Technologies of the ICCB

Chemical Synthesis platform - flexible, economical, scalable, integrated,

enables split-pool diversity-oriented synthesis (DOS)

Screening program - economical, high throughput and flexible.

Informatics- system that is essentially open for all.

ChemBank - fully QC'd central repository of data, technology, how do this, etc...

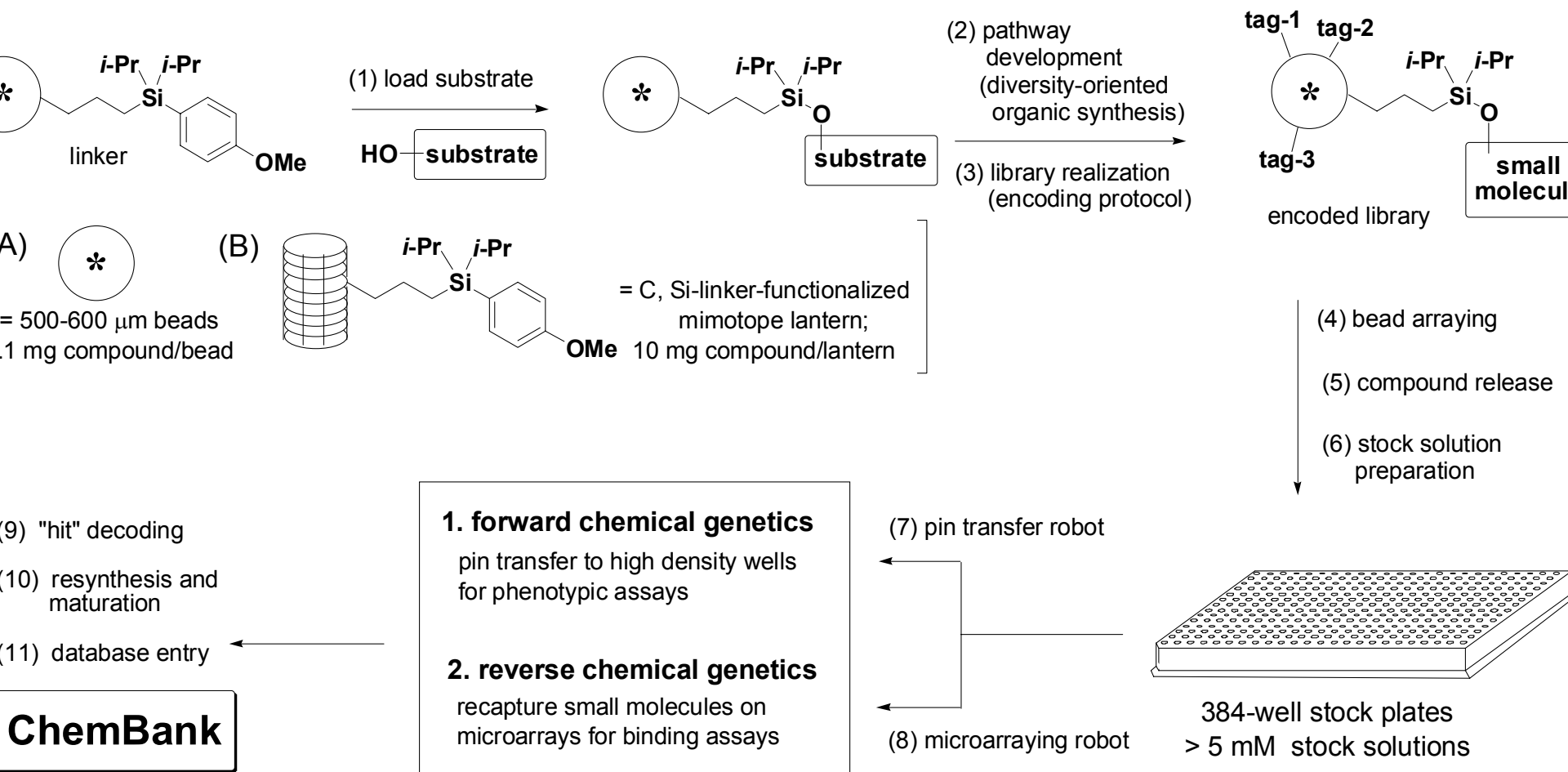
Goals

accomplished economically, efficiently in an academic setting.

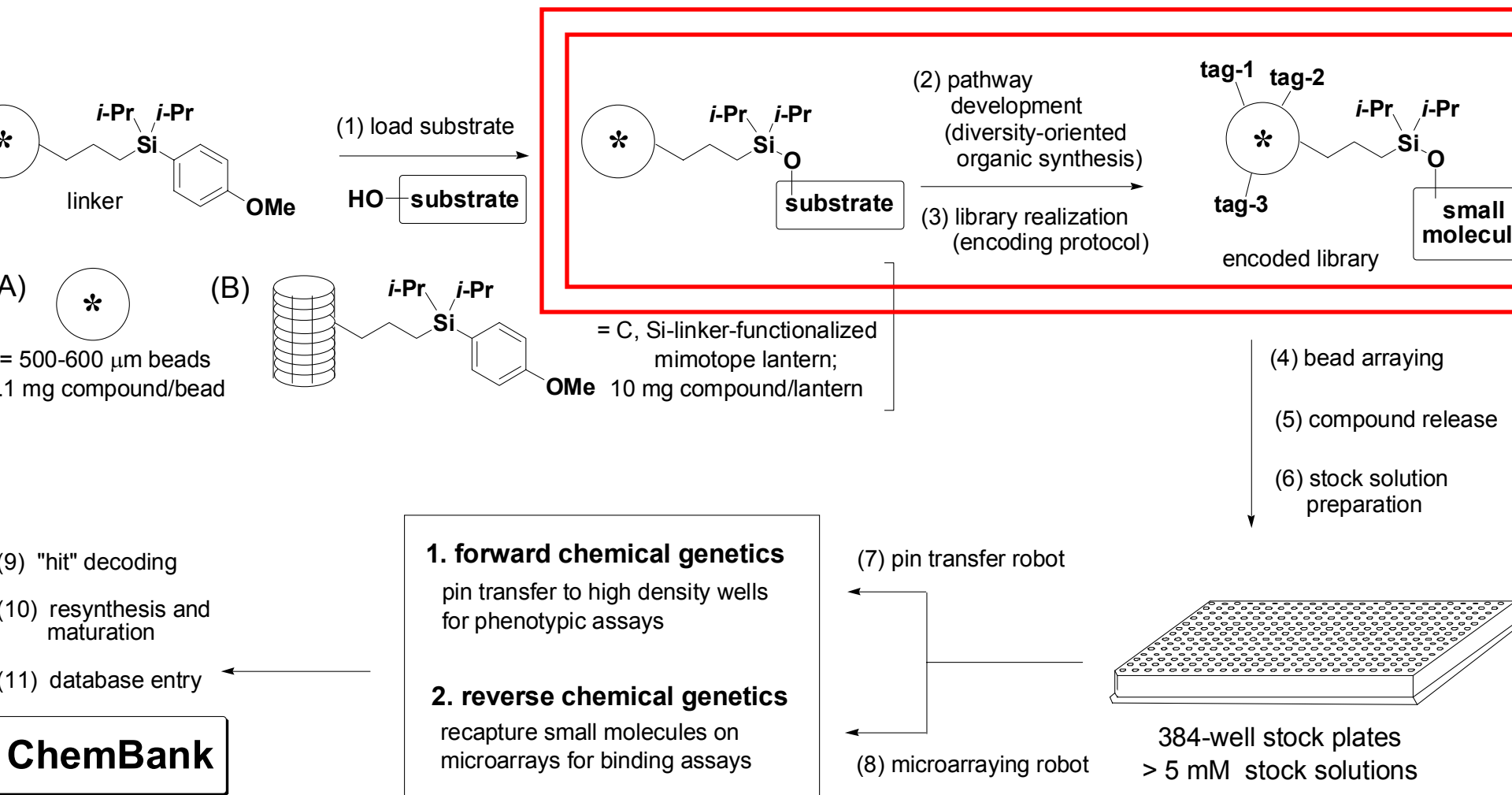
Portable and Publicly accessible

We are not trying to discover drugs.

A technology platform for diversity-oriented synthesis: One bead-one stock solution (Version 2.0)



A technology platform for diversity-oriented synthesis: One bead-one stock solution (Version 2.0)

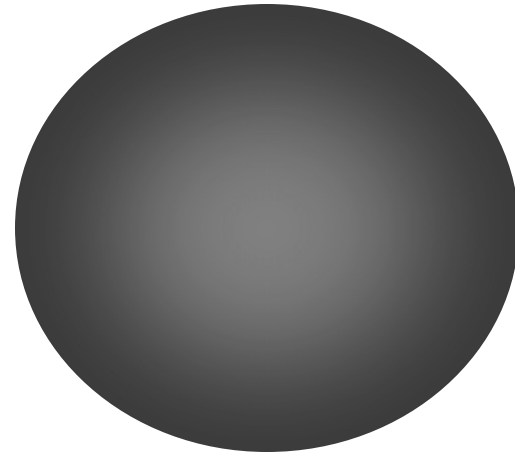


How we got to version 2.0...



80 µm tentagel
100 pmol/bead

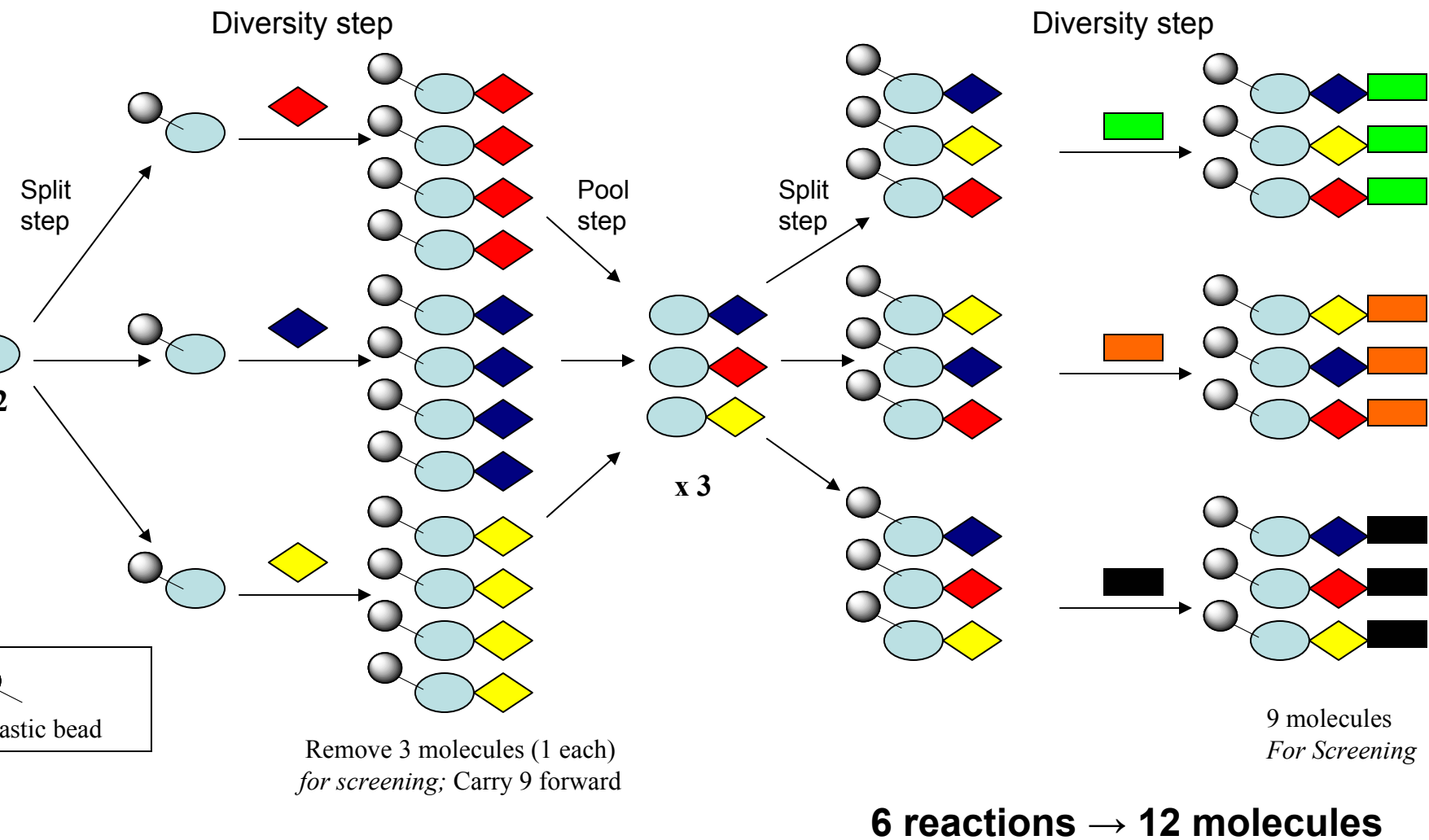
- *Not a robust/efficient Linker system*
- *Arraying beads problematic*
- *Enough for a **single** assay.*
- *Must resynthesize every molecule!*



