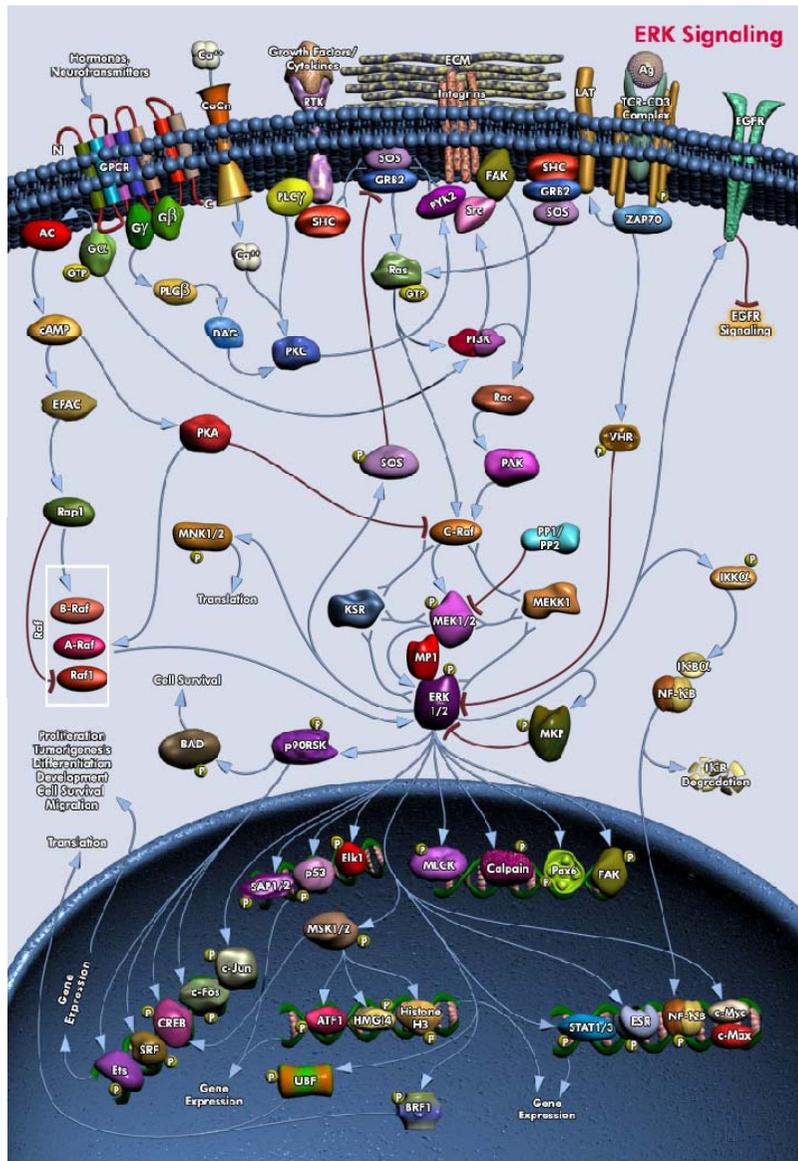


Chemical Biology

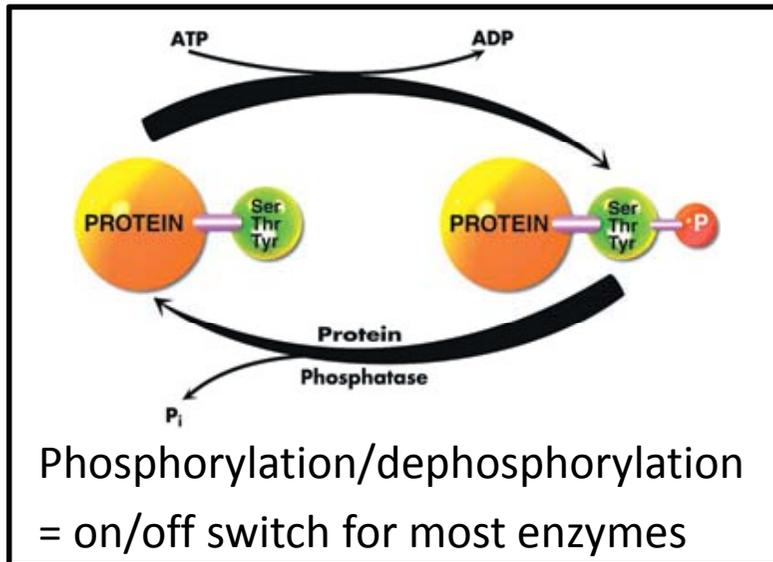


- Uses chemistry to understand biological systems
- small molecules to manipulate protein function



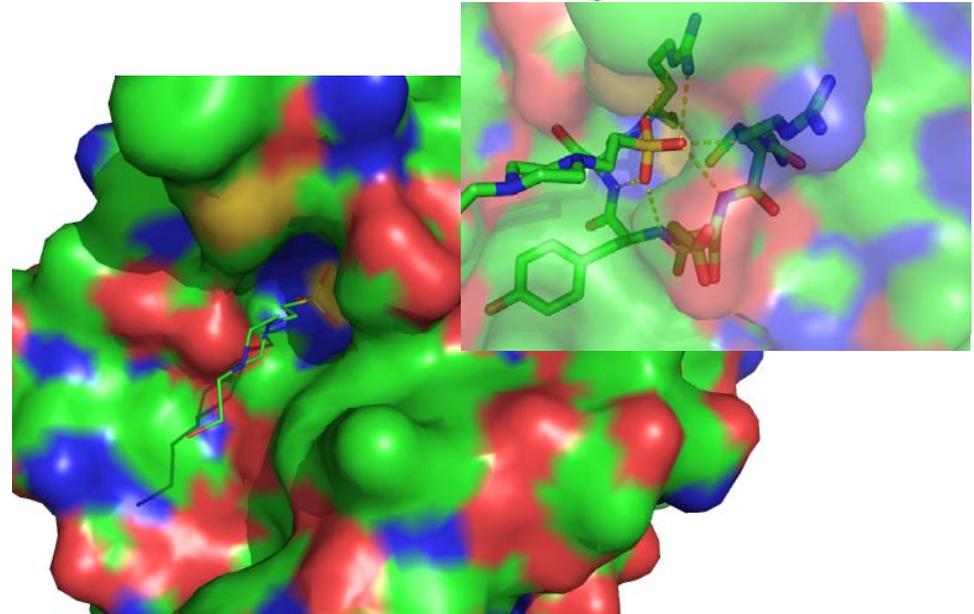
- Phosphorylation of proteins is a fundamental regulatory mechanism, involved in almost all important biological processes.
- abnormal phosphorylation state may lead to many diseases including cancer and diabetes

Dual Specific Phosphatase VHR



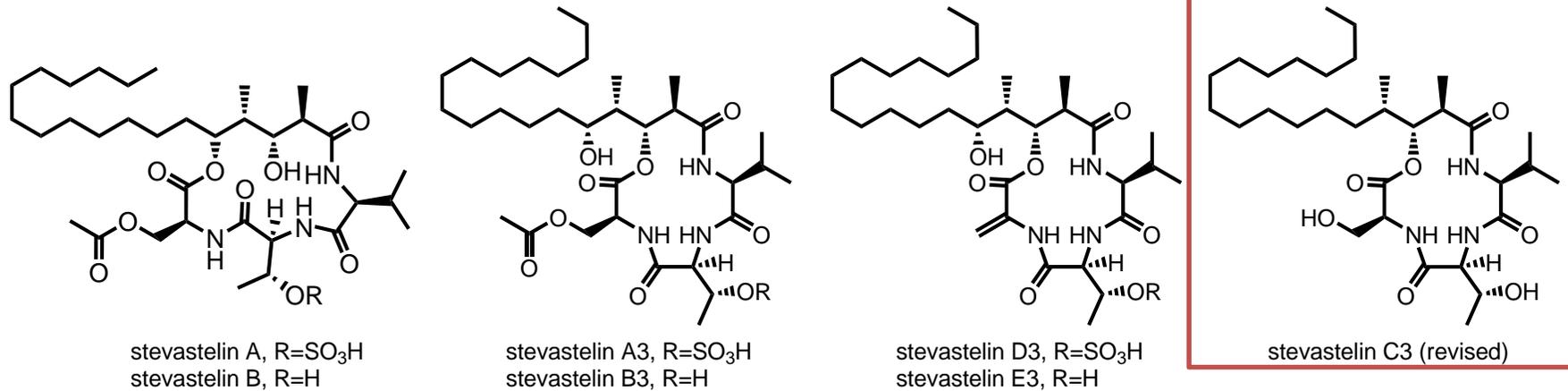
VHR:

- human DSP
- 20 kDa
- substrates: MAPK (ERK, JNK)
- activators: ZAP70, VRK3
- inhibitors: stevastelins,...



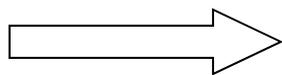
The Mitogen-Activated Protein Kinase Phosphatase *Vaccinia* H1-Related (VHR) Protein Inhibits Apoptosis in Prostate Cancer Cells and Is Overexpressed in Prostate Cancer
Cancer Research **2008**, 68, 9255.

Stevastelins



- isolated from *penicillium* NK374186
- consist of a fatty acid, Ser, Thr, Val
- 13- and 15-membered cyclic depsipeptides
- immunosuppressive activity
- phosphatase inhibition (VHR)

- **mimic** natural substrate
- topological templates which contain **spatially defined functional groups**
- better **oral bioavailability** than linear peptides
- **no enzymatic degradation** of the peptide

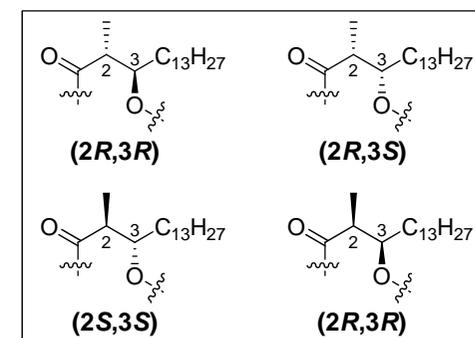
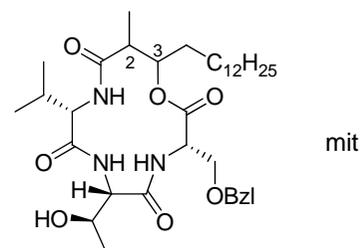


Interesting targets for drug development & investigation of physiological processes

Project Outline

• Synthesis

- diastereomeric cyclodepsipeptides
- phosphorylated cyclodepsipeptides
- altered amino acid sequence



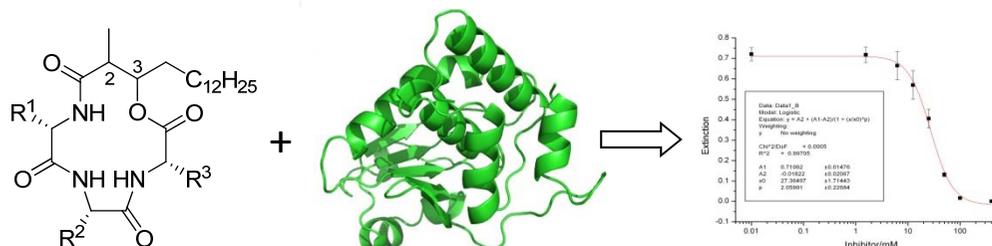
• Conformational Analysis

- influence of stereocenters in the macrocyclic backbone
- influence of phosphorylation of the depsipeptide

