Dynamic Combinatorial Chemistry

in the identification of new host–guest interactions: proof of principle

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MacMillan Group Meeting
October 17, 2001

Lead References: Lehn, J.-M.; Eliseev, A. V. *Science (Washington, DC, U. S.)* **2001**, *291*, 2331-2332.
Lehn, J.-M. *Chem.--Eur. J.* **1999**, *5*, 2455-2463.
Sanders, J. K. M. *Chem. Soc. Rev*. **1997**, *26*, 327.

Conventional combinatorial approach to identification of host–guest interactions

Combinatorial Library

- molecular constituents
- real set
- collection of molecules
- covalent
- non-reversible
- neutral, uninformed
- systematic
- performed by synthesis in the absence of the target
- assayed by high throughput screening
- amplified by independent chemical synthesis

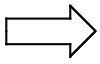
Conventional combichem used to identify molecules of interest ranging from drugs to novel catalysts.

Dynamic combinatorial approach to identification of host–guest interactions

Dynamic or Virtual Combinatorial Library (DCL/VCL):

a set of real or potential compounds which equilibrate under reaction conditions

- molecular or supramolecular constituents
- virtual set
- collection of components
- covalent or non-covalent
- reversible
- instructed
  - ⇨ internally (self-recognition)
  - ⇨ externally (species binding)
    - ⇨ adaptive
- recognition-directed
- self-assembled
- assayed *in situ*
- amplified *in situ*

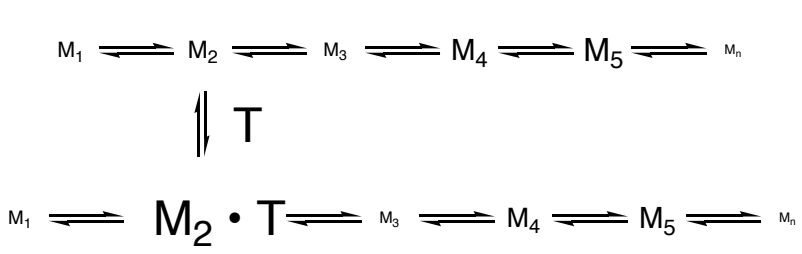


Unifying features of POP research:

- reversible associations
- selection of subunits
- selection of template
- analytical technique
- method for isolation

Dynamic combichem unifies synthesis, screening and amplification steps.

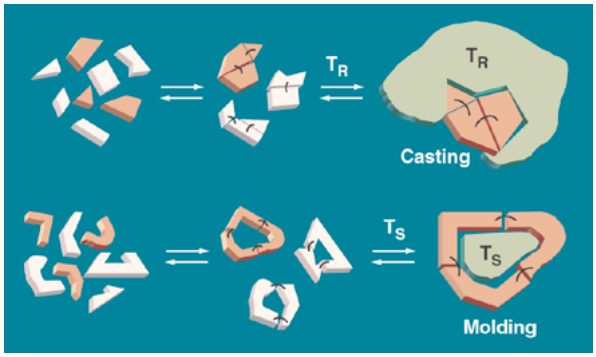
Dynamic combinatorial approach based on Le Châtelier's principle



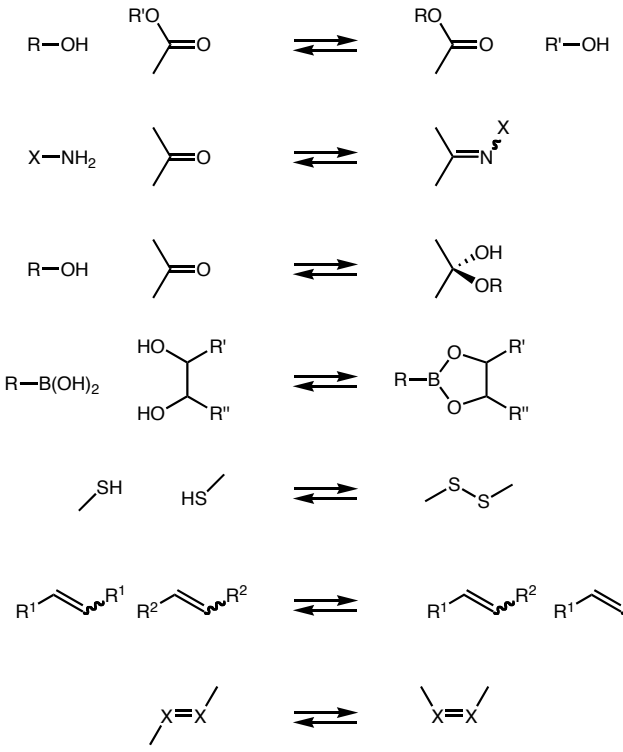
- initial concentrations of library members based on thermodynamic distribution
- addition of template
- equilibrium driven toward members which form favorable associations with template

Two kinds of templating

- **Casting.** A relatively small molecule is formed to fit a large receptor template (e.g. enzyme.)
- **Molding.** A large or even supramolecular assembly is formed to encapsulate a small molecule.



