Application of Physical-Organic Principles for Self-Assembly and for Inhibitor Design

1. Self-Assembly Based Upon the Cyanuric Acid-Melamine Lattice

Harvard University
Christopher T. Seto and George M. Whitesides*

2. Using the Electrostatic Field Effect for the Design of Enzyme Inhibitors

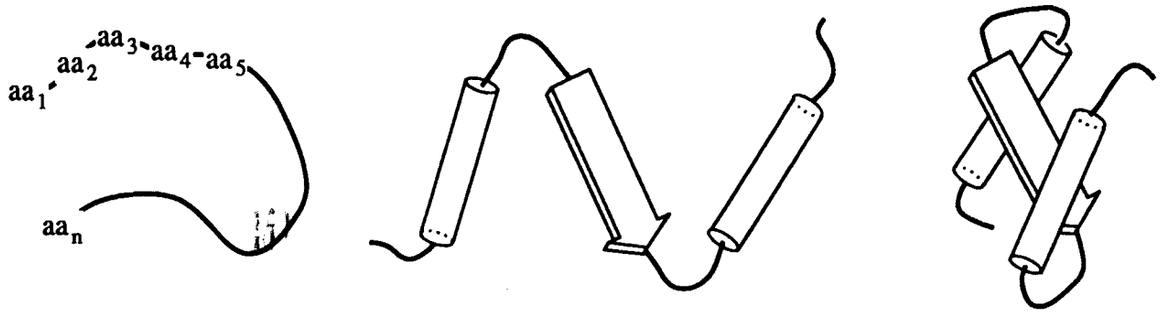
Brown University
Jeffrey L. Conroy, Tanya C. Sanders, and Christopher T. Seto*

Motivations for Investigating Self-Assembly

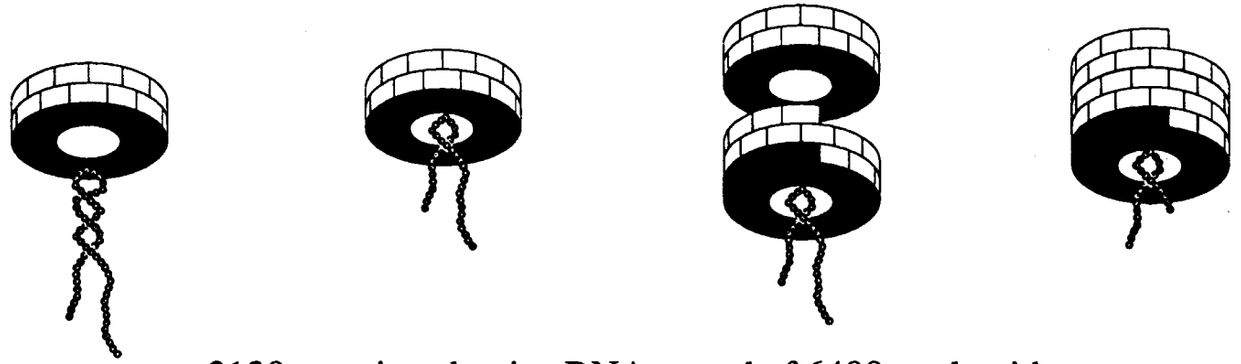
Biological Precedent -- Proteins, t-RNA, Viruses

Develop Synthetic Methodology to Make Macromolecules
Via Non-covalent Synthesis

Proteins

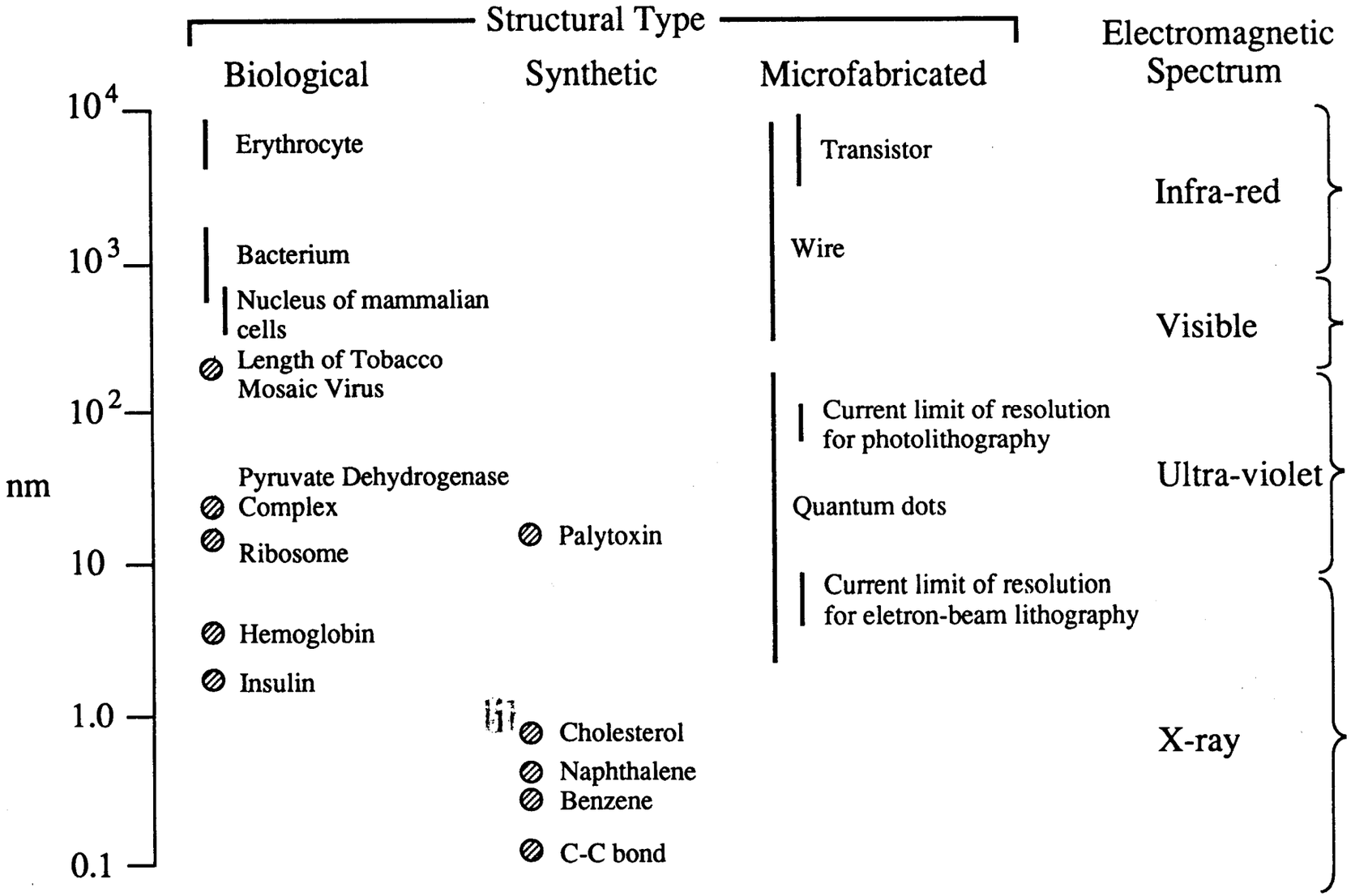


Tobacco Mosaic Virus



2130 protein subunits, RNA strand of 6400 nucleotides

Sizes of Structures from Biology, Synthetic Chemistry, and Microfabrication



Characteristics of Self-Assembly:

Spontaneous -- Equilibrium Conditions

Entropy and Enthalpy are Balanced

Yields Discrete Structures

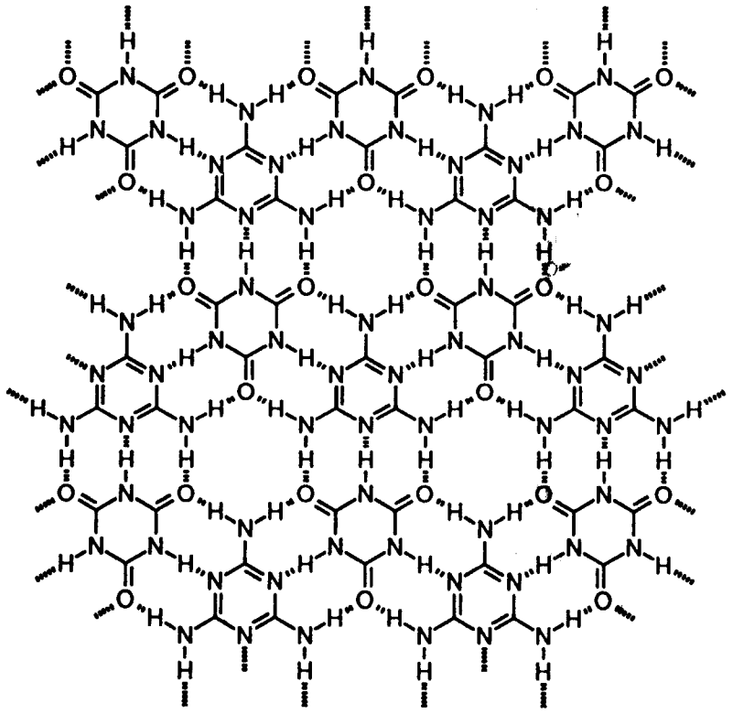
Cooperativity

Goals of the Project:

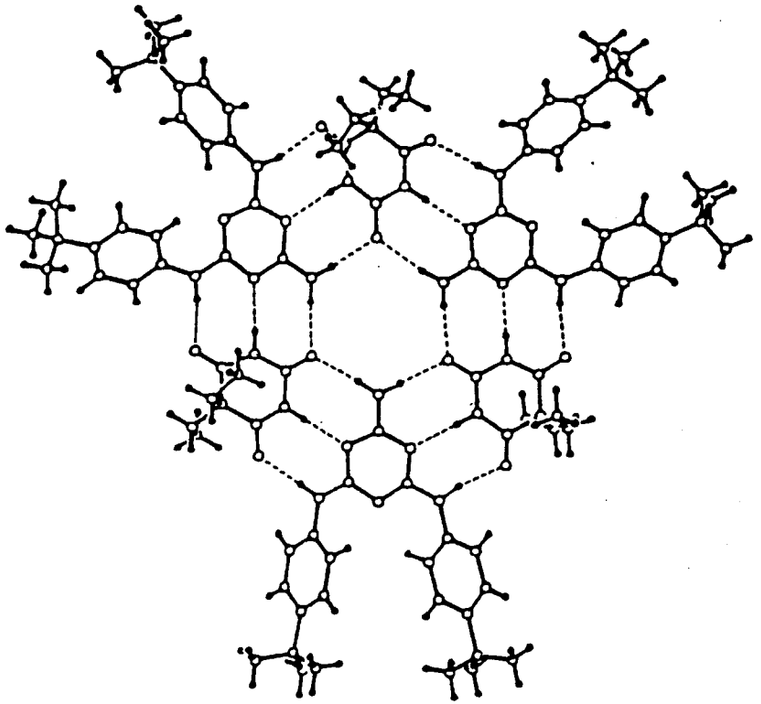
Develop Criteria for the Design of Self-Assembled Structures

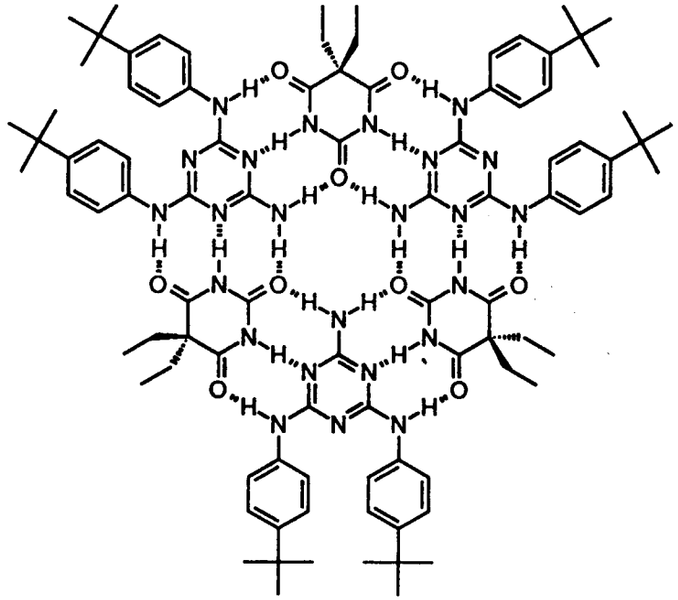
Explore the Thermodynamics of Self-Assembly - What is the Interplay Between Enthalpy (Hydrogen Bonds) and Entropy (Preorganization)?