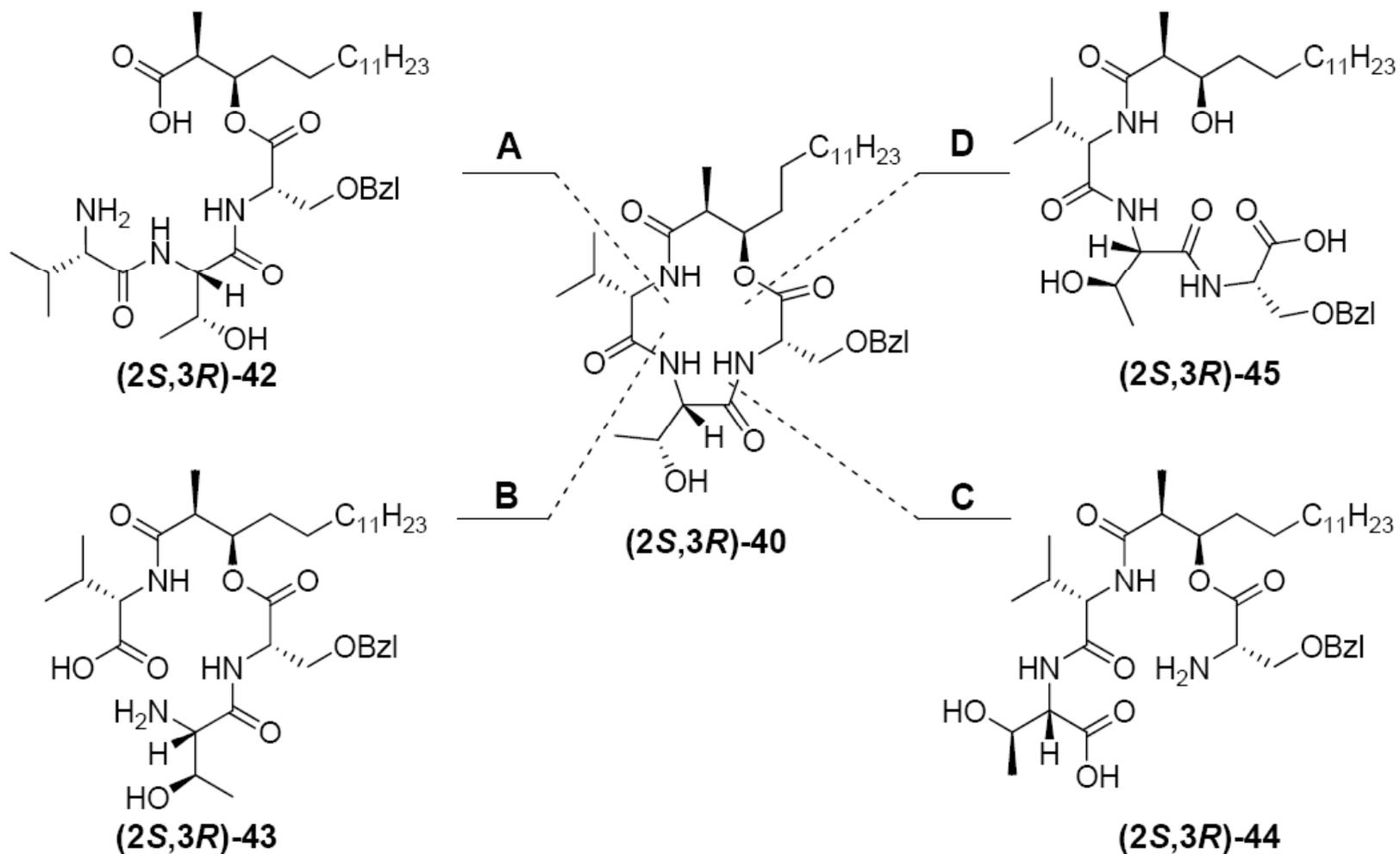


• Biological Investigations

- phosphatase inhibition
- antibacterial activity



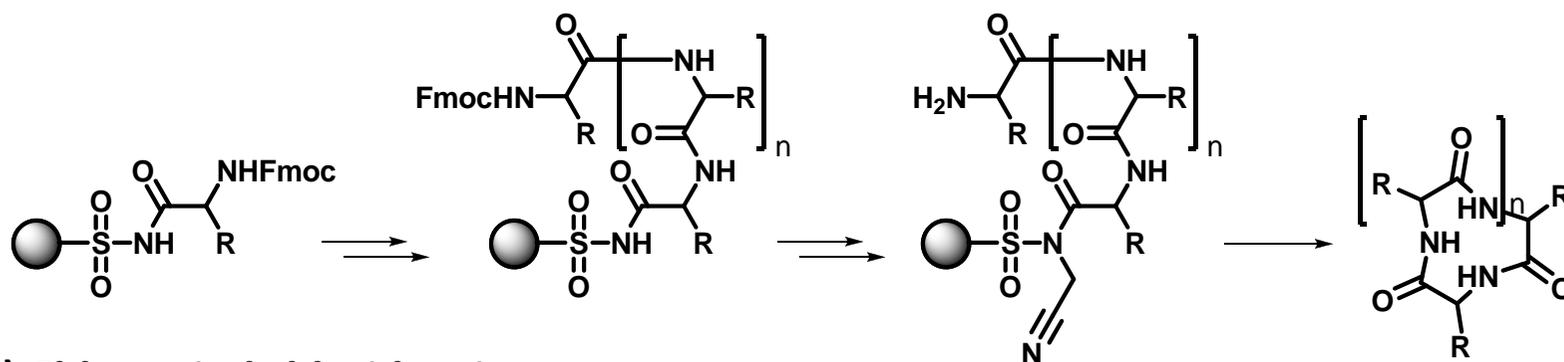
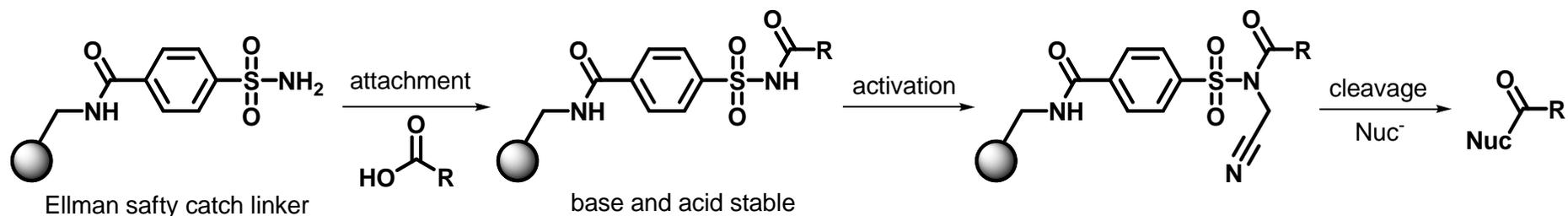
Retro Synthesis



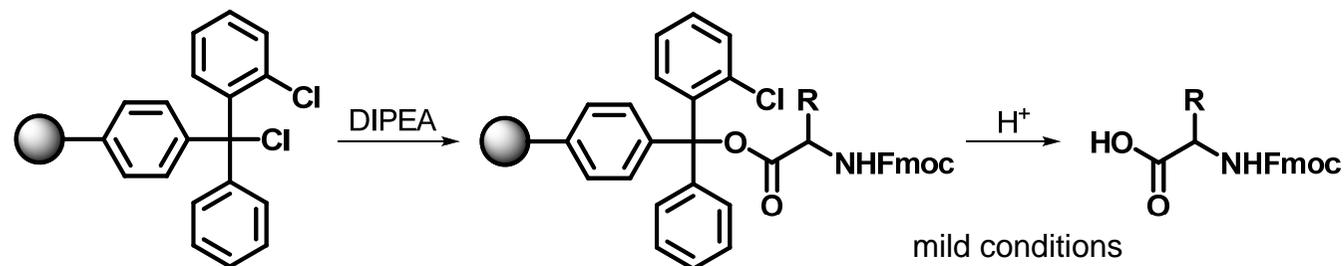
Normally, macrolactamization is favored over macrolactonization.

Solid Phase Synthesis

1) safty-catch linker:

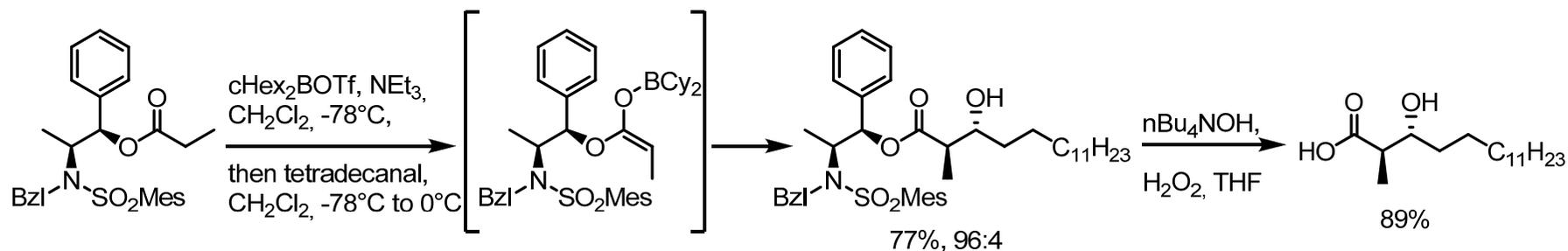
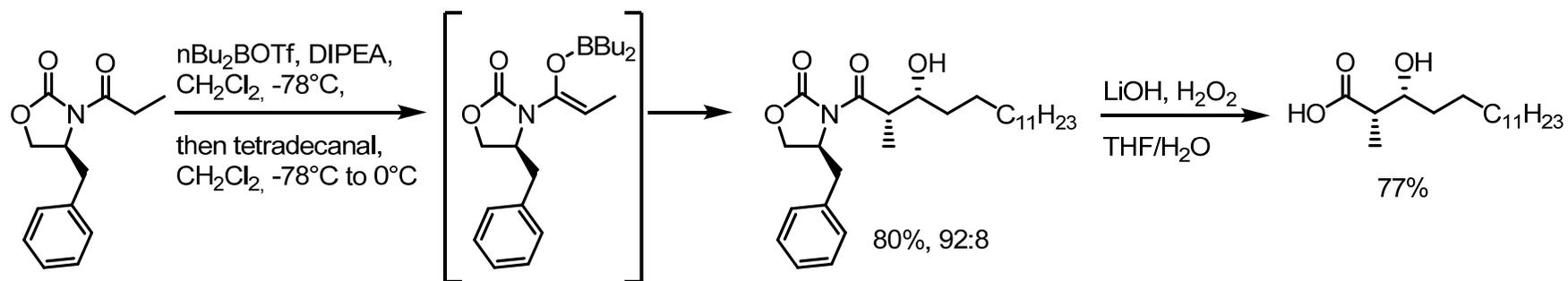
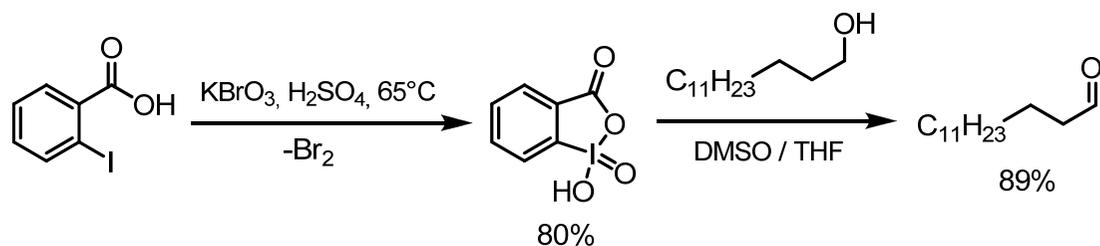


2) Chlorotriptyl chlorid resin:

