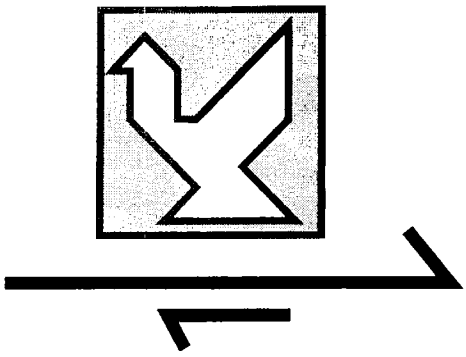
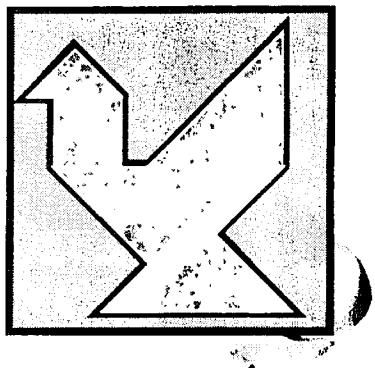
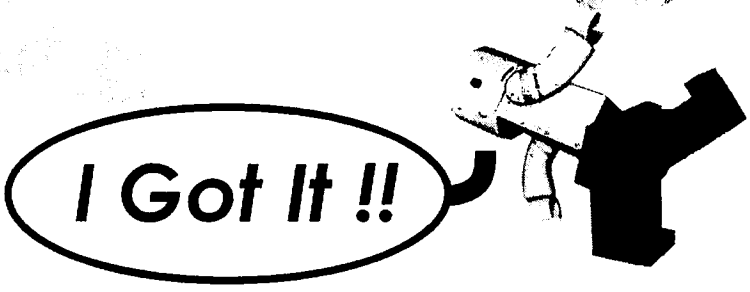
Kounosuke Oisaki (D1 part)

Dynamic Combinatorial Chemistry

~Never-Ending Quest toward High-Performance Chemical Discovery~

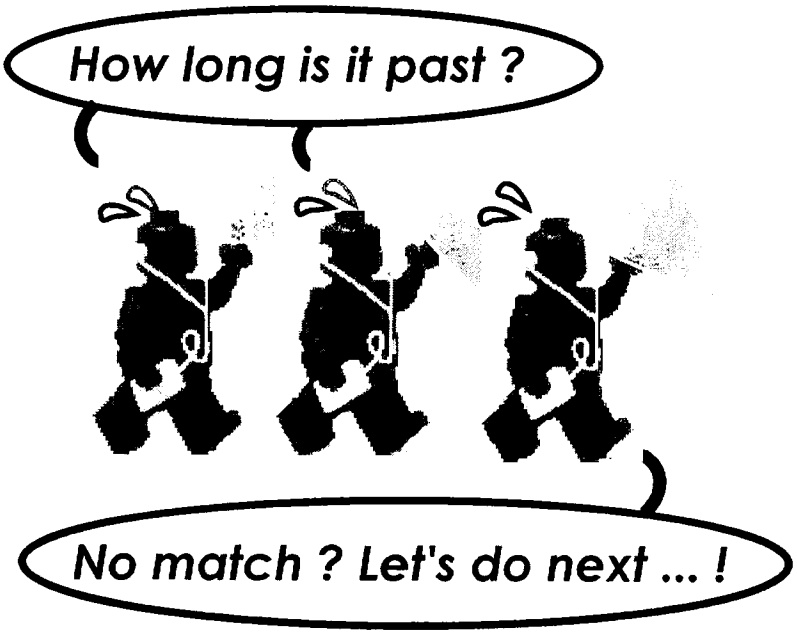


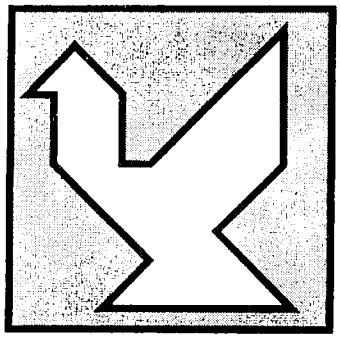




Dynamic Combinatorial Approach

Classical Combinatorial Approach



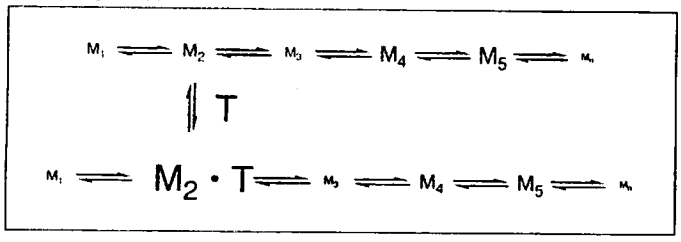


<Contents>

1. Introduction

>> What is "Dynamic Combinatorial Chemistry (DCC)" ??

Scheme 1-1.



Library consists of reversible assembly of starting elements under **thermodynamic** equilibrium.
Addition of template **amplifies** the strongly-binding component *based on Le Chatelier's principle.*

⇩

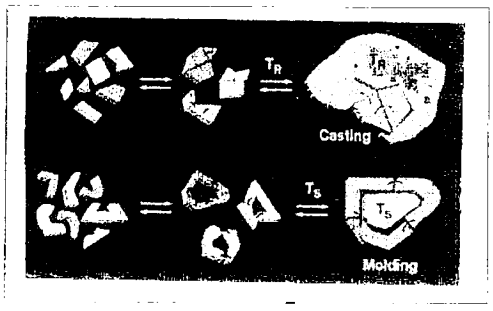
Conceptually New Approach to Identfy Host-Guest Interactions Directly

Table 1-1.

Dynamic / Virtual Library (DCL/VCL)		Combinatorial Library (CL)
virtual, interchanging	Library Set	real, static
present in mixutre	Presence	present alone
just only mixing	Preparation	individually synthesized
molecule / supramolecule	Constituents	molecule
covalent / non-covalent	Bonding	covalent
in situ selection	Assay	individual
HPLC-MS low-resolution	Analysis	Fluorescence etc. high-resolution

⟹ Unified synthesis-screening-amplification process is characteristic.

Figure 1-1. Two kinds of templating



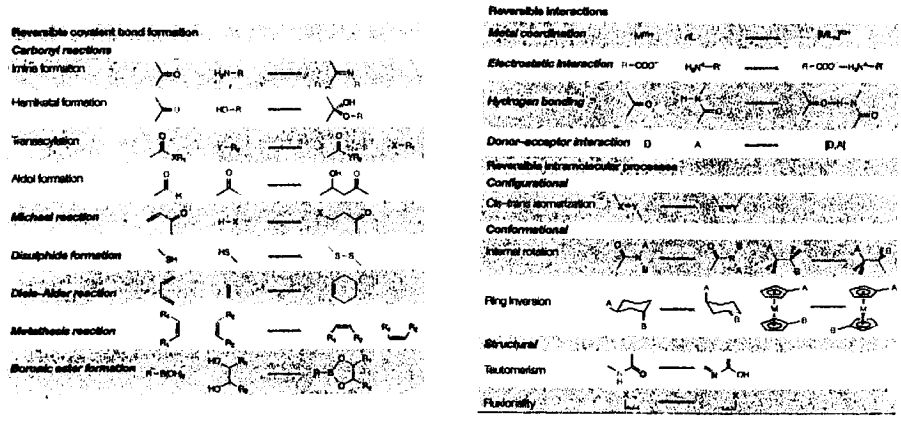
CASTING : relatively small molecule is formed to fit a large receptor template. (i.e. discovery of guest)

⟹ Application to Drug Lead Discovery
(by finding the strongest-binder with enzyme etc.)