Develop Techniques for Characterizing Non-Covalent Structures

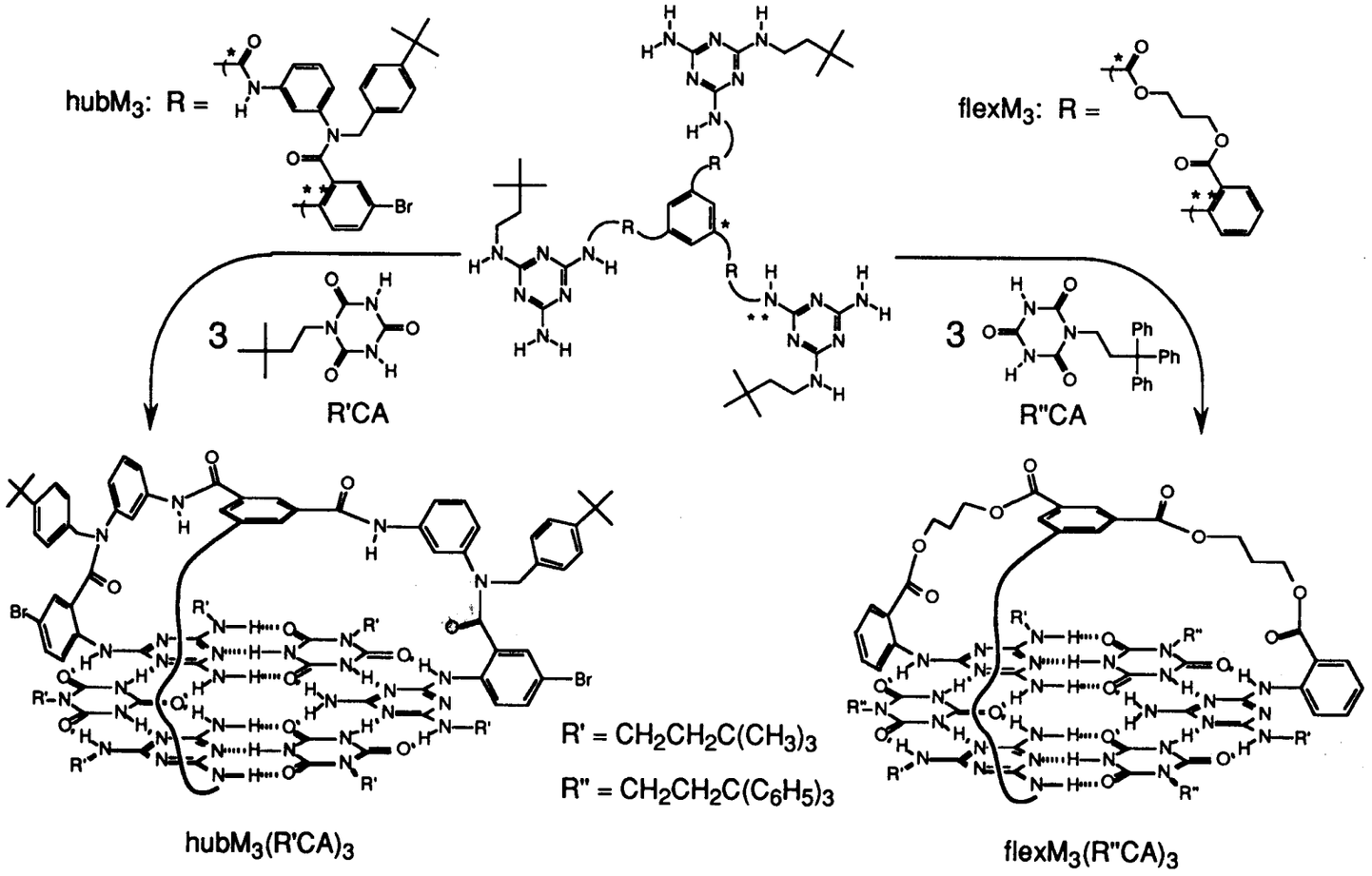


Cyanuric Acid·Melamine



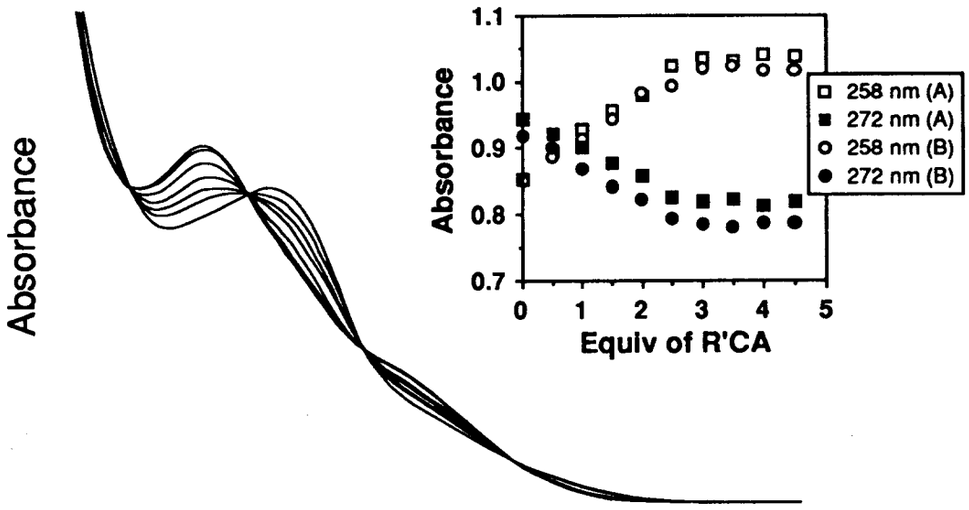


Hub and Spoke Architecture

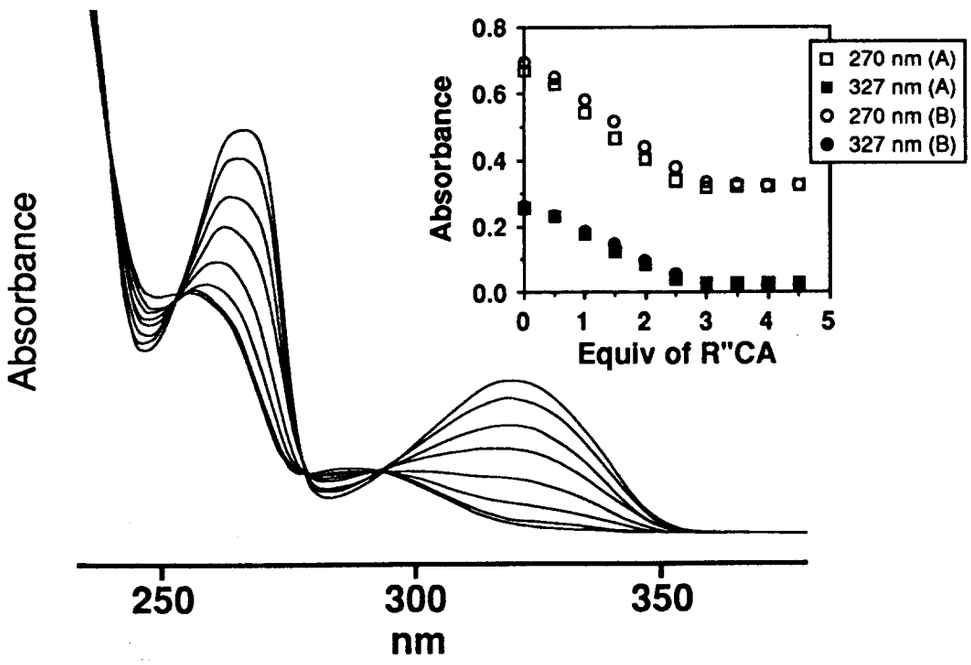


- Limits Loss of Translational and Conformational Entropy

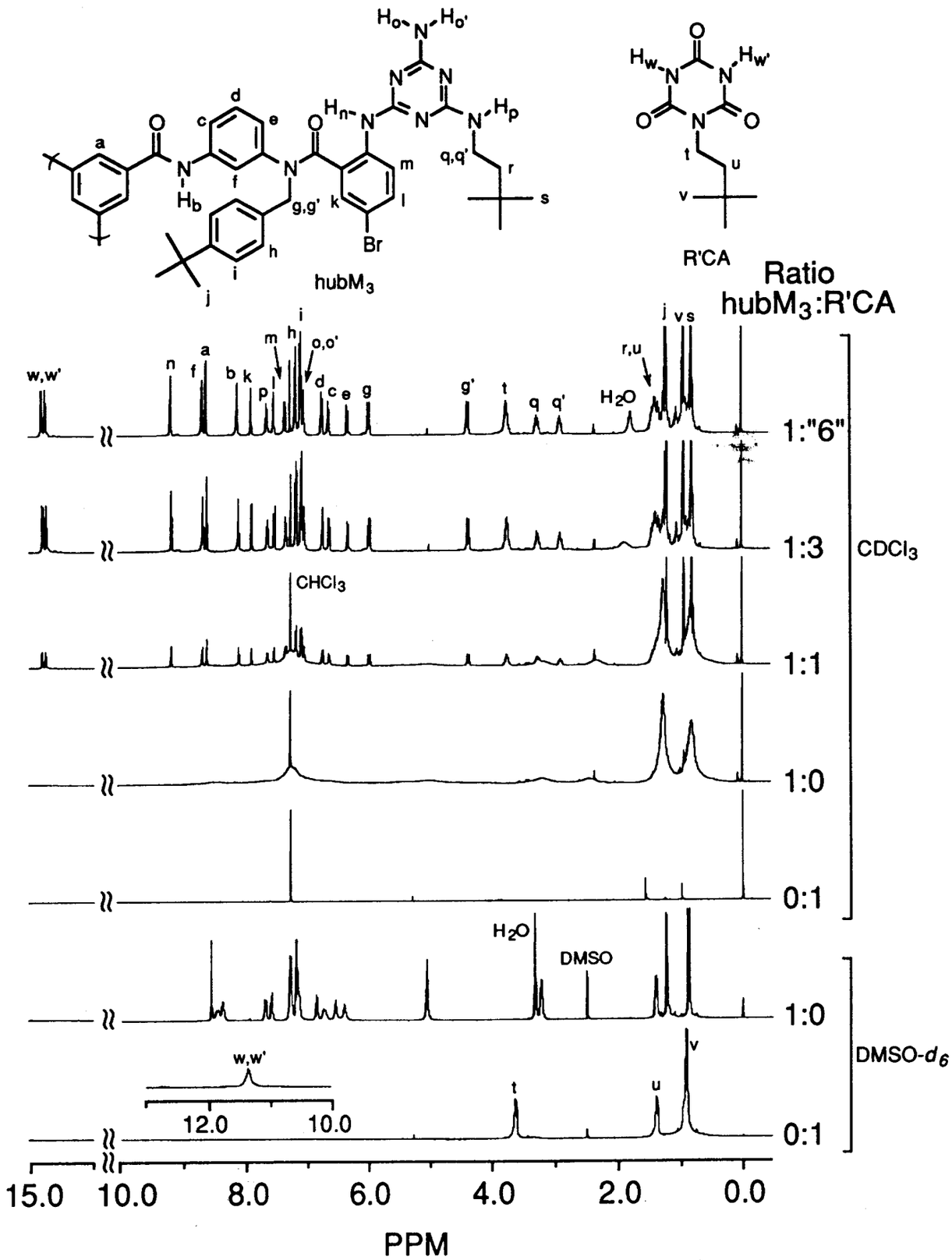
Titration of hubM_3 with R'CA



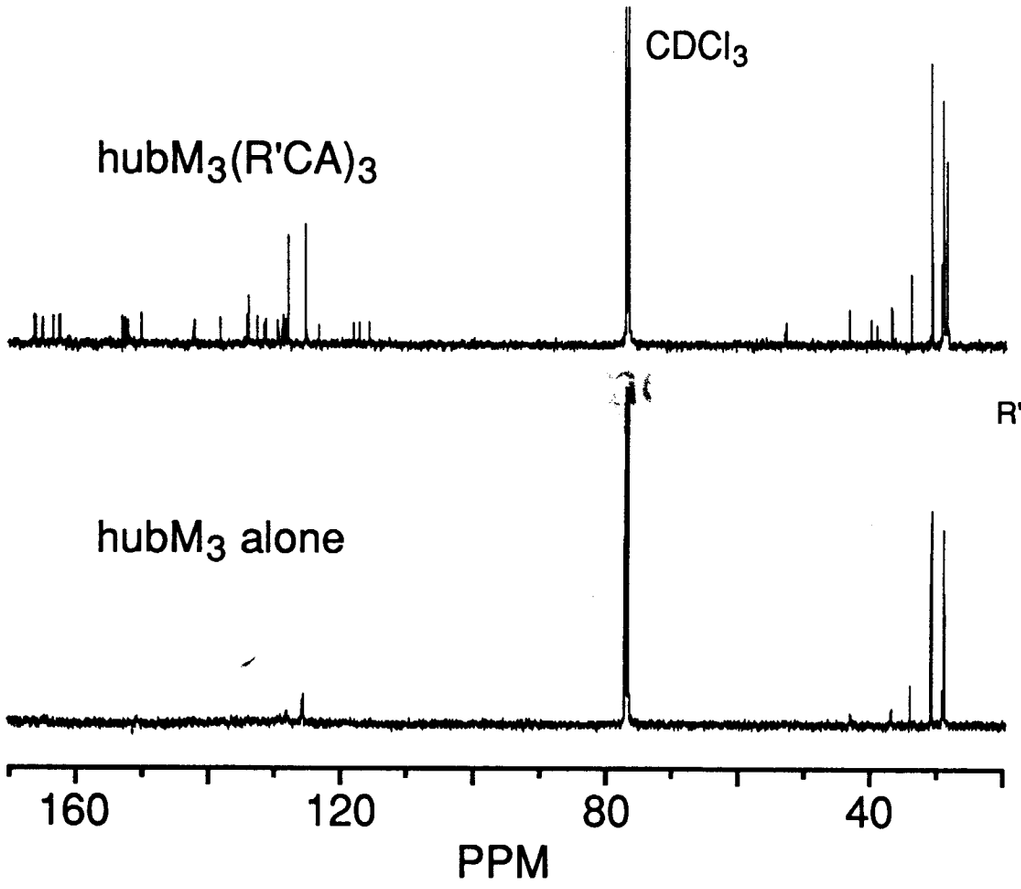
Titration of flexM_3 with R''CA



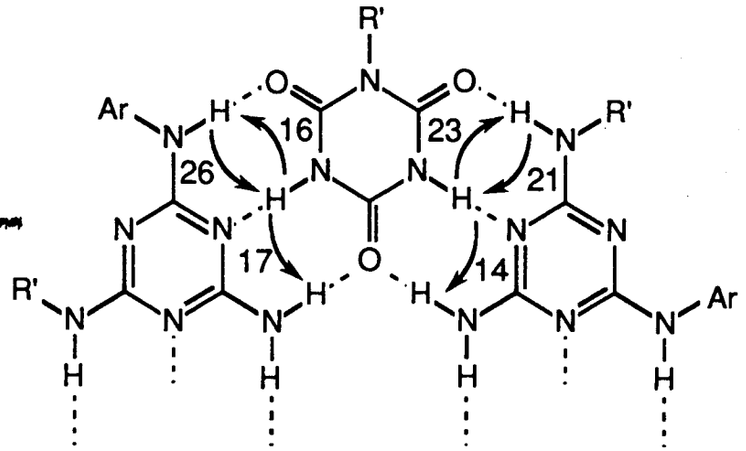
