Chemical Biology of the GPCR's

Assessed through split mix libraries

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Grith Hagel

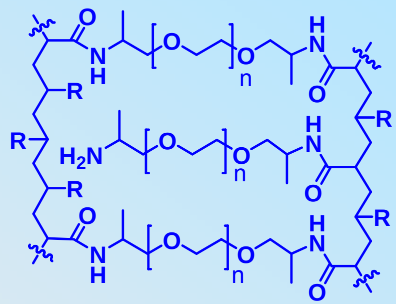
Integrated Solid Phase Combinatorial Chemistry

Design of **solid phase** compound library

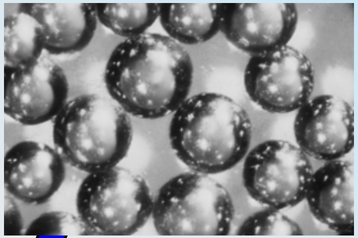
Selection criteria on **solid phase**

Compound analysis on **solid phase**

Integrated Solid Phase Combinatorial Chemistry



PEGA



Resins for SPOCC and molecular recognition

Receptors and assays

Catalysts and assays

Biomolecules and assays

Cellular solid phase assays

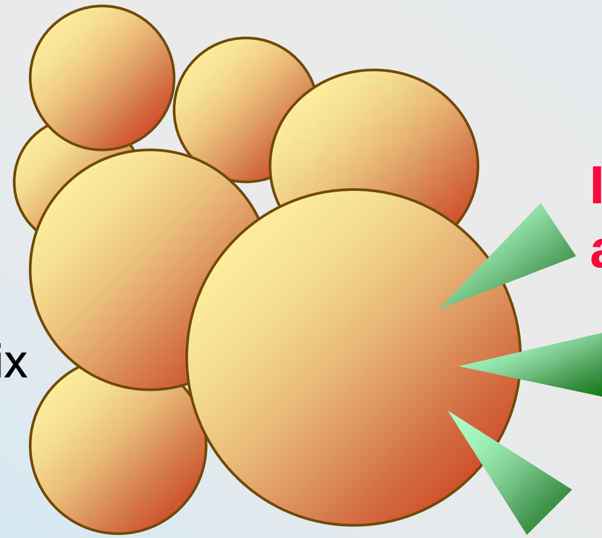
The "One Bead SOME Compounds" Solid Phase Assay

Morten Meldal et al.
Carlsberg Laboratory

A very general assay format:
"One Bead Some Compounds"