600 µm polystyrene
minimally 50 nmol/bead

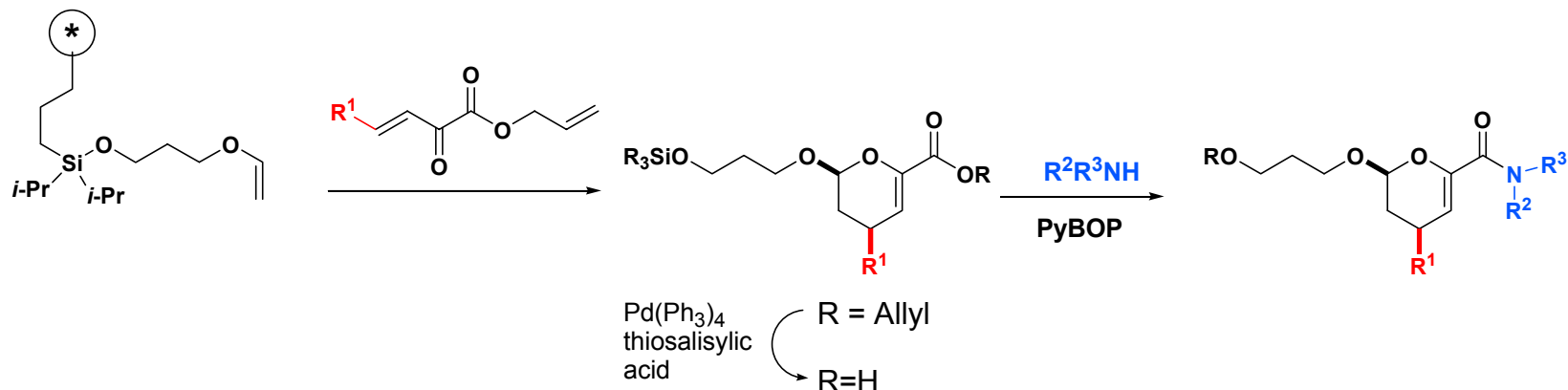
- *Silicon-based Linker system*
- *HF/pyr cleavage system*
- *Single Bead per well arrayer*
- *Arrayed stock DMSO solutions (~ 4 mM)*
- *Enough material for 'cherry-picking'*
- *Realistically, ~ 130 nmol/bead*

Split-Pool Synthesis



Most Libraries to Date are “Mono-skeletal”

The dihydropyran carboxamide (DHPC) library: Stavenger & Schreiber 2001



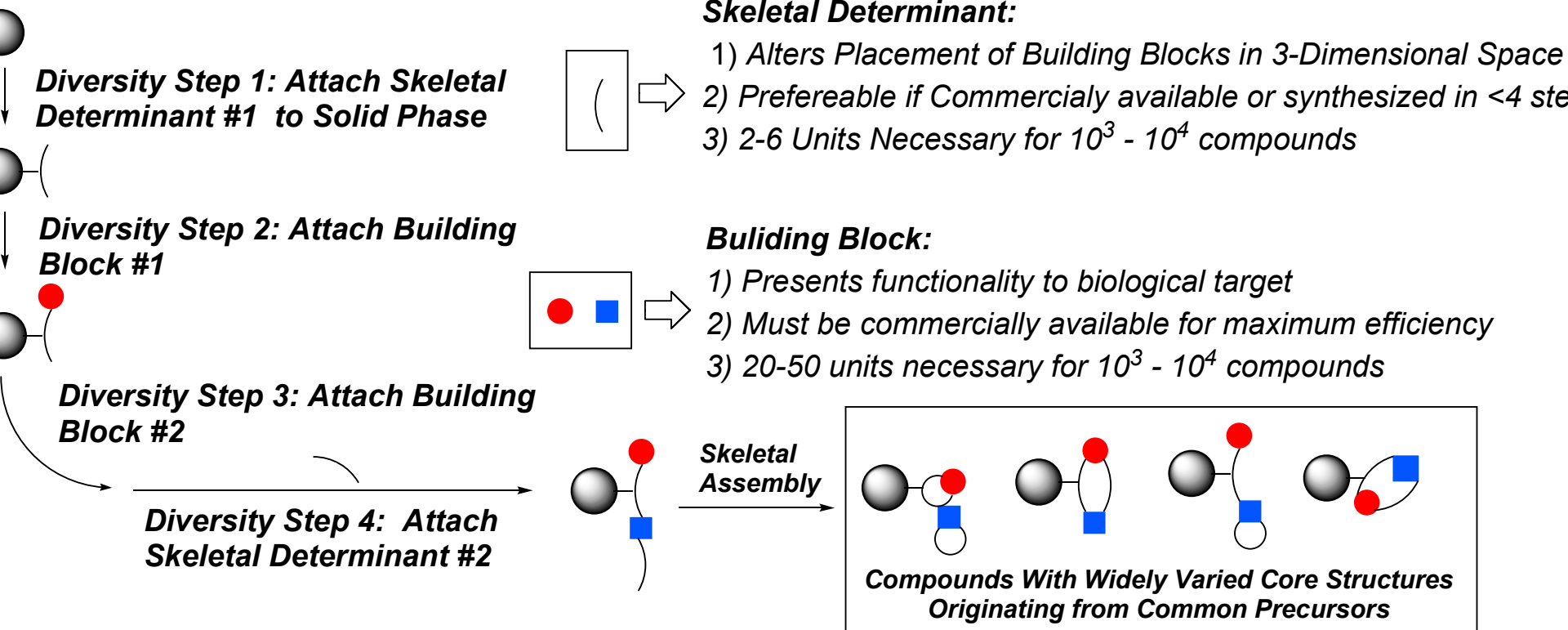
Advantages

- Short, linear synthesis-the number of reactions minimized.
- First Step is enantioselective and catalytic- changing enantiomer of catalyst is an element of diversity

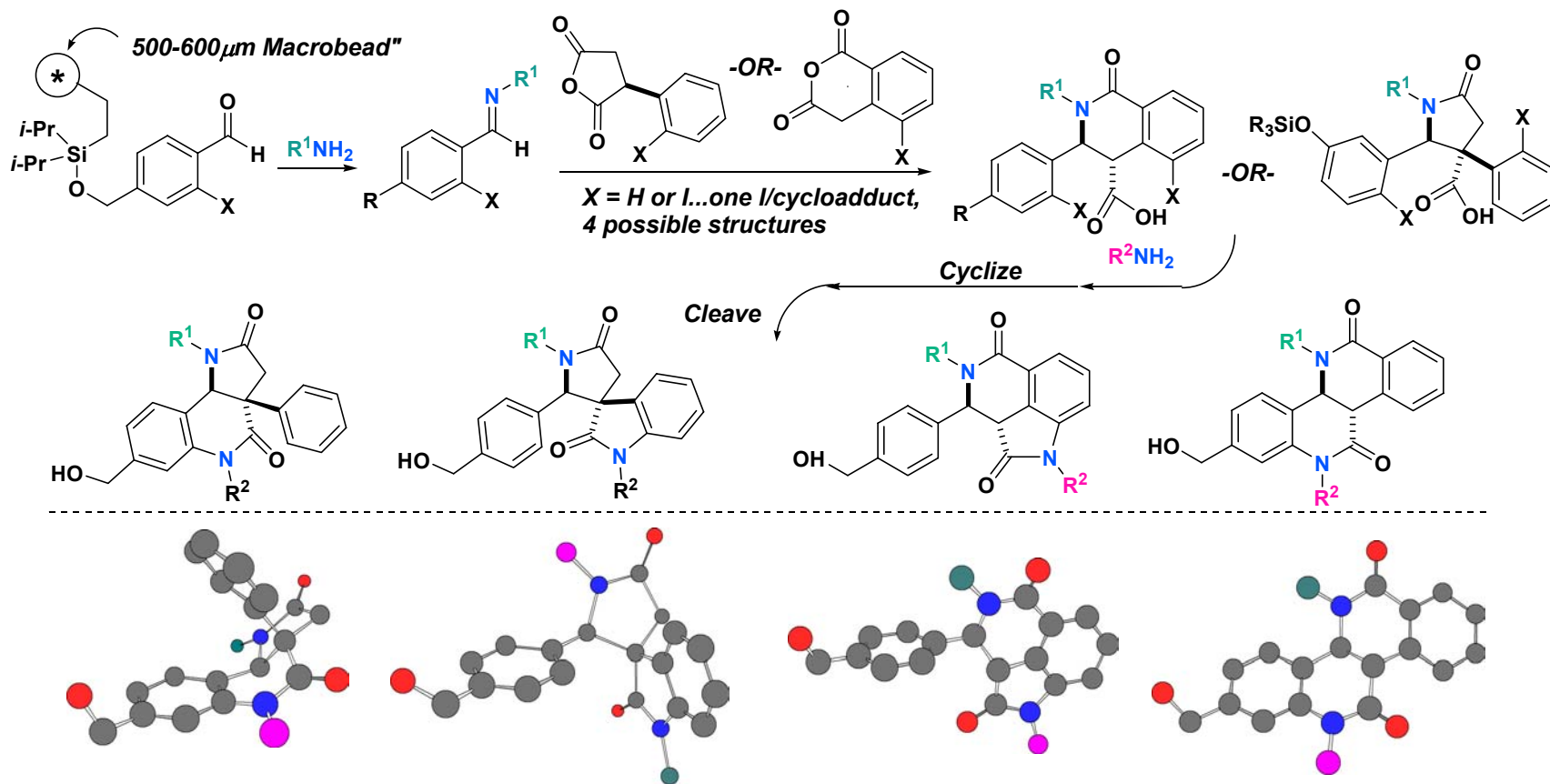
Disadvantages (real and potential)

- Synthesis of building blocks detracts from the efficiency of this library
- Lack of three-dimensional diversity could bias library toward certain targets, away from others

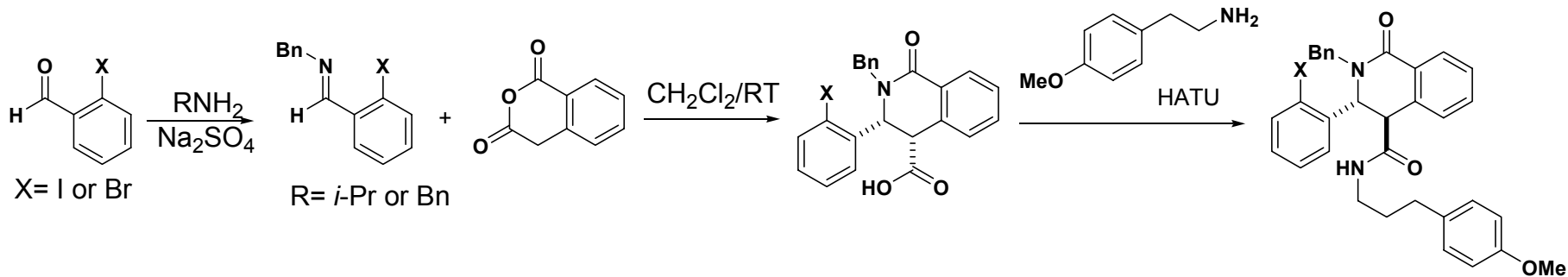
Skeletal Diversity May Offer Greater Variety of Bioactivity from Smaller Libraries



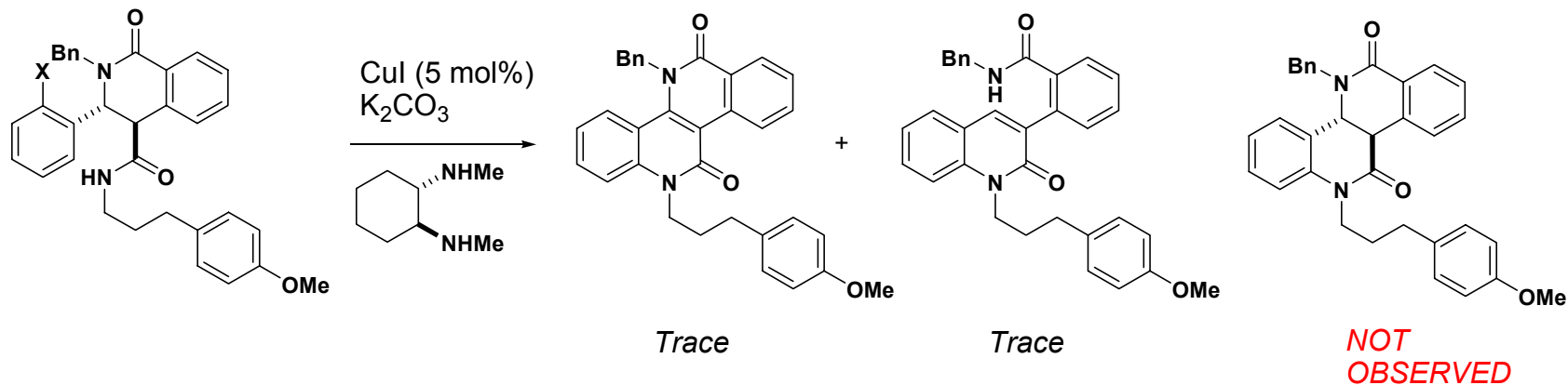
Can a linear Synthetic Sequence Produce Molecules with Different 3-dimensional Structures?