- Solubility studies support the 1:3 stoichiometry

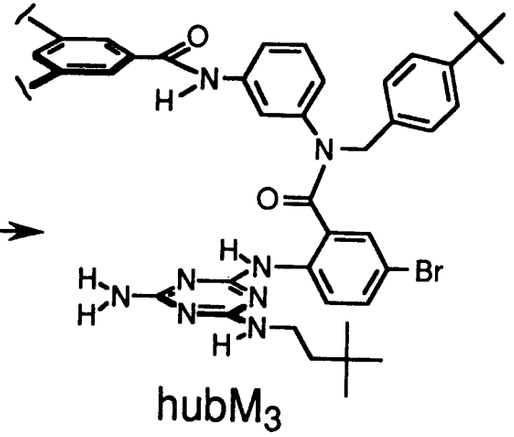

hubM3
R'CA
Ratio
hubM3:R'CA
1:"6"
1:3
1:1
1:0
0:1
CDCl3
CHCl3
H2O
DMSO
DMSO-d6
w,w'
12.0
10.0
15.0
10.0
8.0
6.0
4.0
2.0
0.0
PPM

^{13}C-NMR

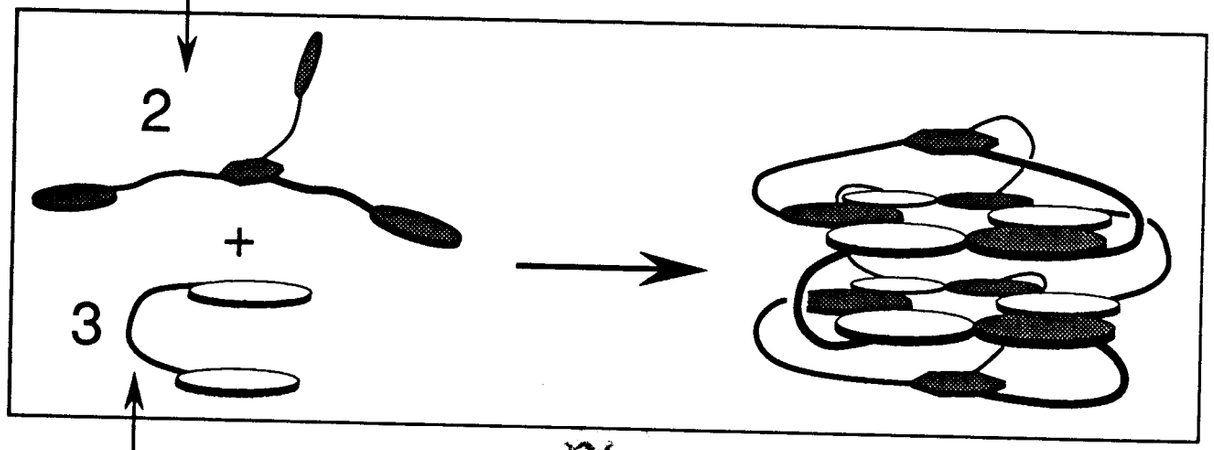

CDCl3
hubM3(R'CA)3
hubM3 alone
160
120
80
40
PPM

NOEs

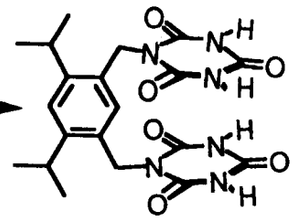

R'
Ar
R'
Ar
16
23
26
21
17
14



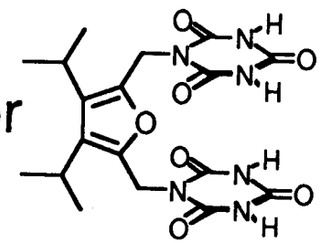
- Solubility, UV, NMR, and NOE studies support the proposed structure