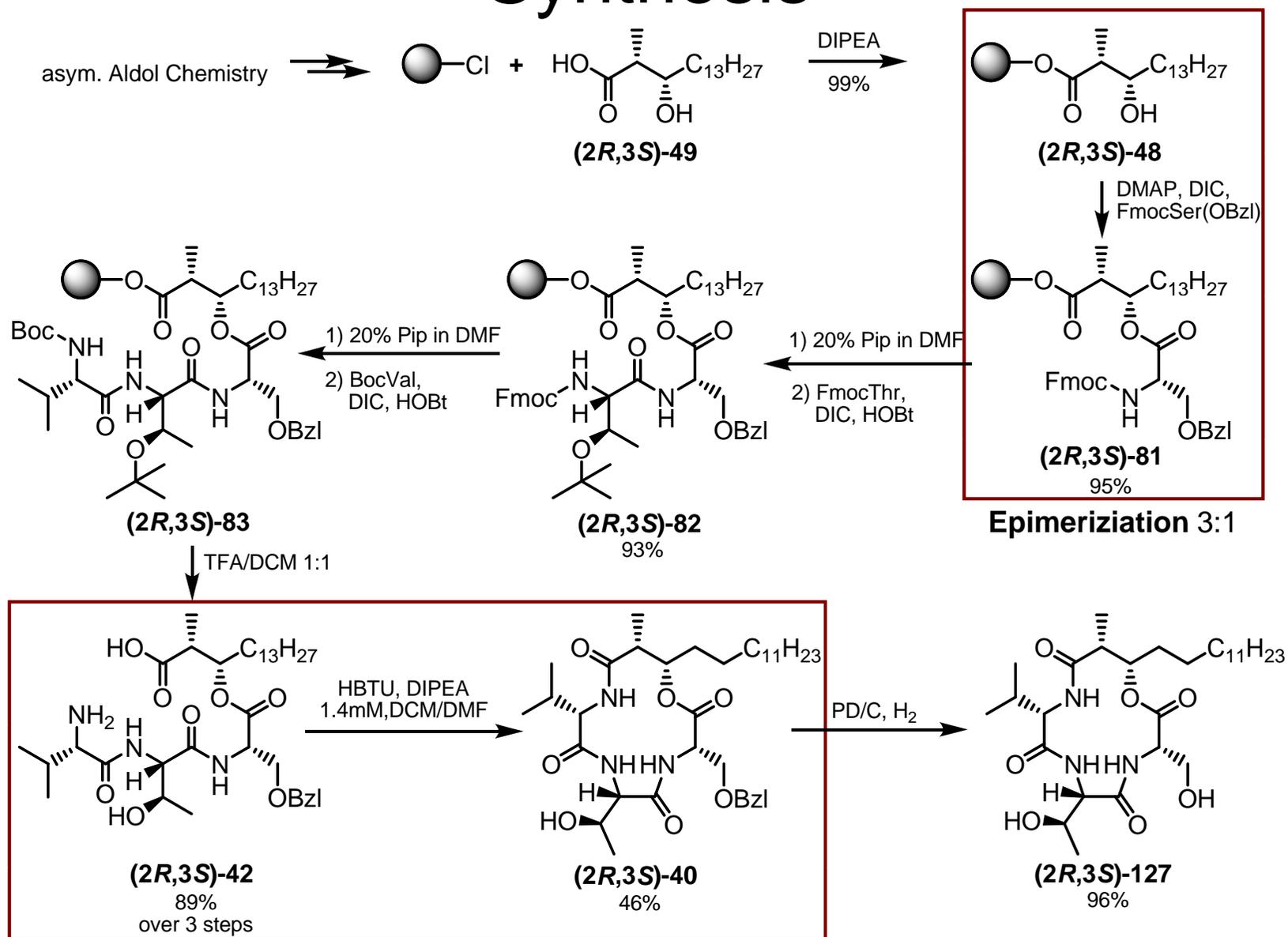


- highly modular
- easy to remove excess reagents
- easy work-up

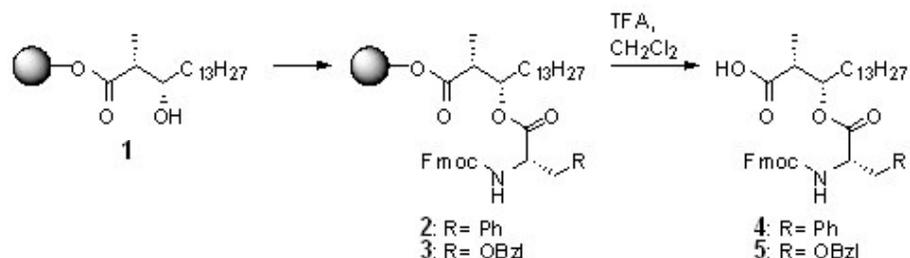
Buliding Block Synthesis



Synthesis



Esterification



R	esterification conditions*	yield	d.r.
1	Ph 5 Eq. DIC, 0.5 Eq. DMAP, RT, 14 h	99%	3:1
2	Ph 5 Eq. DIC, 10 Eq. Imidazole, RT, 14 h	0%	-
3	Ph 5 Eq. DIC, 10 Eq. Collidine, RT, 14 h	0%	-
4	Ph 5 Eq. DIC, 0.2 Eq. DMAP, RT, 4 h	91%	6:1
5	Ph 5 Eq. DIC, 0.2 Eq. DMAP, 0°C, 4 h	90%	9:1
6	Ph 5 Eq. DIC, 0.2 Eq. DMAP, 0°C, 2 h	62%	-
7	Ph 5 Eq. DIC, 0.1 Eq. DMAP, 0°C, 4 h	63%	6:1
8	Ph 5 Eq. EDC, 0.2 Eq. DMAP, 0°C, 4 h	89%	6:1
9	Ph 5 Eq. MSNT, 3.8 Eq. Methylimidazole, RT, 8 h	10%	-
10	Ph 5 Eq. DIAD, 5 Eq. PPh ₃ , RT, 14 h	0%	-
11	Ph 9 Eq. TCBC, 9 Eq. NEt ₃ , 10 Eq. DMAP, 0°C to RT, 14 h	98%	2:1
12	Ph 9 Eq. TCBC, 9 Eq. NEt ₃ , 0.5 Eq. DMAP, 0°C, 4 h	89%	9:1
13	Ph 5 Eq. PyBrop, 0.2 Eq. DMAP, 10 Eq. DIPEA, RT, 14 h	2%	-
14	OBzl 5 Eq. DIC, 0.5 Eq. DMAP, RT, 14 h	98%	5:1
15	OBzl 5 Eq. DIC, 0.2 Eq. DMAP, 0°C, 3 h	89%	8:1
16	OBzl 9 Eq. TCBC, 9 Eq. NEt ₃ , 0.3 Eq. DMAP, 0°C, 4 h	88%	8:1

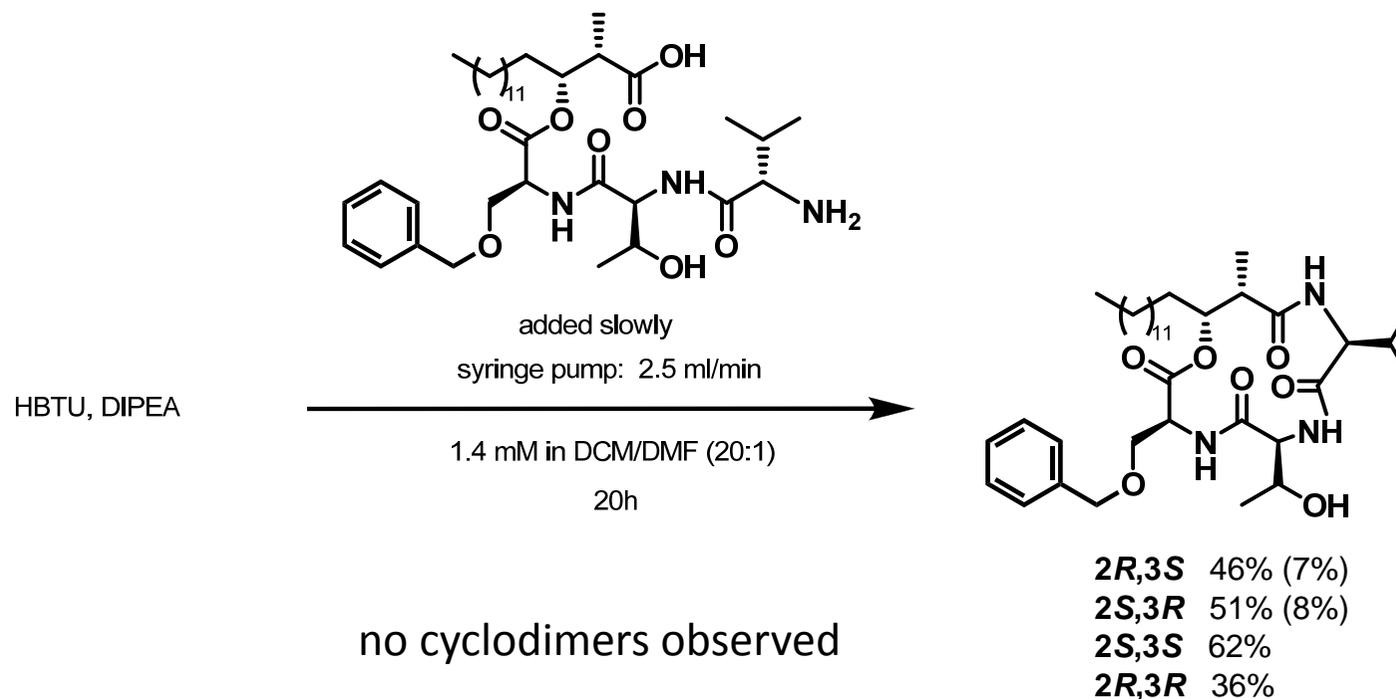
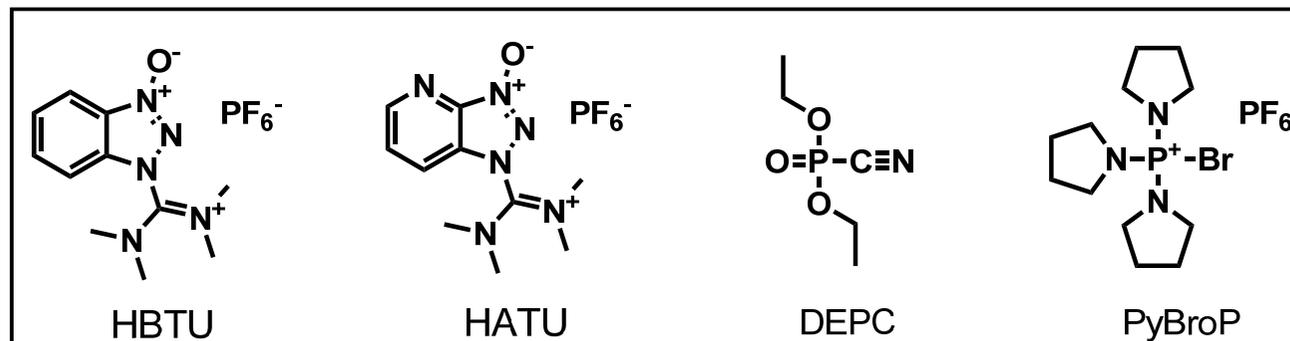
TCBC = 2,4,6-Trichlorbenzoylchlorid;

MSNT = 1-(Mesitylen-2-sulfonyl)-3-nitro-1,2,4-triazole;

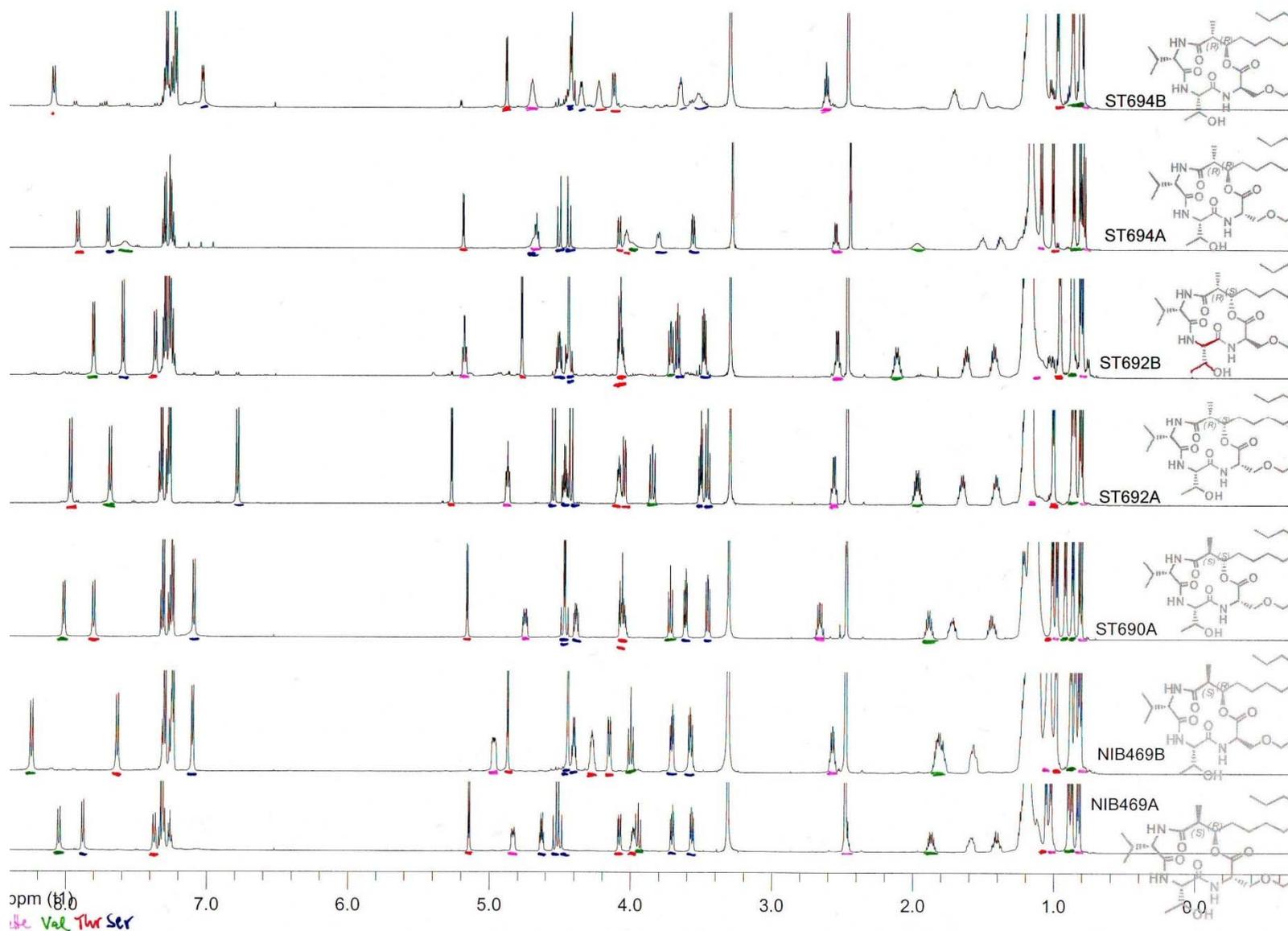
*5 Eq. amino acid
(Fmoc-Phe-OH or Fmoc-Ser(OBzl)-OH)

3:1 \Rightarrow 9:1

Macrocyclization

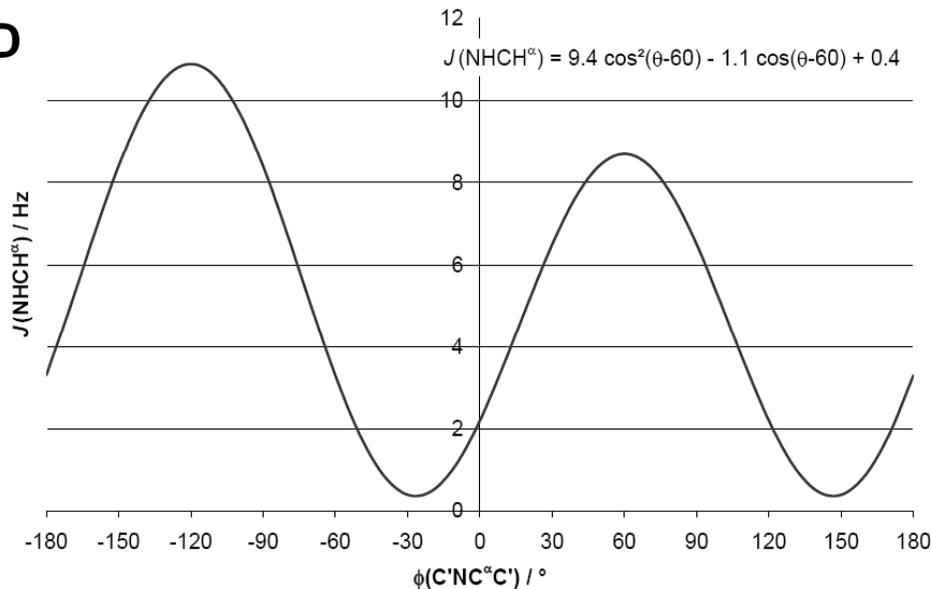


$^1\text{H-NMR}$

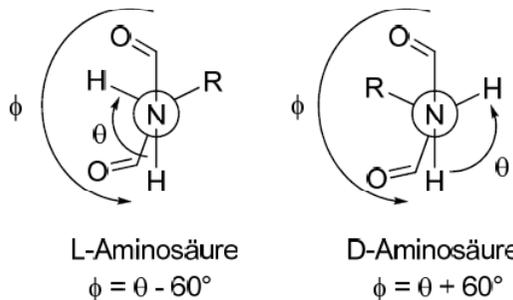
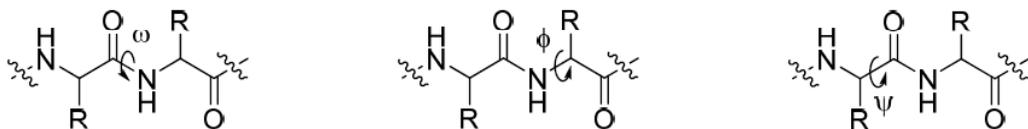