MOLDING : Larger, (supra)molecular assembly is formed to encapsulate a small molecular template. (i.e. discovery of host)

⟹ Application to Catalyst Discovery
(by finding the strongest-binder with transition-state analogues)

Table 1-2. Potentiallv usable interaction for DCC



ref) Lehn, J.-M., et al. *Nature Rev. Drug. Discov.* **2001**, *1*, 26.

2. CASTING for drug discovery

Proc. Natl. Acad. Sci. USA **1997**, *94*, 2106.

Virtual combinatorial libraries: Dynamic generation of molecular and supramolecular diversity by self-assembly

IVAN HUC AND JEAN-MARIE LEHN

Université Louis Pasteur, Institut Le Bel, 4 rue Blaise Pascal, 67000 Strasbourg, France

-Abstract-
Proof of Concept:
DCC approach for drug discovery.

>> Experiment:

Scheme 2-1.

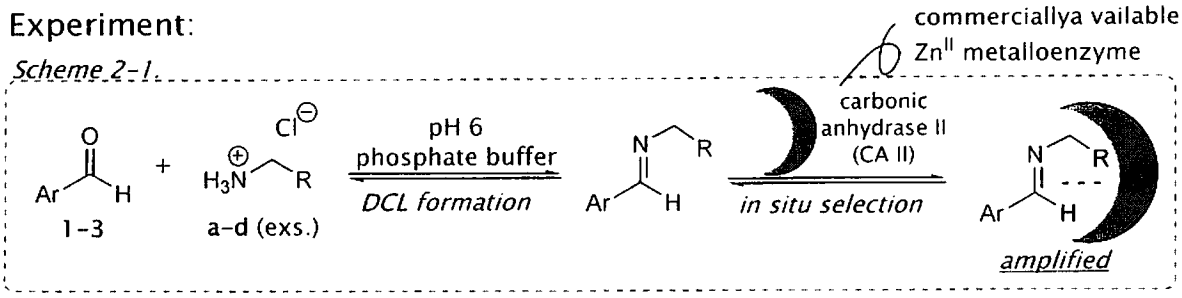
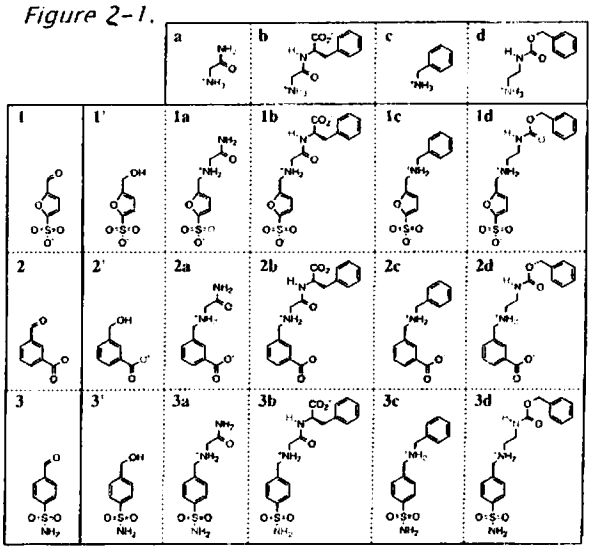


Figure 2-1.

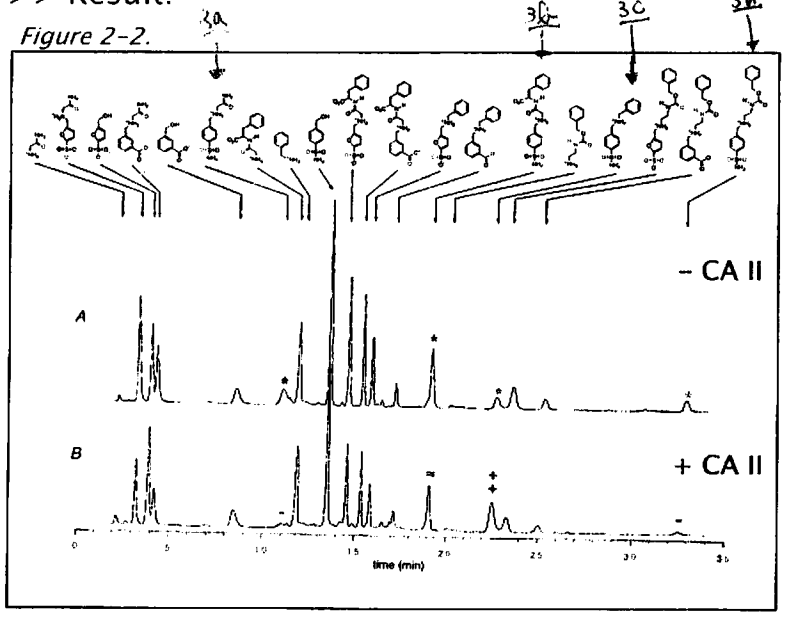


Virtually 3x4 = 12 candidates were generated *in situ*.

para-sulfonamide was known to be essential for inhibition of CA II.

>> Result:

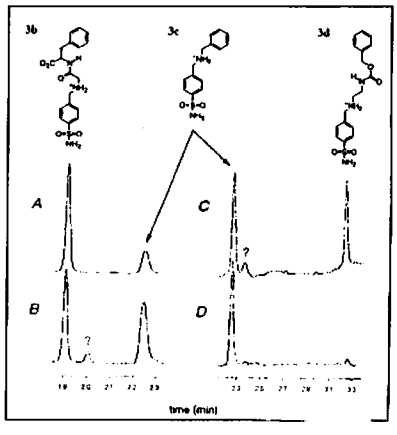
Figure 2-2.



1a-1d, 2a-2d ⟶ no change
3a, 3d ⟶ decreased
3b ⟶ little change
3c ⟶ increased

⇨ family derived from 3 should interact with CA II.

Figure 2-3.



Small-member library experiment also showed amplification of 3c.

Addition of CA II inhibitor (4-sulfamoylbenzoic acid benzylamine) resulted in diminished amplification.

Int. Ed. **2000**, *39*, 3823.

Target-Accelerated Combinatorial Synthesis and Discovery of Highly Potent Antibiotics Effective Against Vancomycin-Resistant Bacteria**

K. C. Nicolaou,* Robert Hughes, Suk Young Cho, Nicolas Winssinger, Christian Smethurst, Harald Labischinski, and Rainer Endermann

Chem. Eur. J. **2001**, *7*, 3824.

Synthesis and Biological Evaluation of Vancomycin Dimers with Potent Activity against Vancomycin-Resistant Bacteria: Target-Accelerated Combinatorial Synthesis

K. C. Nicolaou,*[a] Robert Hughes,[a] Suk Young Cho,[a] Nicolas Winssinger,[a] Harald Labischinski,[b] and Rainer Endermann[b]

-Abstract-
DCC approach to optimize the linker length of vancomycin dimer.

