DESIGN OF NOVEL DYNAMIC LIBRARIES FOR PHOSPHATASE INHIBITION AND THE DISCOVERY OF NEW ANTIPROLIFERATIVE AGENTS

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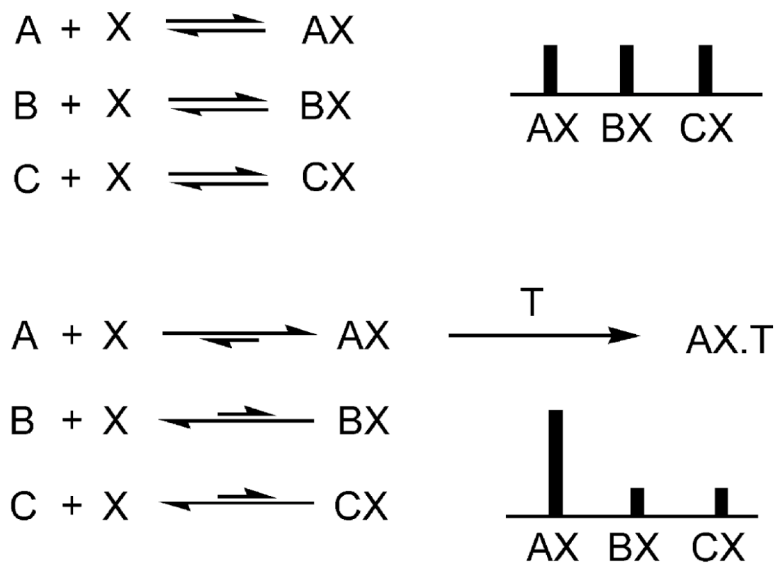
Dynamic Combinatorial Chemistry (DCC) combines synthesis and screening in a single-step approach to the discovery of new functional molecule.

J-M. Lehn and *A. Eliseev*

The key feature of DCC is the Dynamic Combinatorial Library (DCL) in which each library member is assembled from building blocks that are connected through reversible bonds. As a result of this reversibility, all library members are interconverting to give a distribution that is under thermodynamic control. Thus addition of a guest molecule, or template, that can selectively bind to one receptor in the library will serve to increase the concentration of that host at the expense of others in the DCL.

Science, **2001**, 2331

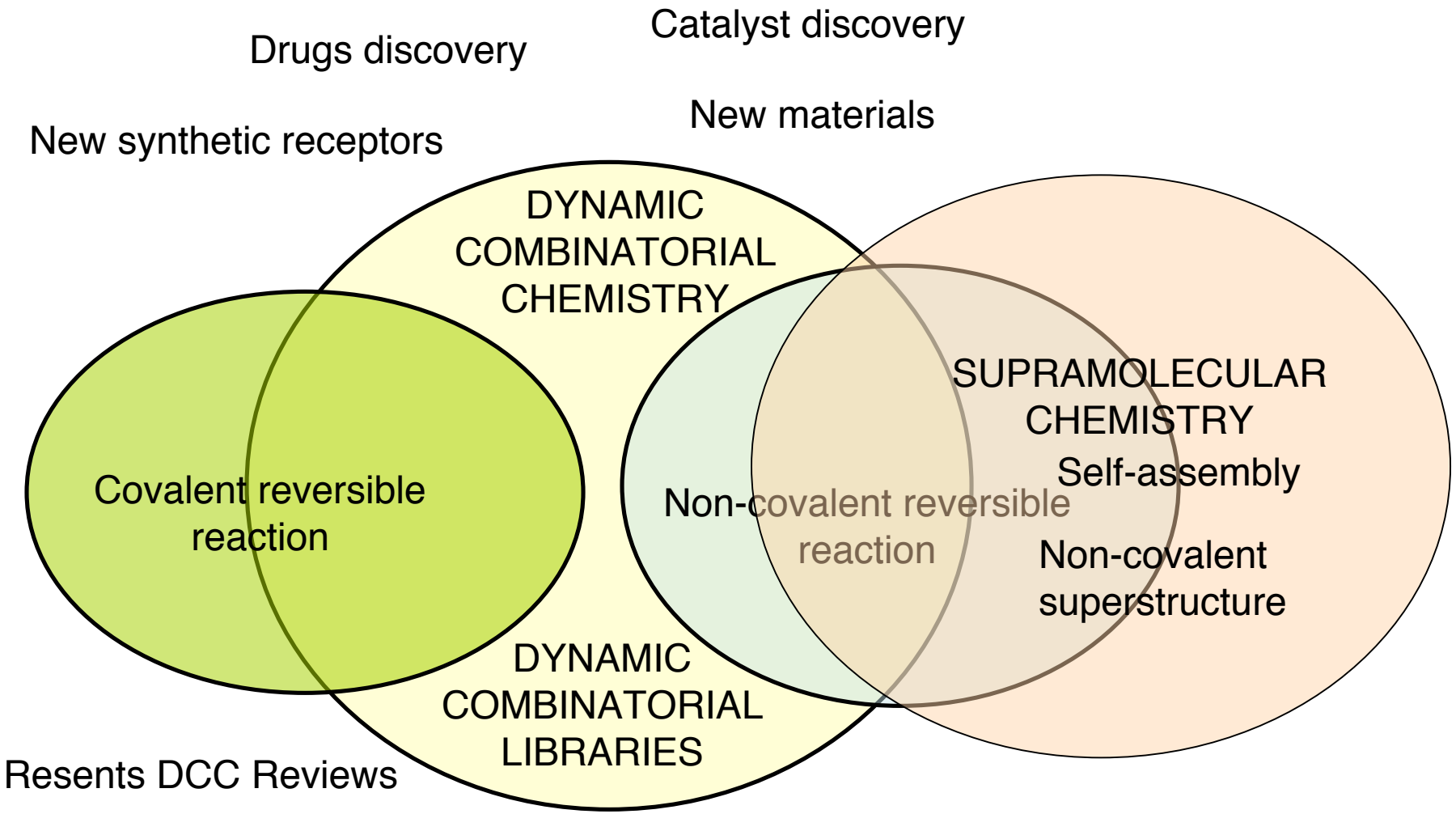
(DCC) concept



Reversible reaction performed with limiting amount of **X** generate a mixture of compounds **AX, BX, CX**. So the library product distribution is thermodynamically controlled and responsive to external influences.

Binding of **AX** to a molecular trap **T** causes perturbation of the equilibrium involving **A** and **X**, giving overall amplification of **AX** at expense of the other library members

Tetrahedron, 60 (2004) 771



Lehn, J-M et al. *Science* **2002**, 295, 2400
Otto, S. *Curr. Opin Drug. Disc.* **2003**, 6, 509.
Sanders, J. et al. *Curr. Opin Chem. Biol*. **2002**, 6, 321
Lehn, J-M et al. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, 99, 4763
Sanders, J. et al. *Drug Discov. Today* **2002**, *7*, 1117

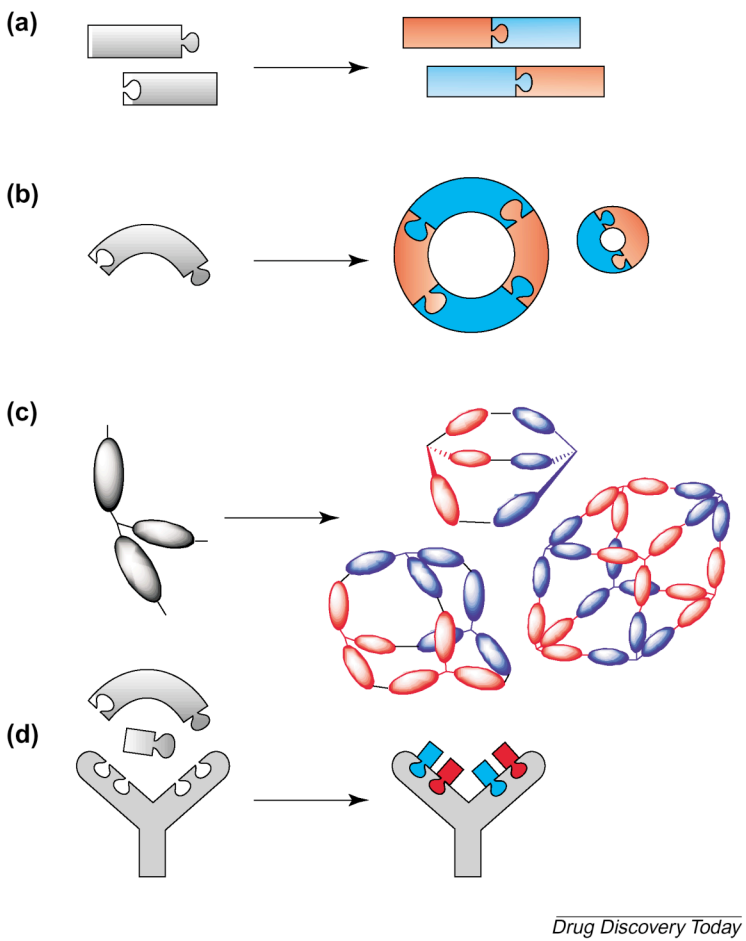
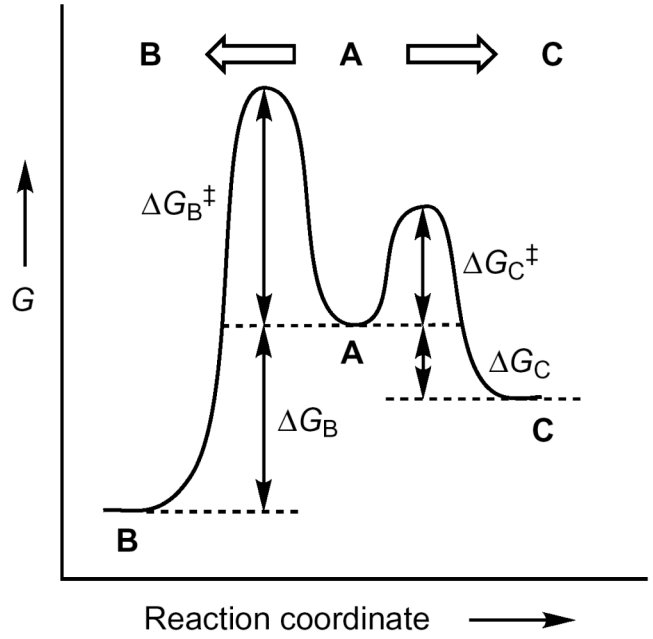


Fig. 2. Libraries made from building blocks with different numbers of functional groups (valency) available for reversible bond formation. **(a)**Mono-functionalized building blocks generate libraries of dimmers, **(b)**di-functionalized building blocks give access to macrocycles, and **(c)**tri-functionalized building blocks generate libraries of capsule-like molecules. **(d)** Mixing building blocks with different valencies further extends the range of possible architectures.