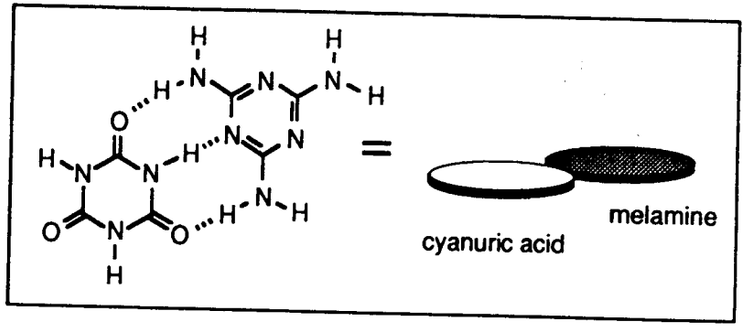


n

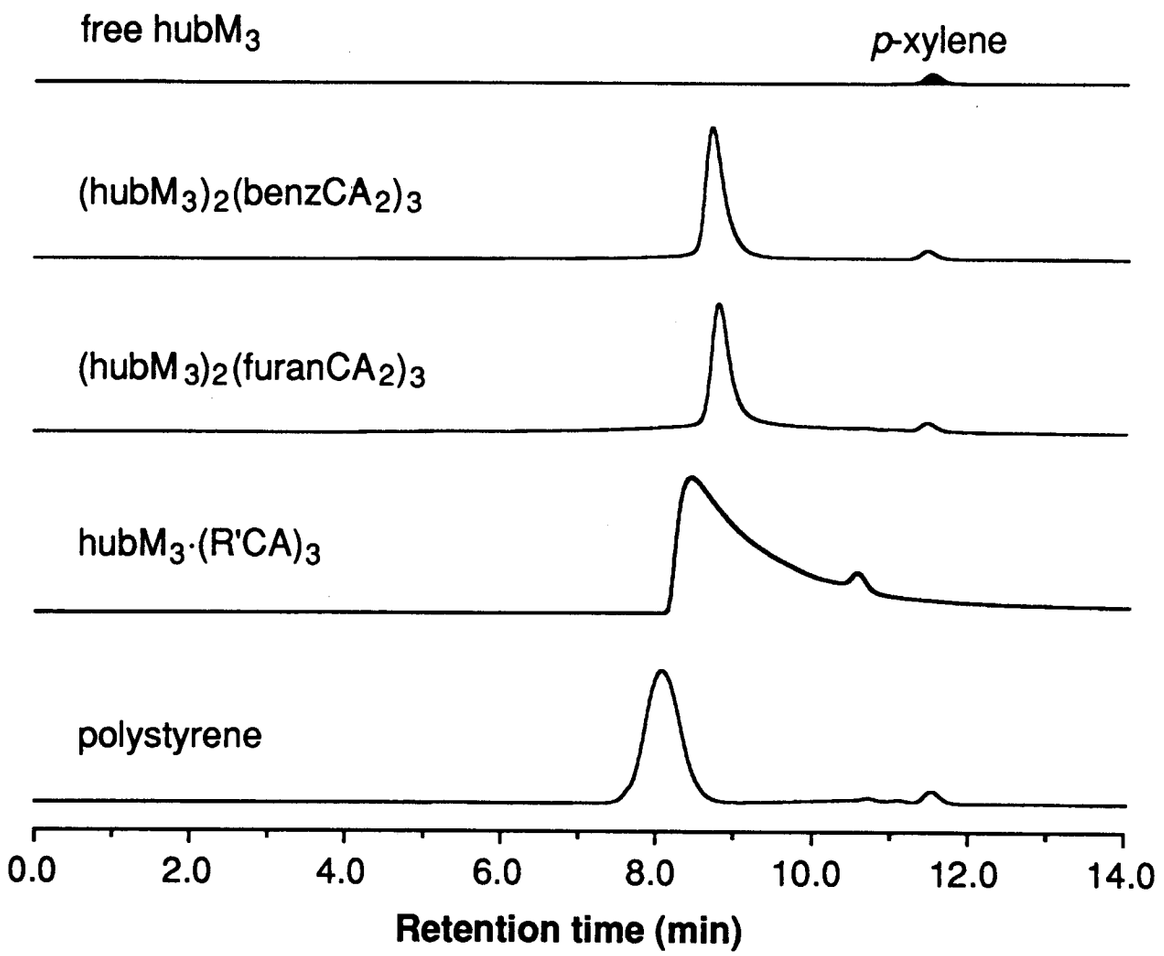


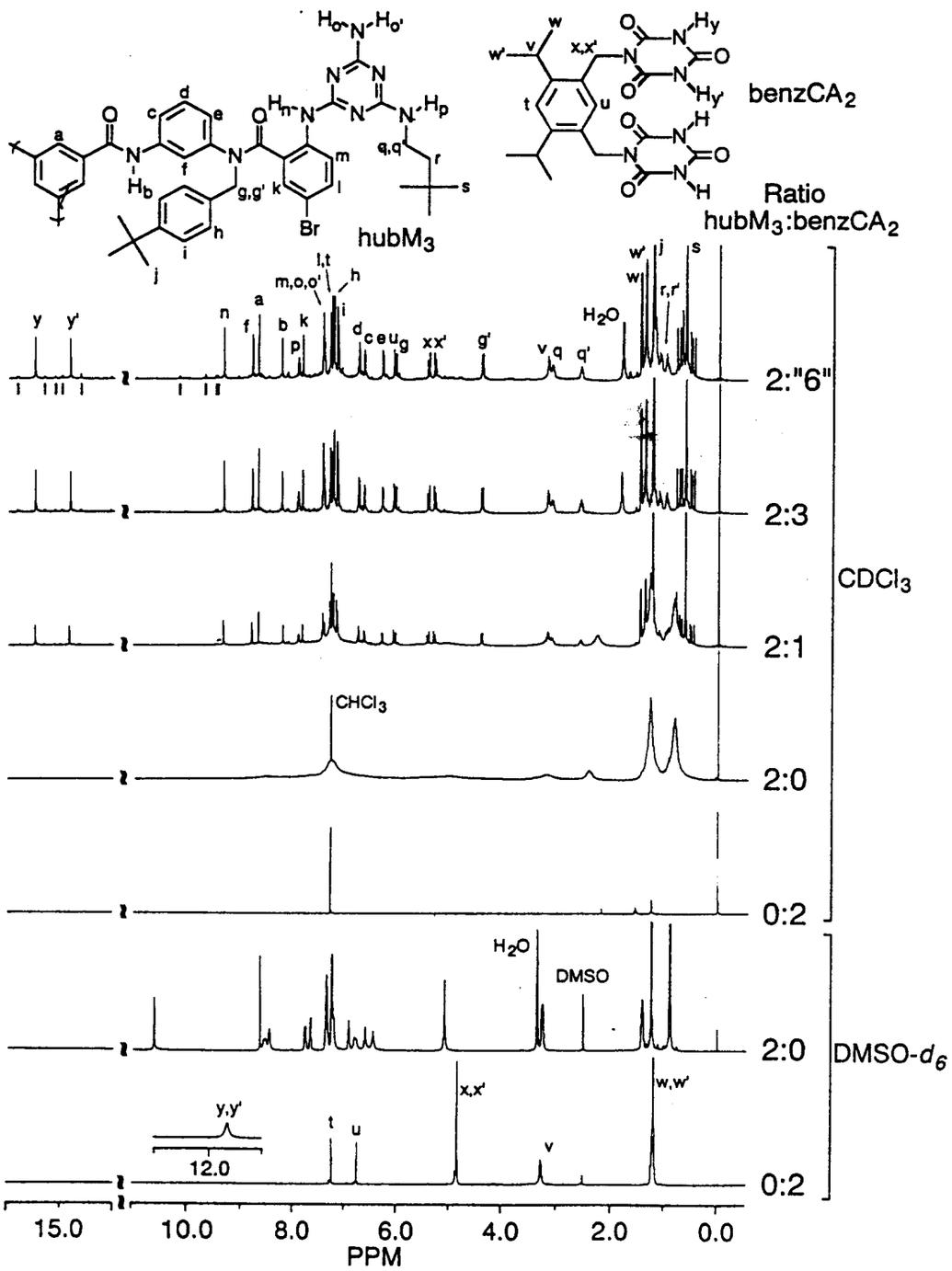
or



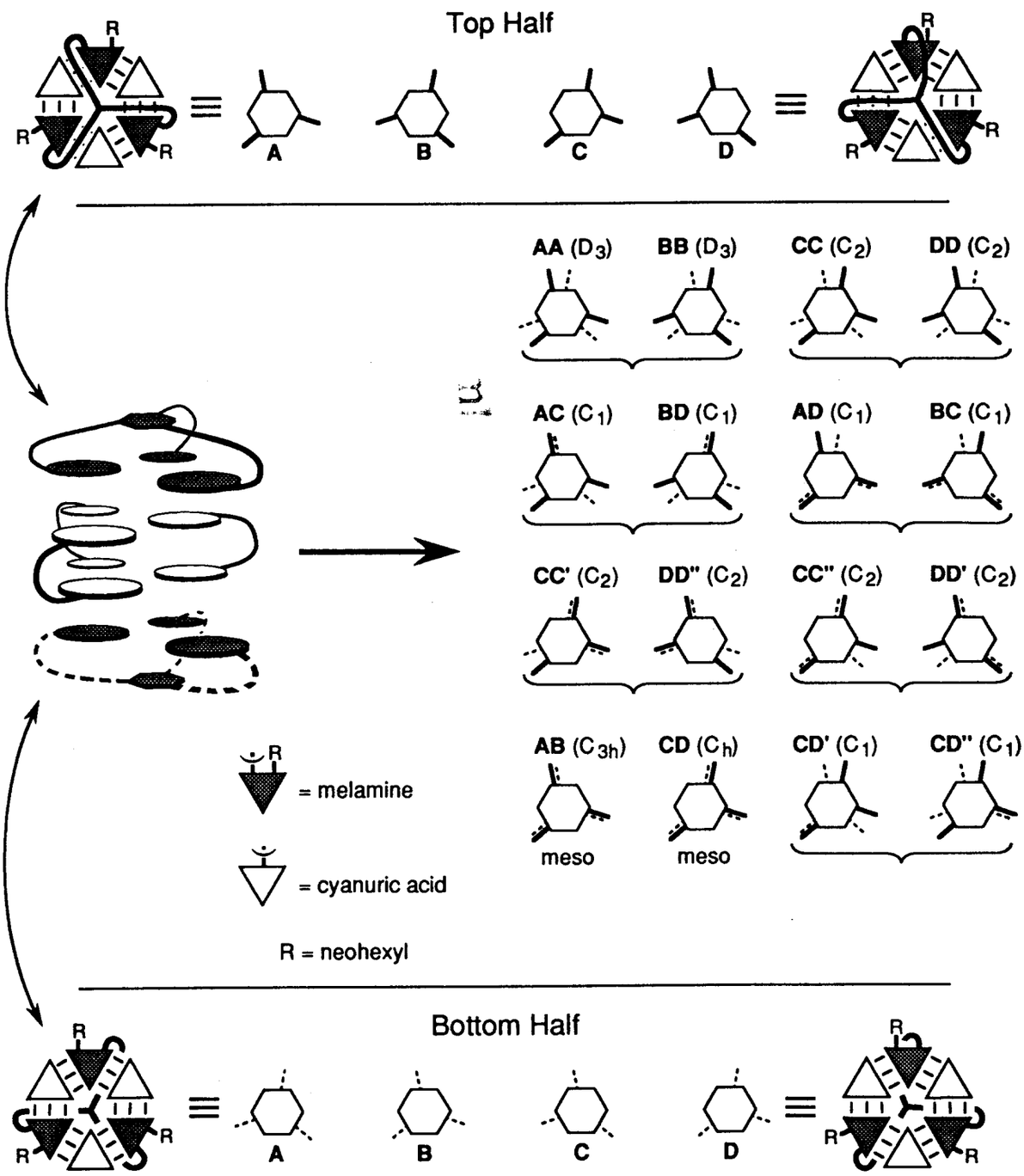


Gel Permeation Chromatography




hubM3
benzCA2
Ratio
hubM3:benzCA2
2:"6"
2:3
2:1
2:0
0:2
CDCl3
CHCl3
H2O
DMSO
2:0
0:2
DMSO-d6
y,y'
12.0
x,x'
w,w'
t
u
v
15.0
10.0
8.0
6.0
4.0
2.0
0.0
PPM

16 Possible Configurational Isomers



Demonstrated Self-Assembly as a Strategy for Synthesis

Sphere ~ 30Å in diameter, 5 molecules, 36 hydrogen bonds
MW ~ 5500

Discrete, Stable Structure

ΔH = 1.3 kcal/mol/hydrogen bond

Characterization

^{1}H, ^{13}C NMR
GPC, VPO, UV, solubility

Extentions of this Work

Complexity - Assembly of 10 molecules into a single aggregate
Specificity - Assembly of three different types of molecules
Enthalpy - 54 hydrogen bonds
Developed mass spectrometry as a tool for characterization