>> Background and Concept:

Figure 2-4.

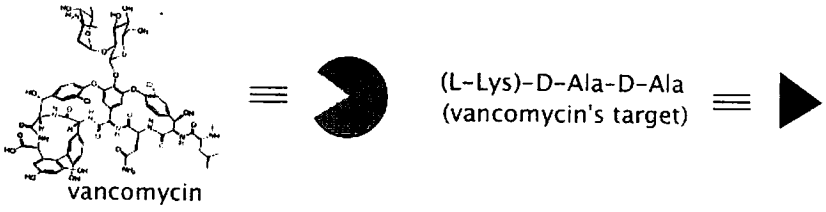
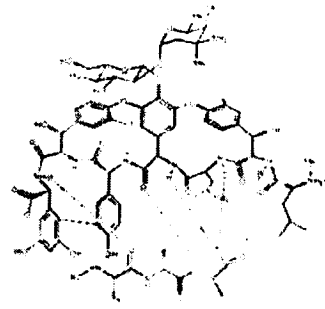
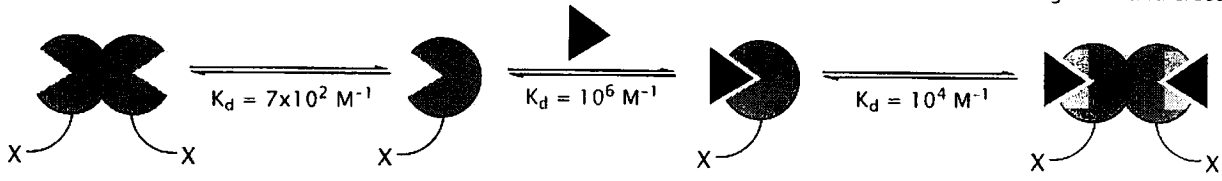


Figure 2-5. antibiotic mechanism of vancomycin



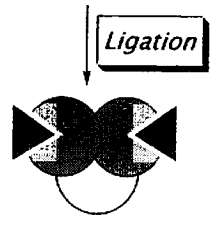
Vancomycin binds to the terminal Lys-D-Ala-D-Ala fragment of growing peptidoglycan biosynthetic precursor, and inhibits the cell wall growth and cross-linking.

Scheme 2-2.



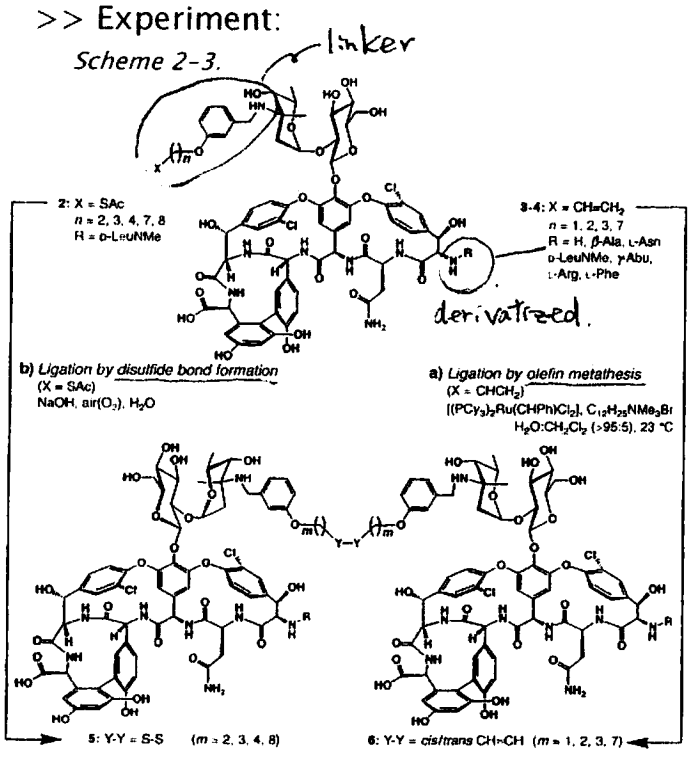
It is known that back-to-back dimerization of vancomycin strengthen the hydrogen-bonding interaction with target.
Vancomycin bound to its target has a greater dimerization constant (ca. 10^4 M) than vancomycin itself.

⟹ If linker length is proper, ligation should be conductable via stable dimer ?
More active vancomycin dimer is possibly discovered ?



>> Experiment:

Scheme 2-3.

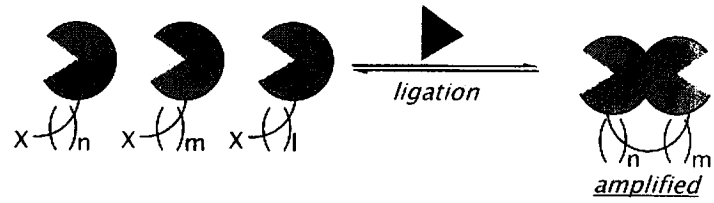


Olefin metathesis or disulfide formation was selected for reversible, bioorthogonal ligation reactions.

Reaction was accelerated in the presence of target, that suggested ligation occured preferably via bounded dimer.

Some termini-modified vancomycin derivatives are also used for DCC to compare the effect.

Scheme 2-4.

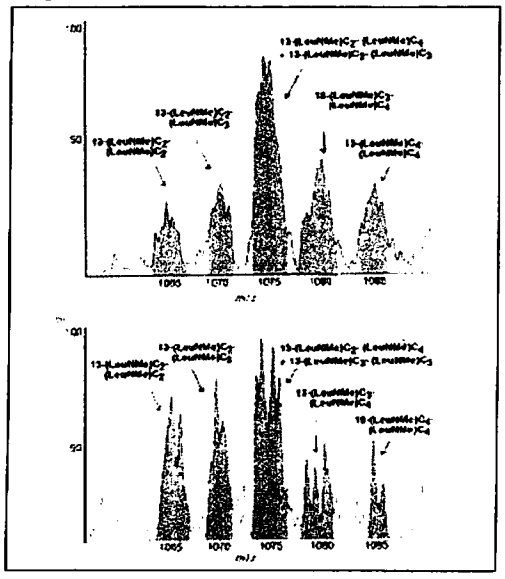


amplified

analyzed by ESI-MS

>> Result:

Figure 2-6.