Furlan, R; Otto, S.; Sanders, J. *Drug Discov. Today* **2002**, *7*, 1117

Dynamic Covalent Chemistry
Reactions carried out reversibly under equilibrium conditions.

Dynamic Covalent Chemistry



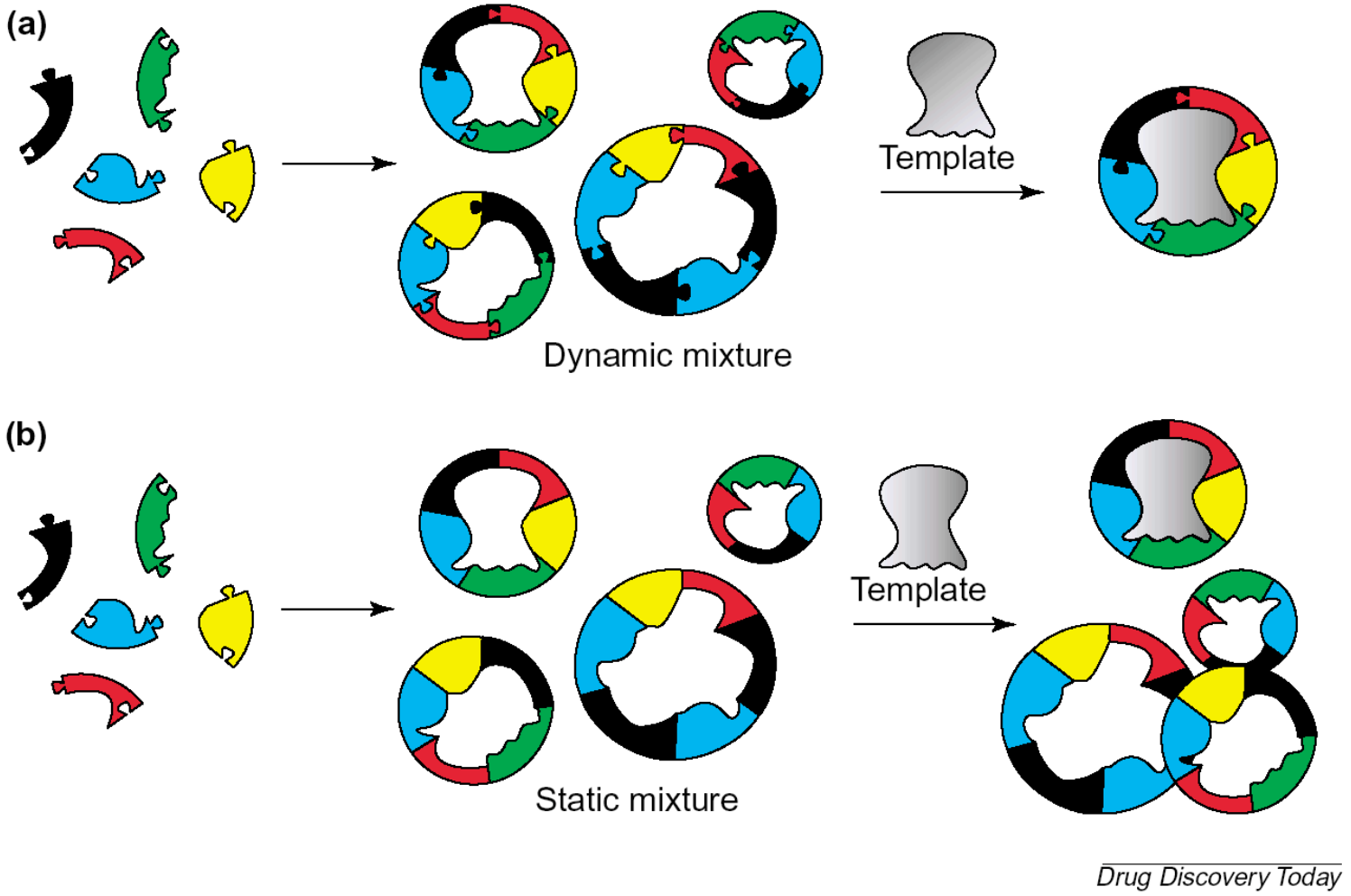
Free energy profile illustrating kinetic (**A** to **C**) *vs.* thermodynamic (**A** to **B**)

Most covalent chemistry is irreversible and so occurs under kinetic control while most supramolecular and dynamic covalent chemistry is reversible and so occurs under thermodynamic control

$\Delta G^o = \Delta H - T\Delta S = -RT\ln K$ (**1**) Thermodynamic process

Rowan, S.J; Cantrill, S.; Cousins, G. Sanders, J.; Stoddart, F. *Angew. Chem. Int. Ed. Engl.* **2002**, 41, 898.

(a) Dynamic combinatorial chemistry versus
(b) Traditional combinatorial chemistry.



Furlan, R; Otto, S.; Sanders, J. *Drug Discov. Today* **2002**, *7*, 1117

Comparison of Traditional Combinatorial Libraries with Dynamic Combinatorial Libraries

Traditional Combinatorial Libraries	Dynamic Combinatorial Libraries
• Concentrations unaffected by recognition events • Selected compounds need to be synthesized independently • Complex topologies are difficult to access	• Recognition can induce amplification • Selected compounds can be isolated from the library • Complex topologies are easier to access
• Many irreversible reaction available	• Limited number of reversible reaction
• Insolubility of some library members of no consecuence	• All library members need to be soluble
• Stepwise control over individual reaction step	• Limited control because of reversibility

Furlan, R; Otto, S.; Sanders, J. *Drug Discov. Today* **2002**, *7*, 1117

Processes Involved In a DC approach:

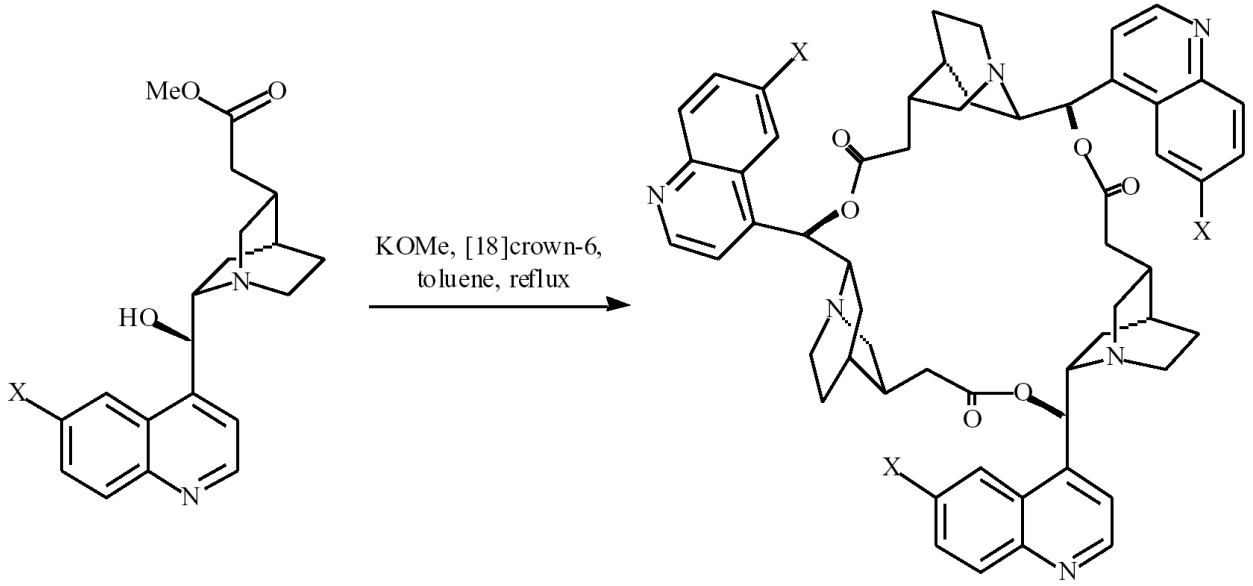
1- Preparation of the mixture of inter-converting molecules
2- Amplification of the best binder through non-covalent interaction with a template
3- Isolation (or re-synthesis of the best binder**)**

The useful reaction suitable for DCC :

- Should be reversible under physiological conditions (for drug discovery).
- All the components must be soluble under physiological Conditions.
- Should not interfere with the biological functional groups.
- Stop the reaction for analyses.