Reversible chemical reactions consitute basis of fluxionality

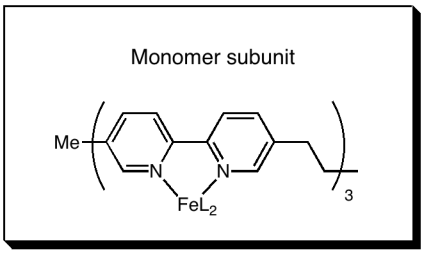


Also: Diels-Alder, conjugate addition, metal coordination, electrostatic interaction, bond rotation, ring inversion, tautomerism

Lehn, J.-M. *Chem.--Eur. J.* **1999**, *5*, 2455-2463

Roots in supramolecular self-assembly:

Trimericbipy-Fe cryptand templated for different counterions

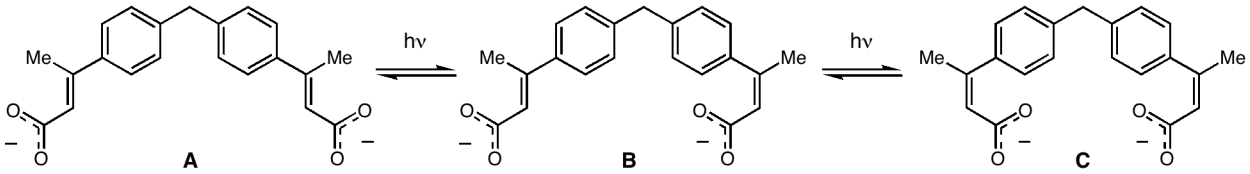


Equilibration based on reversible Fe-bipy complexation.

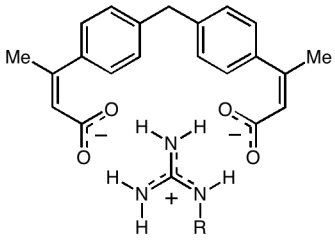
Lehn, J.-M., et. al., *Angew. Chem. Int. Eng, Ed.* **1996**, *35*, 1838.

Figure: Sanders, J. K. M. et. al. *Curr. Opin. Chem. Biol.* **2000**, *4*, 270-279

Elementary examples: three member library based on π-bond isomerization



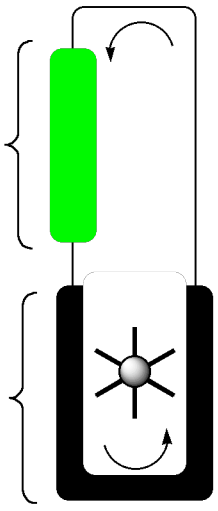
(***trans, trans***) (***trans, cis***) (***cis, cis***)