Library member: 6
$(LeuNMe)C_2$, $(LeuNMe)C_3$, $(LeuNMe)C_4$
Ligation: metathesis
Target: Ac_2-L-Lys-D-Ala-D-Ala

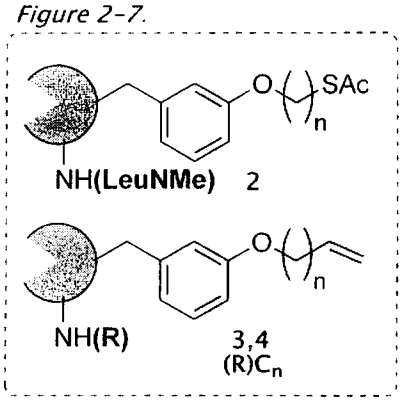
- target

⇨ statistical mixture (1:2:3:2:1)

+ target

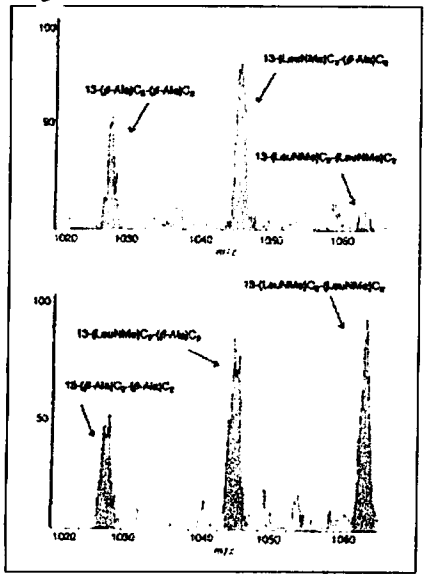
⇨ C_2-C_2 and C_2-C_3 combination was amplified.

Figure 2-7.



Both disulfide bond and olefins.
16 atom between the N atom on suger was optimum.

Figure 2-8.



Library member: 3
$(LeuNMe)C_2$, $(\beta\text{-Ala})C_2$
Ligation: metathesis
Target: Ac_2-L-Lys-D-Ala-D-Ala

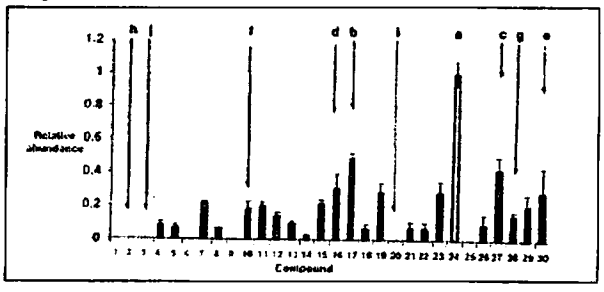
- target

⇨ $(\beta\text{-Ala})C_2$ homodimer was major.

+ target

⇨ $(LeuNMe)C_2$ homodimer was amplified.

Figure 2-9.



Library member: 36
$(LeuNMe)C_2$, $(LeuNMe)C_4$,
$(\beta\text{-Ala})C_2$, $(\beta\text{-Ala})C_4$,
$(Asn)C_2$, $(Asn)C_4$, $(H)C_2$, $(H)C_4$
Ligation: metathesis
Target: Ac_2-L-Lys-D-Ala-D-Ala

⇨ $(LeuNMe)C_2$ homodimer [a] > [b] > [c] > --- > [g] >> [h]-[j]

Table 2-1.

Table 1. Antibacterial activity (MIC: μg mL⁻¹) of selected vancomycin dimers against vancomycin susceptible strains, a vancomycin intermediate resistant strain, and a vancomycin-resistant strain.

[Rank]Compound	Strain Sa8250[a]	Sp670[a]	27266[a]	L4002[a]	48Na[a]	Mu50[b]	L4001[c]
tetracycline	3.13	50	100	100	> 100	50	50
vancomycin	0.4	0.4	3.13	0.8	0.8	3.1	> 100
olefinic dimers							
[a] 6-(LeuNMe)C_2-(LeuNMe)C_2	< 0.03	< 0.03	0.125	0.25	0.25	1	2
[b] 6-(LeuNMe)C_2-(β-Ala)C_2	< 0.03	0.125	0.25	0.25	1	4	8
[c] 6-(LeuNMe)C_2-(LeuNMe)C_3	< 0.03	< 0.03	1	1	4	8	4
[d] 6-(β-Ala)C_2-(β-Ala)C_2	1	0.5	4	2	2	4	8
[e] 6-(LeuNMe)C_2-(LeuNMe)C_4	4	8	> 16	16	16	> 16	> 16
[f] 6-(β-Ala)C_2-(β-Ala)C_3	0.25	0.25	1	0.25	2	4	> 16
[g] 6-(Asn)C_2-(Asn)C_2	4	8	16	8	16	> 16	> 16
[h] 6-(H)C_2-(H)C_2	2	4	16	16	> 16	> 16	> 16
[i] 6-(Asn)C_2-(Asn)C_2	8	> 16	> 16	> 16	> 16	> 16	> 16
[j] 6-(H)C_2-(H)C_2	8	4	16	8	16	> 16	> 16
disulfide dimers							
5-(LeuNMe)C_2-(LeuNMe)C_2	< 0.03	< 0.03	1	1	8	8	1
5-(LeuNMe)C_2-(LeuNMe)C_3	< 0.03	< 0.03	2	1	4	8	2
5-(LeuNMe)C_2-(LeuNMe)C_3	0.125	0.06	4	2	8	8	2

[a] Vancomycin-susceptible strains (Sa8250 and Sp670 are *Streptococcus pneumoniae* strains; 27266 and L4002 are *Enterococcus faecalis* strains; 48N is a multiresistant *Staphylococcus aureus* strain (MRSA)). [b] Vancomycin intermediate resistant strain (MU50 is a *Staphylococcus aureus* strain). [c] Vancomycin-resistant strain (L4001 is a *Enterococcus faecium* strain).

# 48Na = MRSA (multiresisitant)
Mu50 = VISA (Vancomycin intermediate resistant)
L4001 = VRE (Vancomycin resistant)

Newly diveloped vancomycin dimer has strongest activity, even to VRE.

Biological activity of a-j is almost consistent with the tendency of amplification (i.e. adequecy of tether).

3. MOLDING for catalyst discovery

Angew. Chem. Int. Ed. **2003**, *42*, 1270.

Dynamic Combinatorial Libraries

Selection and Amplification of a Catalyst from a Dynamic Combinatorial Library**

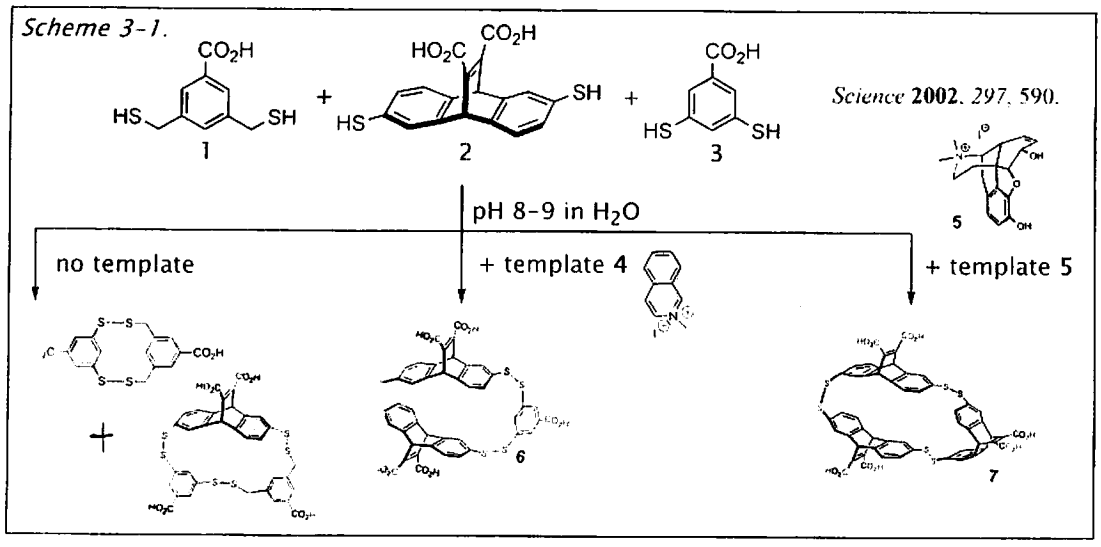
*Barbara Brisig, Jeremy K. M. Sanders, and Sijbren Otto**

-Abstract-
Host molecule developed by DCC approach accelerated the specific reaction.