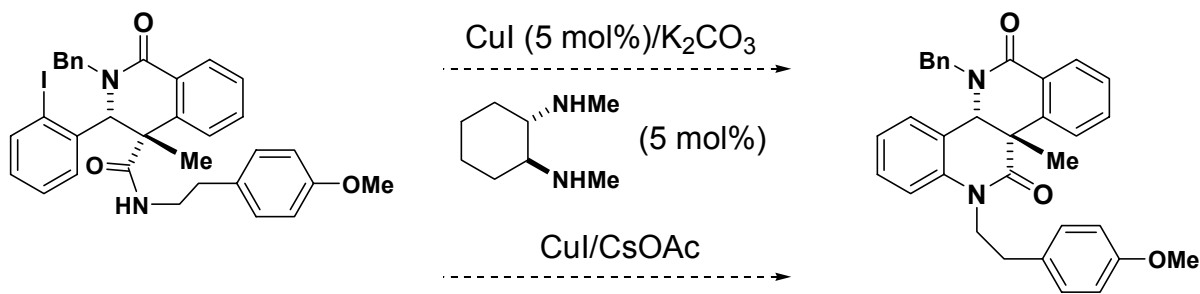
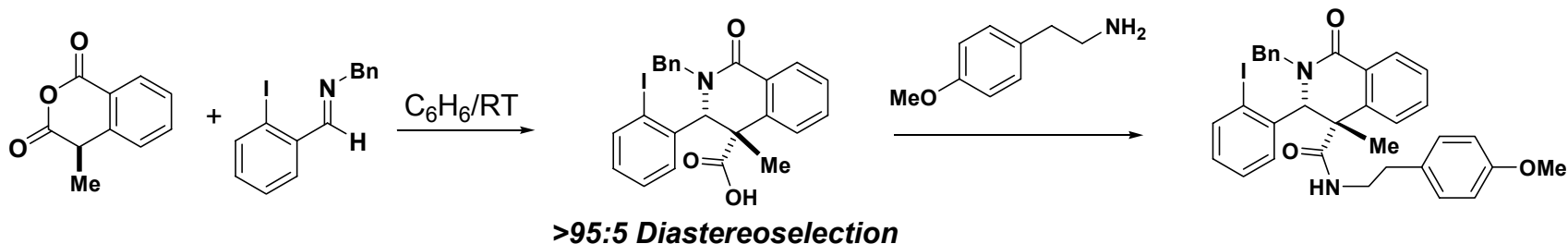
Pathway Development



85% Yield
>95:5 diastereoselection



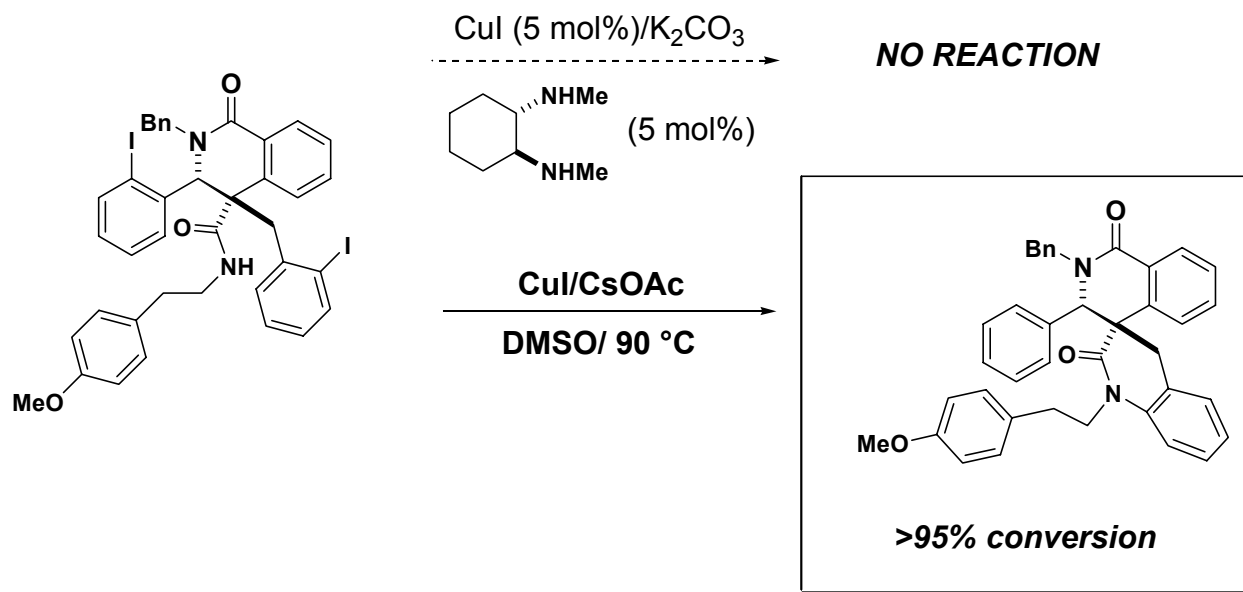
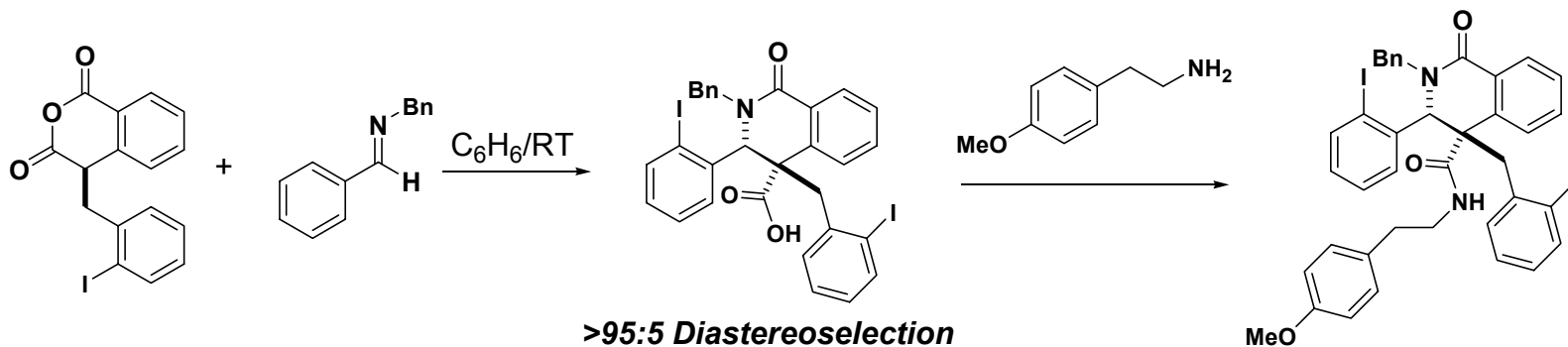
Pathway Development



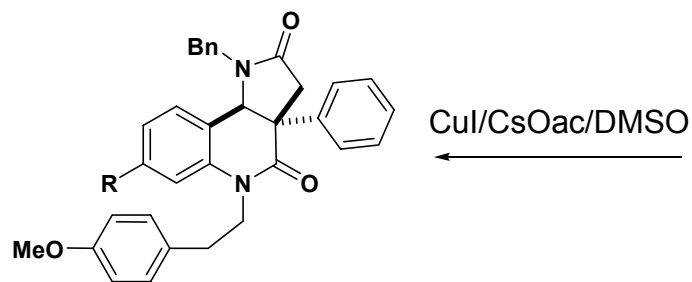
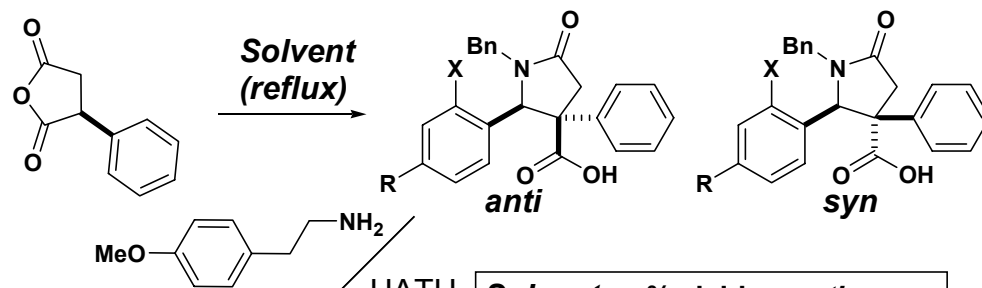
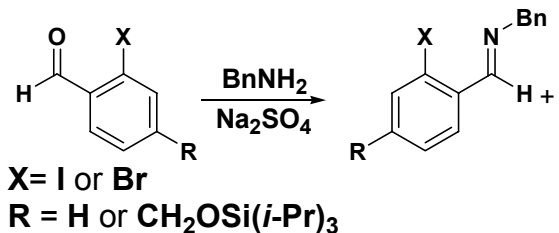
Buchwald Reaction: Klapars, A.; Huang, X.; Buchwald, S. L. "A General and Efficient Copper Catalyst for the Amidation of Aryl Halides." *J. Am. Chem. Soc.* **2002**, *124*, 7421-7428.

Tokuyama Reaction: Yamada, K.; Kubo, T.; Tokuyama, H.; Fukuyama, T. "A mild copper-mediated intramolecular amination of aryl halides." *Synlett* **2002**, 231-234.