Developed models for predicting relative stability of aggregates

Protease Inhibitors are Important in a Variety of Medicinal Applications

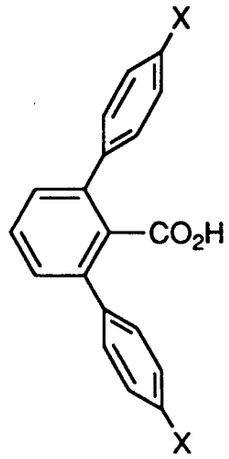
Cysteine Proteases	Biological Importance
Cathepsins B & L	Rheumatoid Arthritis Cancer Metastasis Muscular Dystrophy
Serine Proteases	
Thrombin, Factors VII, IX, X, XII	Blood Coagulation
Plasmin, Urokinase Tissue Plasminogen Activator	Fibrinolysis
Human Leukocyte Elastase	Emphysema, Cystic Fibrosis
Dipeptidyl Peptidase IV (CD26)	Immunosuppression, AIDS

Through-Space Electronic Interactions

Used to Control Equilibria...

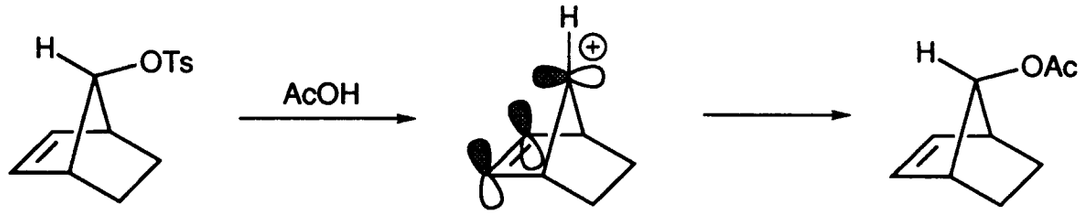
X = -NO_2, -CN, -Br, -Me, -OMe, etc.

Roberts & Moreland, 1953



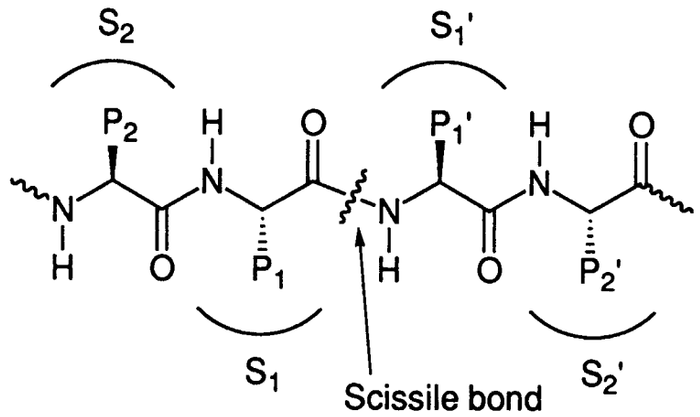
Chen & Siegel, 1993

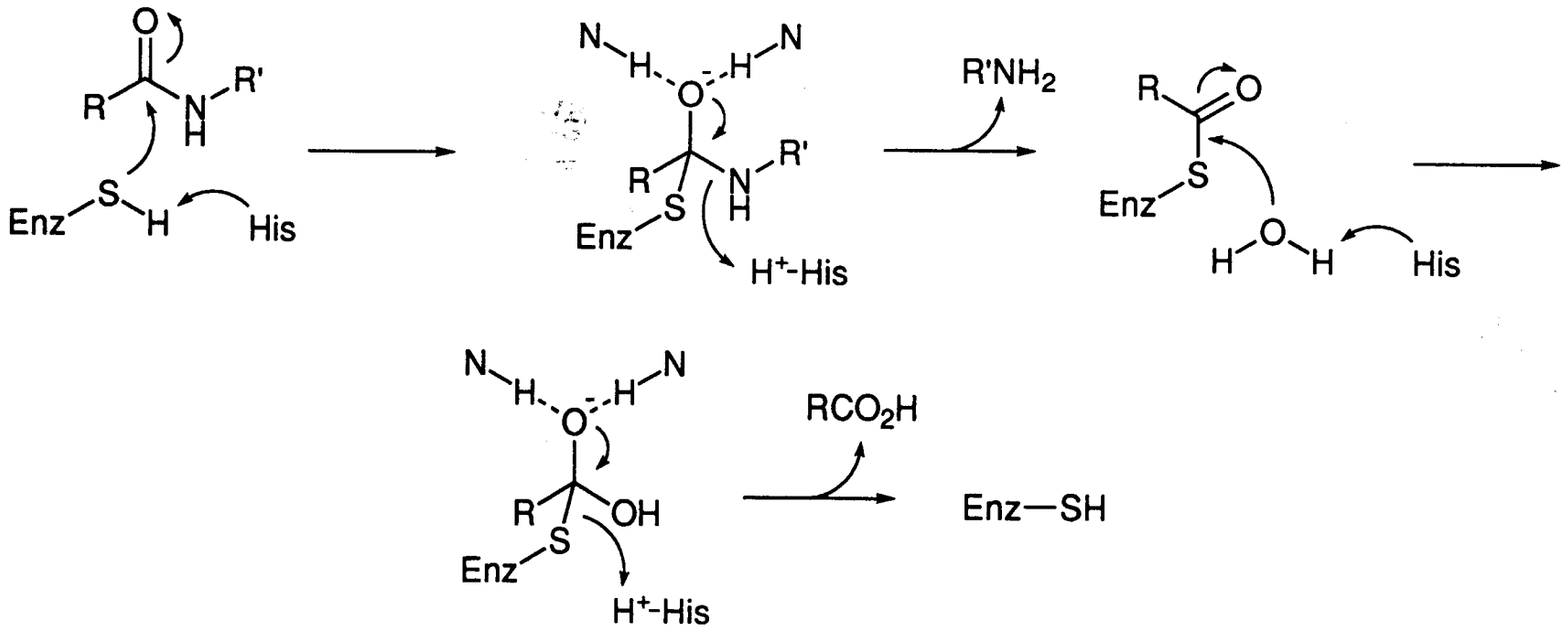
...And Reaction Rates and Stereochemistry