>> Background and Concept:

Authors had already discovered disulfide-exchange MOLDING system.
Different host was amplified depending on the hydrophobic quarternary ammonium template.

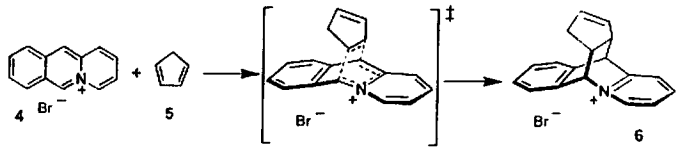
Scheme 3-1.



⟹ If ammonium template is stable transition-state analogues (TSA), catalyst can be developed ?

Model reaction: Diels-Alder reaction of acrizinium bromide

Scheme 3-2.



Authors speculated product 6 is similar to transition-state, so 6 was selected as stable TSA.

>> Experiment and Result:

Scheme 3-3.

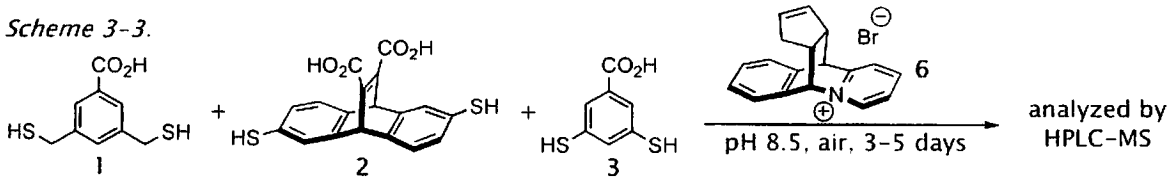
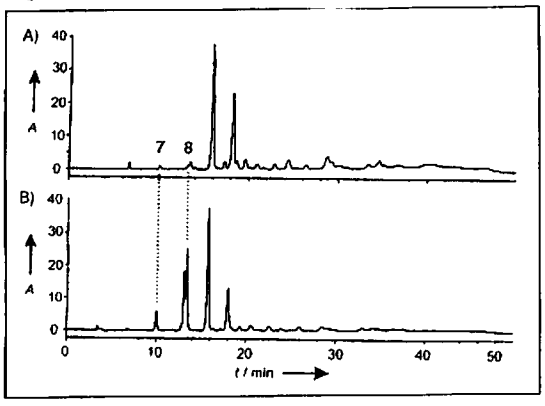


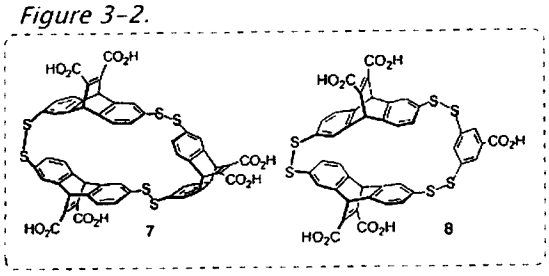
Figure 3-1.



no template

addition of 6

Figure 3-2.



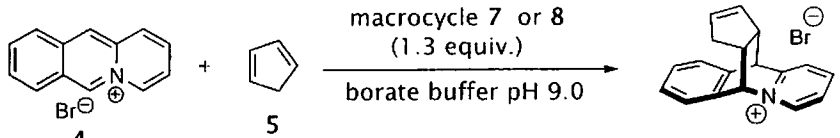
Template amplified macrocycle **7** and **8**.
8 is obtained non-diastereoselectively.

Tabl.e 3-4.

		7	8
4	K_1 [M^{-1}]	1.3×10^5	6.4×10^5
(SM)	ΔG° [kJ mol^{-1}]	−29.1	−33.1
	ΔH° [kJ mol^{-1}]	−23.7	−40.6
	$T\Delta S^\circ$ [kJ mol^{-1}]	5.4	−7.5
6	K_1 [M^{-1}]	2.4×10^5	3.9×10^5
(TM)	ΔG° [kJ mol^{-1}]	−30.7	−31.9
	ΔH° [kJ mol^{-1}]	−25.8	−38.5
	$T\Delta S^\circ$ [kJ mol^{-1}]	4.9	−6.6

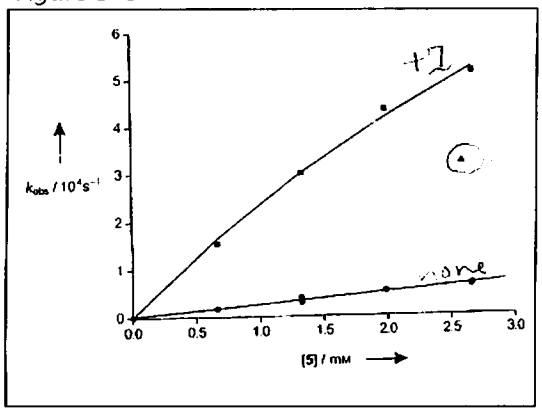
Binding tendency (K_1) is
7: 4 < 6
8: 4 > 6
⟹ **8** is inactive promoter ?
... actually, it is.

Scheme 3-4.



reaction was monitored by UV/Vis spectoroscopy (4 has absorption at 396nm).

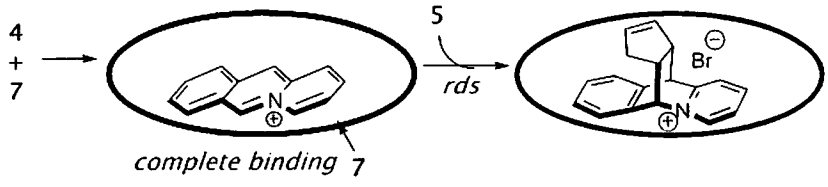
Figure 3-3.



8 didn't act as promoter.

7 induced a modest acceleration effect.

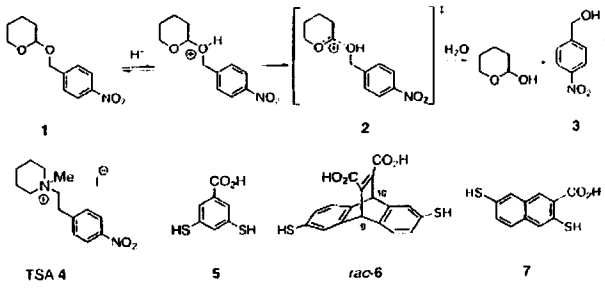
Reaction is pseudo-first order depending on [5].
⟹ Probable mechanism is...



Authors didn't try to use substoichiometric amount of 7... (due to strong product inhibition)
But when 1 equivalent of **6** is added in reaction, still acceleration effect is observed.
⟹ possibility of catalyst turnover ??