receptor–template complex

Selection:
silica-bound
arginine

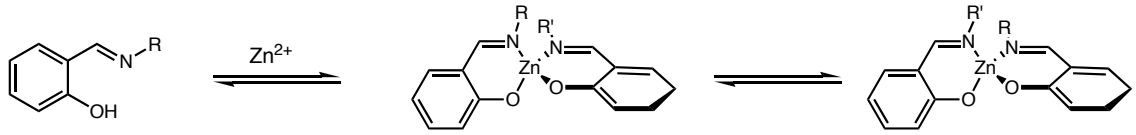
Mutation:
light source

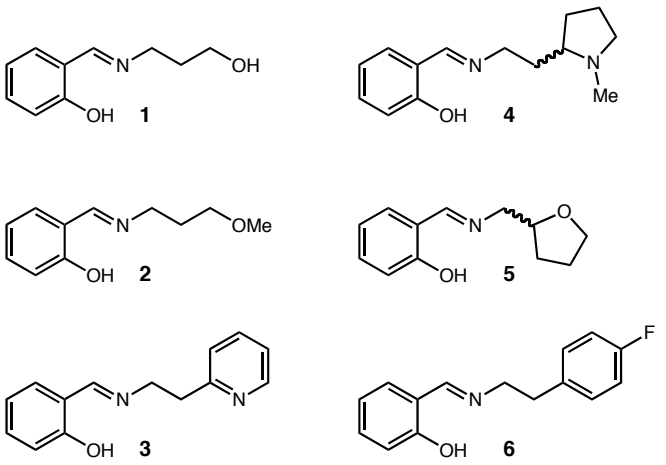


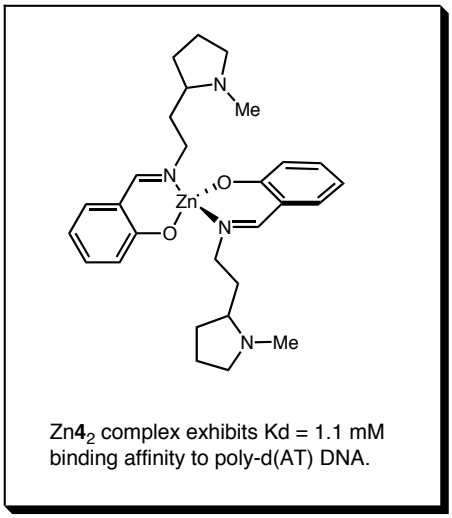
	%A	%B	%C
equilibrated w/o template	69	28	3
in solution after 30 cycles	23	29	48
on column after 30 cycles (54%)	2	13	85

Eliseev, A. V.; Nelen, M. I. *J. Am. Chem. Soc.* **1997**, *119*, 1147-1148.

Elementary examples: Miller's DNA-binding Zn^{2+} salen complexes





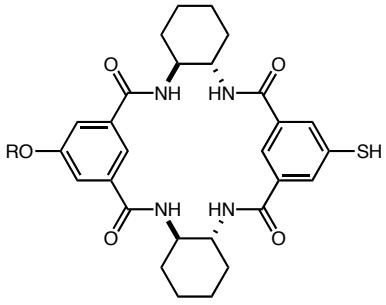


$Zn\mathbf{4}_2$ complex exhibits Kd = 1.1 mM binding affinity to poly-d(AT) DNA.

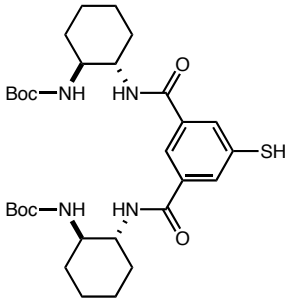
When eluted over an affinity column of immobilized poly-d(AT) DNA in the presence of Zn^{2+}, significantly decreased amounts of **4** were recovered.

Miller, B. L., et. al. *Tet. Lett.* **1997**, *38*, 8639-8642.

"Informed" 3-member DCL used shows bias for homodimers in presence of template



ASH



BSH

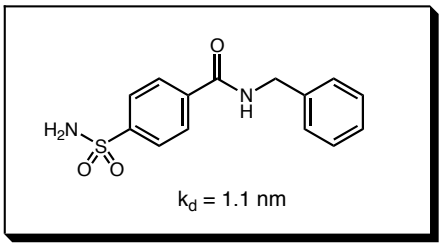
- A-SS-A linked to fluorophore and screened against library of 3375 *N*-acetyl tripeptides.
- Ac-(D)-Pro-(L)-Val-(D)-Val-PS was found to bind favorably to A-SS-A (binding constant ~10^4-10^5).
- A mixture of the two monomers are dimerized in the presence and absence of template.

	A-SS-B	**B-SS-B**	**A-SS-A**
Absence of tripeptide-PS:	43%	57%	
Presence of trpeptide-PS:	15%	85%	
Solution phase	13%	85%	10%
Resin phase	2%	0%	75%

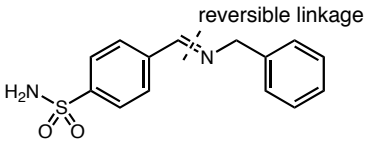
In the presence of cognate peptide, equilibrium shifts to favor homodimers. A-SS-A can be isolated in 97% purity by simple wash cycle.

Still, W. C., et. al. *J. Org. Chem.* **1998**, *63*, 9045.

Raising the bar: template directed amplification of a carbonic anhydrase (CA) inhibitor