NMR experiments

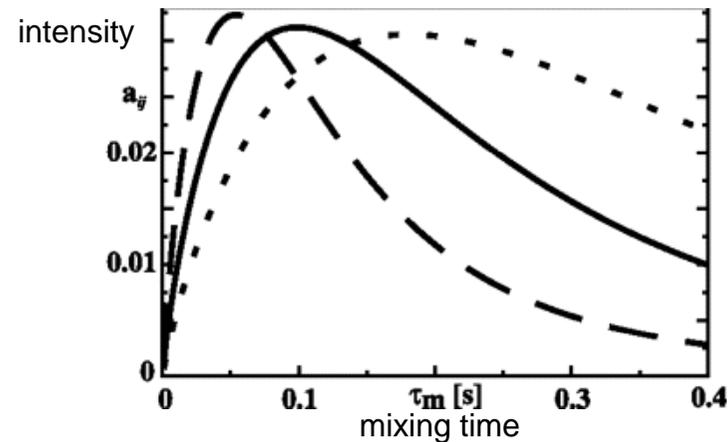
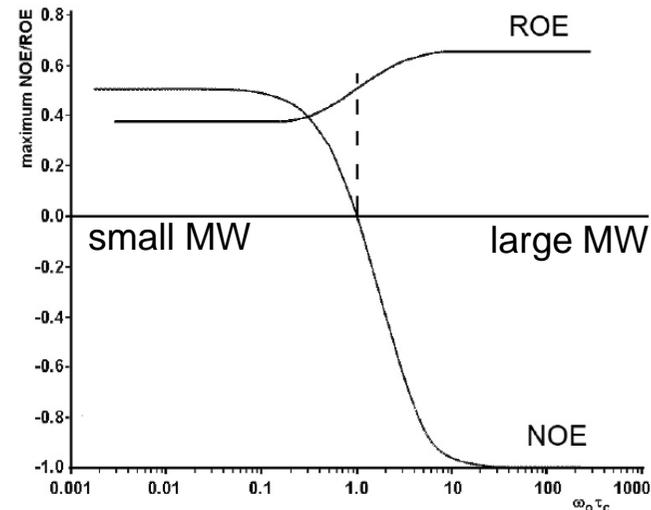
1D



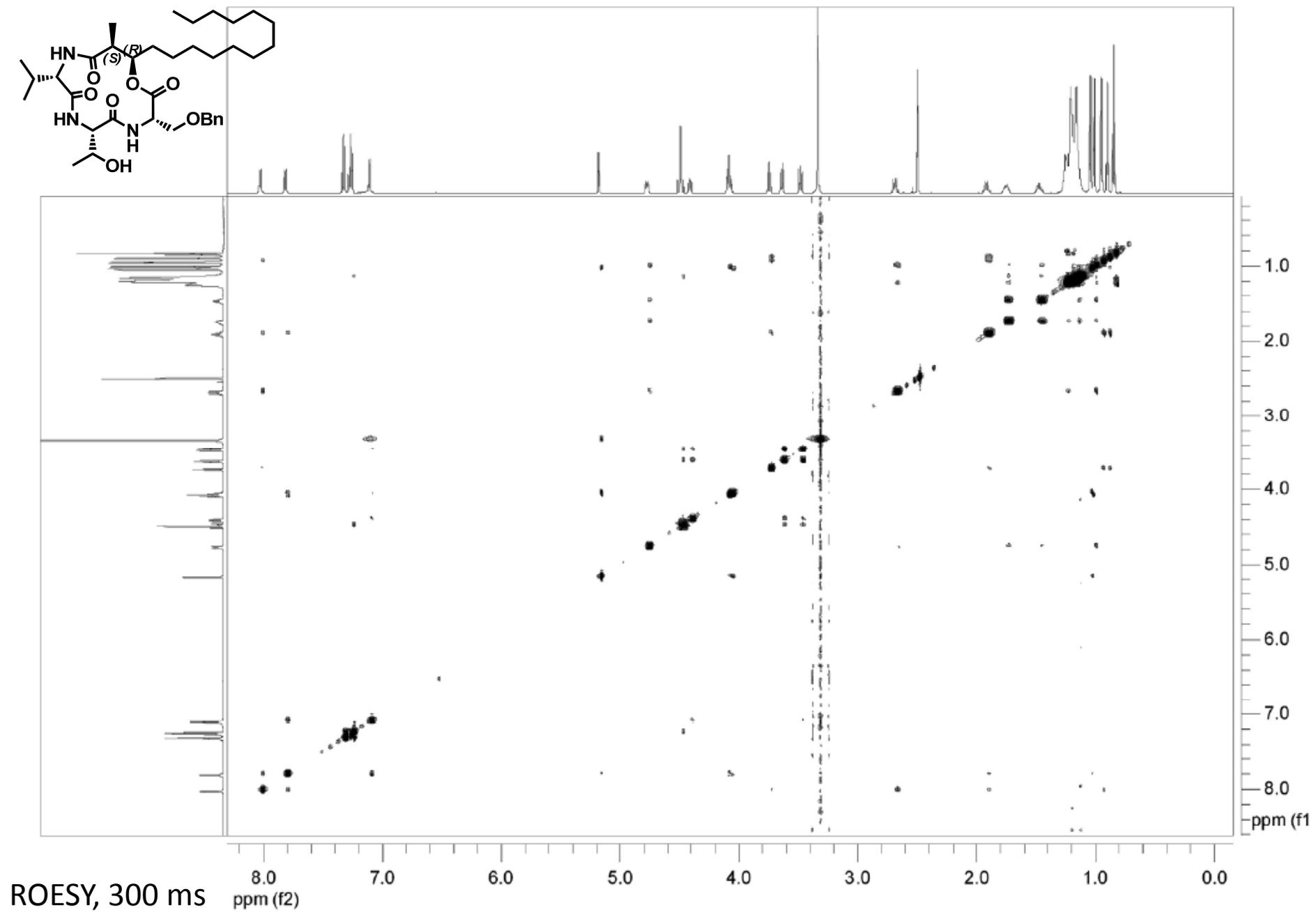
Karplus gives torsion angle (C'NC^αC')



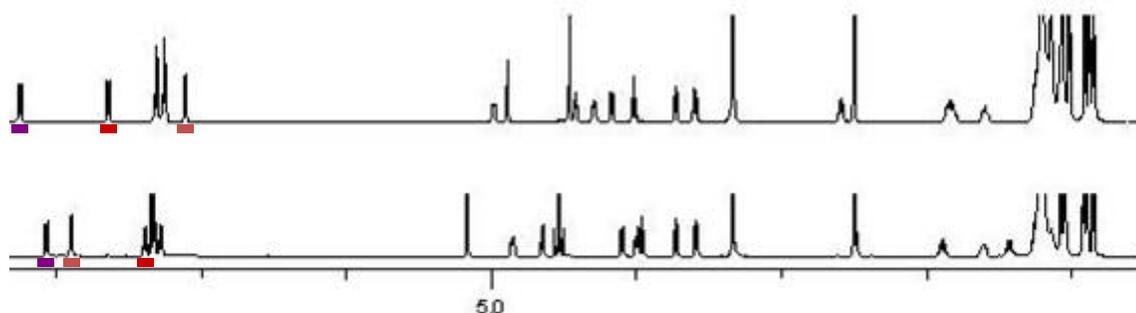
2D



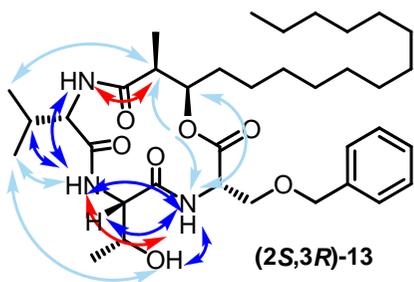
$$r_{ij} = r_{ref} \sqrt[6]{\frac{V_{ref}}{V_{ij}}}$$



Conformational Analysis



¹H-NMR:
 sharp signals
 large coupling constants
 chemical shifts differ



↔ starker NOE 1.8 - 2.3 Å
↔ mittlerer NOE 2.3 - 3.5 Å
↔ schwacher NOE 3.5 - 5.0 Å

ROE-signals per macrocycle:

- ~ 40 cross peaks
- 25 intraresidual contacts
- 13 sequential contacts
- 1 medium range contact

20-30 distances for force field calculations

Force field calculations:

OPLS force field with GB/SA solvation model

TNCG minimization, LMOD method

with NMR-restraints

without restraints



lowest energy conformation



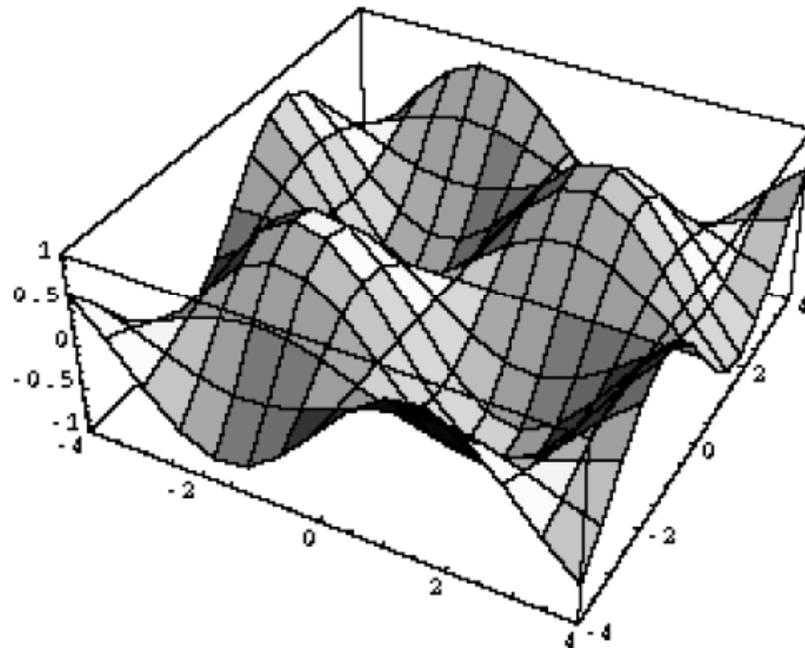
control and flexibility

Computational Studies

1. Calculate Energy (OPLS force field parameters):

- Optimal bond length, angle,...is given
- Harmonic and disharmonic potentials are used

$$E = E_{\text{bond length}} + E_{\text{bond angle}} + E_{\text{torsion angle}} + E_{\text{electrostatic}} + E_{\text{van der Waals}}$$



Potential hyper surface

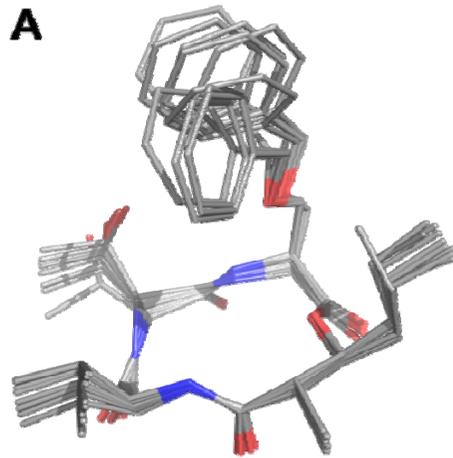
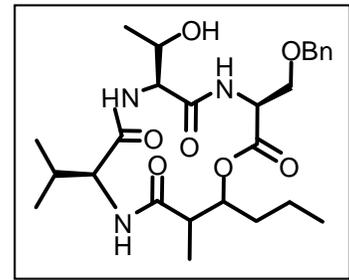
2. Minimize Energy (TNCG):

- Find minimum next to calculated energy
- Use 1st & 2nd derivative

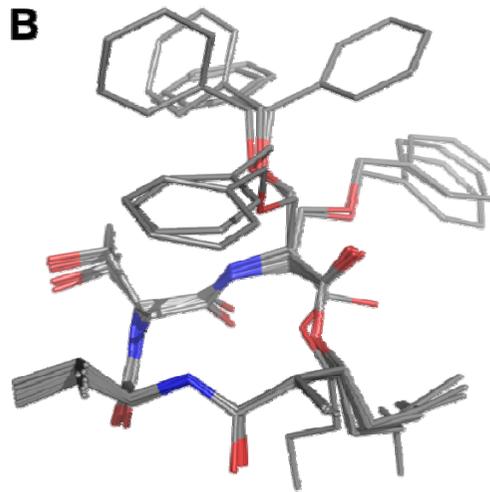
3. Find Next Minimum (LMO):