Trans-esterification

Base catalyzed-esterification



1a X=OMe
1b X=H

X=OMe
X=H

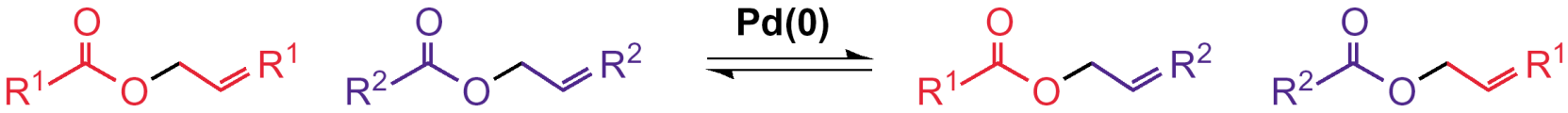
Fig. (4). Quantitative reversible formation of a cyclic trimer of cinchonidine derived hydroxy-ester **1**.

Sanders, J. et al. *JOC*, **1998**, 63, 1536.

Harsh conditions,
template effects observed

Trans-esterification

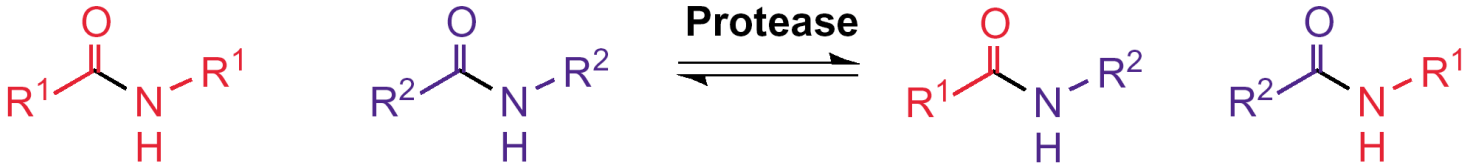
Pd catalyzed-esterification of allyl esters



Used for the templated synthesis of cyclic porphyrin dimmers under reversible conditions.

Amatore, et al. *Chem. Eur. J.* 1999, 5, 466

Peptide-bond exchange

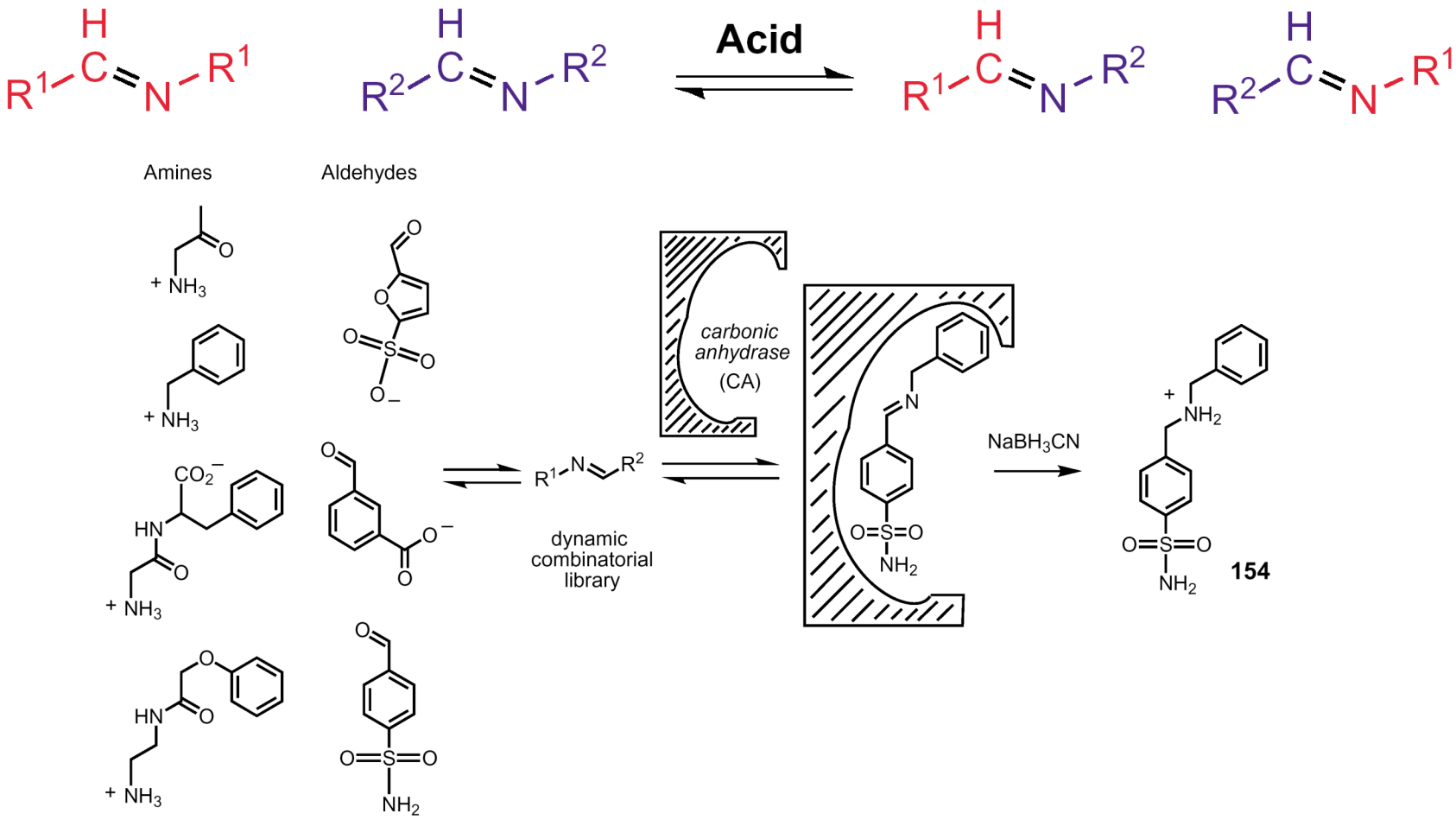


The peptides were incubated with low specificity proteases under reversible conditions, using an antibody as a molecular trap obtained weak amplification.

Venton, D. L. et al. *Biopolymers* **1996**, 40, 617

Shift base exchange

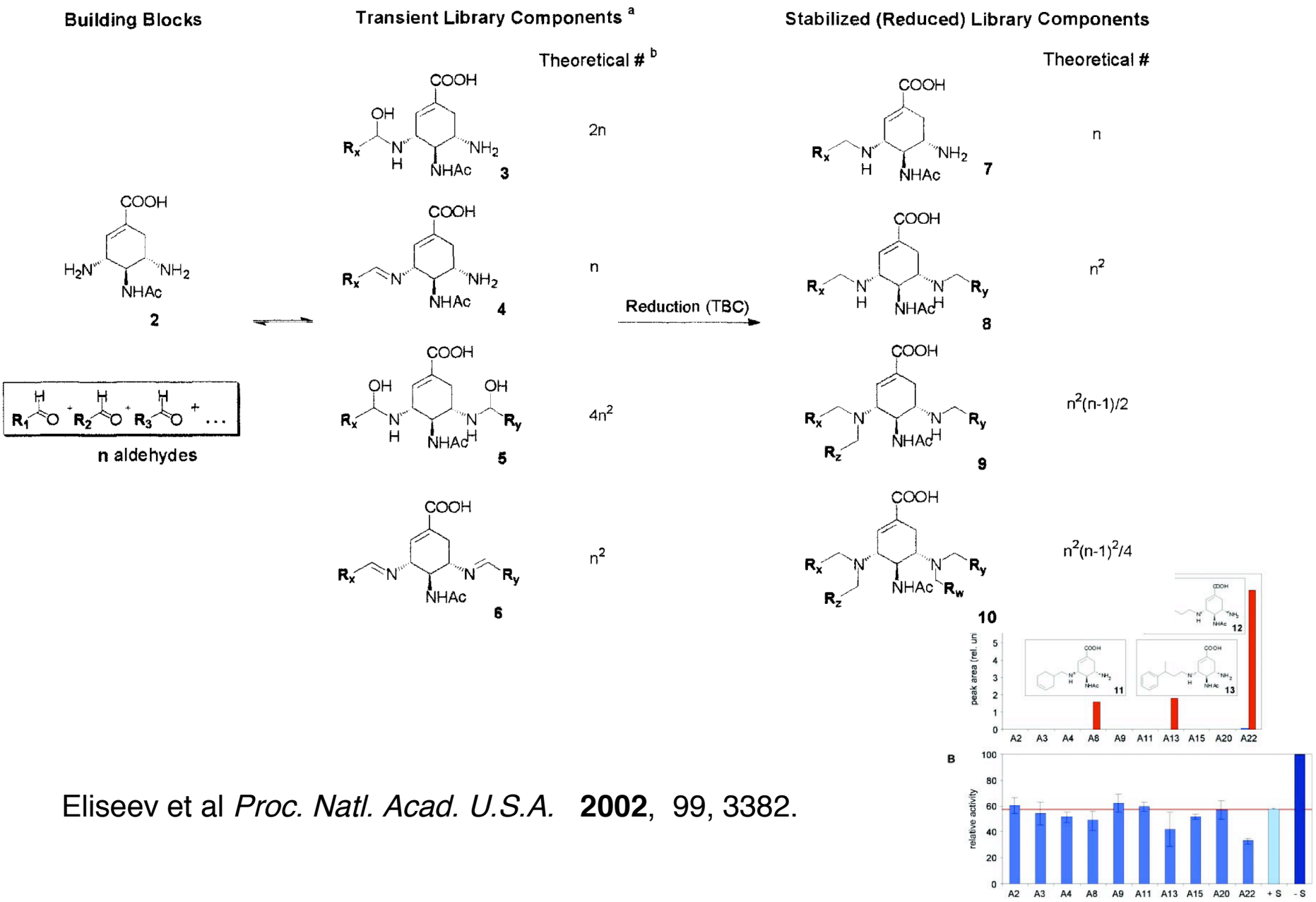
Lenhn, J-M. et al *Proc. Natl. Acad. U.S.A.* **2000**, 98, 1347.