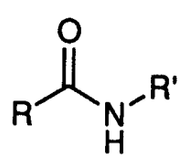
Winstein, Shatavsky, Norton, & Woodward, 1955

Mechanism of Cleavage by Cysteine Proteases

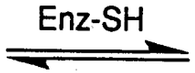


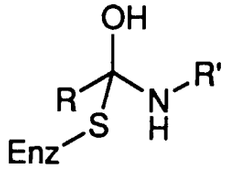


Other Reversible Cysteine Protease Inhibitors

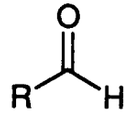


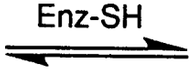
Peptide Substrate

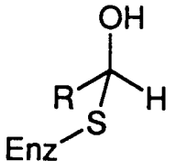




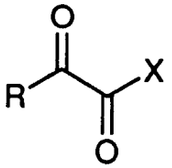
Tetrahedral Intermediate

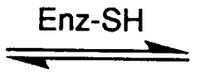




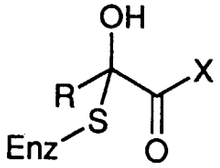


Hanzlik, Jacober & Zygmunt, 1991
Cheng, Keitz, Jones, 1994
Smith, et al, 1988

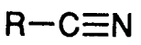


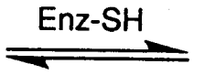


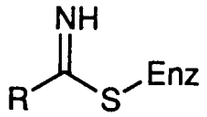
$X = OR', NHR', CH_3$



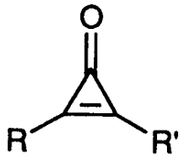
Hu & Abeles, 1990







Moon, Coleman & Hanzlik, 1986
Liang & Abeles, 1987

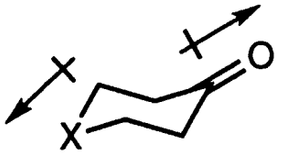


?

Ando & Morinaka, 1993

4-Heterocyclohexanones Are More Electrophilic Than Standard Ketones

- Through-space electrostatic repulsion destabilizes the ketone



X = O, S, SO, SO_2, $^+NH_2$

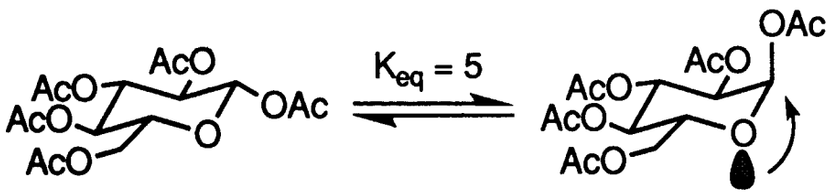
Burkey & Fahey, 1985
Das & Thornton, 1993

- Ring strain

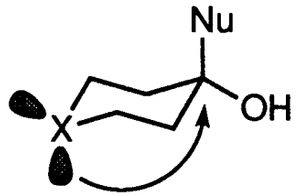
Wiberg, Morgan, Maltz, 1994

- Transannular anomeric effect - Stabilizes the hemithioketal

Cieplak, 1981

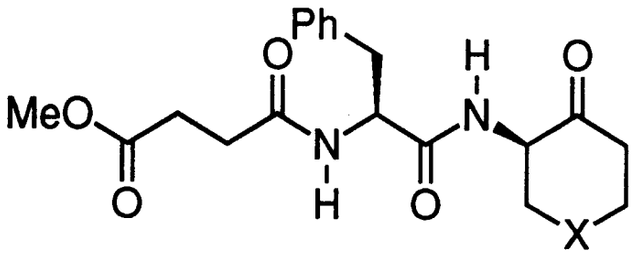


1:1 AcOH:Ac_2O, 0.1 M H_2SO_4, 25 °C



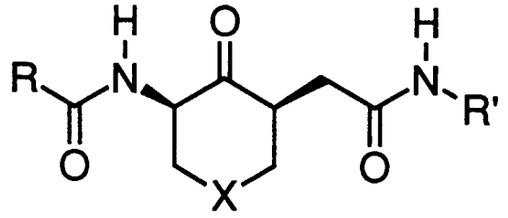
Delocalization of lone pair electrons into the C-Nu antibonding orbital (n_X-$\sigma^*_{C\text{-}Nu}$ overlap)

Papain Inhibitors Based Upon 4-Heterocyclohexanones

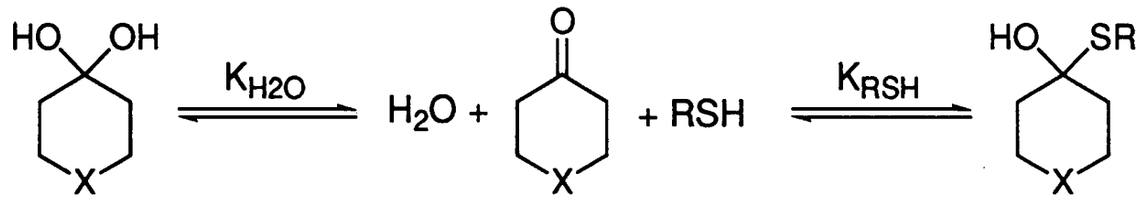


X = CH_2, O, S, SO, SO_2, NH_2^+

- Potency controlled by through-space interactions between the ketone and X-groups
- Designed to give a reversibly formed hemithioketal with the active site cysteine
- Papain is specific for Phe at P2
- Can be modified to interact with both the S and S' subsites
- Conformationally constrained inhibitors



Model System: Addition of Thiol to Ketones in Aqueous Solution



X	10^3 K_{H2O} (M^{-1})	K_{RSH} (M^{-1})	$K_{RSH,app}$ (M^{-1})
CH_2	0.81	0.22	0.21
S	9.0	1.5	0.99
O	8.0	1.8	1.3
NH_2^+	176	27.6	2.7
SO	68	11.7	2.5
SO_2	298	60.2	3.5
Other Ketones - Burkey & Fahey, 1983			
CH_3COCH_3	0.023	0.0052	0.0052
CH_3COCO_2H	31	58	22
$CH_3COCO_2CH_3$	45	71	20

$$K_{RSH,app} = \frac{[\text{hemithioketal}]}{[\text{ketone+hydrate}][\text{thiol}]} = \frac{K_{RSH}}{1 + K_{H2O}[H_2O]}$$

Sanders & Jencks, 1968

RSH = $HSCH_2CH_2CO_2H$

What is the Relationship Between X and K_{eq}?