- Low frequency distortions (vibrations)
- using Hesse matrices
- 2nd derivatives → saddle points

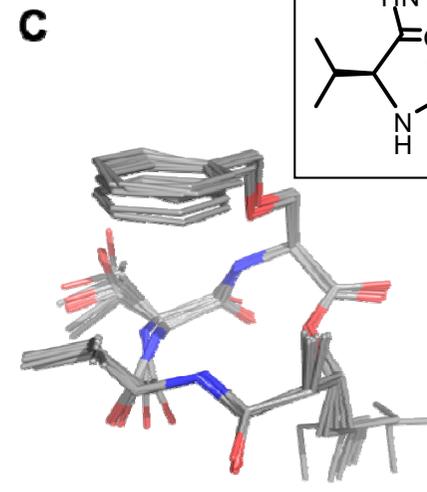
Conformational Analysis



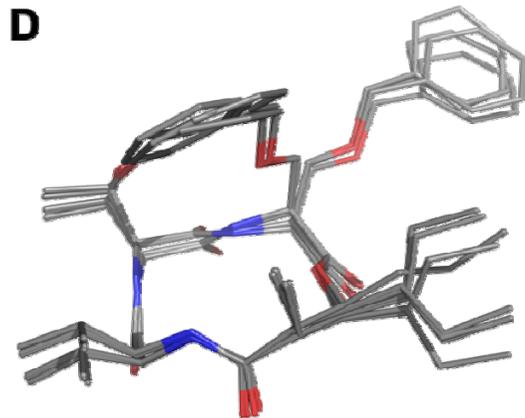
2S,3S



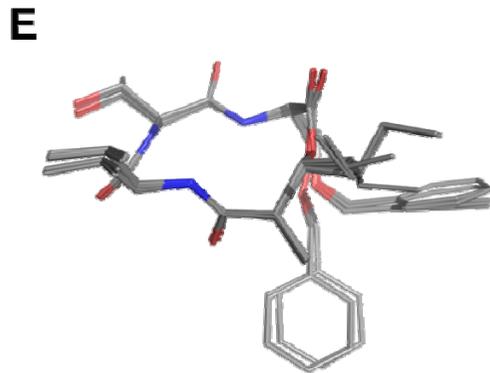
2S,3R



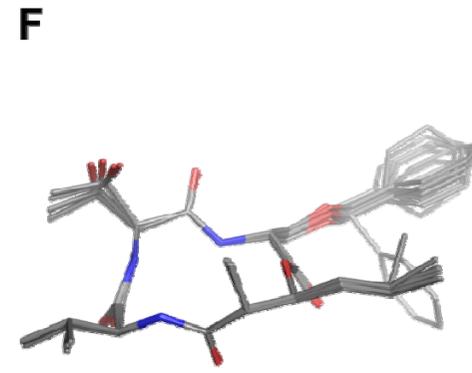
2R,3R



2R,3S



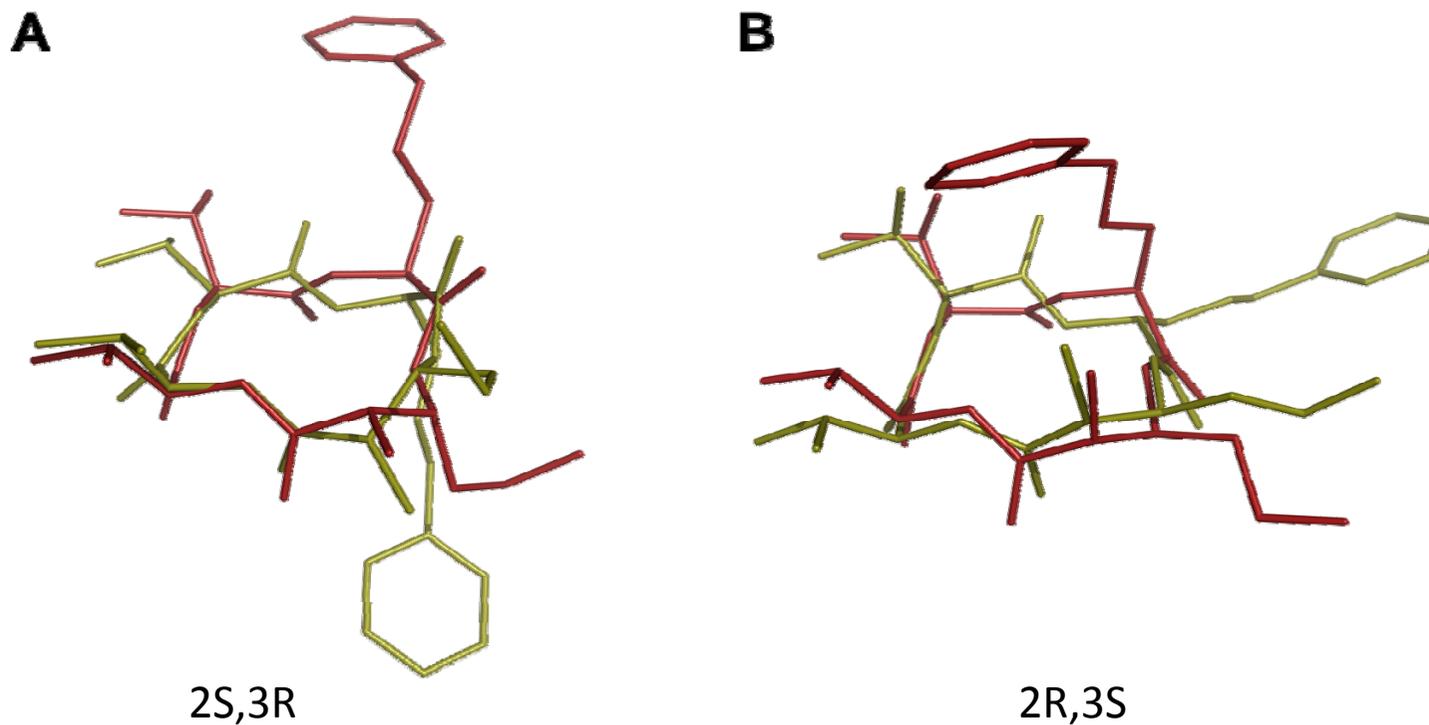
2S,3R D-Ser



2R,3S D-Ser

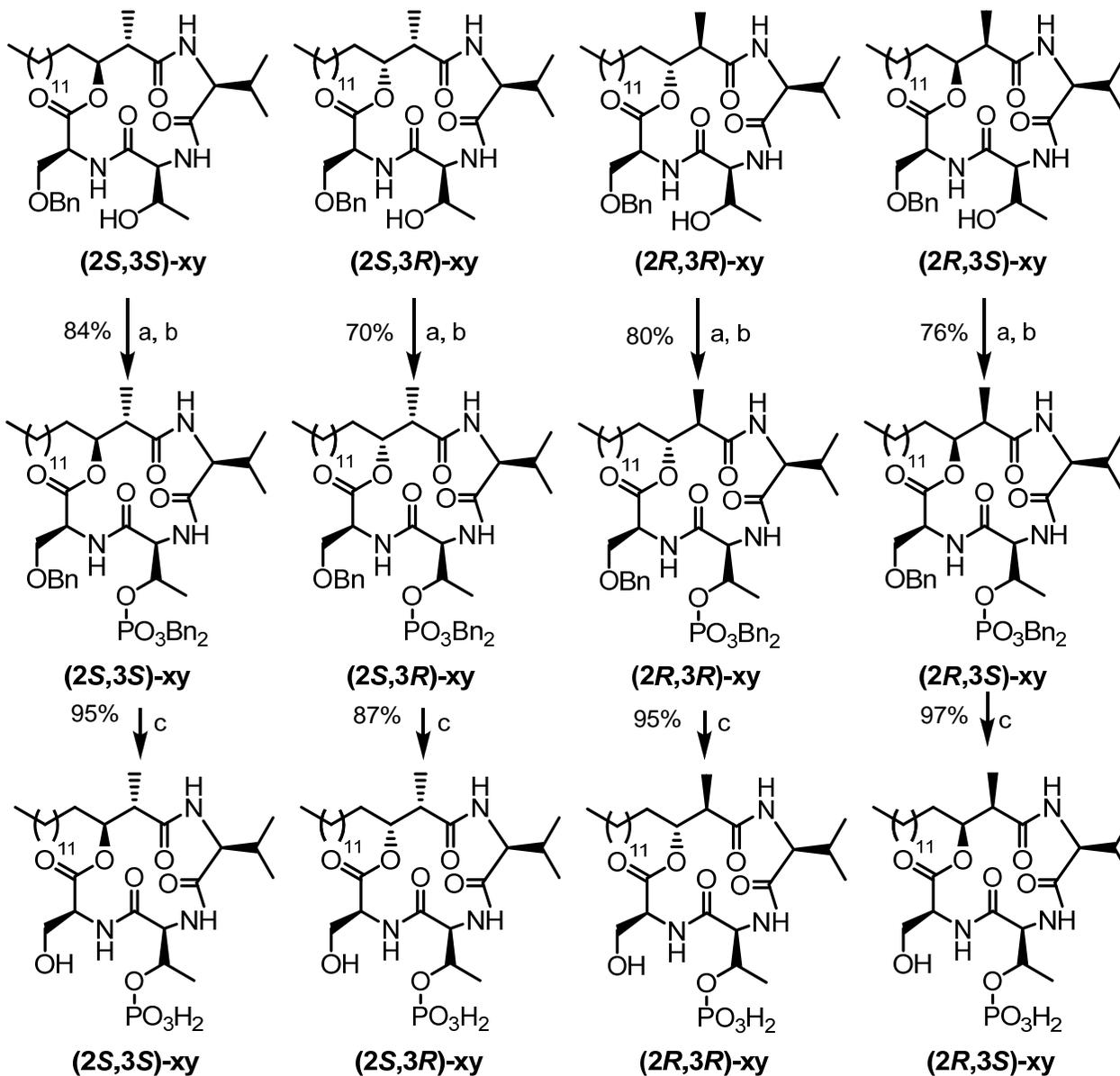
→ peptidic part determines overall geometry

Effect of D-Ser on Conformation

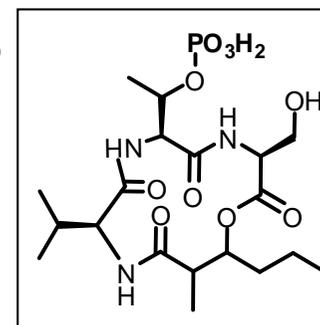
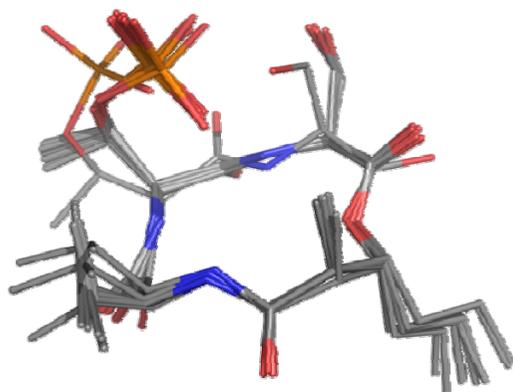