In 2005, authors also published out the same approach for development of acetal hydrolysis promoter.

Scheme 3-5. *New. J. Chem* **2005**, *29*, 1001.



4 is designed as a cationic TS analogue.

homotrimer of **6** is amplified in the presence of **4** in DCL.

2.1-fold acceleration of hydrolysis was observed in the presence of homotrimer of **6** .

4. Analytical Advance

At present, the most problematic point of DCC is, <u>low-resolution in analysis</u>.

In large-membered library, usually, **many proper candidates should be generated.**
HPLC separation and detection should become difficult due to insufficient amplification.

According to theoretical model, in a random population, mean binding constant can only be increased to a limited degree (ca. 10^2 fold).

⟹ Some experimental procedure are reported to overcome these analytical problems.

ChemBioChem **2001**, *2*, 438.

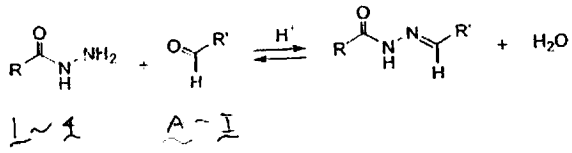
Dynamic Deconvolution of a Pre-Equilibrated Dynamic Combinatorial Library of Acetylcholinesterase Inhibitors

Taridaporn Bunyapaiboonsri,[a] Olof Ramström,[a] Sophie Lohmann,[a] Jean-Marie Lehn,*[a] Ling Peng,[b] and Maurice Goeldner[b]

-Abstract-
Proof of concept: "Dynamic deconvolution"

>> Experiment:

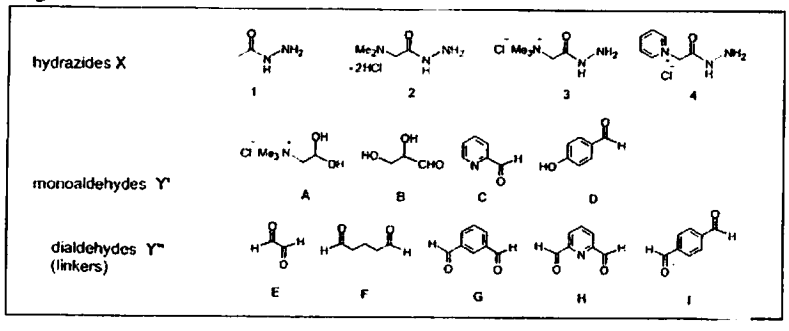
Scheme 4-1.



Reversible acylhydrazone formation constructed DCL.

This library was applied to search acetylcholine esterase (AChE) inhibitor.

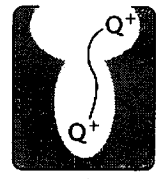
Figure 4-1.



X-Y' or X-Y''-X is generated in DCL.

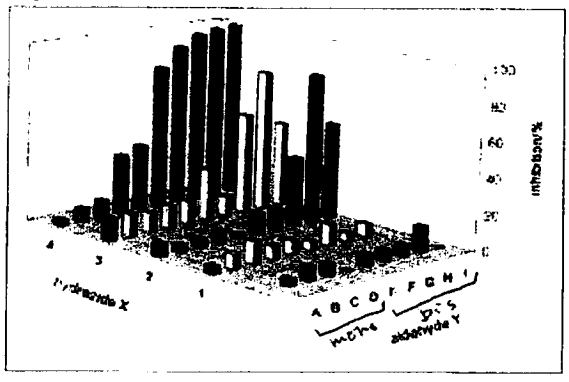
X-Y''-X is designed to adapt two recognition site, located at the bottom and at the mouse.

Figure 4-2.



AChE

Figure 4-3.



Individual assay proved **4-I-4** is the most potent binder. Bis(ammonium) structure is important for strong affinity.

>> "Dynamic Deconvolution" is...

(1) Assay the whole mixture of DCL

1 +...+ 4 + A +...+ I —(pH 4 NaOAc buffer, rt, 1 week)→ generated DCL → aliquot is directly subjected to AChE inhibition assay

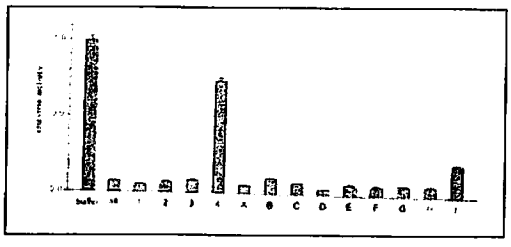
(2) Assay the sub-DCL that lacks specific component

- 2 +...+ 4 + A +...+ I (absence of 1) —(pH 4 NaOAc buffer, rt, 1 week)→ generated sub-DCL → aliquot is directly subjected to AChE inhibition assay
- 1 + 3 +...+ 4 + A +...+ I (absence of 2) —(pH 4 NaOAc buffer, rt, 1 week)→ generated sub-DCL → aliquot is directly subjected to AChE inhibition assay
- ⋮
- 1 +...+ 4 + A +...+ H (absence of I) —(pH 4 NaOAc buffer, rt, 1 week)→ generated sub-DCL → aliquot is directly subjected to AChE inhibition assay

(3) If specific removal resulted in decrease of activity, it should be important component. In this case, assay is required 1 + 4 + 9 = 14 times (< 66 virtual constituents).

>> Result:

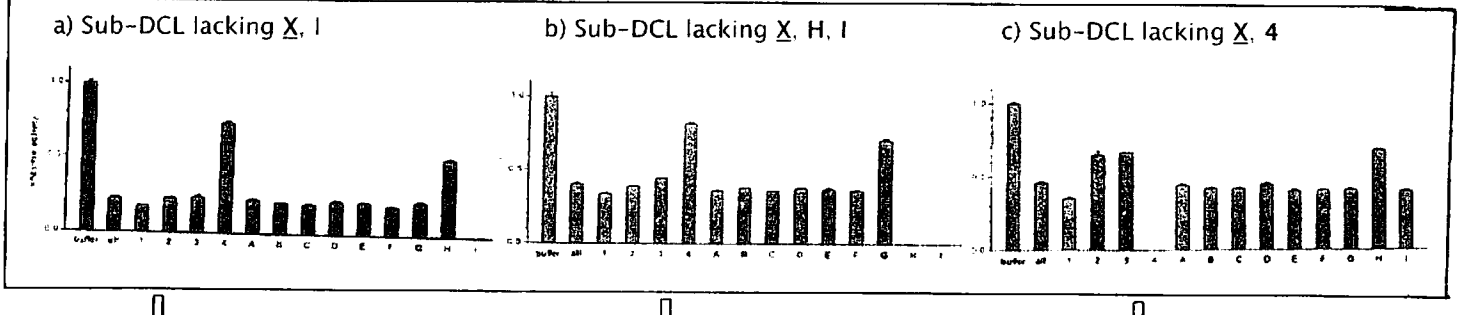
Figure 4-4.



1-component (X) removed Sub-DCLs were tested.

⟹ removal of 4 and I is crucial for library inhibition activity.