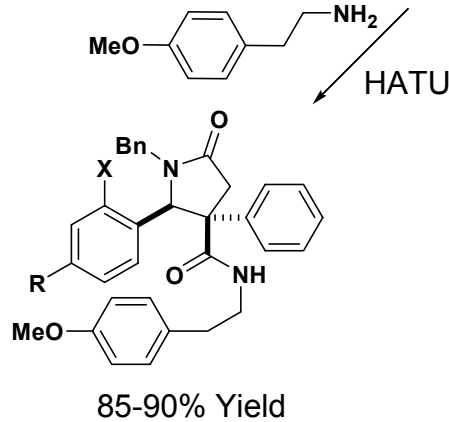
Pathway Development



Pathway Development

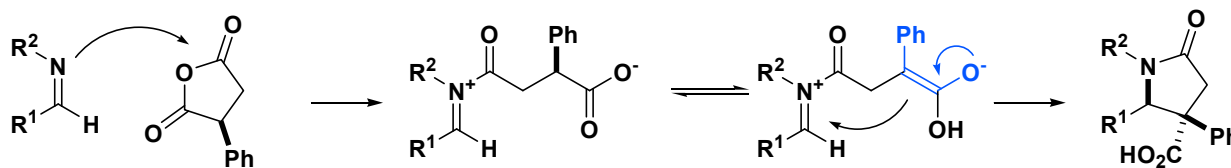
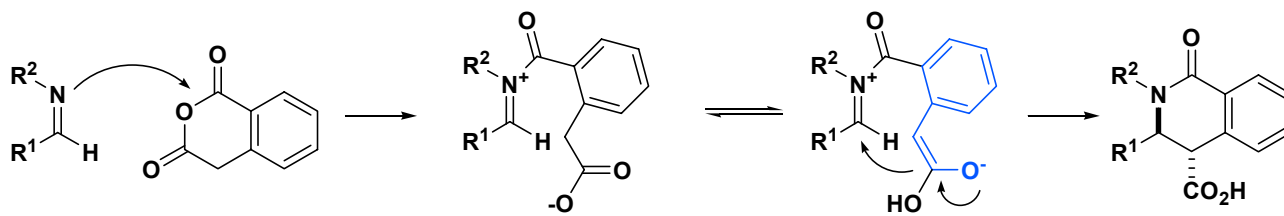
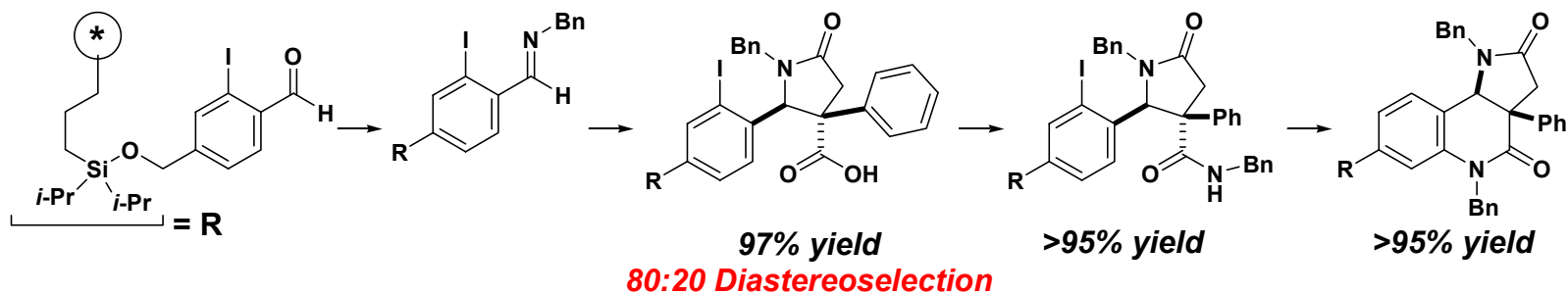


$\text{X} = \text{Br}$, No Reaction
 $\text{X} = \text{I}$, $\text{R} = \text{H}$, 95% yield
 $\text{X} = \text{I}$, $\text{R} = \text{CH}_2\text{OSi}(i\text{-Pr})_3$, 95% yield

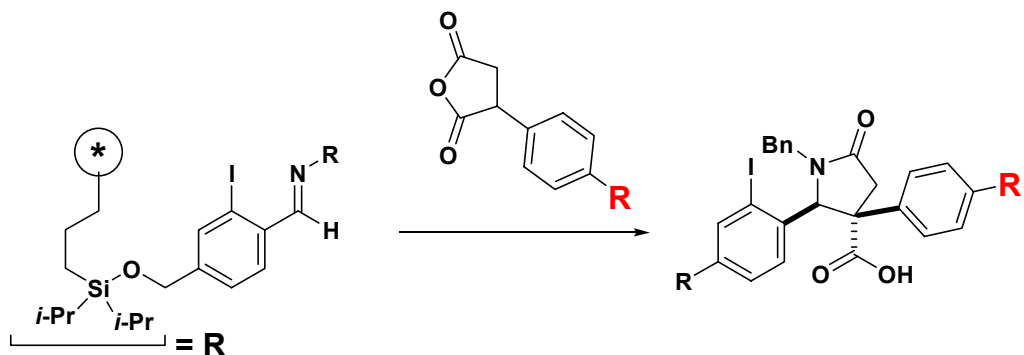


Solvent	% yield	<i>anti</i> : <i>syn</i>
CH ₂ Cl ₂	80	83 : 17
CHCl ₃	90	83 : 17
EtOAc	88	75 : 25
THF	85	70 : 30
EtOH	79	70 : 30
DMF	Decomp.	—
CH ₃ CN	92	65 : 35
Benzene	94	75 : 25
Toluene	93	90 : 10

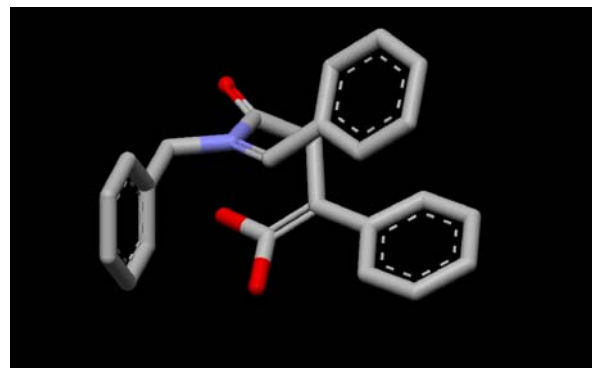
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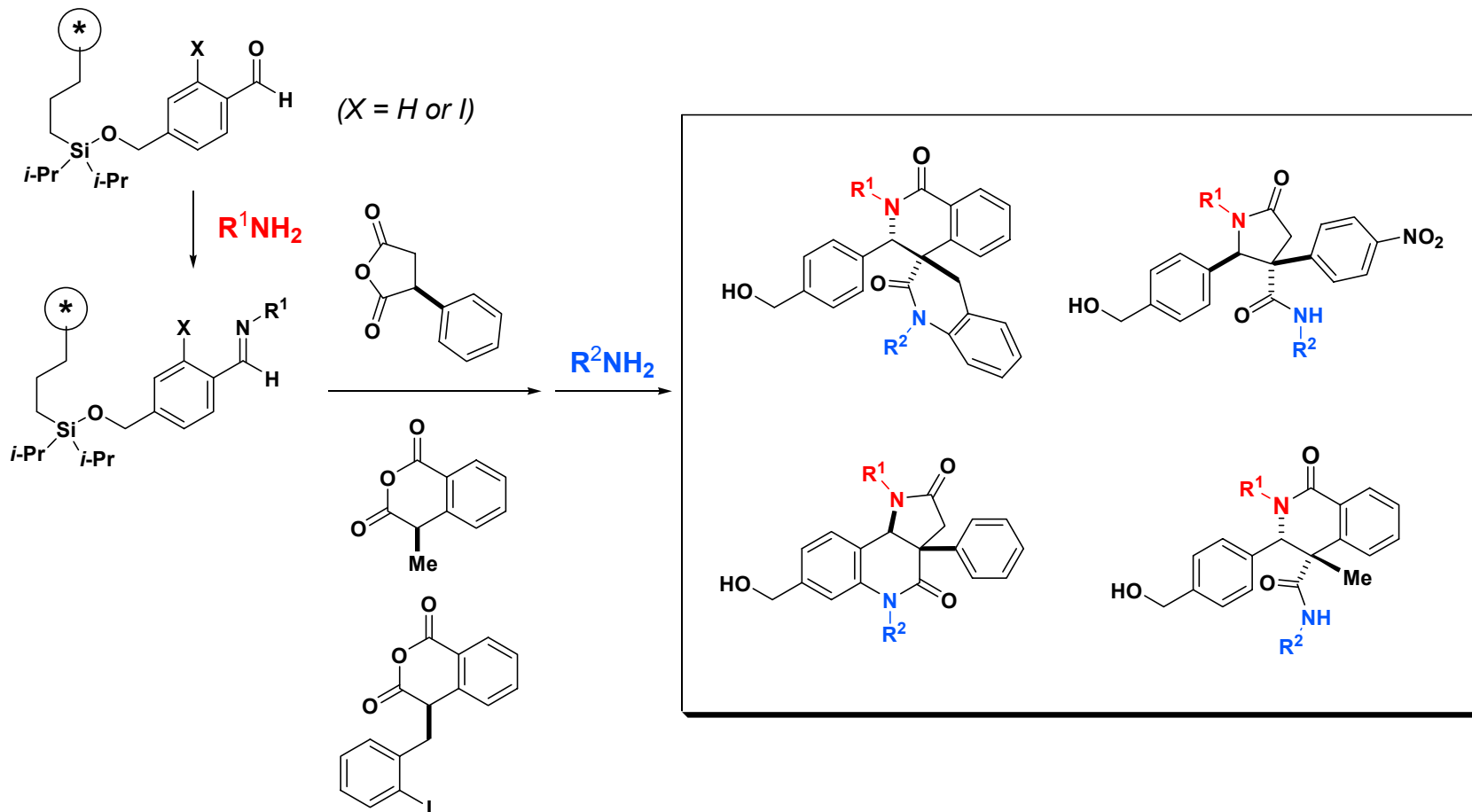
Pathway Development



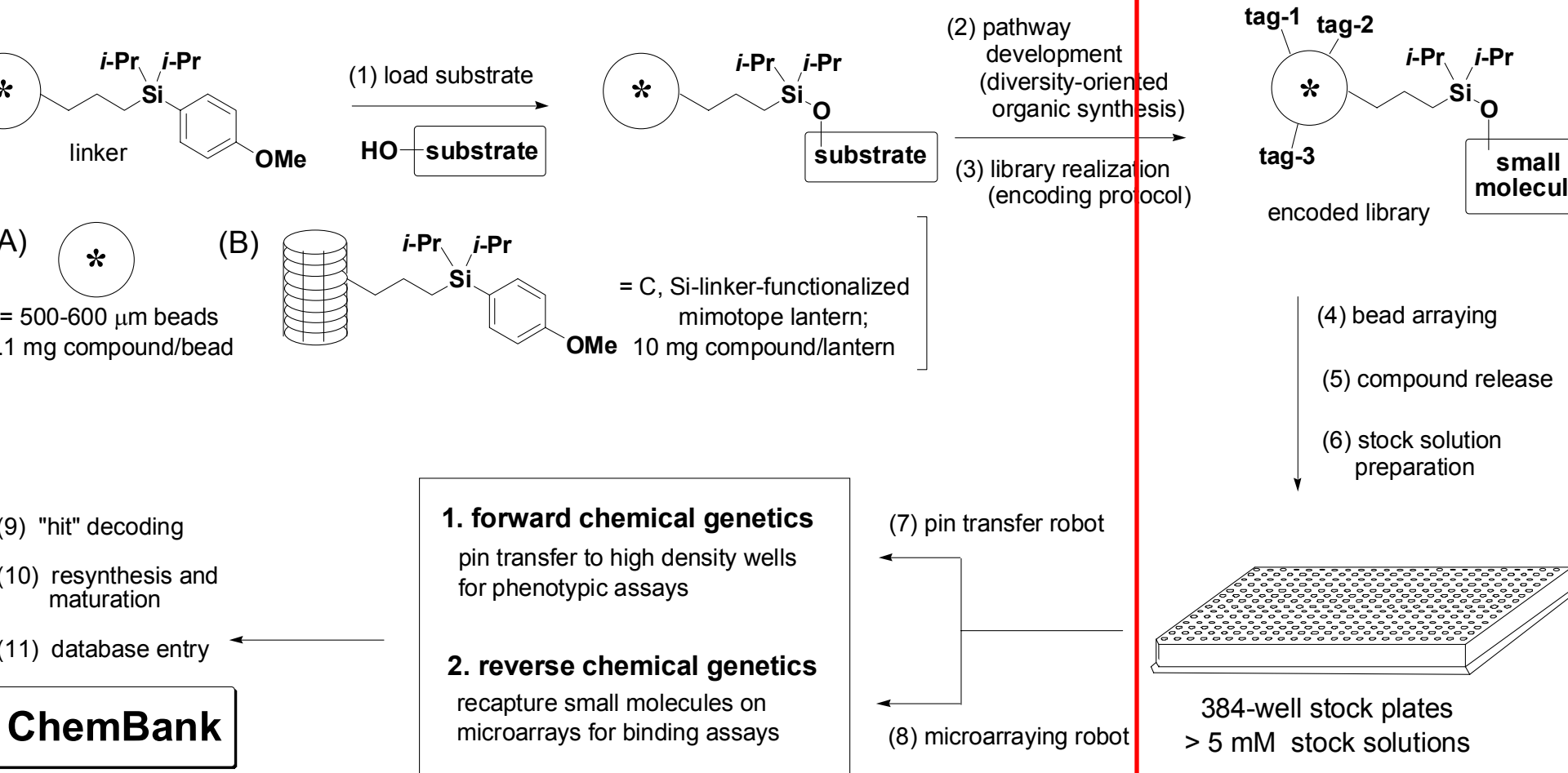
anhydride R	SM	Product (anti : syn)
H	—	>95 (76 : 24)
OMe	16	84 (80 : 20)
Cl	<5	>95 (71 : 29)
CF₃	<5	>95 (69 : 31)
NO₂	—	>95 (>95 : 5)