L-Ser: red
D-Ser: olive

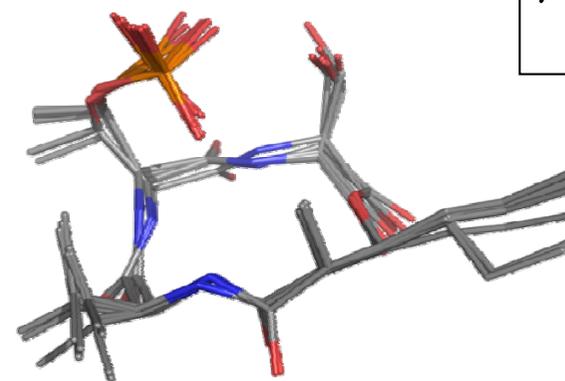
Phosphorylation



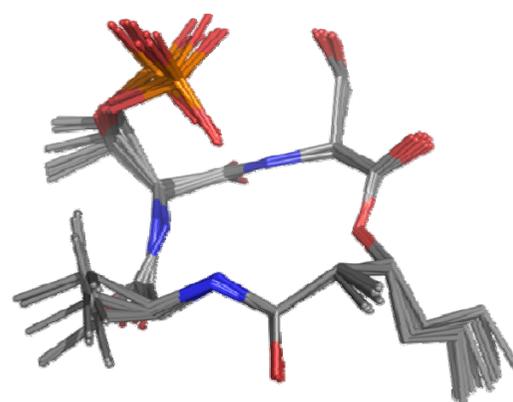
Phosphorylated Depsipeptides

**A**

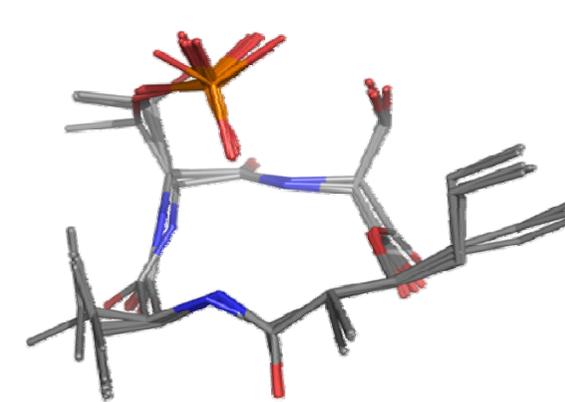
2R,3R

B

2R,3S

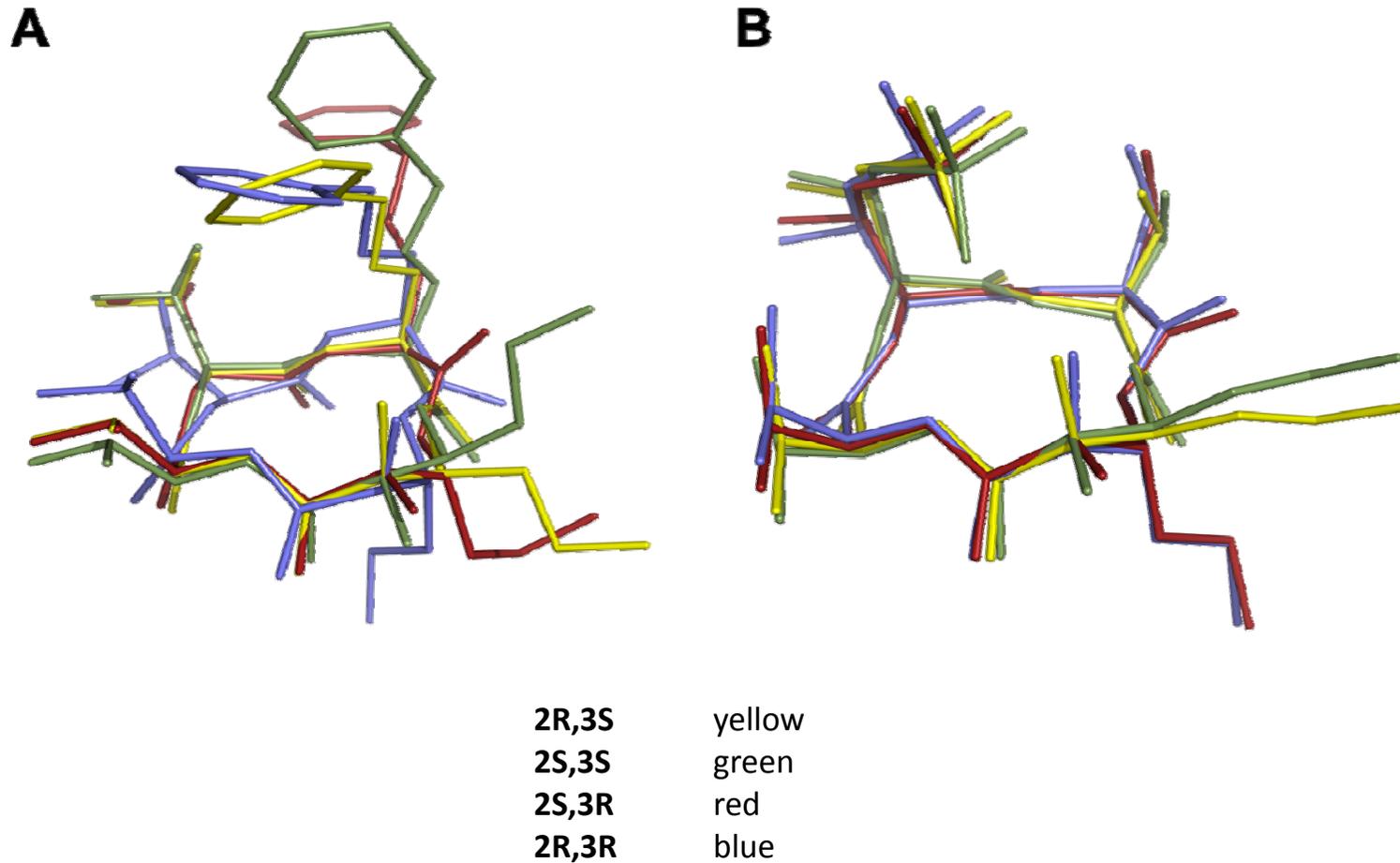
C

2S,3R

D

2S,3S

Benzylated vs. Phosphorylated Cyclodepsipeptides

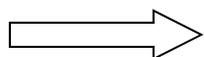


Flexibility

entry	cycle	E_{\min} / kJ mol ⁻¹	conformations	cluster
1	(2 <i>S</i> ,3 <i>S</i>)-Bn	-292.1	132	38
2	(2 <i>S</i> ,3 <i>R</i>)-Bn	-289.8	87	14
3	(2 <i>R</i> ,3 <i>R</i>)-Bn	-287.4	117	37
4	(2 <i>R</i> ,3 <i>S</i>)-Bn	-290.7	150	32
5	(2 <i>S</i> ,3 <i>S</i>)-PO4	-1023.2	346	106
6	(2 <i>S</i> ,3 <i>R</i>)-PO4	-1021.5	959	147
7	(2 <i>R</i> ,3 <i>R</i>)-PO4	-1017.5	1111	115
8	(2 <i>R</i> ,3 <i>S</i>)-PO4	-1015.1	754	246

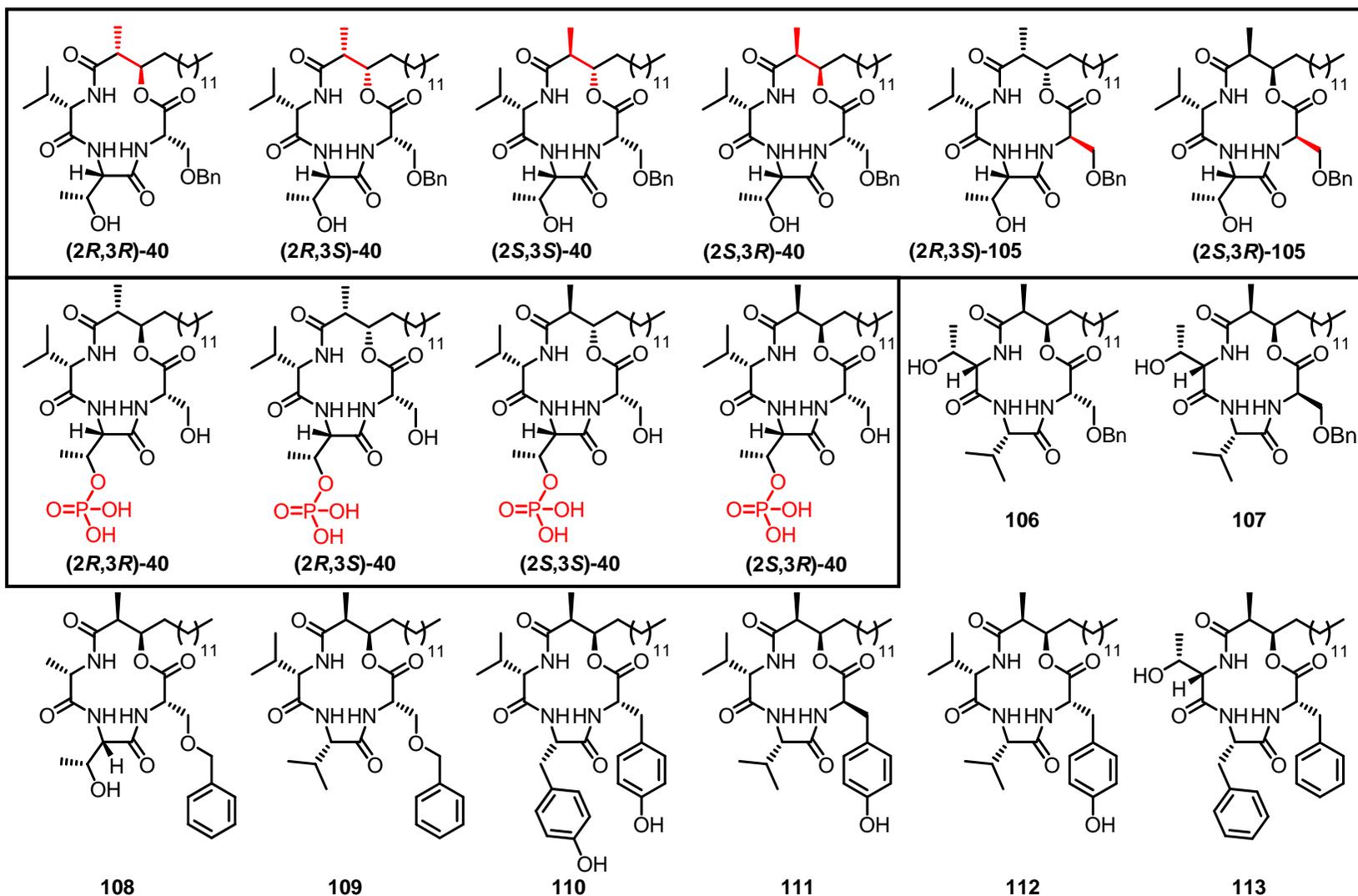
in 50 kJ/mol from the global minimum

Phosphorylated structures are much more flexible.

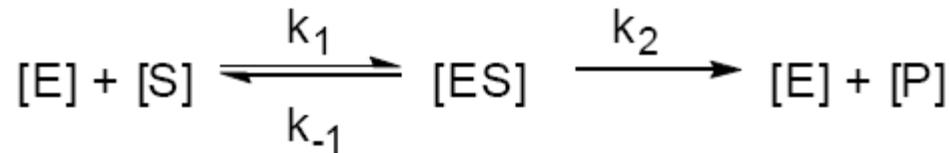


Are there any consequences on biological activity?

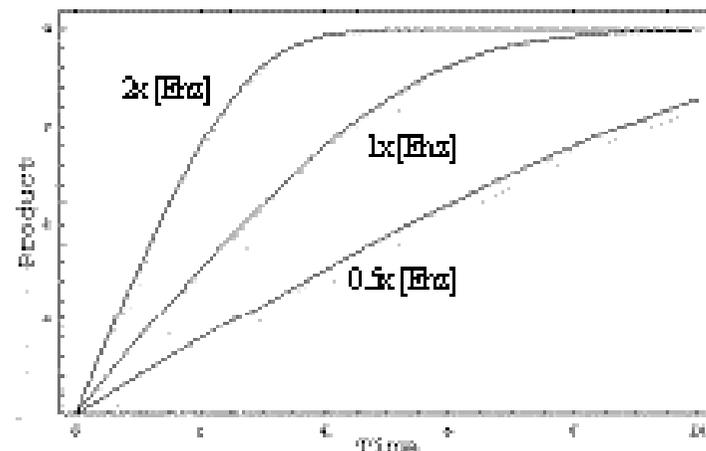
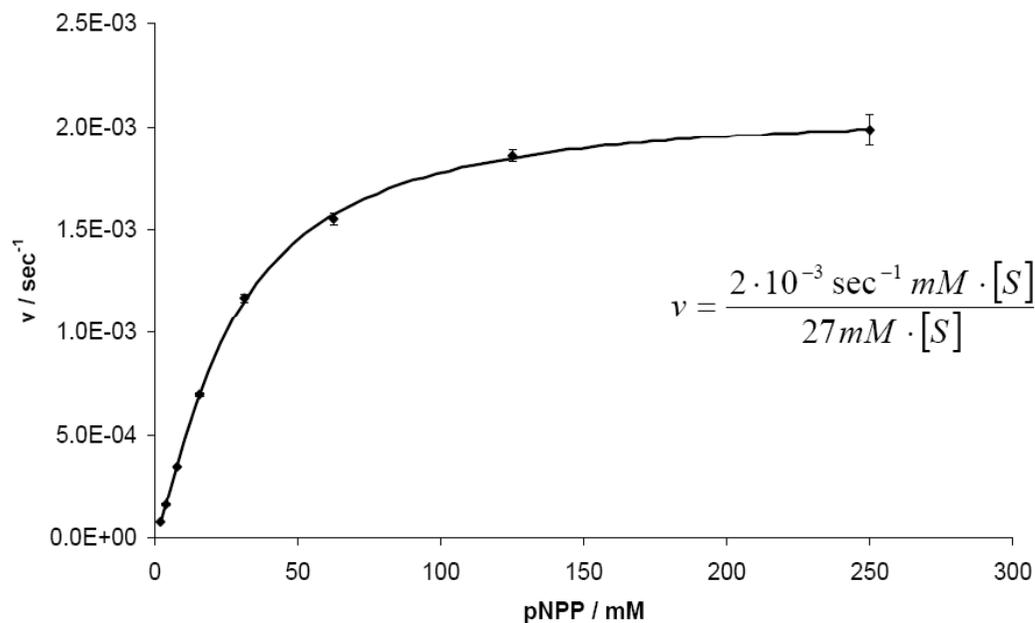
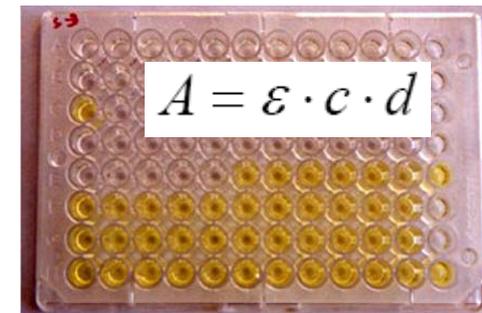
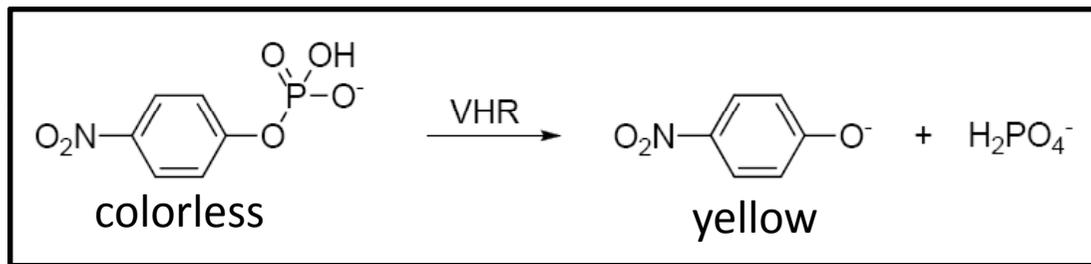
Synthesized Cyclodepsipeptides