known inhibitor of carbonic anhydrase II



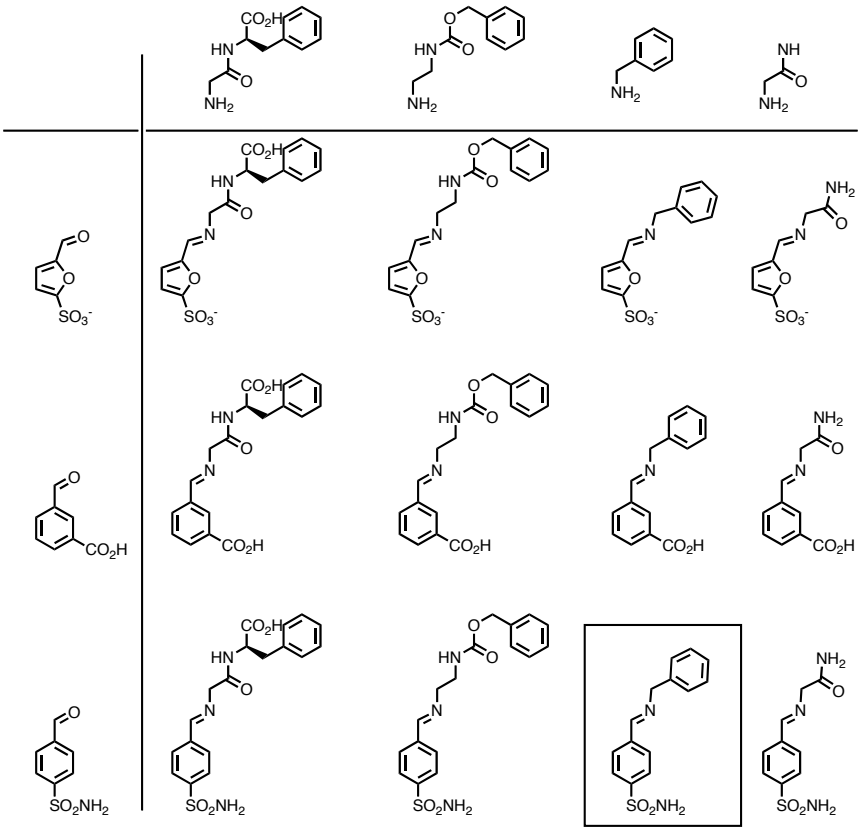
imine isostere

• Purpose: to make a VCL of imines in the presence of CAII and look for amplification of known inhibitor motif

Challenge		Strategy
Bond equilibration under physiological conditions	⇨	Transimination, pH 6
Switch off equilibration process after templating	⇨	$NaBH_3CN$ reduction of imines
Minimize uninformed thermodynamic bias	⇨	Only aryl aldehydes; keep divergent functionality away from bond forming site
Characterize library	⇨	HPLC/MS

Hasenknopf, B.; Lehn, J.-M.; Boumediene, N.; Dupont-Gervais, A.; Van Dorsselaer, A.; Kneisel, B.; Fenske, D. *J. Am. Chem. Soc.* **1997**, *119*, 10956-10962.

Components of Lehn's carbonic anhydrase-templated iminium VCL

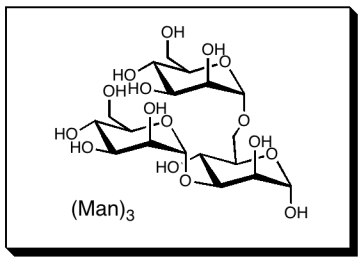


Results of Lehn's carbonic anhydrase-templated iminium VCL

pre-template

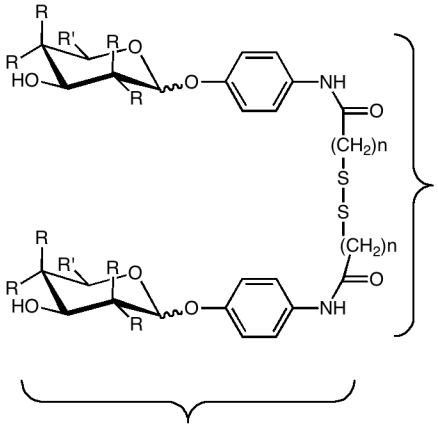
post-template

Pseudo-trisaccharide identification and amplification via selective binding to sepharose bound Con A



Natural Substrate for Concanavalin A

Substrate Analogs



Flexible auxiliaries function in role of central mannose.