Summary



A technology platform for diversity-oriented synthesis: One bead-one stock solution (Version 2.0)



Bead Arrayer

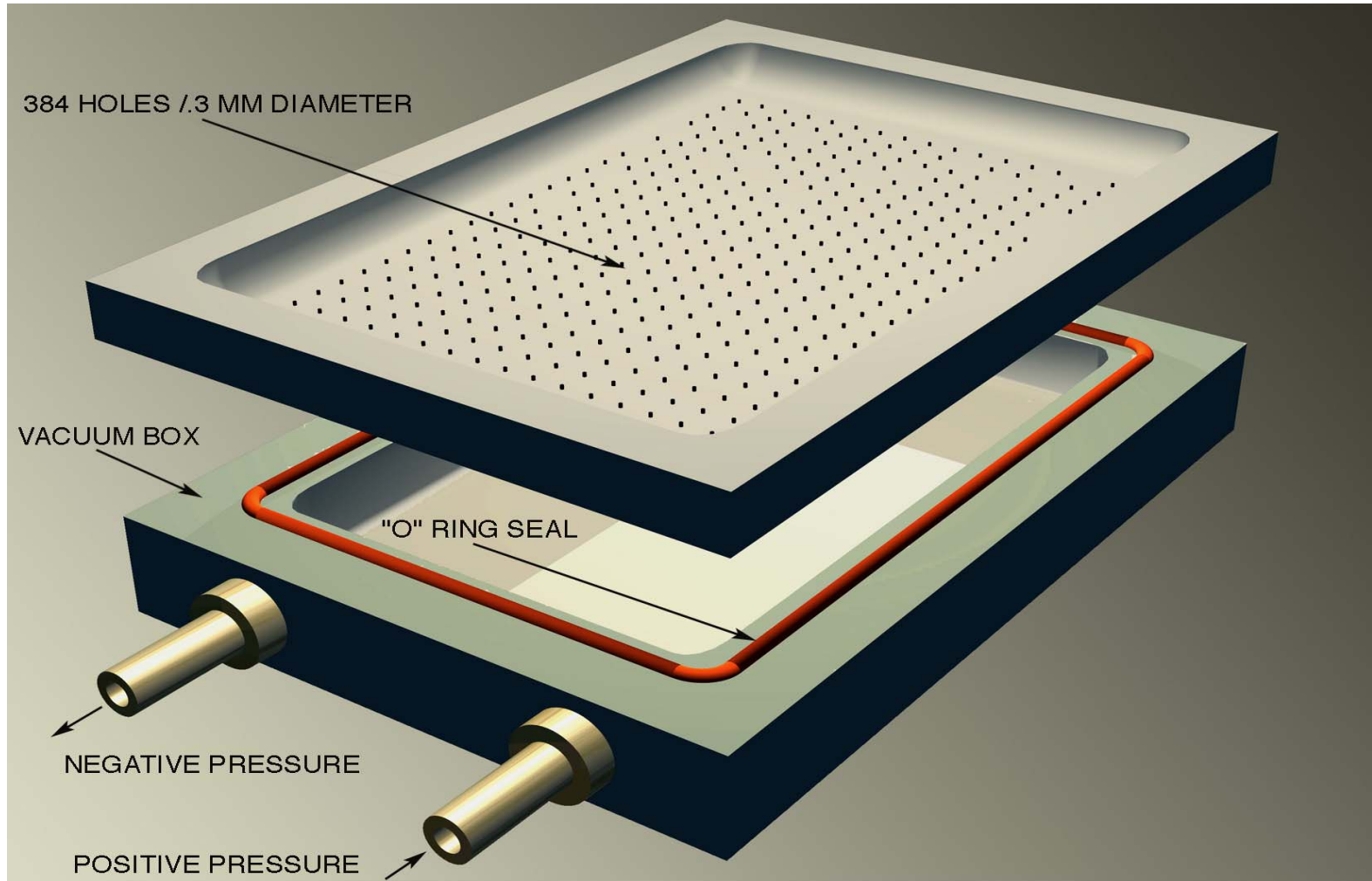
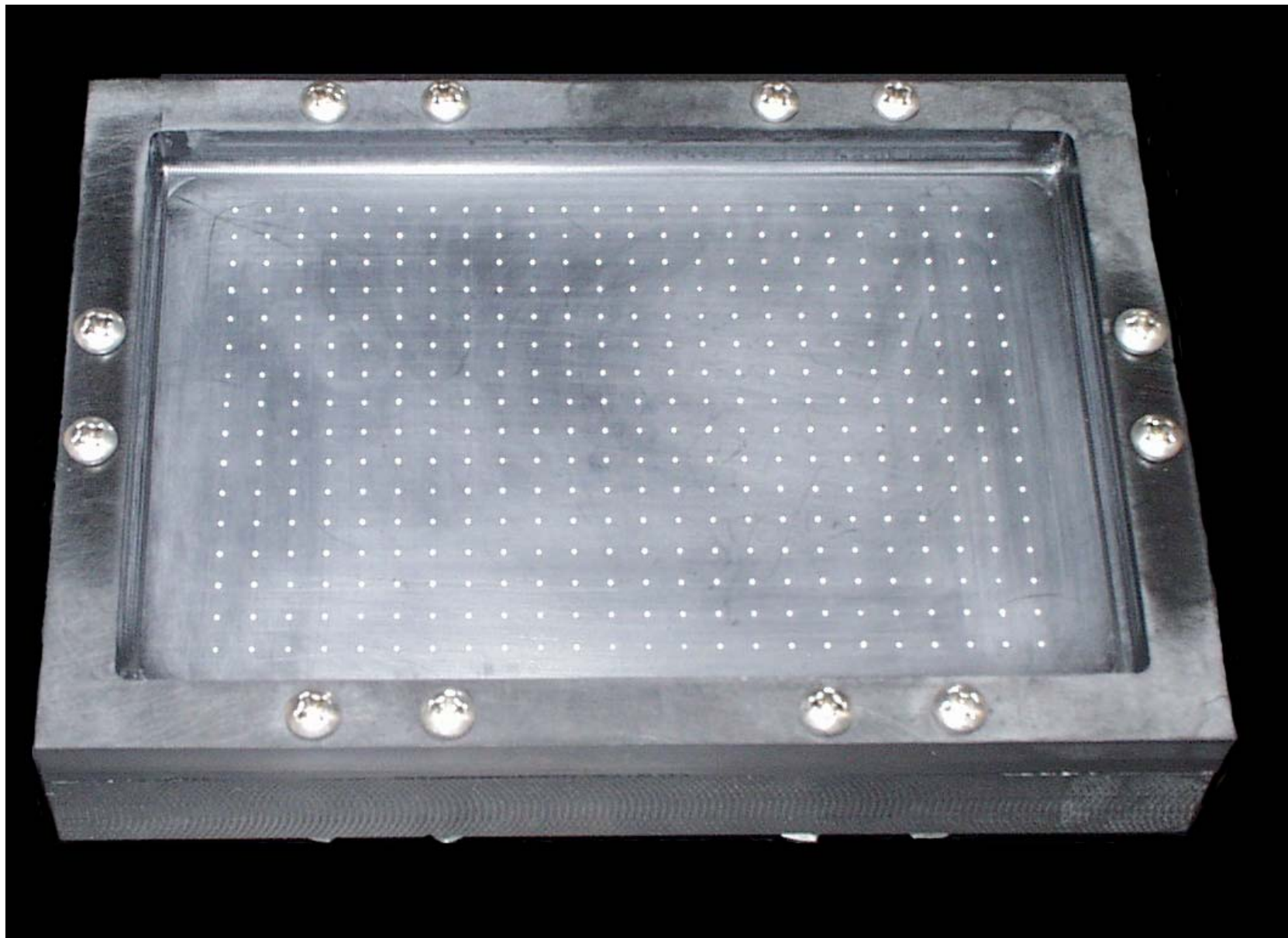