Enzyme Assays



$$K_m = \frac{k_{-1} + k_2}{k_1}$$



Initial rate:

less than 10% product are formed

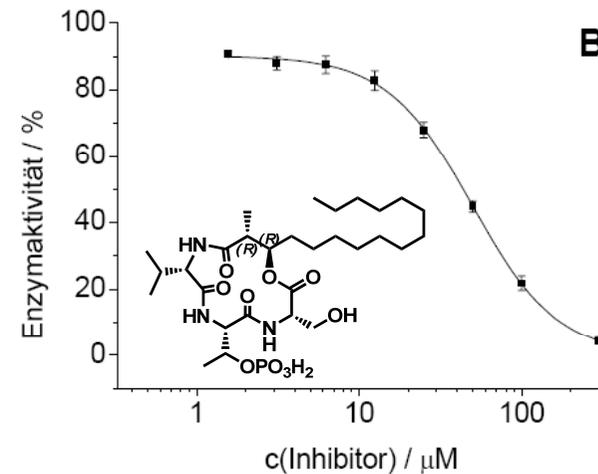
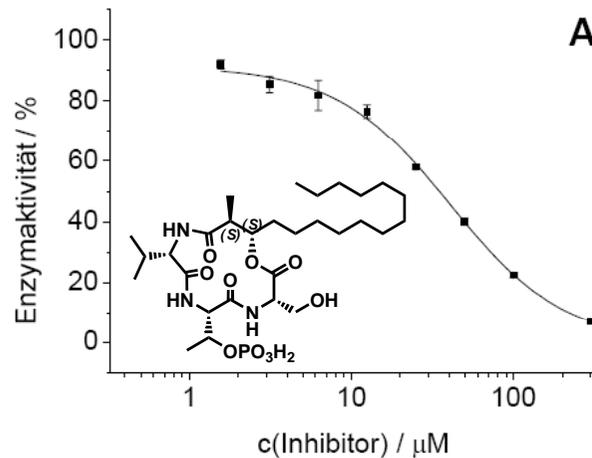
→ $[S] \approx [S]_0$, nearly no reverse reaction

IC₅₀ Determination

Measure enzyme activity at different inhibitor concentrations.
 IC₅₀ = [I] at which half of the original enzyme activity is reached.

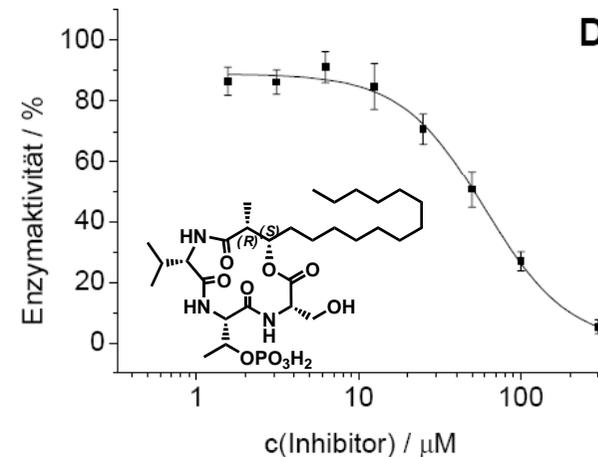
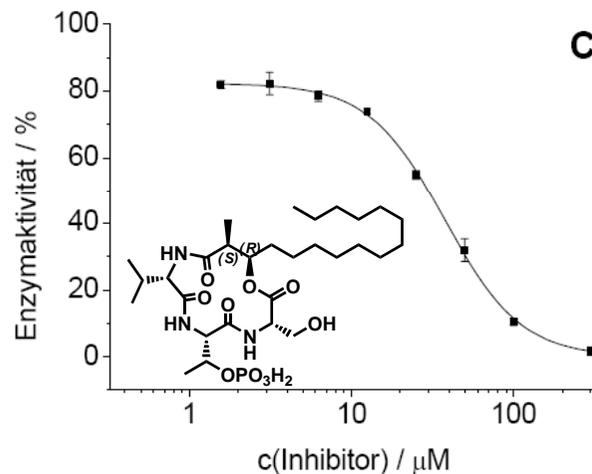
$$K_i = \frac{IC_{50}}{1 + [S] / K_m}$$

IC₅₀(VHR) = 41 μM
 Ki (VHR) = 30 μM



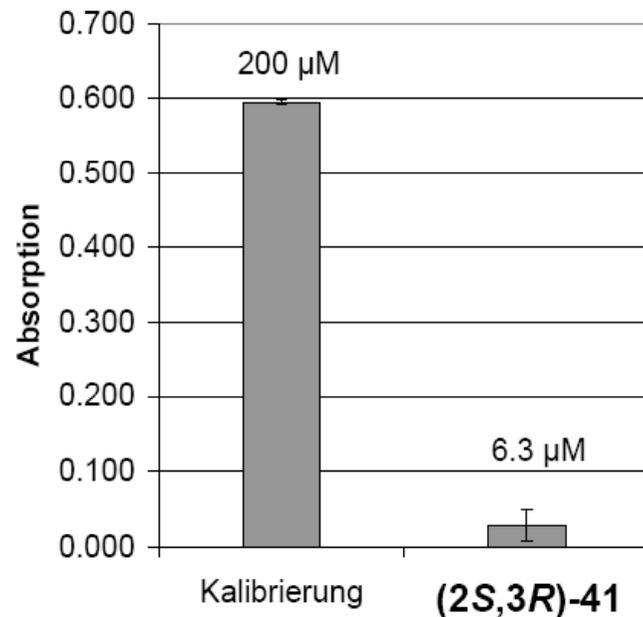
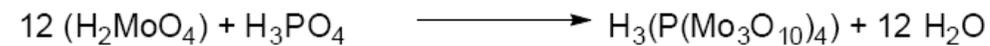
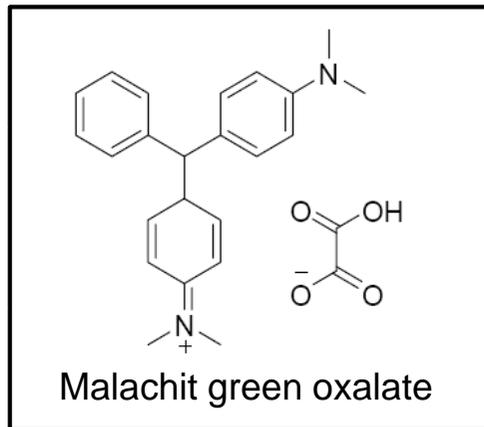
IC₅₀(VHR) = 50 μM
 Ki (VHR) = 36 μM

IC₅₀(VHR) = 38 μM
 Ki (VHR) = 28 μM



IC₅₀(VHR) = 60 μM
 Ki (VHR) = 44 μM

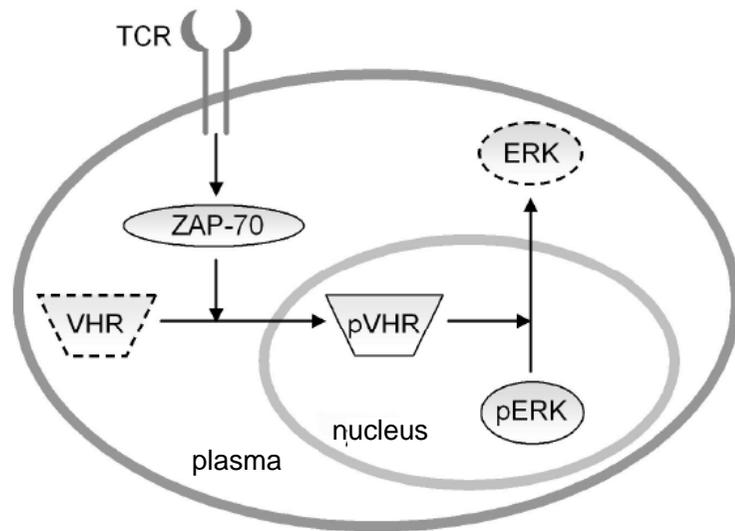
Dephosphorylation of Inhibitor



Using my assay conditons:
3% dephosphorylation of inhibitor

➔ stable inhibitor

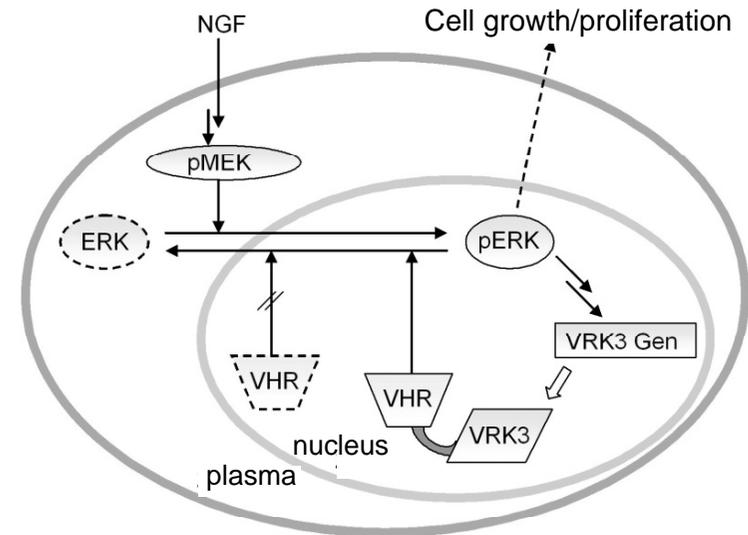
VHR In The Cell



ZAP70 activation of VHR:

- Kinase ZAP70 phosphorylates VHR
- active VHR dephosphorylates pERK
- ERK is inactive

immunosuppressant



VRK3 activation of VHR:

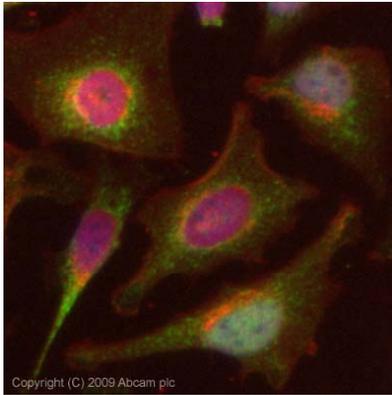
- Kinase VRK3 binds to VHR
- active VHR dephosphorylates pERK
- ERK is inactive

cancer treatment



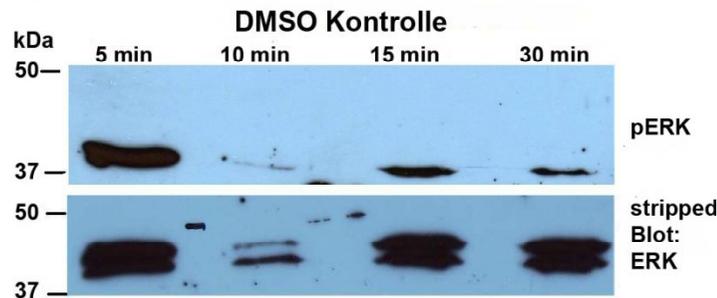
Investigate phosphorylation state of ERK

Cellular studies

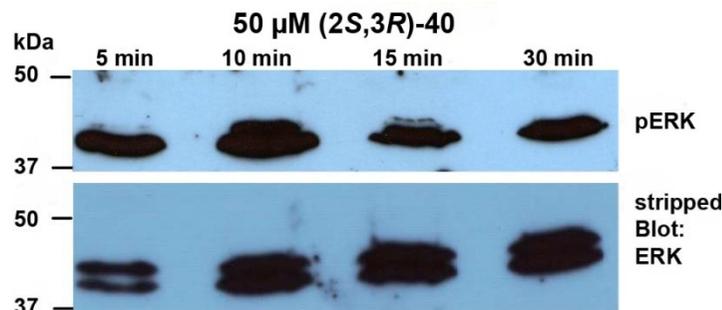
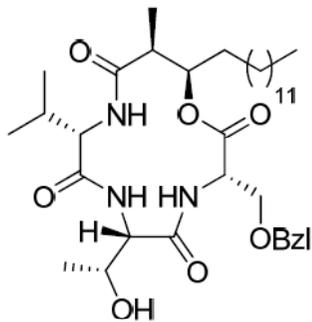


Cervical cancer cells from Henrietta Lacks, who died from cancer in 1951.