Scheme 72. Formation of a DCL of imines from four amines and three aldehydes.

Imines are sensitive to hydrolysis for practical porpouses requieres reduction of the products to amines. Such covalent modification change geometry and electronics and may affect binding properties of the target.
Some amplification effect using carbonic anhydrase as a template.

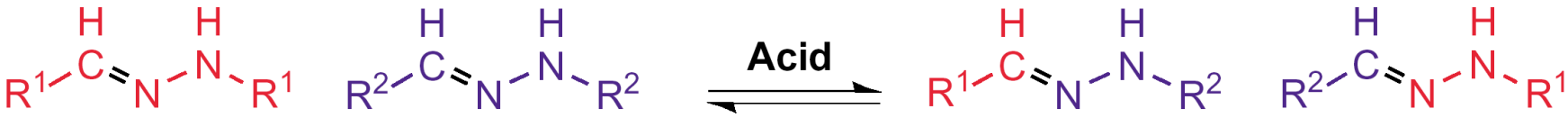
Identification of neuraminidase from potentially DCLs



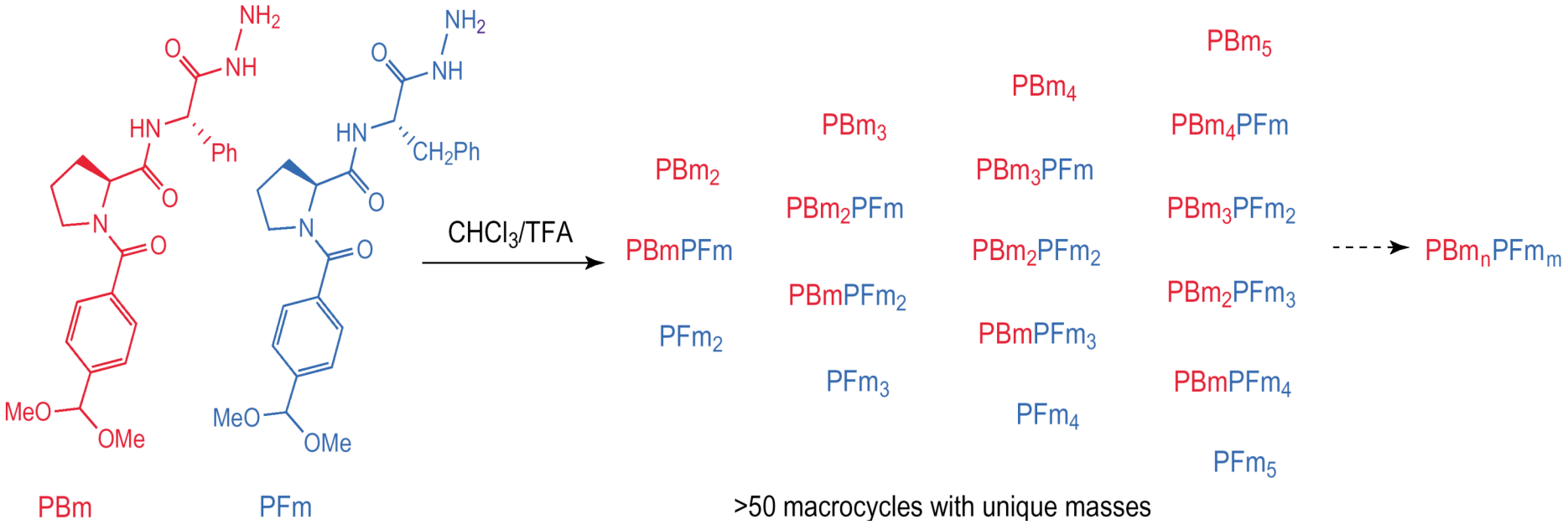
Eliseev et al *Proc. Natl. Acad. U.S.A.* **2002**, 99, 3382.

Hydrazone exchange

Hydrazones and oximes are structurally related to imines but they are hydrolytically more stable.



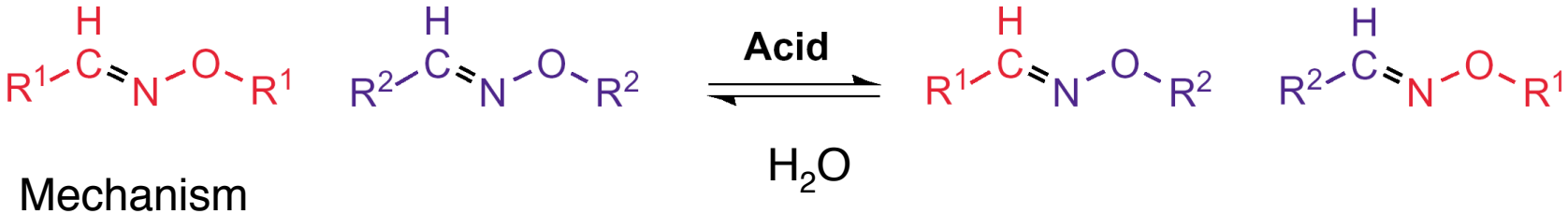
Hydrazone -based DCL, generation of macrocycles



Preparation of receptor-like molecule using Li^+
Analysis by ESI-FTICR-MS

Sanders, J. K. M. et al. *Chem. Comm.* **1999**, 1575.

Oximes exchange



Mechanism

Eliseev, A. et al *J. Phys. Org. Chem*. **1999**, 12, 357

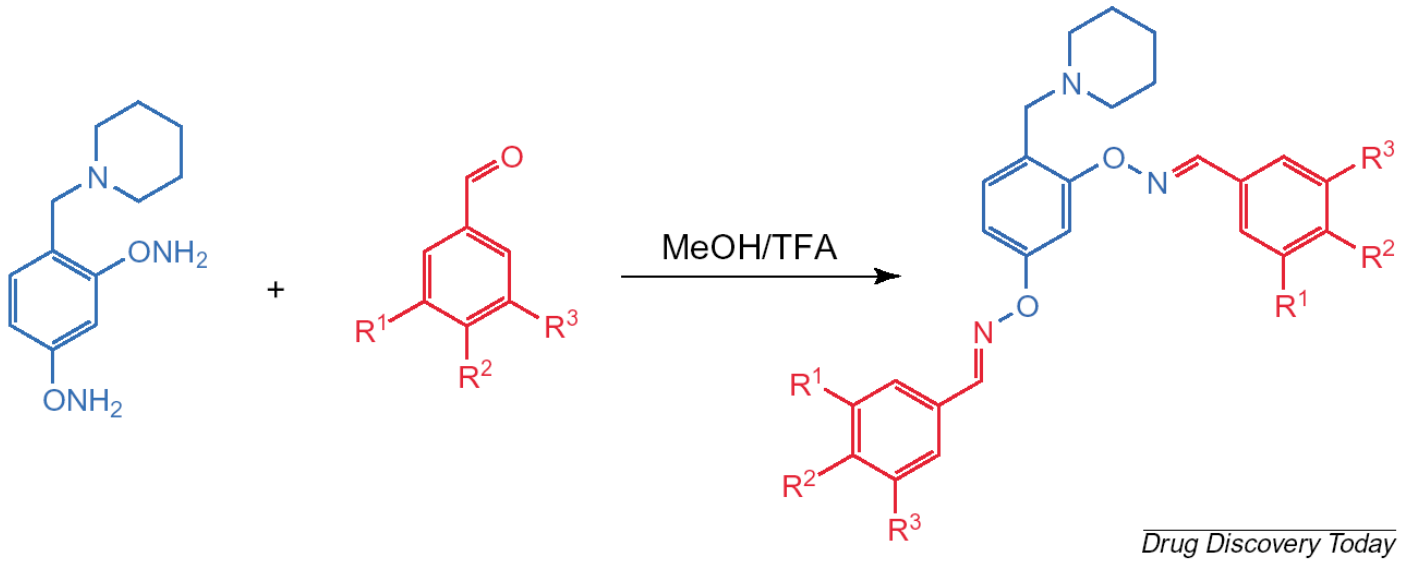
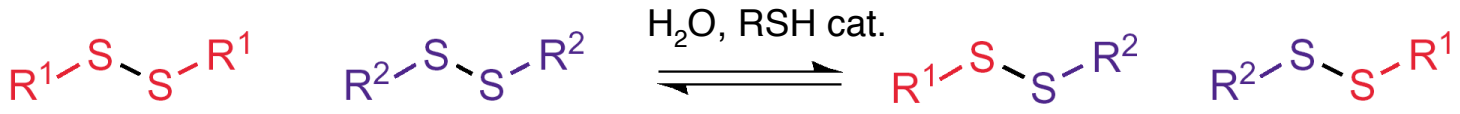
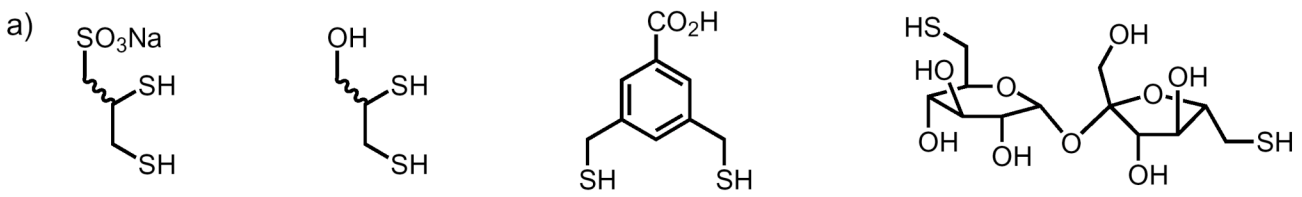


Figure 4. A scaffold-based approach for a dynamic combinatorial library based on oxime exchange. The reaction was carried out at room temperature. R^1, R^2 and R^3 are different combinations of H, OH, Me, OEt, OMe, OAc, and NHAc. Red and blue colouring is used to distinguish the different building blocks.