Illustration By: Jim Horn

Prof. Randy King, Les Walling

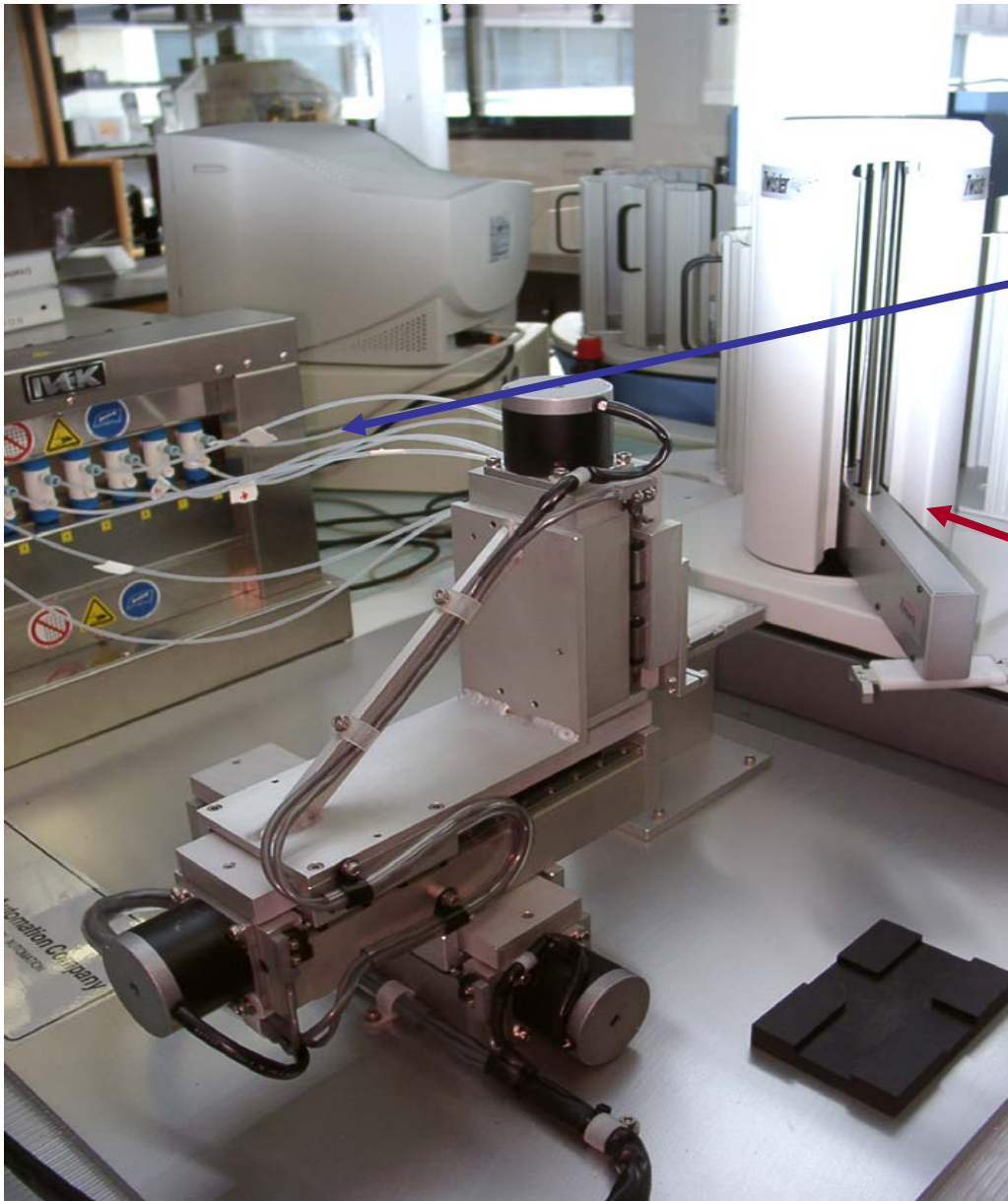


HF Dispenser

HF Pump system



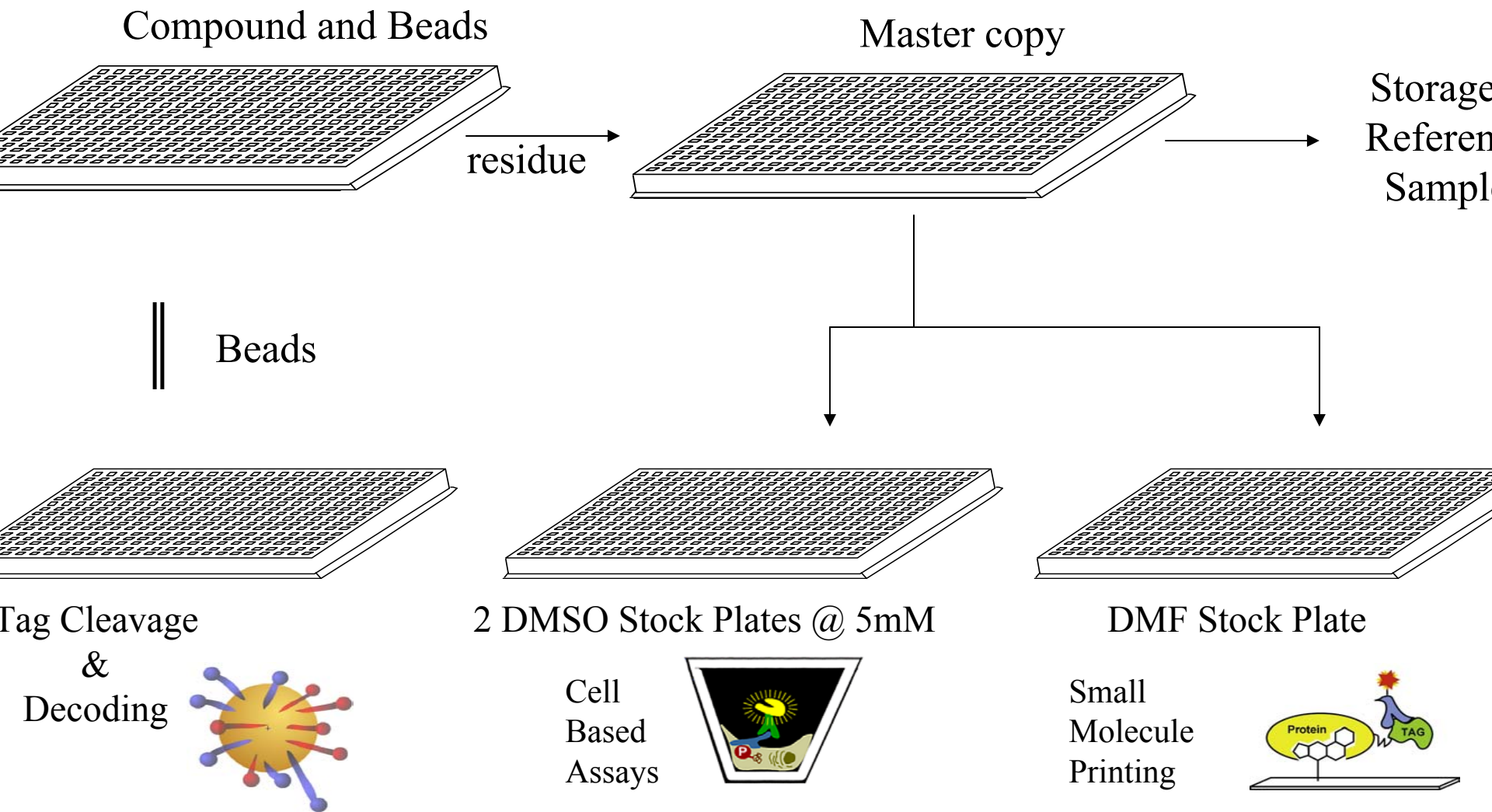
Stacking Unit



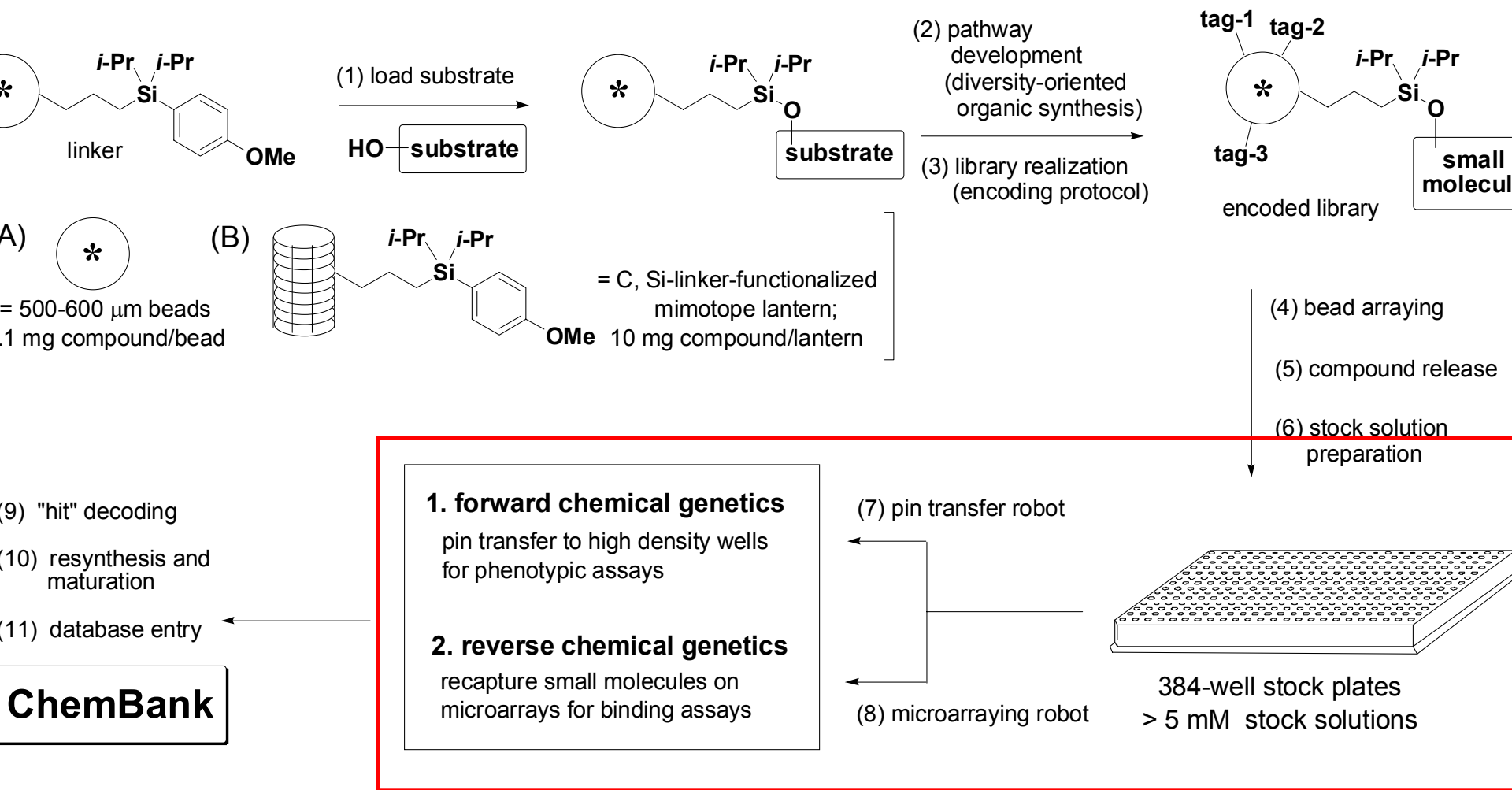
Self-Contained Cleavage System



Library Formatting

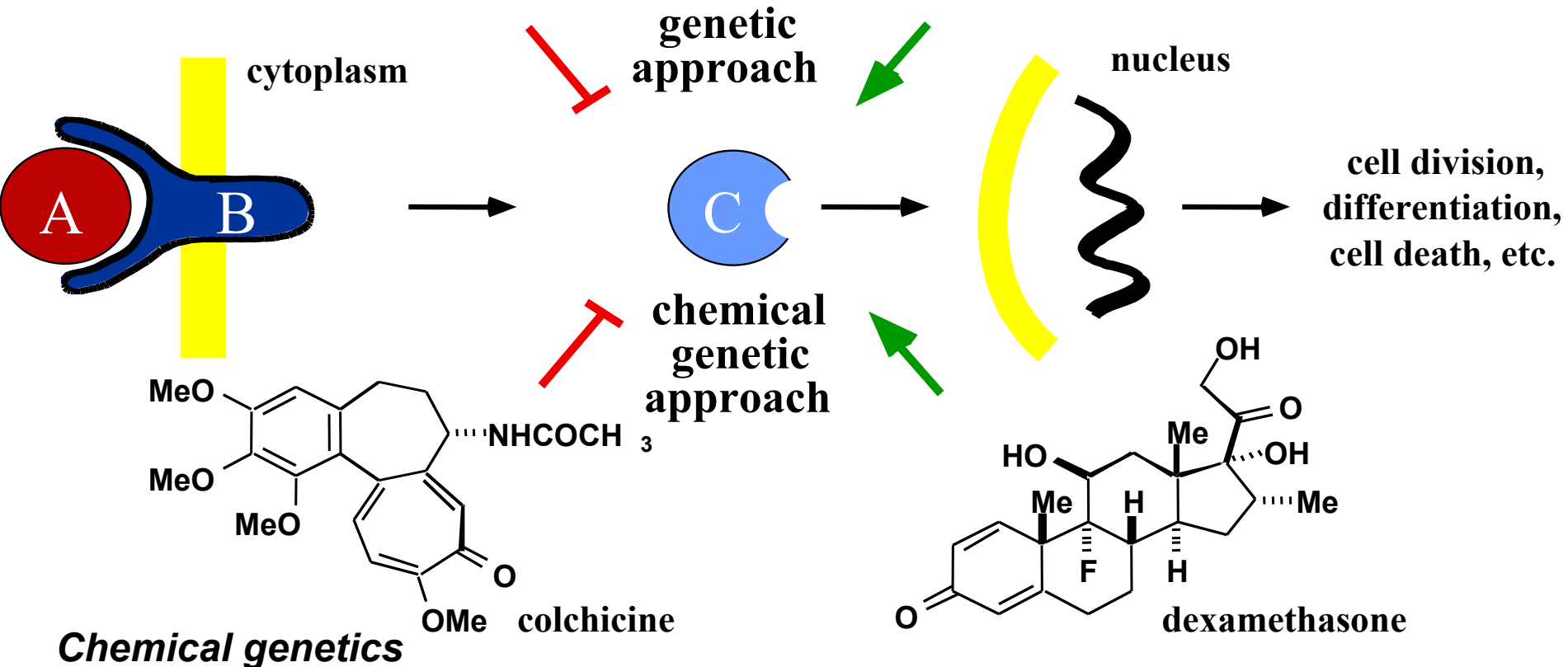
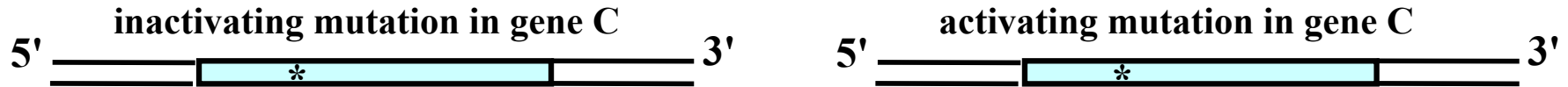


A technology platform for diversity-oriented synthesis: One bead-one stock solution (Version 2.0)