Phosphorylation state of ERK:
 -incubated HeLa-cells + inhibitor (DMSO)
 -stimulated with serum
 -lysed and blotted



Fast dephosphorylation of pERK.
 (at constant total ERK)

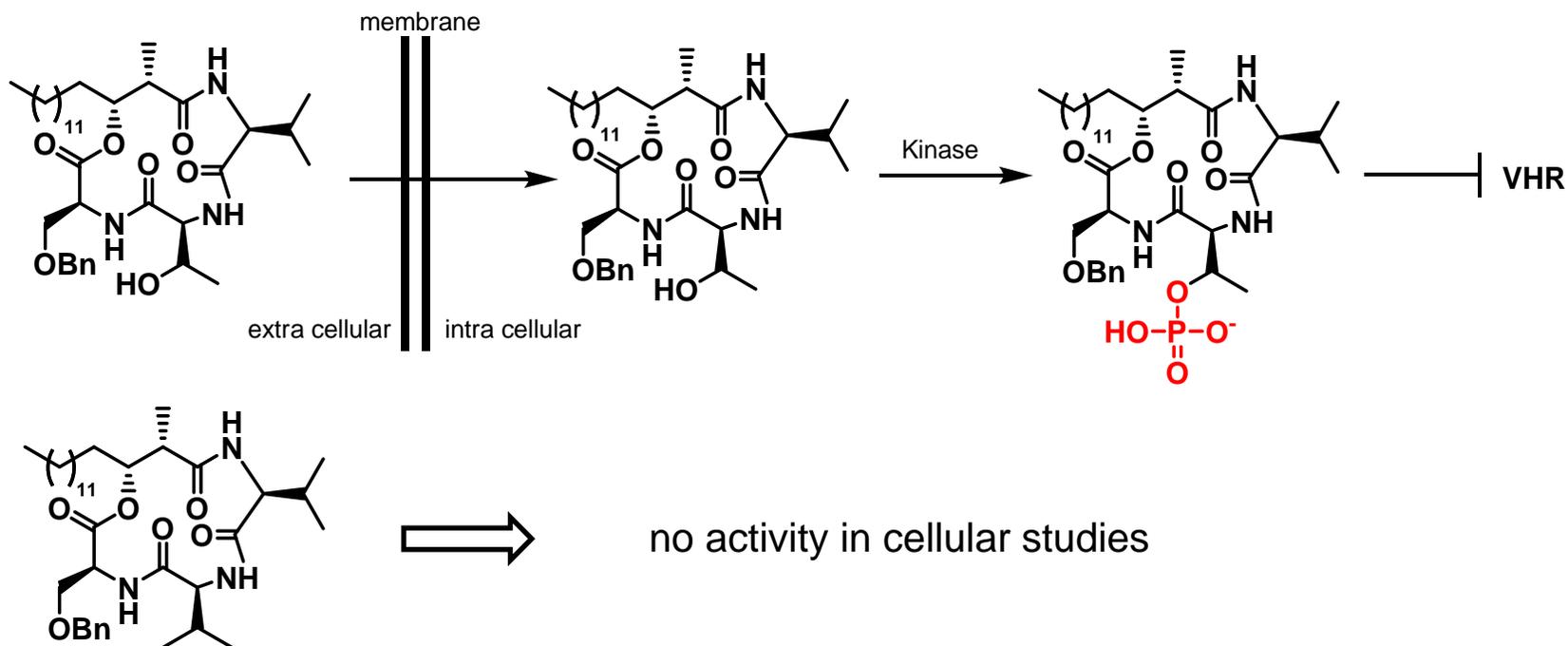


Inhibited pERK-Dephosphorylation.
 (at constant total ERK)

- inactive in VHR assay
- active in cell

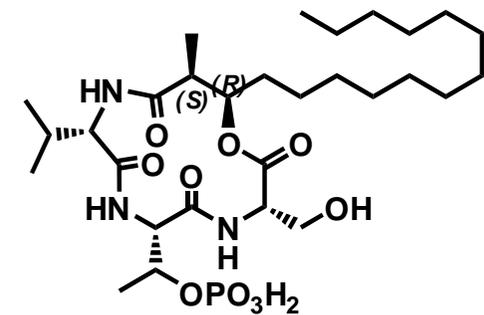
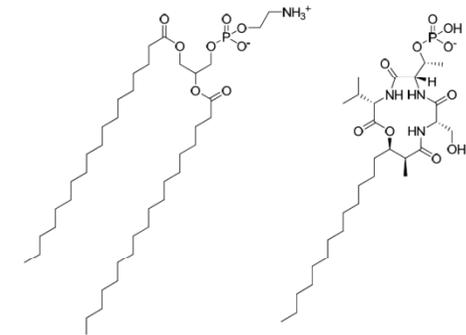
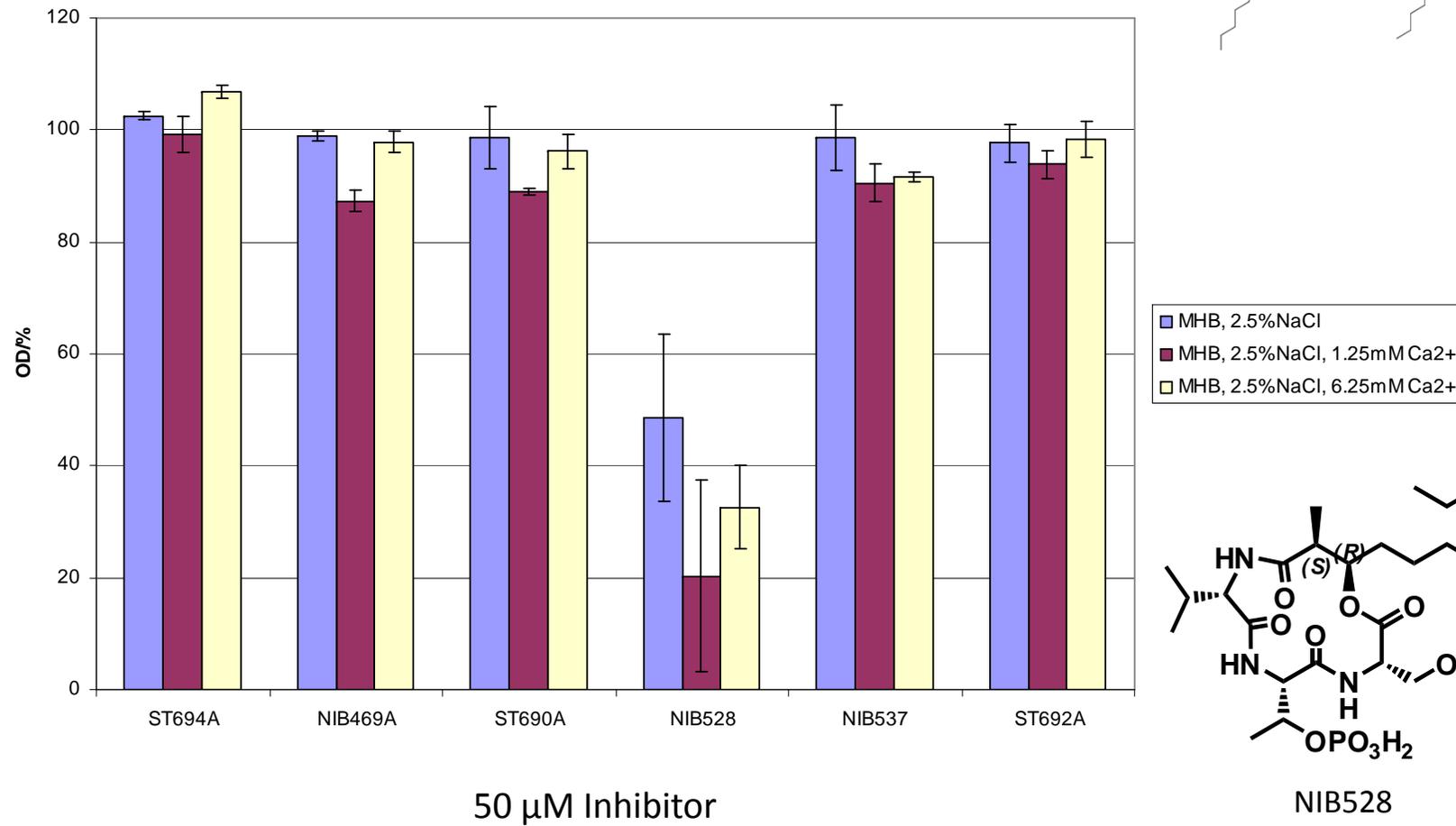
Possible Explanations

- **The compound is targeting a different enzyme, which also dephosphorylates ERK.**
other MKPs have to be tested
- **The compound inhibits VRK3 or ZAP70 (VHR activators).**
Nearly no ZAP70 expression in HeLa cells. Only VRK3 could be inhibited.
- **The compound gets activated (e.g. phosphorylated) in the cell.**
phosphorylated structures have been shown to be active



Antibiotic Screen

Bernhard

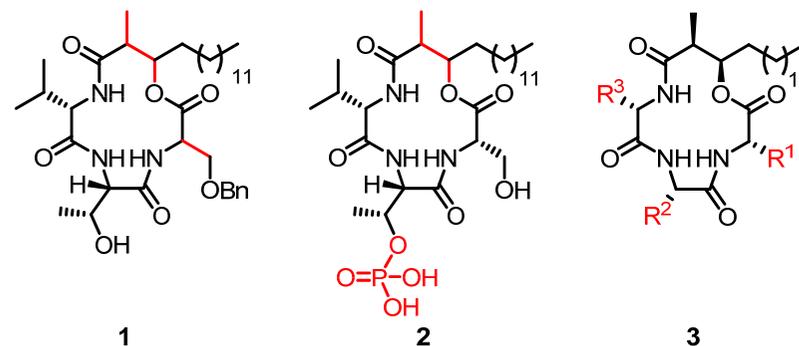
Staphylococcus aureus

NIB528

Summary

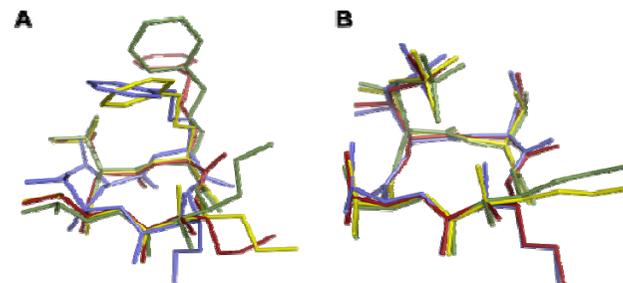
Synthesis of Stevastelin C3 Analogs

- high yielding synthesis of Stevastelin C3 Analogs
 - 6 diastereomeric cyclodepsipeptides
 - 4 phosphorylated cyclodepsipeptides
 - modified amino acid sequence



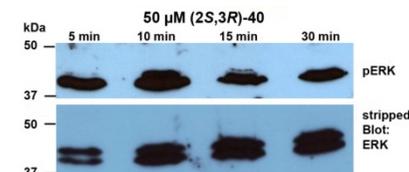
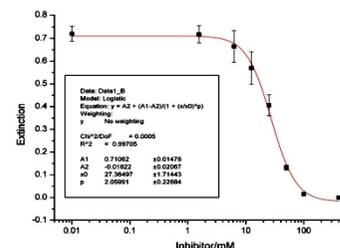
Conformational Analysis

- cyclodepsipeptides can serve as rigid templates
- peptidic part determines geometry
- Phosphorylation controls conformation of the peptidic part



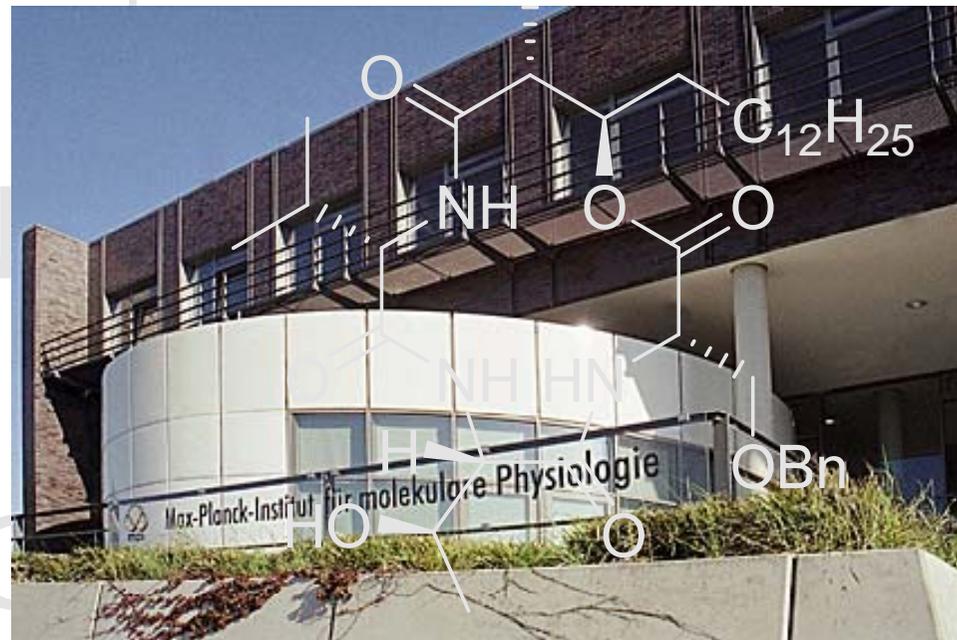
Biological Investigations

- Phosphorylation is essential for VHR inhibition
- unphosphorylated analogs lead to inhibition of ERK dephosphorylation
- weak antibacterial activity



Acknowledgement

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- MPG



PTP & DSP Mechanism