- Hammett Equation $\log K_x/K_H = \rho\sigma$ Hammett, 1937

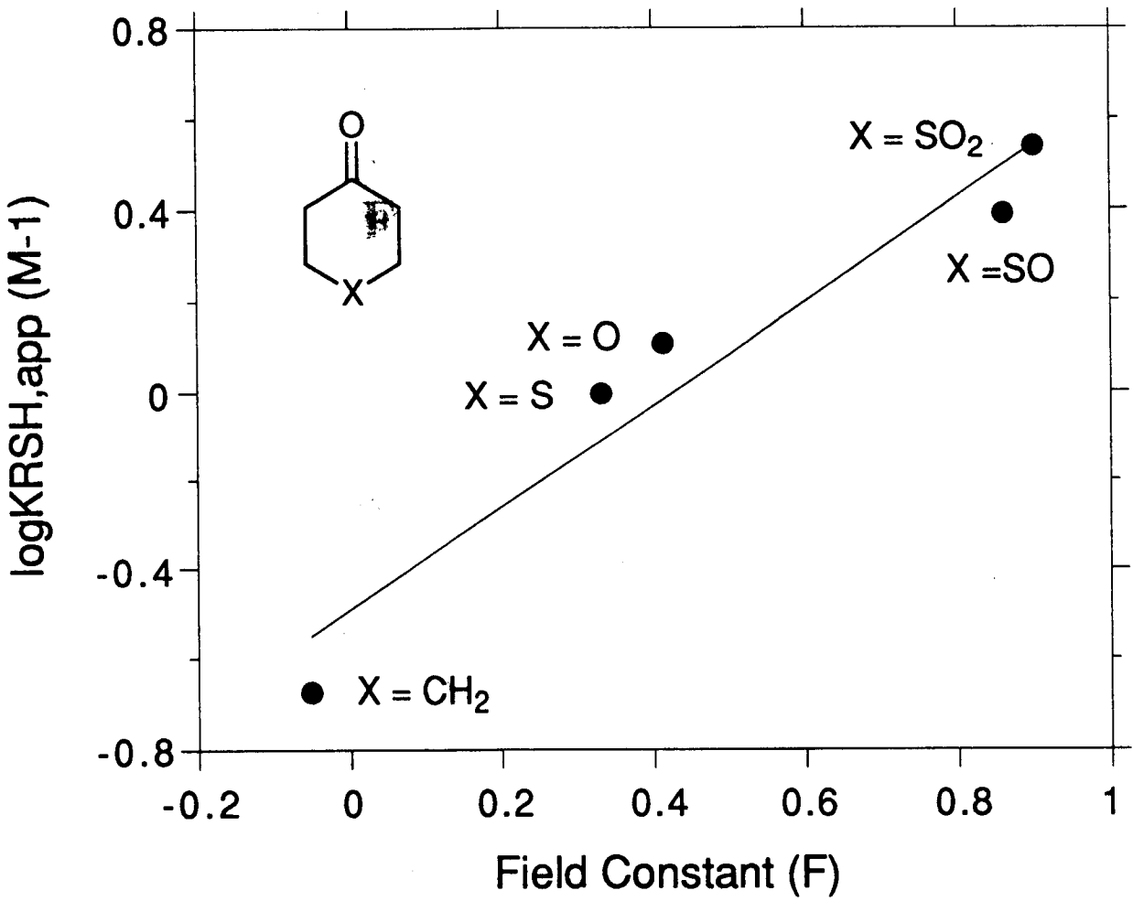
  reaction constant — substituent constant

- Generalized Hammett Equation Swain & Lupton, 1968, 1983

$$\log K_x/K_H = \rho(f\,\mathbf{F} + r\,\mathbf{R})$$

F = Field substituent constant
R = Resonance substituent constant
f & *r* = Weighing factors specific for a particular reaction
Assumes sterics are not important

$K_{RSH,app}$ Correlates Well With F



- Approximate X groups with $-CH_3$, $-SCH_3$, $-OCH_3$, $-SOCH_3$, $-SO_2CH_3$
- NH_2^+ omitted because no corresponding F value is available

What Does This Correlation Mean?

- Interaction between X and ketone is best described as a through-space electrostatic repulsion

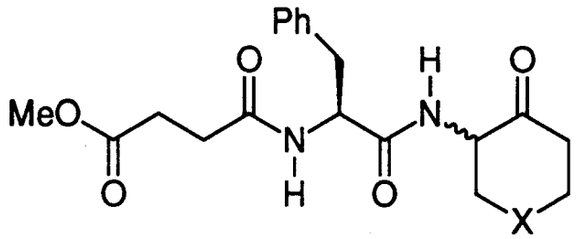
- Resonance effects, differences in ring strain, and the transannular anomeric effect are minor influences

- For $K_{RSH,app}$ vs. **F**, slope = 1.1

  For  slope = $\rho f = 0.49$

  4-Heterocyclohexanones respond approximately twice as strongly to the field component of X

Inhibition of Papain by 4-Heterocyclohexanones



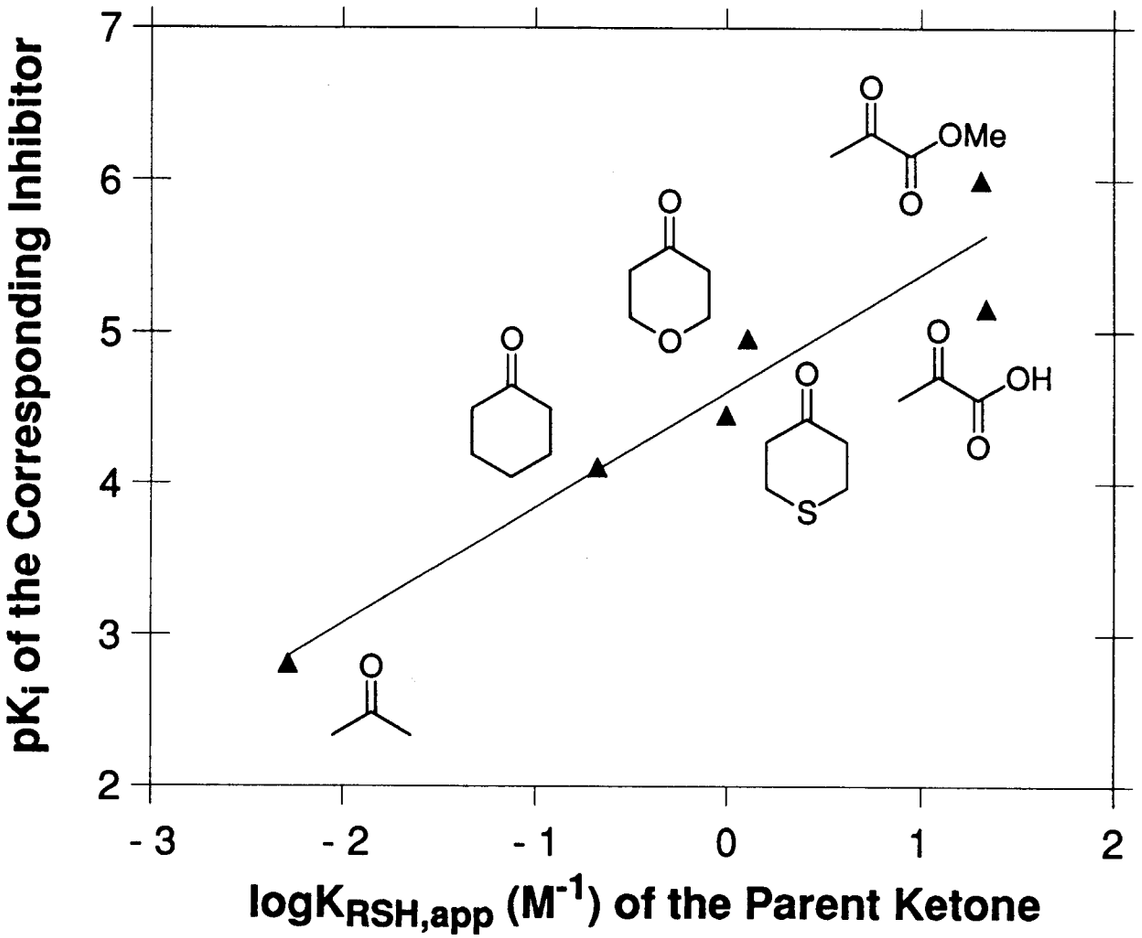
X	K_i (μM) More Potent Diastereomer	K_i (μM) Less Potent Diastereomer
CH_2	78	3200
S	35	390
O	11	3300
NH_2^+	350[a]	
SO	?	?
SO_2	?	?

Other Ketones - Hu & Abeles, 1990; Lowe et al, 1977

AcPhe-$NHCH_2COMe$	1550
ZPhe-$NHCH_2COCO_2H$	7
ZPhe-$NHCH_2COCO_2Me$	1

[a]Assayed as a mixture of diastereomers. This compound racemizes under the assay conditions.

Correlation Between $\log K_{RSH,app}$ and pK_i



$pK_i = 0.8 \log K_{RSH,app} + 4.6$, correlation coefficient = 0.96

Conclusions

- Addition of thiol to ketone in aqueous solution is a good model of the enzymatic reaction
  -- $K_{RSH,app}$ can be used to predict K_i

- The ketone, and not the hydrate, is the active form of the inhibitor

- Through-space electrostatic interactions are a useful and predictable design element for bioorganic chemistry

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