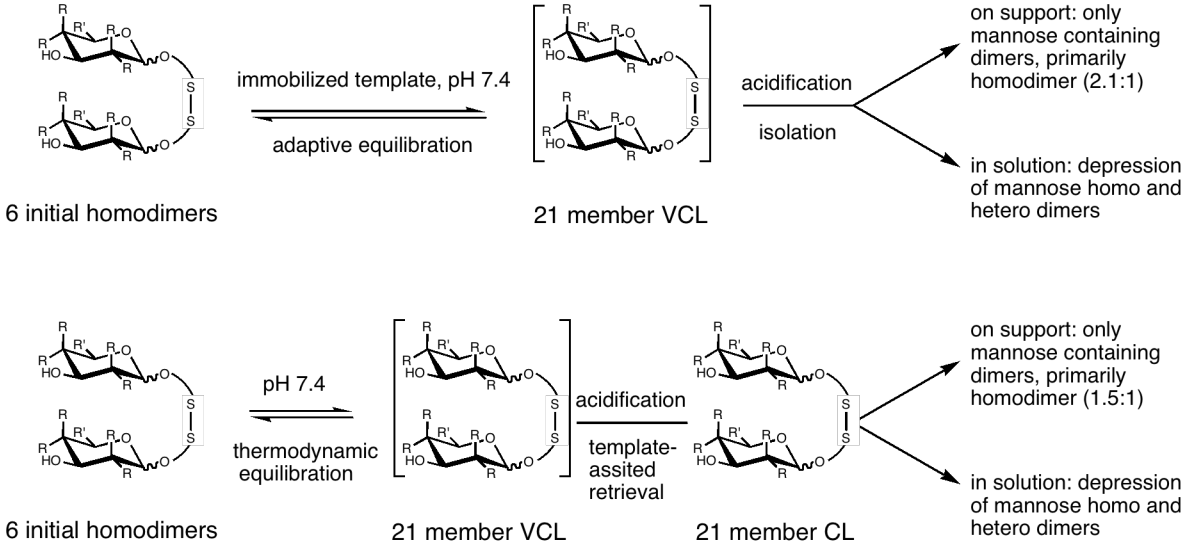
Disulfide bonds allow for interconversion between dimers.

Shallow enzyme binding pocket forgives obvious linker differences.

With R = H, OH and R' = H CH_2OH and tether lengths of 2 or 3 methylenes, a real library of 6 carbohydrate dimers was formed.

Ramstrom, O.; Lehn, J.-M. *ChemBioChem* **2000**, *1*, 41-48.

Two approaches toward identification and isolation:



• Addition of template during equilibration conditions allows for amplification of favorable ligands. (Adaptive effect)

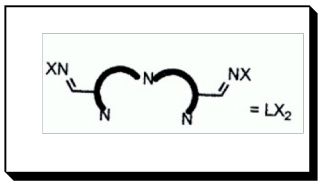
• Addition of template to pre-equilibrated library is less selective but obviates need for compatability with rxn conditions.

Ramstrom, O.; Lehn, J.-M. *ChemBioChem* **2000**, *1*, 41-48.

Double-level orthogonal dynamic combinatorial libraries:

A general scheme for ion coordination/transimination

$M^{red} + LX_2 + LY_2$



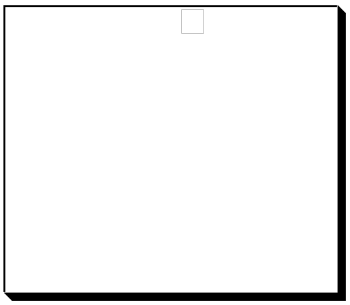
• Oxidation state of metal center functions as ligand on/off switch.

• pH and amine concentration (or oxime or hydrazine) regulate transimination.

Eliseev, A. V.; Lehn, J.-M. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 1347.

Double-level orthogonal dynamic combinatorial libraries.

Reduced to practice: ligand lability of Co^{2+} and Co^{3+} complexes

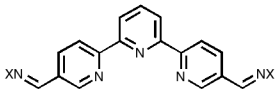


$Co(L_{11})_2^{2+}$ $Co(L_{22})_2^{2+}$ $\underset{\text{rate}_k \approx 1{,}300\ M^{-1}\ s^{-1}}{\overset{30\%\ H_2O/CH_3CN}{\rightleftharpoons}}$ $Co(L_{11})_2^{2+}$ $Co(L_{11})(L_{22})^{2+}$ $Co(L_{22})_2^{2+}$

DCL of symmetrical complexes

- Co^{3+} exchange 1/2 life @ 25 °C ≈ 1 month ==> negligible
- interestingly, metal ligand exchange based on excess ligand is also slower (~15 $M^{-1}s^{-1}$)
- complete scrambling is possible via hydrazone exchange at pH 3, 60 °C

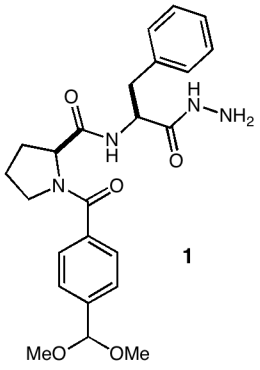
L_{xx} =



X = OMe, OEt, OBn, NHCOMe, NHCOBn, NHCO3Py, NHCO4HP

Eliseev, A. V.; Lehn, J.-M. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 1347.