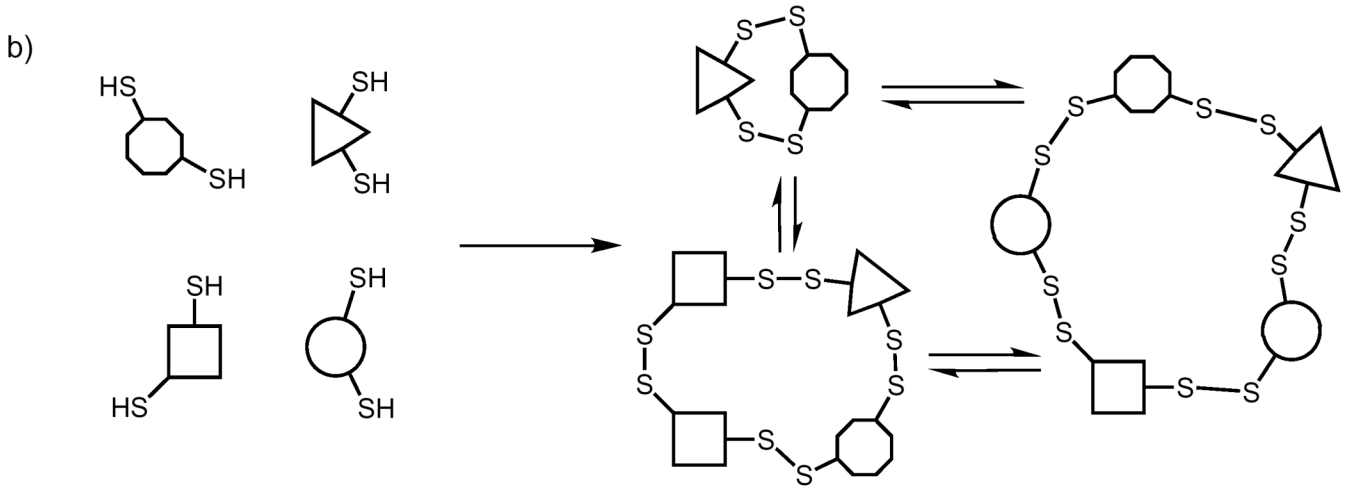
Eliseev, A. et al *J. Comb. Chem*. **1999**, 1, 199

Disulfide exchange





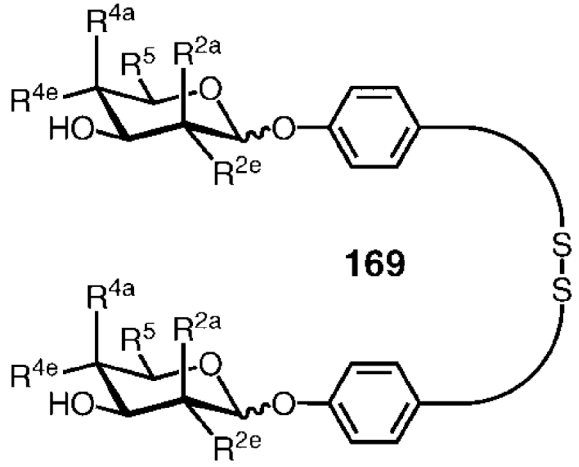
Switched-off slightly ac. cond.



Scheme 79. a) Dithiols **170**–**173** used by Otto et al.[184] to form a DCL of disulfide macrocycles. b) Schematic representation of DCL formation and exchange.

Sanders, J. K. M. et al. *J. Am. Chem. Soc.* **2000**, 122, 12063

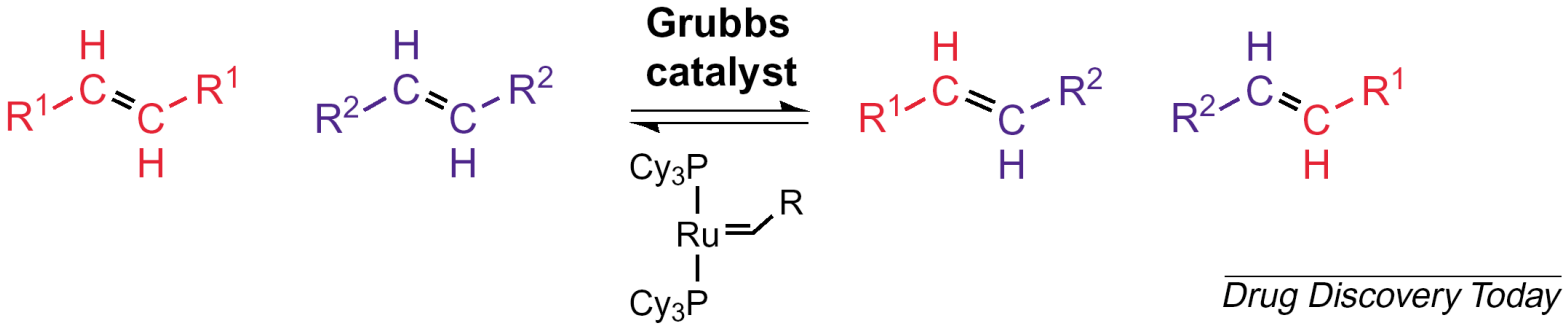
Lehn & co-workers prepare a library of sugar dimmers in H_2O and observe some amplification using Concavalin A as a template.



Compound	α/β	R^{2a}	R^{2e}	R^{4a}	R^{4e}	R^5
Man/Man	α	OH	H	H	OH	CH_2OH
GalC2/GalC2	β	H	OH	OH	H	CH_2OH
GalC3/GalC3	β	H	OH	OH	H	CH_2OH
Glc/Glc	β	H	OH	H	OH	CH_2OH
Ara/Ara	β	H	OH	OH	H	H
Xyl/Xyl	β	H	OH	H	OH	H

Lenhn, J-M. et al. *ChemBioChem* **2000**, 1, 41.

Olefin metathesis



Drug Discovery Today

Cross metathesis of 2 di-substituted olefins can generate 20 different comp.

Brändli, C.and Ward, T. R.; *Helv, Chim. Acta.* **1998**, 81, 1616

Libraries of Vancomycin dimmers in H_2O with templating effect in presence of the peptide Ac2-L-Lys-D-Ala- D-Ala.

Nicolau, K. C.et al. *Angew. Chem. Int. Ed.* **2000**, 39, 3832.