Classical Genetics/Chemical Genetics

Classical genetics



Collaborative Screening at the ICCB

patterned after forward genetics

patterned after reverse genetics

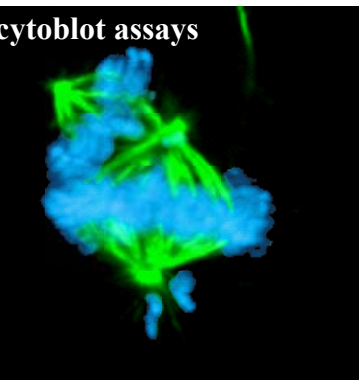


phenotypic screening

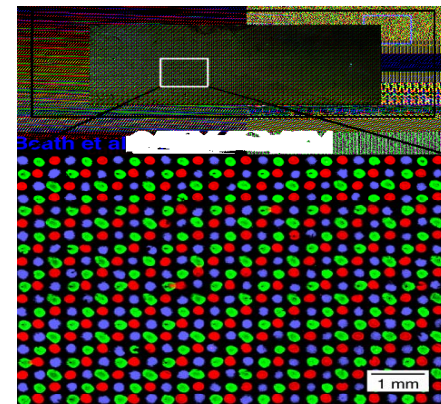
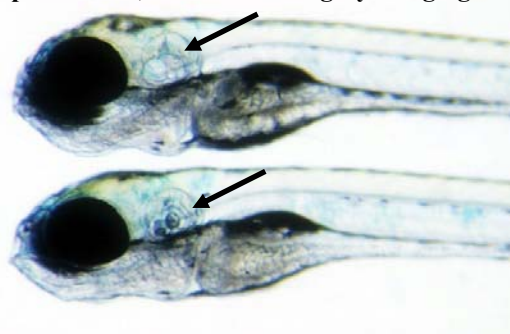
• screen collections of structurally complex and diverse small molecules in search of a particular cellular/organismal phenotype.
• ICCB hosts investigators

proteomic screening

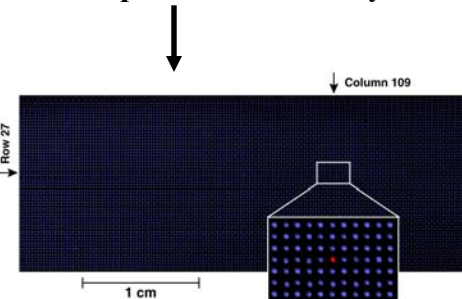
• modify cellular/organismal function of targeted protein with a small molecule, search broadly for the resulting phenotype.
• ICCB will distribute microarrays



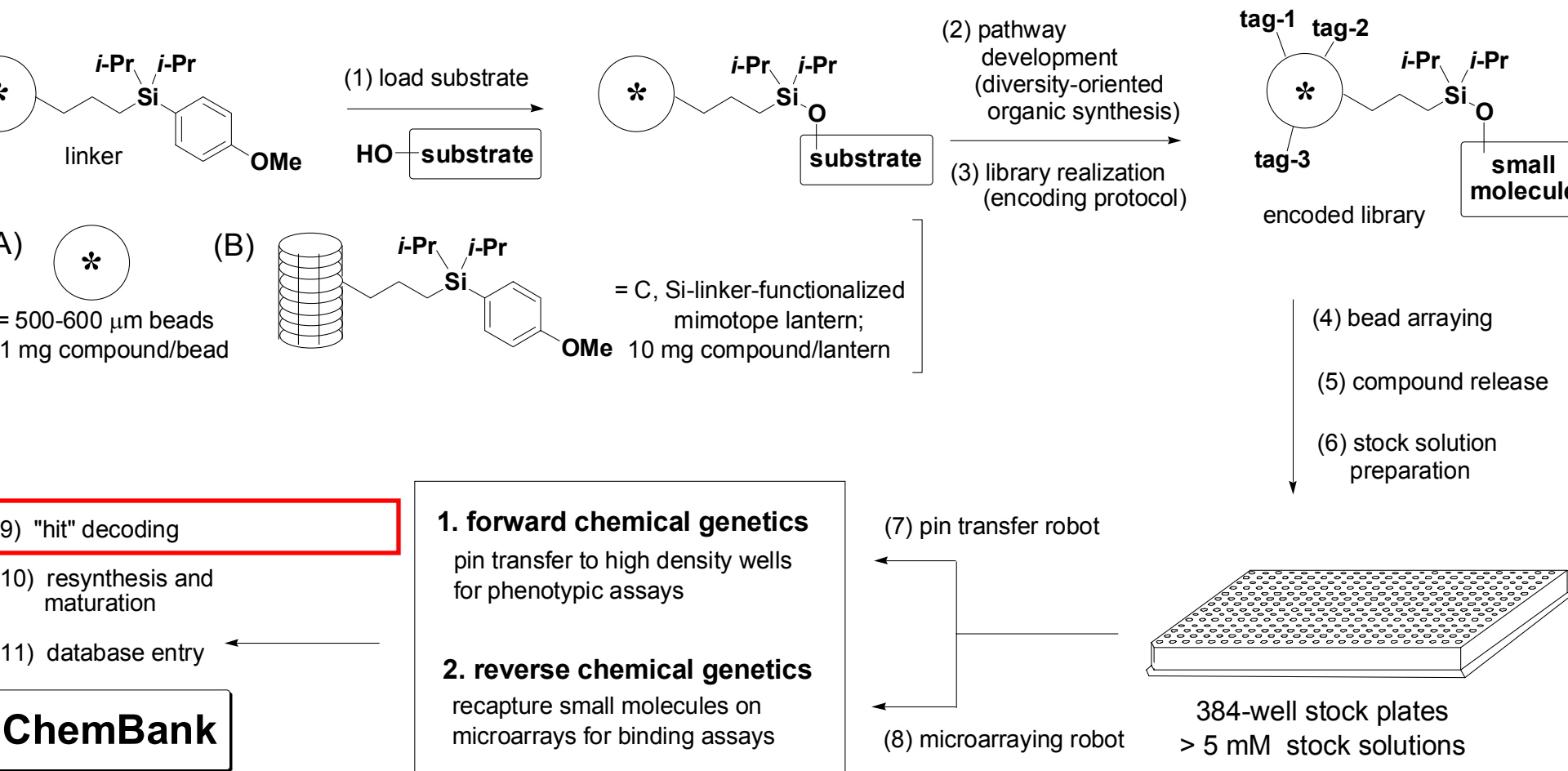
htp zebrafish, worm screening-by-imaging



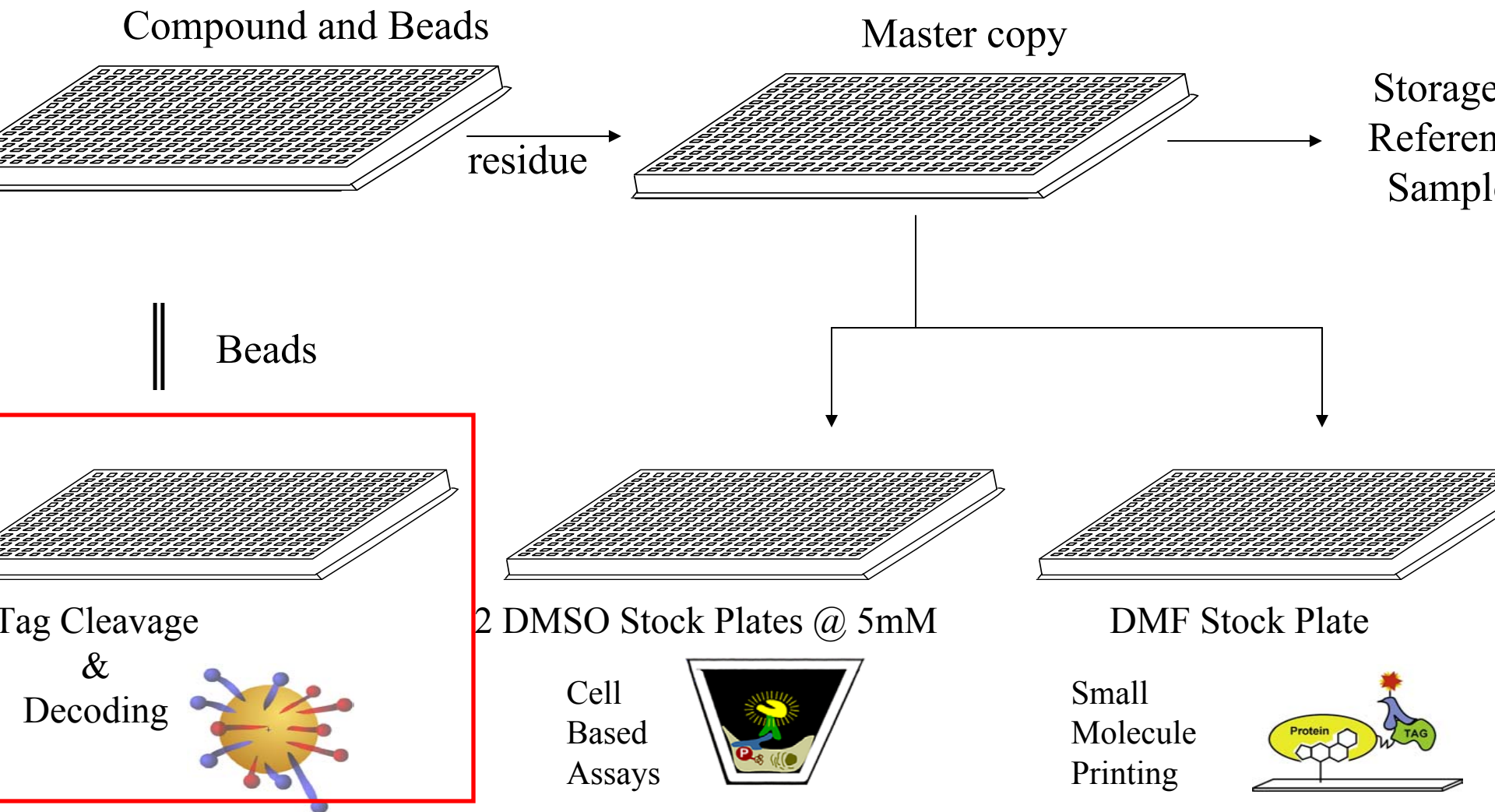
← small molecule and protein microarrays



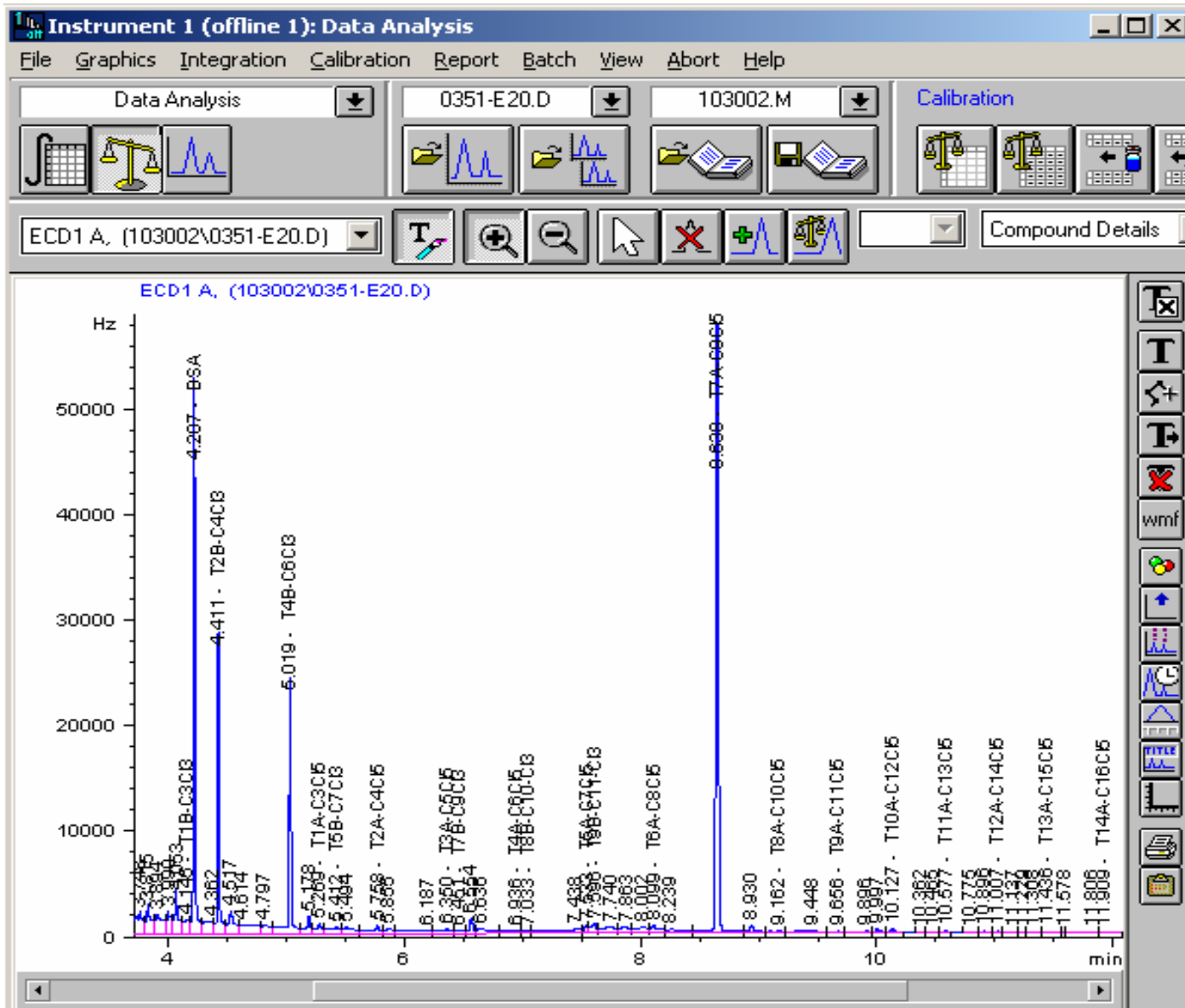
A technology platform for diversity-oriented synthesis: One bead-one stock solution (Version 2.0)