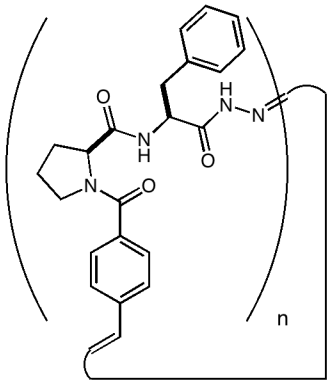
Pseudo-peptide cyclic oligomers



1

TFA

high dilution, DCM

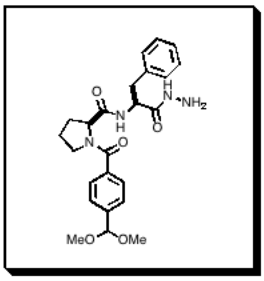


Proline used for geometrical constraint (β-turn enforcement)

- DCL at equilibrium, without template favors formation of cyclic oligomers with 2-5 repeating subunits. (a)
- On addition of 18-crown-6, HPLC trace is dominated by species **6** which is the monomer unit **1** in deprotected form. (b)
- MS dominated by 6 + 18-crown-6 + H^+.
- Original equilibrium quantities can be restored by the addition of KBr. (c)

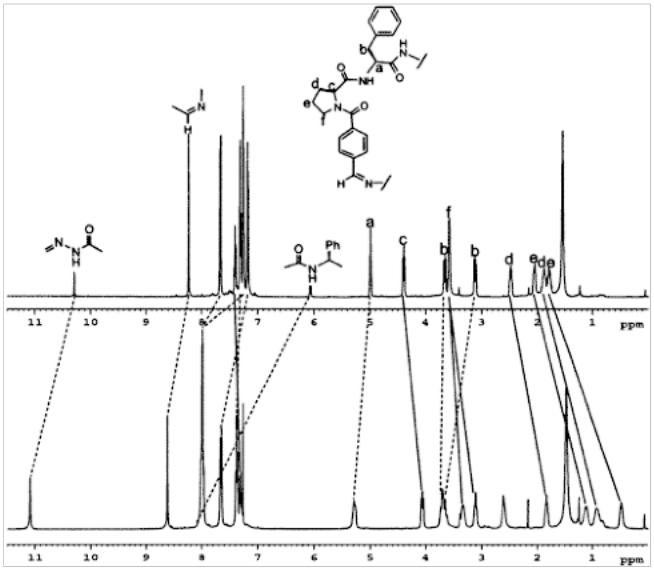
Furlan, R. L. E.; Cousins, G. R. L.; Sanders, J. K. M. *Chem. Commun. (Cambridge)* **2000**, 1761-1762.

Amplification and induced fit of pseudo-peptide cyclic oligomers



• Equilibrium shifted toward trimer on addition of inorganic salts

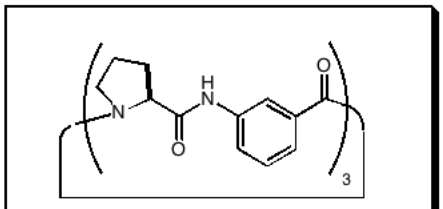
• No change observed with NR_4^+ iodides or KI, RbI, CsI



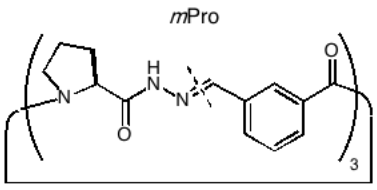
• NMR of isolated trimer and trimer in the presence of lithium shows dramtic shits throughout entirety of oligomeric structure.

Furlan, R. L. E.; Ng, Y.-F.; Otto, S.; Sanders, J. K. M. *J. Am. Chem. Soc.* **2001**, *123*, 8876-8877.

Molecular amplification of pseudo-peptide cyclic oligomers



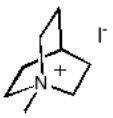
Kubik's peptide

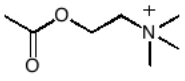


*m*Pro

hydrazone analog

Substrates:





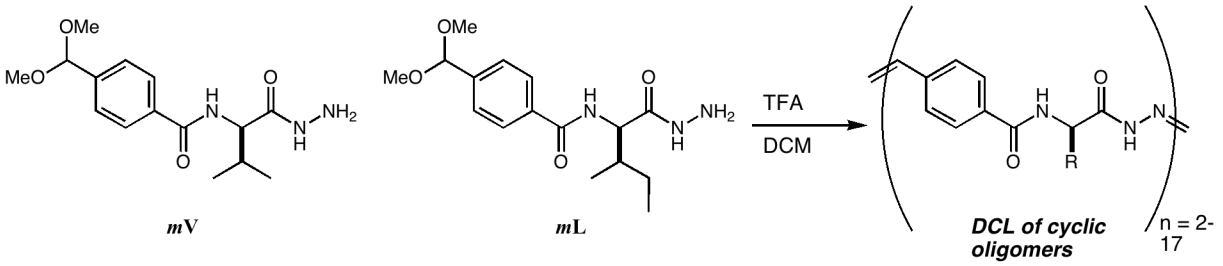
	N-methyl quinuclidium salts	acetyl choline
Binding constant Kubik's peptide:	42200 M^{-1}	11000 M^{-1}
Binding constant hydrazone:	150 M^{-1}	230 M^{-1}

• Kubik's trimeric cyclic peptide is known to have binding affinity for quaternary ammonium ions: quinuclidium and acetyl choline.