Boronic ester formation

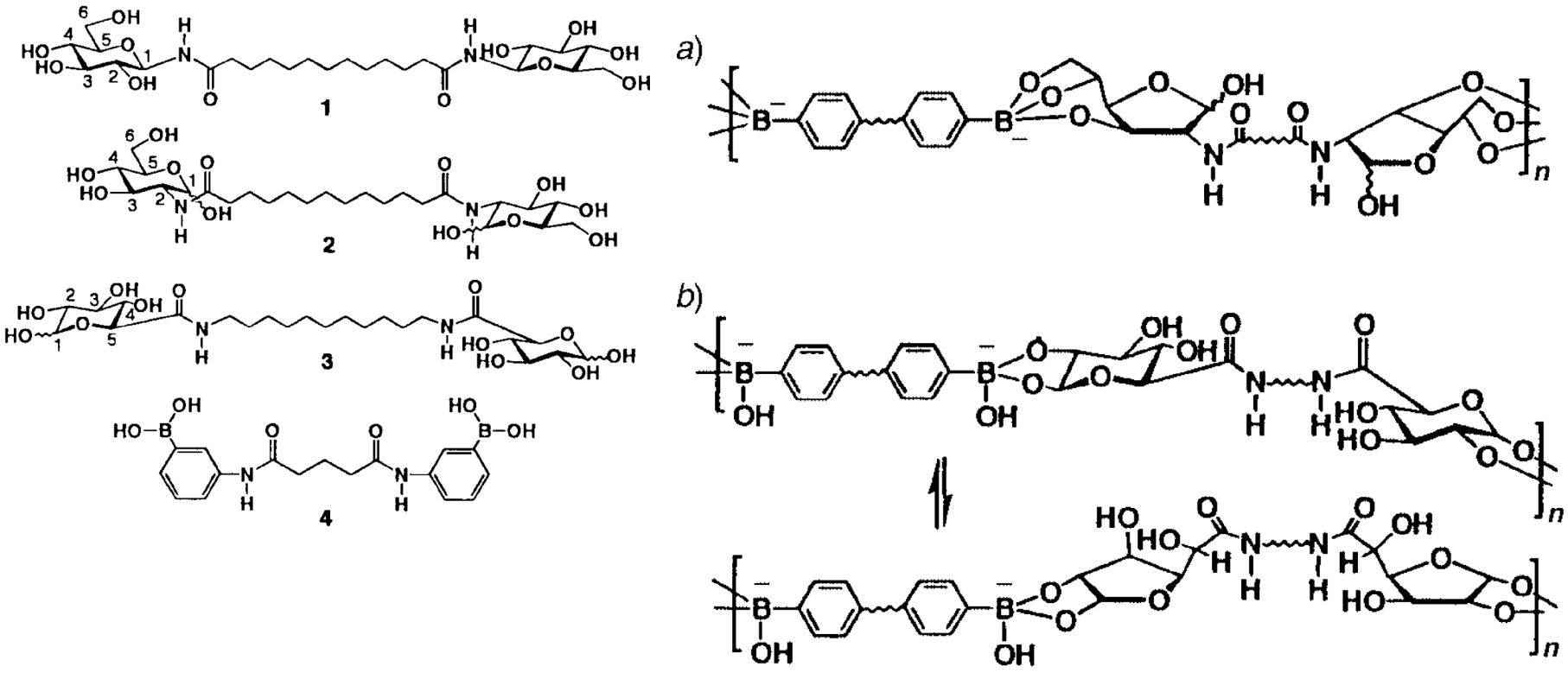
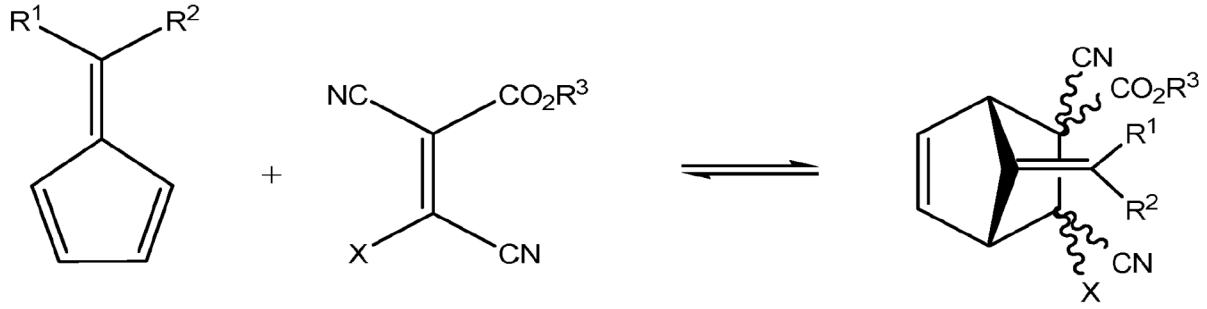


Fig. 2 Possible structures of the polymer main chains formed from (*a*) the mixture of **2** and **4**; (*b*) the mixture of **3** and **4**.

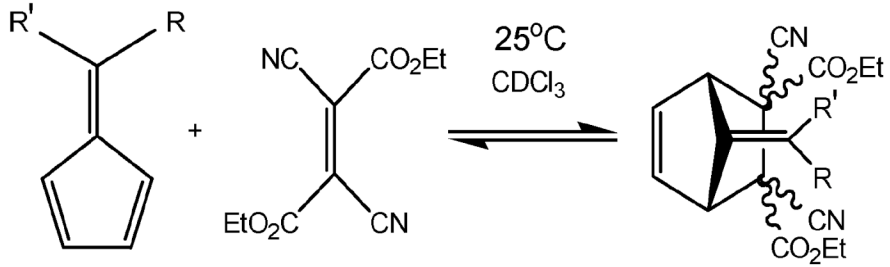
Shimizu et al. *Chem. Com*. **2000** 10, 881

Reversible Diels-Alder reaction

Lenhn, J-M. et al. *Org. Lett* **2005**, 7, 15.



Scheme 1. Reversible Diels−Alder Reactions between Fulvenes **1**−**9** and Diethyldicyanofumarate **10**



1 R = R' = Me **10**

2 R = Me; R' = Et

3 R = R' = Et

4 R = Me; R' = CH_2CH_2OH

5 R = Me; R' = CH_2CH_2OAc

6 R = Me; R' = $CH_2CH_2O_2CCH_2T$

7 R = Me; R' = $(CH_2)_3CO_2H$

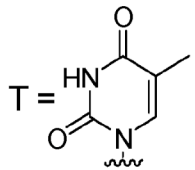
8 R = Me; R' = $(CH_2)_3CONHBz$

9 R = Me; R' = $(CH_2)_3CONH$-(L)$CH(CH_2Ph)$-CO_2Me

(**n**,**10**)

n = **1**-**9**

T =

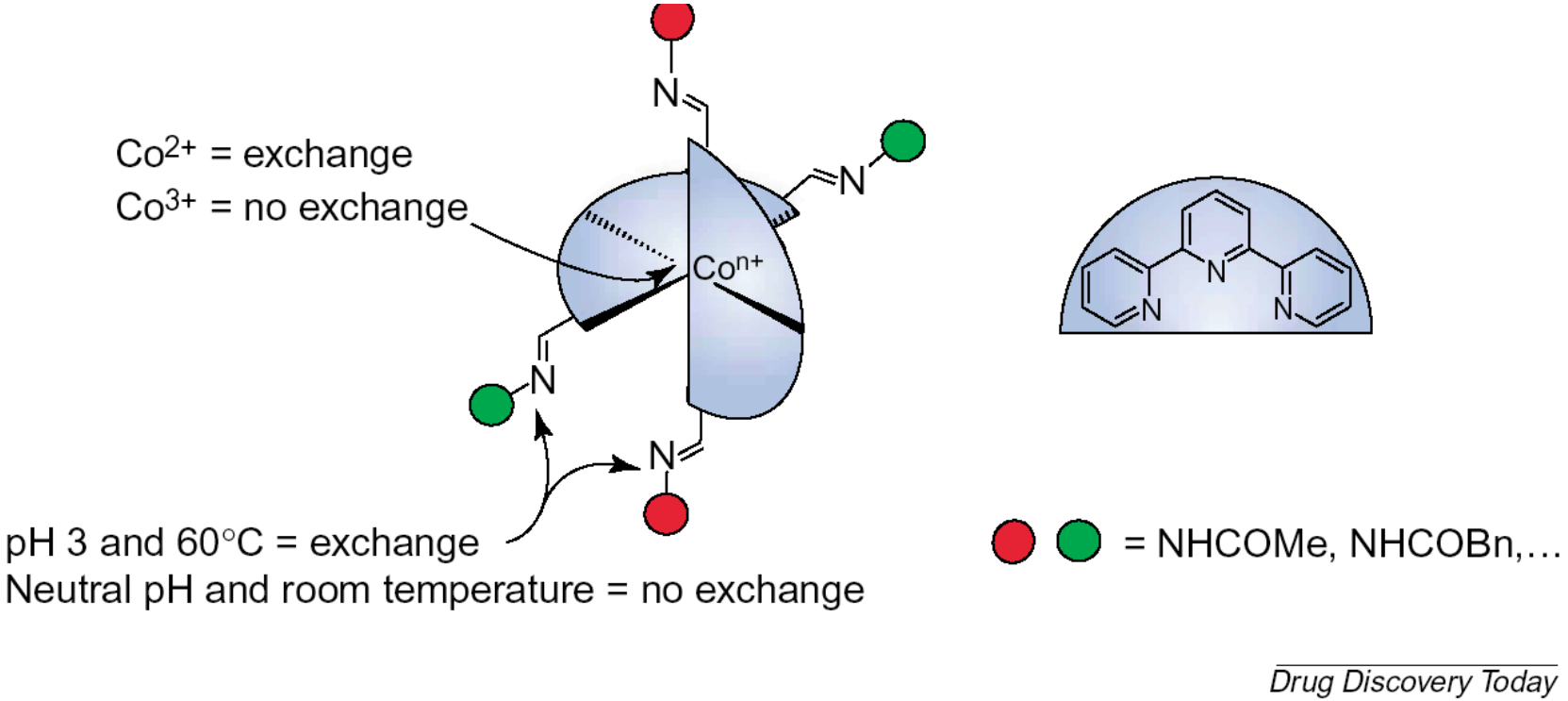


- 3-D structure from planar comp
- Belong to a self-contained RR
- Easy generate structural and functional diversity

Orthogonal libraries

Goral et al. *Proc. Natl. Acad. U.S.A.* **1997**, 94, 2106

Hydrazones are incorporated into the tridentate ligands that bind to a central Co^{+n} ion.