Figure 4-5. sequencial dynamic deconvolution to determine "Next important"

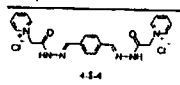
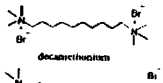
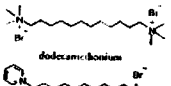
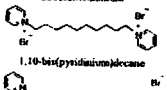
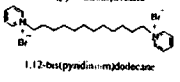


⇩ Next importants are 4 and H

⇩ Next importants are 4 and G

⇩ Next importants are 2, 3 and H, but not so crucial

Table 4-1

Compound	$n^{[a]}$	IC_{50} (nM)
 4-I-4	14	2.30 ± 0.09
 decamethonium	10	1063 ± 42
 dodecamethonium	12	93.2 ± 2.3
 1,10-bis(pyridinium)decane	10	25.8 ± 1.4
 1,12-bis(pyridinium)dodecane	12	16.0 ± 1.3

Obtained best inhibitor 4-I-4 is more potent than other kinds of bis(ammonium) inhibitors, probably due to additional binding effect of linker region.

J. Am. Chem. Soc. **2002**, *124*, 5692.

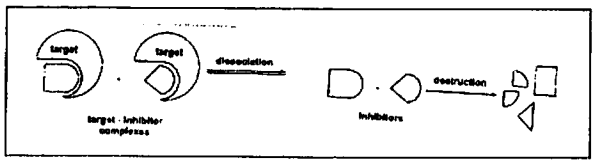
Amplification of Screening Sensitivity through Selective Destruction: Theory and Screening of a Library of Carbonic Anhydrase Inhibitors

Jeremy D. Cheeseman, Andrew D. Corbett, Ronghua Shu, Jonathan Croteau, James L. Gleason,* and Romas J. Kazlauskas*

-Abstract-
Incorporation of irreversible destruction step makes the existence ratio more distincive.
Easier analysis of *in situ* selection dynamic library is probably possible.

>> Concept and Theoretical Treatment:

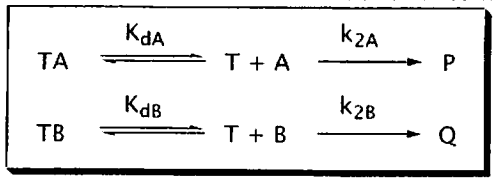
Scheme 4-2. Assumed model



Unbound / weak inhibitor is preferencially destroyed.

Bounded / strong inhibitor is remained.

Scheme 4-3. Theoretical model of 2-component selection



$$S = \frac{\ln[(1-C)(2/(1+R))]}{\ln[(1-C)(2R/(1+R))]}$$

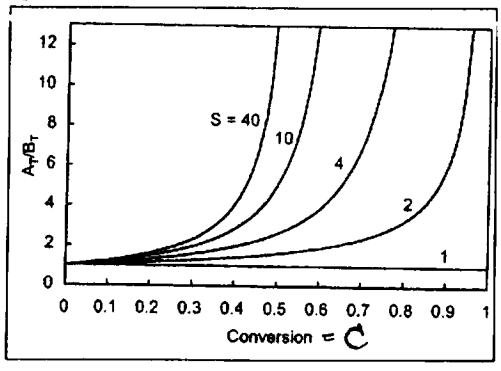
where $C = 1 - \frac{[A_T] + [B_T]}{[A_T]_0 + [B_T]_0}$ (conversion)

$$R = \frac{[A_T]}{[B_T]} \quad \text{(ratio)}$$

if $k_{2A} = k_{2B}$, $S = \frac{K_{dB}}{K_{dA}}$ (selectivity)

($[A_T]$, $[B_T]$ is the total concentration of A and B)

Figure 4-6.

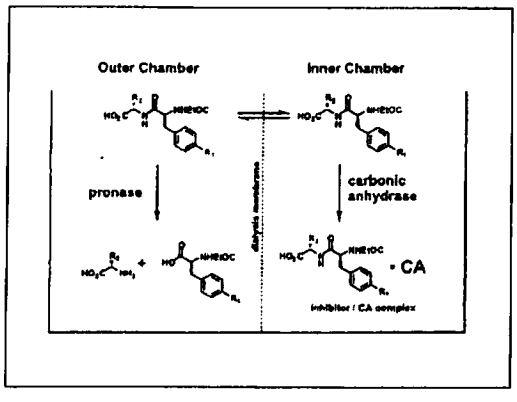


If S > 1 (A is more stronger inhibitor than B).
As the conversion (C) approaches 1, ratio (R) dramatically increased.

⟹ Possibilty for easy analysis of similar binding DCLs.

>> Proof of Concept:

Figure 4-6.

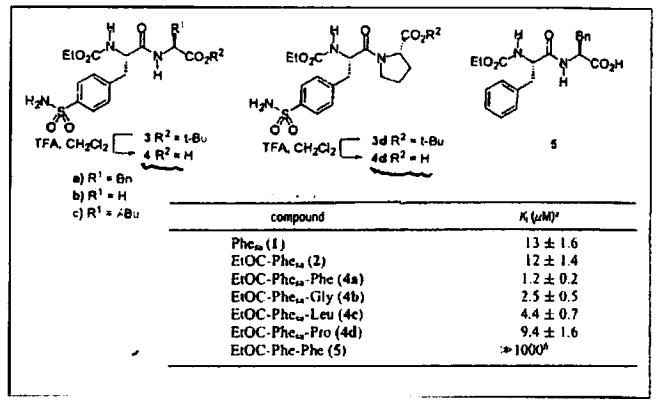


Inner : binding by carbonic anhydrase (CA)
Outer : non-selective destruction by Pronase

Dialysis prevents diffusion of protein,
but small molecule (<10kDa) can move between chambers.

Dipeptide posessing *para*-sulfonamide moiety is chosen as inhibitors.
All compounds have very similar binding constant.

Table 4-2.

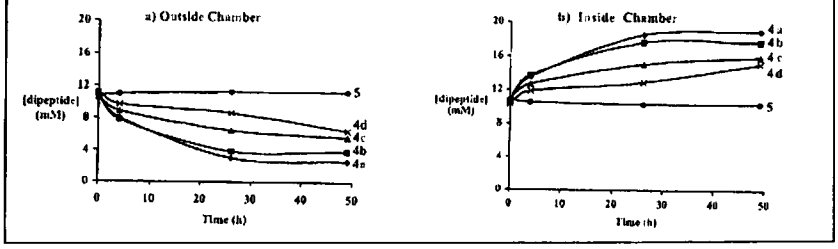


compound	K_i (μM)[a]
Phe_{aa} (1)	13 ± 1.6
EtOC-Phe_{aa} (2)	12 ± 1.4
EtOC-Phe_{aa}-Phe (4a)	1.2 ± 0.2
EtOC-Phe_{aa}-Gly (4b)	2.5 ± 0.5
EtOC-Phe_{aa}-Leu (4c)	4.4 ± 0.7
EtOC-Phe_{aa}-Pro (4d)	9.4 ± 1.6
EtOC-Phe-Phe (5)	≥1000[b]

>> Result:

Figure 4-7.