Library Formatting



Typical GC run for decoding solution



Manual Decoding Process



Chemistry



Beads



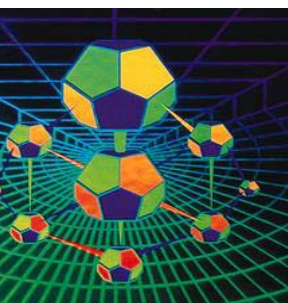
GC/LCMS



Visual analysis &
manual data entry



Excel file

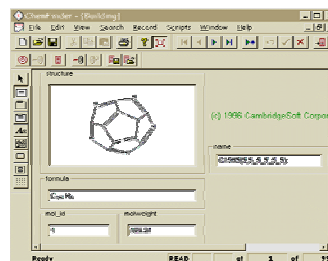


Afferent



	0.701	0.830	0.780	0.707
10	0.744	0.798	0.770	0.84
31	0.754	0.833	0.806	0.83
46	0.770	0.807	0.807	0.77
93	0.323	0.879	0.718	0.80
97	0.331	0.894	0.750	0.80
97	0.756	0.802	0.749	0.80

SDF file



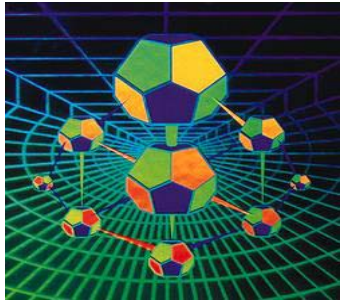
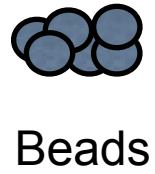
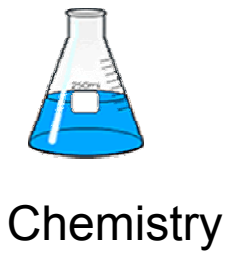
ChemFinder



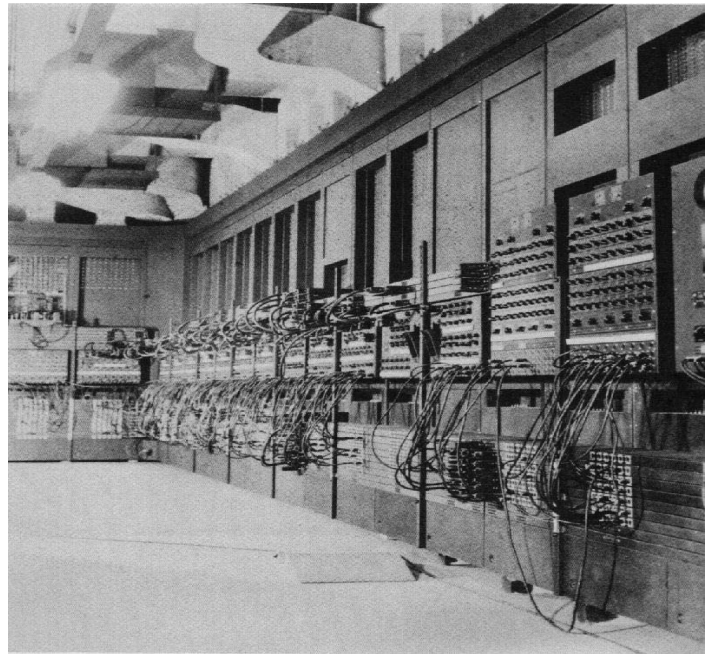
Disadvantages of Manual Decoding

- Visual analysis, manual data entry and maintenance of the data is slow and laborious
- Excel is not suited to storing data large amounts of decoding data
- The process is so time-consuming that generally only a small subset of compounds (those that hit in a screen) are actually decoded

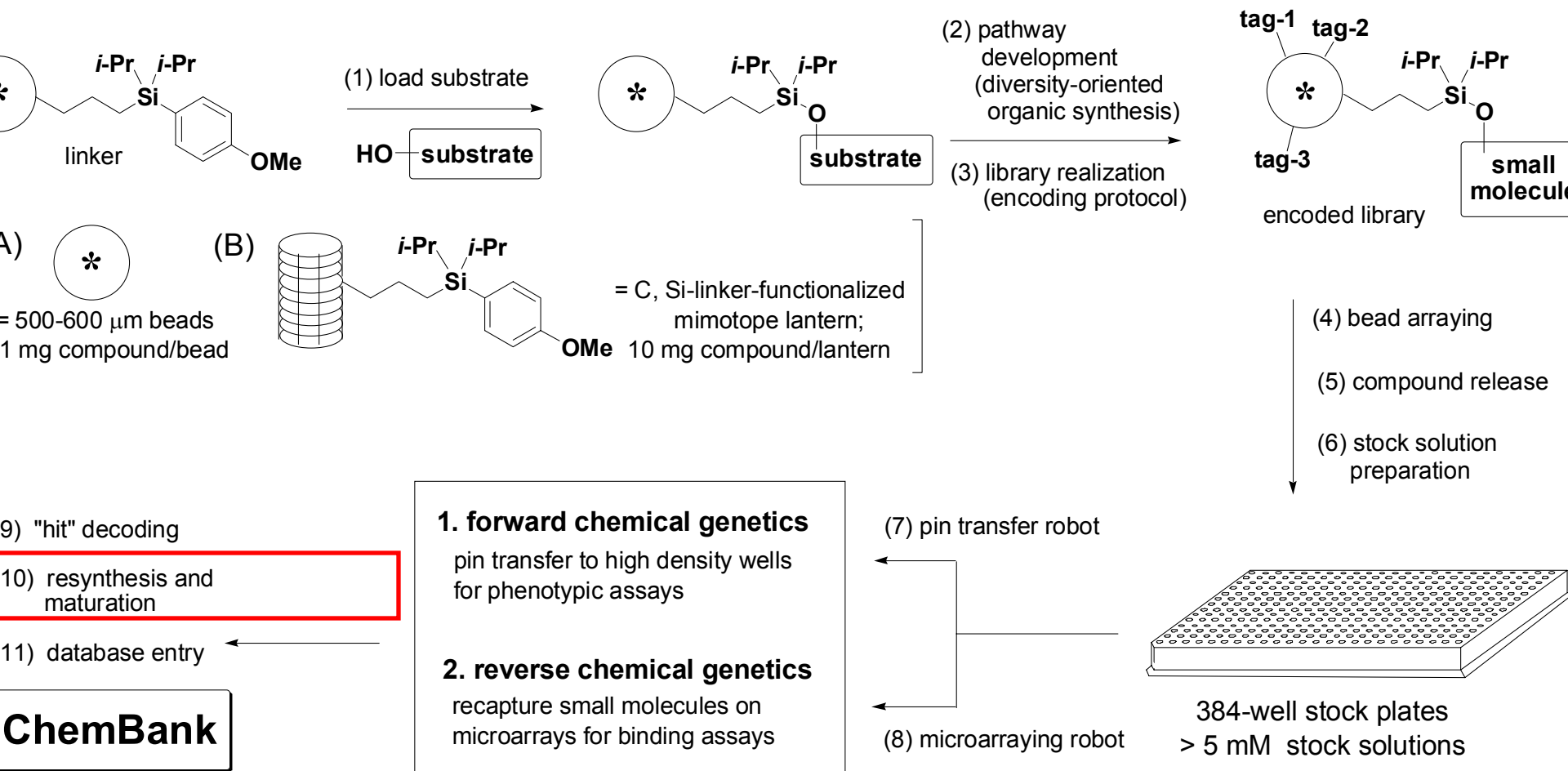
Can Chemical Encoding be integrated with Library Enumeration into an Automated Process?



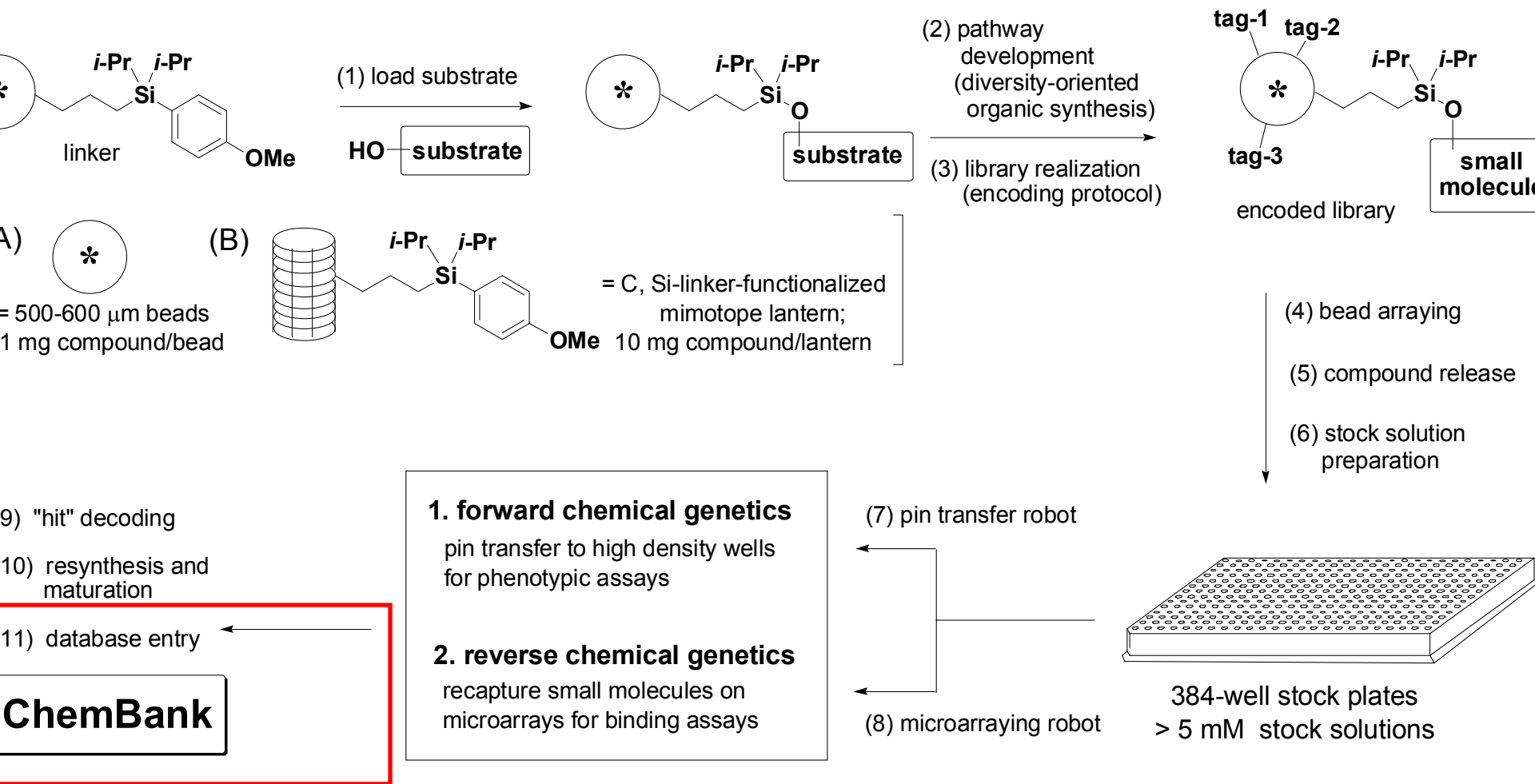
20	0.731	0.830	0.783	0.770
10	0.744	0.798	0.770	0.84
31	0.754	0.833	0.806	0.83
46	0.770	0.807	0.807	0.77
93	0.323	0.879	0.718	0.80
97	0.331	0.894	0.750	0.80
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Acknowledgments

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ICCB Laboratories

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Feng Laboratory
Mitchison Laboratory

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Daylight



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