• In a DCL which favors the dimer over trimer (88:11) of subunit *m*Pro, the 230 M^{-1} binding affinity to AcCh reversed the preference to (14:86).

Cousins, G. R. L.; Furlan, R. L. E.; Ng, Y.-F.; Redman, J. E.; Sanders, J. K. M. *Angew. Chem., Int. Ed.* **2001**, *40*, 423-428.

Expensive toys: analysis of a DCL of pseudopeptide oligomers by ESI-FTICR-MS/MS

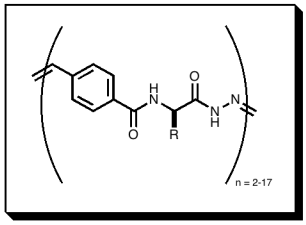


Expected array of oligomeric species

Poulsen, S.-A.; Gates, P. J.; Cousins, G. R. L.; Sanders, J. K. M. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 44-48.

Analysis of a DCL of pseudopeptide oligomers

Tetramer: the simplest non-degenerate case



Two possible orders for V_2L_2 oligomer:

1 **2**

Mass fragmentation pattern for **1**:	V–V	V–L	V–L	L–L	1 : 2 : 1
Mass fragmentation pattern for **2**:	V–L	V–L	V–L	V–L	0 : 4 : 0
Net fragmentation pattern:					1 : 6 : 1

- Similar, but more complex, analyses can be performed on larger oligomers and DCL systems.
- Deviation from ideal ratio can give incite into connectivity as well as composition.

Poulsen, S.-A.; Gates, P. J.; Cousins, G. R. L.; Sanders, J. K. M. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 44-48.

Low-tech/high-concept analysis of DCL

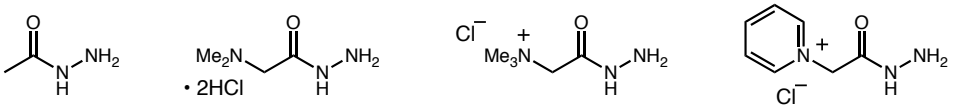
Dynamic deconvlution strategy based on enzyme inhibition

Step 1: Selection of template/assay

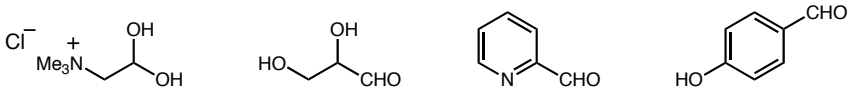
- Acetylcholinesterase activity and inhibition can be easily monitored spectrophotometry

Step 2: Construction of a suitable DCL

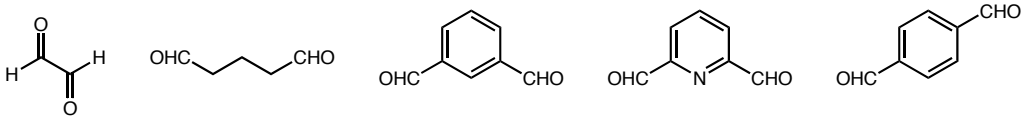
acylhydrazines:



monoaldehydes:



dialdehydes:
(linkers)

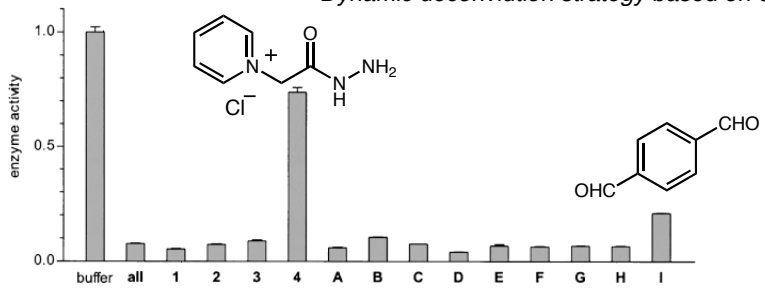


- All constituents are water soluble and showed negligible inhibition as free hydrazines or aldehydes
- Up to 66 possible different species from a small set (13) components