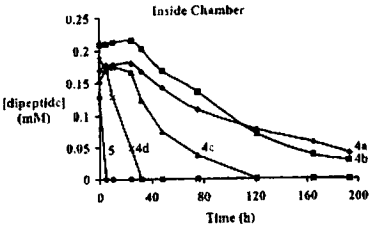
absence of Pronase



Strong binder moved to inside (CA) chamber.
(binding: 4a > 4b > 4c > 4d >> 5)
but existence ratio is < 2 ⟹ **difficult to analysis directly.**
(But because this is small set, concentration is possible to be monitored by reverse-phase HPLC analysis)

Figure 4-7.

presence of Pronase



after 120 h, weak-binding inhibitor is completely destructed.
after 200 h, <u>4a/4b = 3.8 / 1</u>
(larger than K_i ratio = 2.1/1.)
This result matched theoretical value well.

Angew. Chem. Int. Ed. **2004**, *43*, 2432.

Selective Binding

Pseudodynamic Combinatorial Libraries: A Receptor-Assisted Approach for Drug Discovery**

Andrew D. Corbett, Jeremy D. Cheeseman, Romas J. Kazlauskas, and James L. Gleason**

-Abstract-
Expansion of previous concept to pseudo-reversible combinatorial library (pseudo-DCL).

Scheme 4-4.

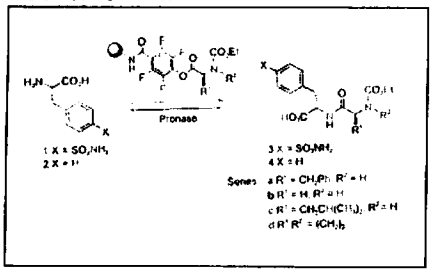
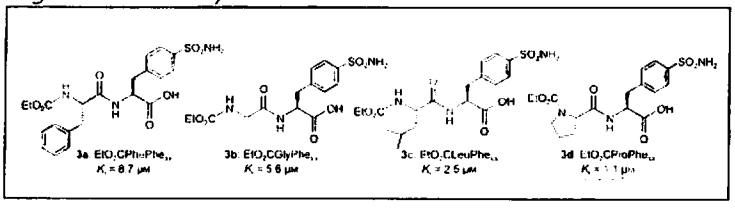
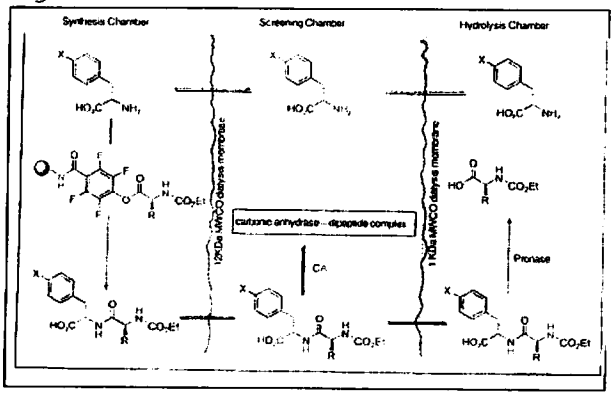


Figure 4-8. library members



Scheduled addition of Tantagel-suppoted activated ester realized **pseudo-dynamic combinatorial library.**(= combination of irreversible synthesis and destruction)

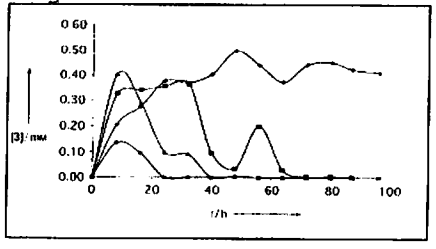
Figure 4-9.



3-compartment reaction vessel is designed.
(synthetic chamber is added to previous system)

Fresh resin with activated ester is added in cycle of 16 h.
(8, 12h were also tested, but too short for this system)

Figure 4-10. Result



after 100h,
<u>3d/3c = >100/1</u>
(K_i ratio= 2.3/1)

5. Future Prospect

>> Overview and Present Problems

Still at the stage of proof of concept (especially in MOLDING system).

Development of the concept and methodology to expand much larger library, is important.

Development of more bioorthogonal library that should be applicable to sensitive biomolecules.

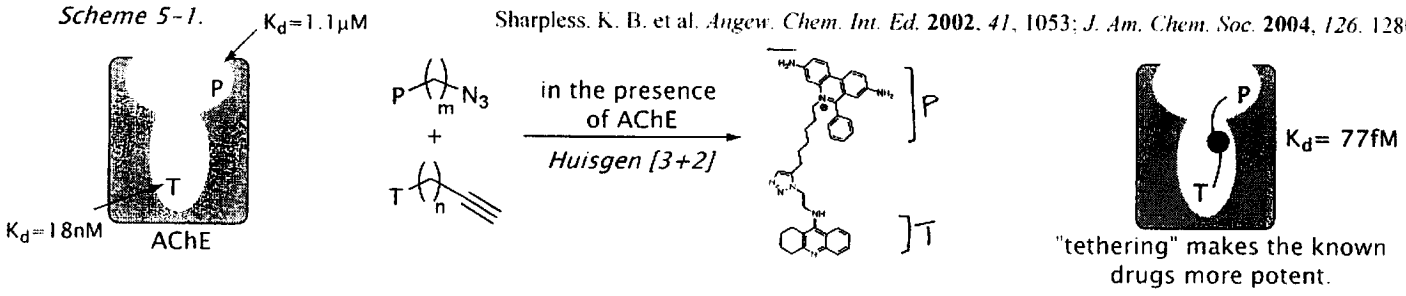
Requirement of stoichiometric amount of template is inherently problematic.

>> Inductive concepts

DCC concept is very attractive: various new concept should be induced from these reports.

> Fragment-based drug discovery

Scheme 5-1.

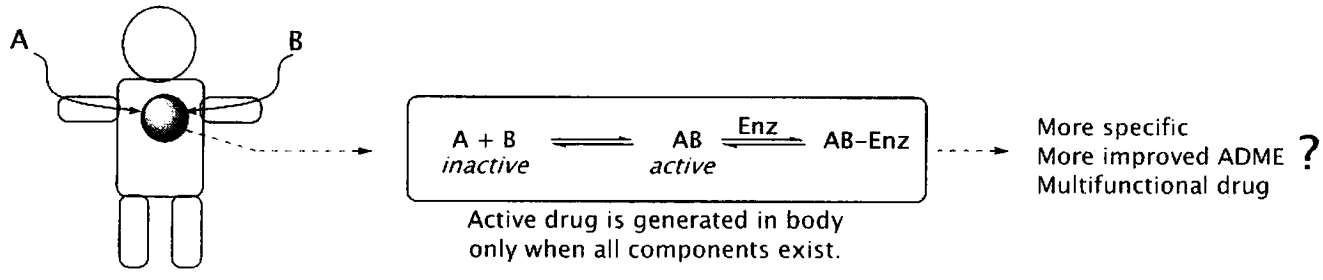


Sharpless, K. B. et al. *Angew. Chem. Int. Ed.* **2002**, *41*, 1053; *J. Am. Chem. Soc.* **2004**, *126*, 12809.

"tethering" makes the known drugs more potent.

> Separated dosing of drug components

Scheme 5-2.



Active drug is generated in body only when all components exist.

> Protein Manifacturing by Selective Reagent / Catalysis

Scheme 5-3.