Two Orthogonal because: 1. Exchange with ligands around Co^{+2} center
2. Hydrazone exchange

EXAMPLES OF TEMPLATING EFFECT

Targets: receptors
small molecules
ions
enzymes

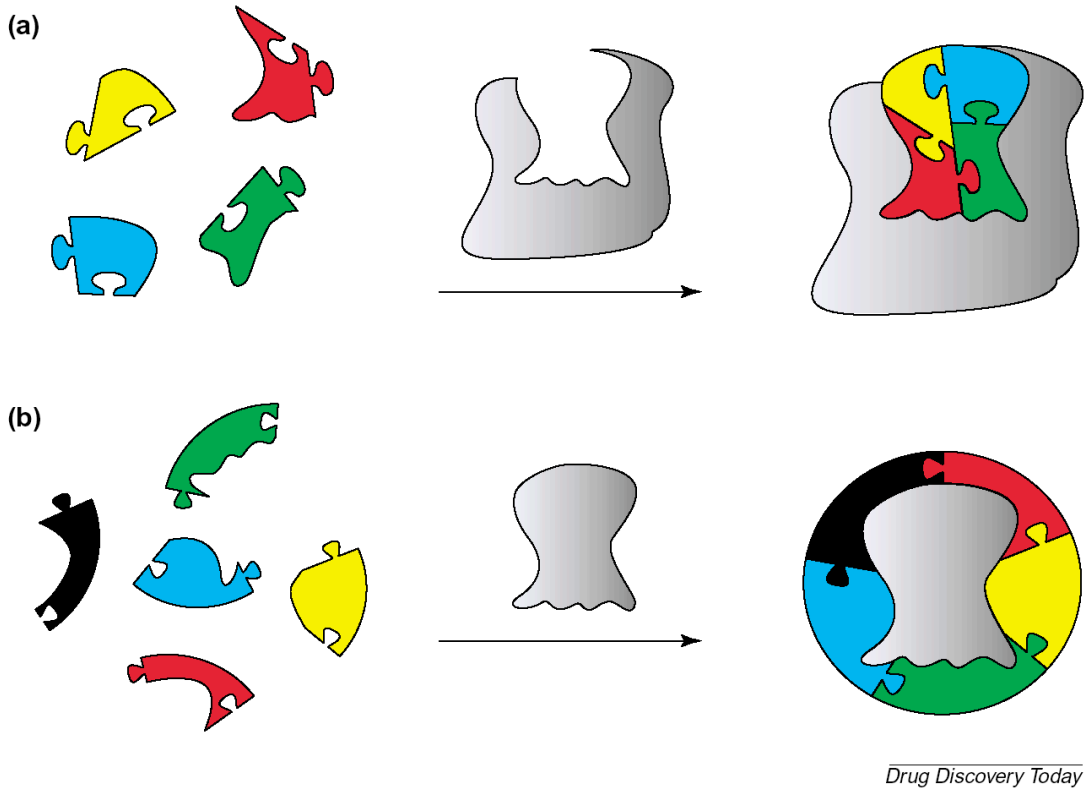
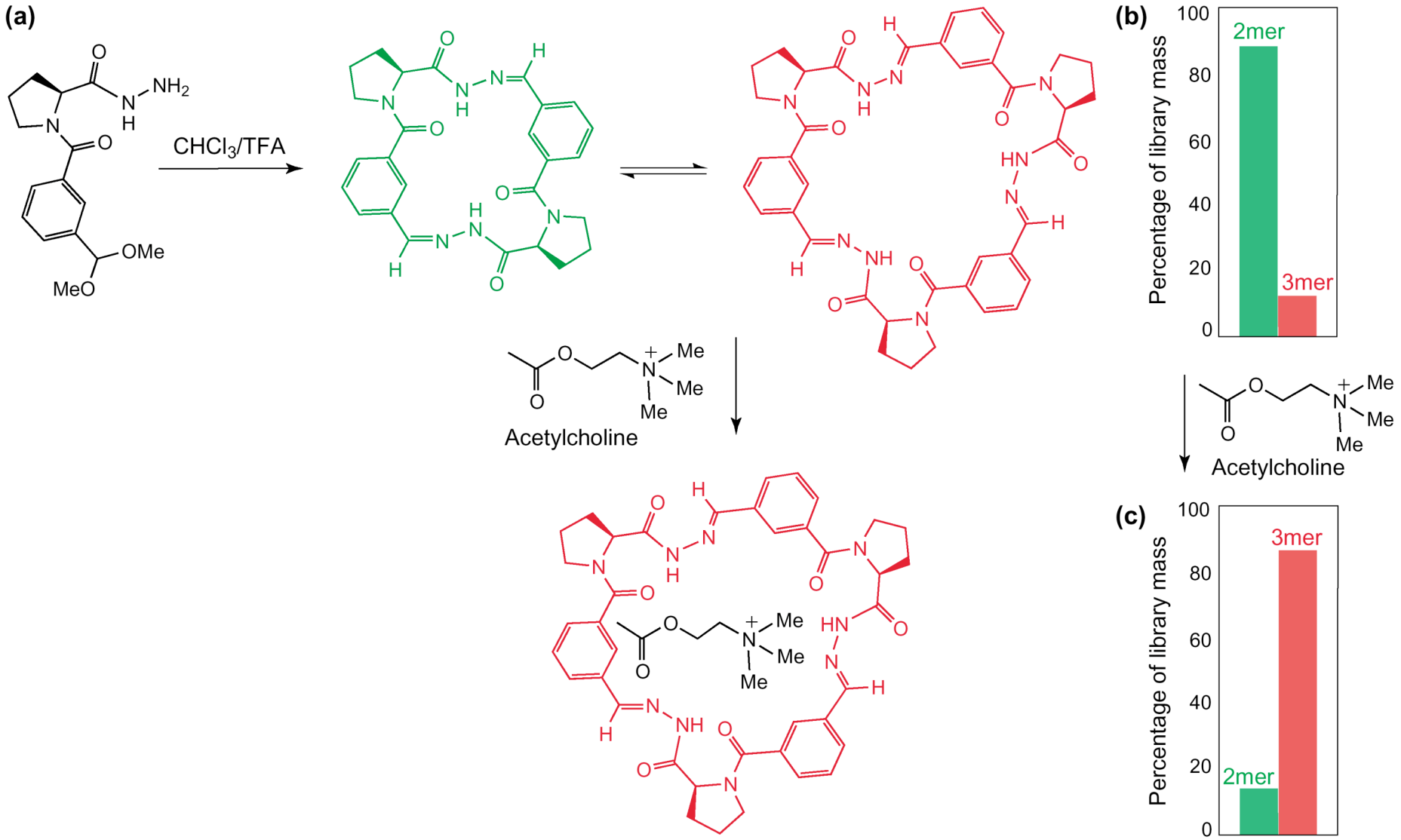
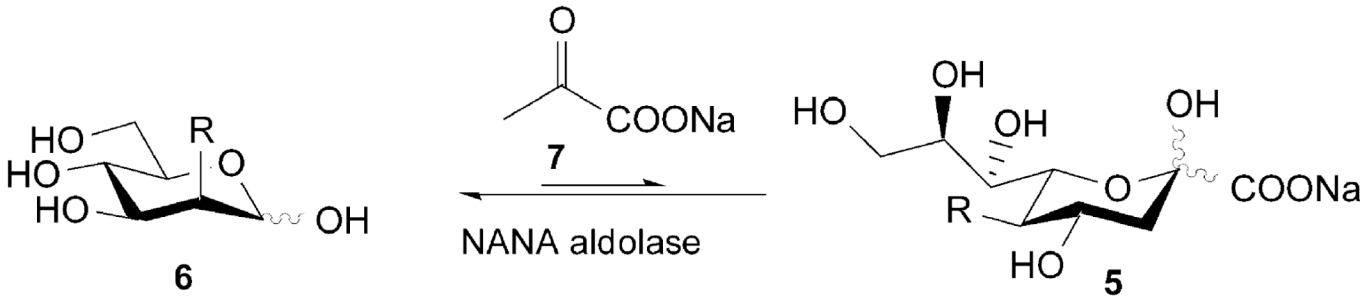


Figure 8. Dynamic combinatorial libraries can be templated by **(a)** receptor molecules or **(b)** ligand molecules.

Acetylcholine as a template induces amplification of the trimmer

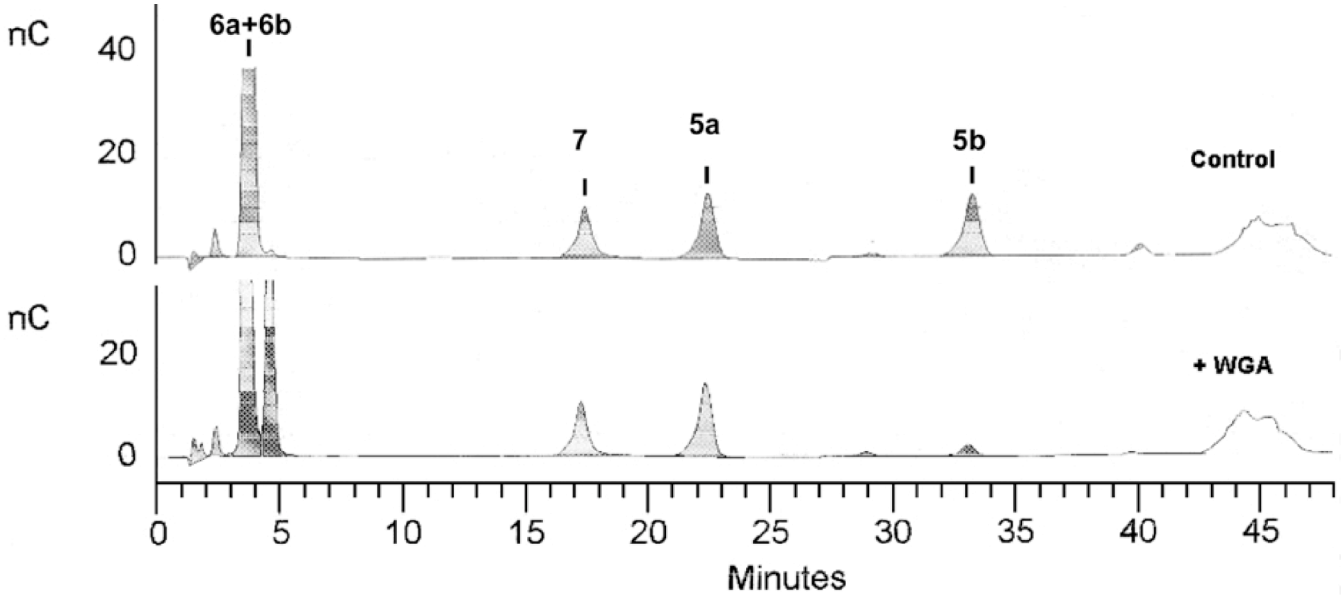


Generation of a dynamic combinatorial library using sialic acid aldolase and in situ screening against wheat germ agglutinin