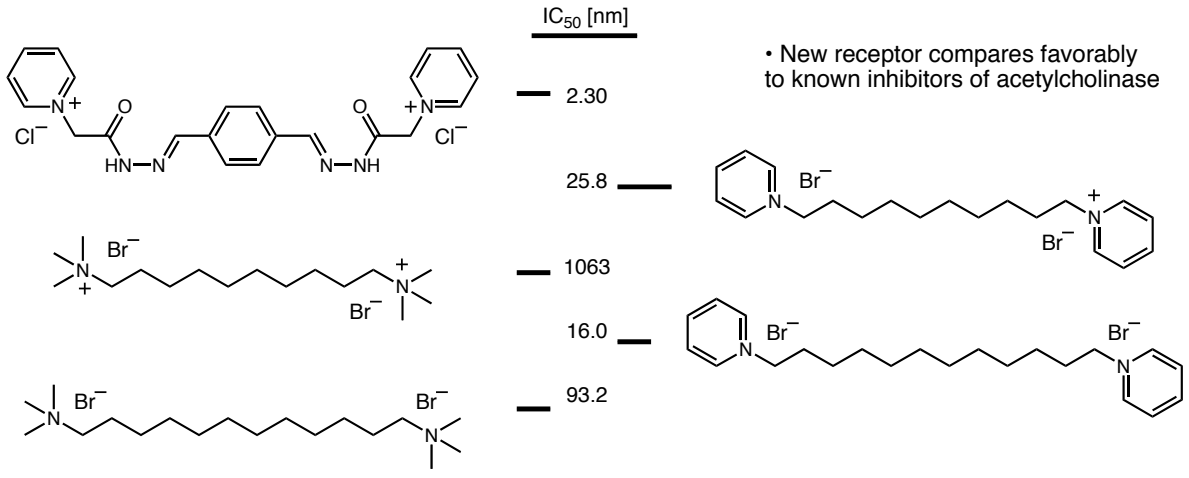
Bunyapaiboonsri, T.; Ramstrom, O.; Lohmann, S.; Lehn, J.-M.; Peng, L.; Goeldner, M. *ChemBioChem* **2001**, *2*, 438-444.

Low-tech/high-concept analysis of DCL

Dynamic deconvlution strategy based on enzyme inhibition



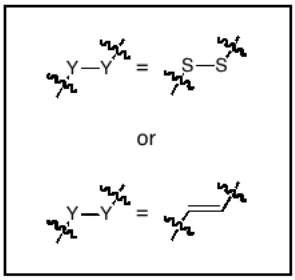
- Each bar corresponds to omission of a given component.
- Hydrazine 4 and dialdehyde I seem to be most important in inhibition.



- New receptor compares favorably to known inhibitors of acetylcholinase

Bunyapaiboonsri, T.; Ramstrom, O.; Lohmann, S.; Lehn, J.-M.; Peng, L.; Goeldner, M. *ChemBioChem* **2001**, *2*, 438-444.

Effect of templating on vancomycin dimerization



Homodimerization of m = 1 and m = 3 substrates, via olefin methathesis

- Dimerization of vancomycin leads to increase potency.
- Dimerization with various tether lengths in the presence of template should be faster and select for more effective binders.
- Clear preference was found for short tether lengths when equilibration was carried out in presence of template.
- Analogs with up to 12x activity against susceptible strains and up to 100x activity against resistant strains were identified.

Nicolau, K. C. *Angew. Chem., Int. Ed.* **2000**, *39*, 3823-3828.

Is DCC doomed from the start?

A theoretical analysis

Assumptions:

- Binding affinities among a random population of aptamers are reasonably described as being normally distributed in log *K*.
- Any reasonably defined population of a noncovalent association will have a maximum typical stability range of 5-6 orders of magnitutde in the equilibrium constant, resulting in a standard deviation of about 1 log *K* unit.
- The mean will be determined by the inherent features of the population.
- The standard deviation, however is presumably controlled by the range of forces available from non-covalent interactions

Connors, K. A. *Chem. Rev.* **1997**, *97*, 1325-1357.

Conclusions:

- In a random population, the mean binding constant can only be increased to a limited degree (ca. 2 orders of magnitude) by addition of a template.
- Iterative templating to get around this problem will be plagued by exponentially decreased yields.
- Selection and amplification will be required for true chemical evolution.
- DCC may be useful in generating lead compounds, but never in generating practical quantities of desired binders.

Moore, J. S. *Org. Lett.* **2000**, *2*, 915-918.

Summary

• Dynamic combinatorial libraries provide access to large numbers of real and virtual compounds with little synthetic effort

• DCC research is still in the proof of principle stage

• New reversible molecular associations are being explored

• New methods for the analysis of increasingly complex DCLs are being developed.

• The goal of DCC research is to rapidly define new host-guest interactions important in biomedical applications and catalyst discovery.