6a R = NHAc
6b R = OH

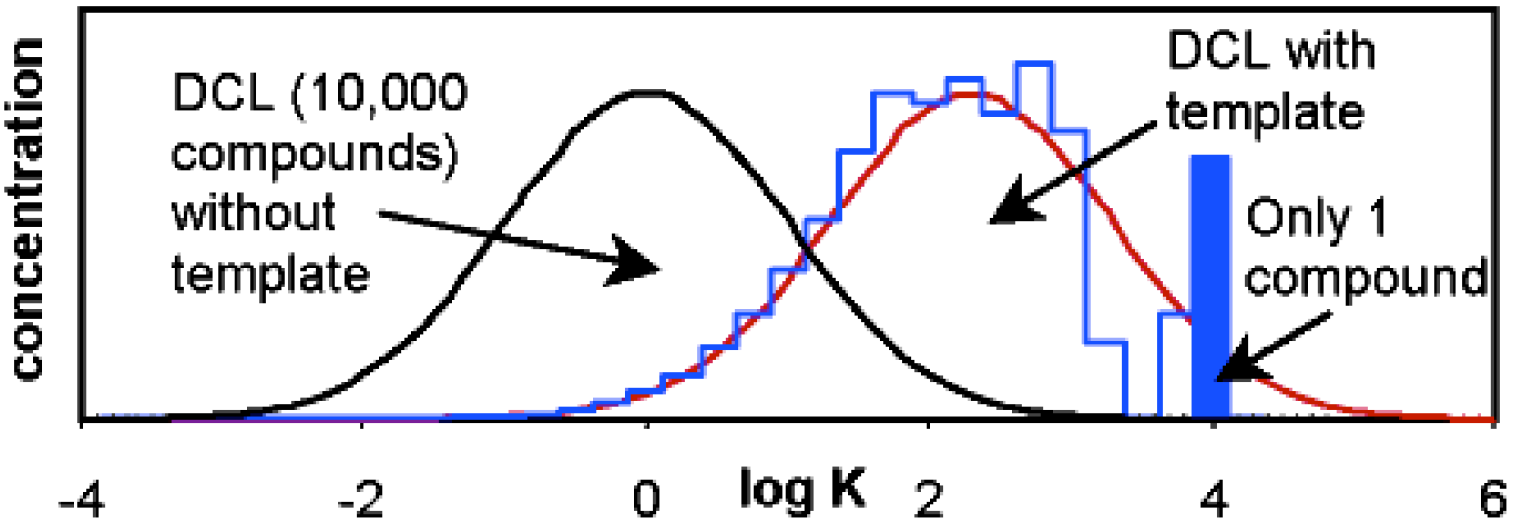
5a R = NHAc
5b R = OH



Lins, et al. *Tetrahedron*, **2004**, 60, 771

Theoretical Studies and Simulations

* What are the limits to the size of effective dynamic combinatorial libraries?

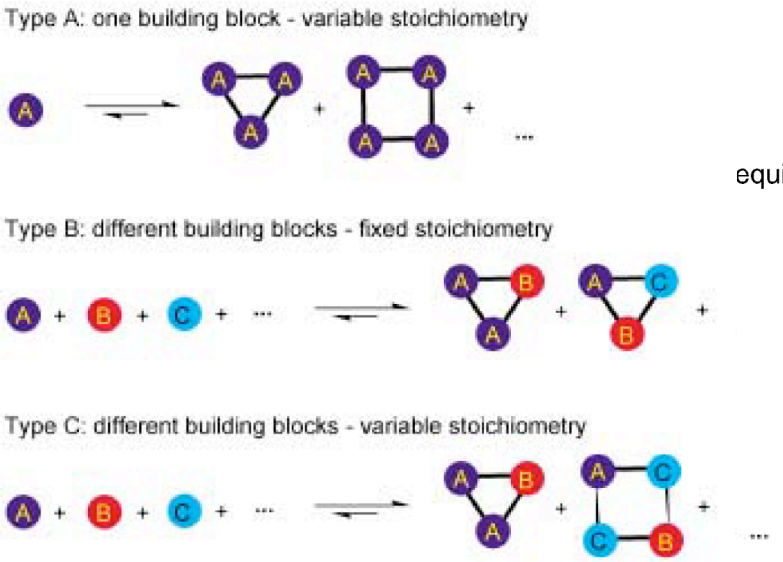


10^6 compounds host-guest binding can induce amplification of strong binders

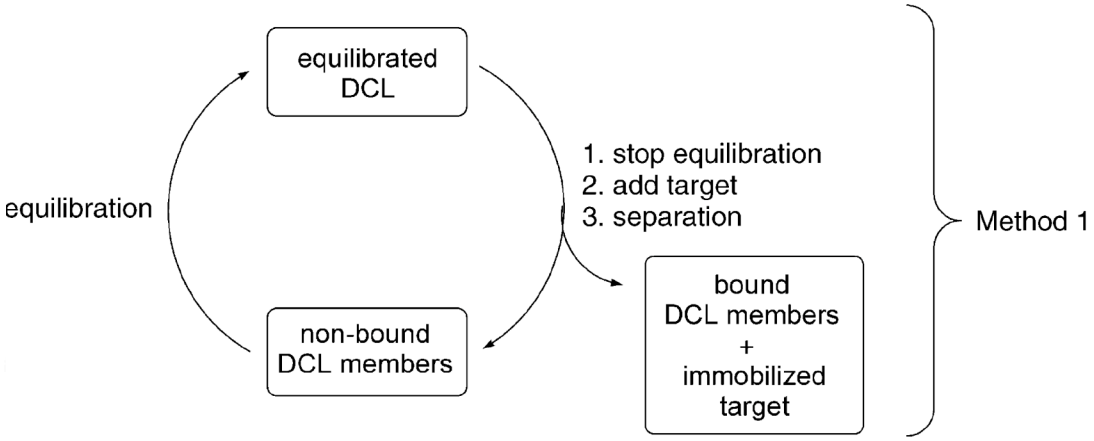
J. K. M. Sanders, et al. *Organic Letters*, **2004**, 6, 1825-1827.

* Do it always amplify the compound with the highest affinity?

NO



Scheme 1. Different types of dynamic combinatorial libraries obtained by assembly of reactive building blocks.



Scheme 7. Iterative procedure for the selection of DCL members with a high affinity to an immobilized target ("Method 1").

Severin, et. al. *Chem. Eur. J.*, **2004**, 10, 2565

* Do it always amplify the best compounds?

Correlation between host-guest binding strength and amplification in simulated dynamic combinatorial libraries.

They shown that under some conditions, it is likely that the best library member will be amplified the most - but that under different conditions, it is more likely that some other library member (usually one that still binds quite well to the template) will be better amplified.

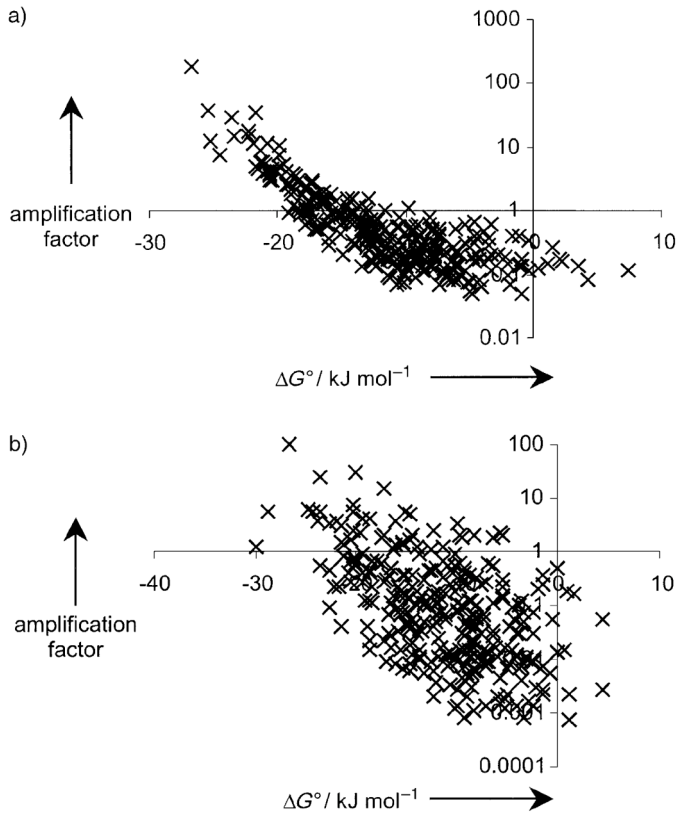


Figure 1. The relationship between amplification and free energy of binding for all of the hosts in two randomly-generated DCLs, that differ only in the way the binding constants are distributed over the various hosts. In both DCLs, the total concentration of the building blocks and the concentration of the template is 10 mM. R^2 values for the correlation between free energy and the logarithm of the amplification factor (taking only significantly amplified hosts into account) are 0.72 and 0.24, respectively.

Two round screening 1. Equimolar amount of template, 2. Reduce the concentration of template

J. K. M. Sanders, et al. *Chem. Eur. J.*, **200**