PEG-Based resin

Split-Mix
Library

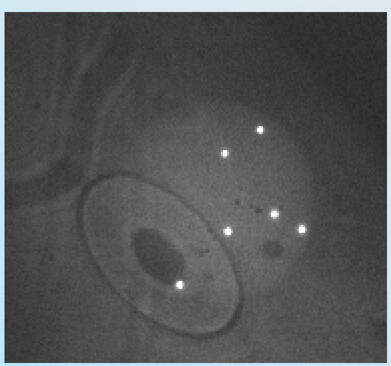


Indicator, property modifier
auxhiliary molecule

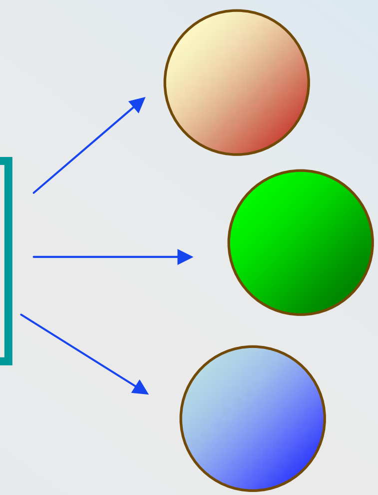
Identity tag

Reactive library component

Assay container
~0.1 µL/bead

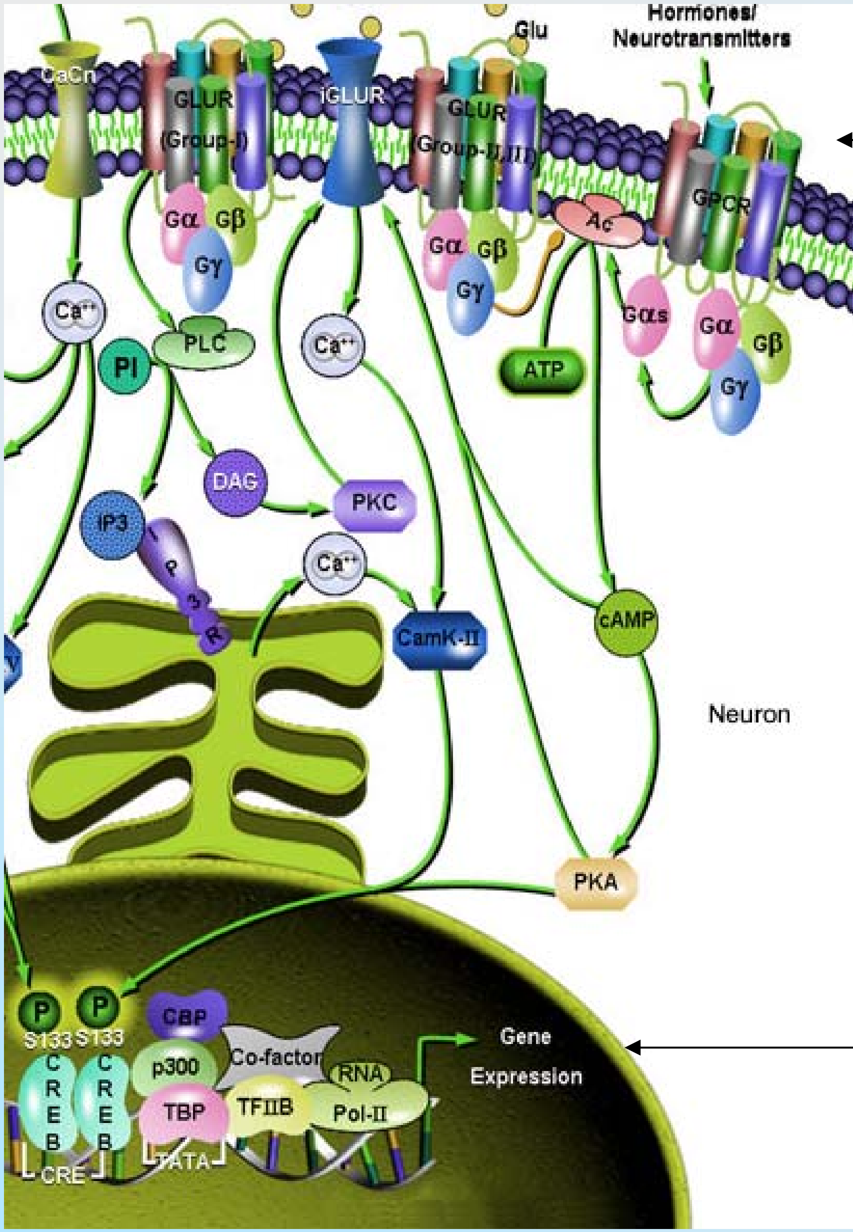


Enzyme reaction
Chemical reaction
Cellular interaction?



Morten Meldal et al.
Carlsberg Laboratory

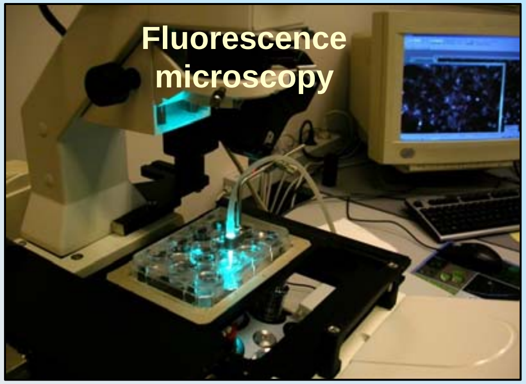
Casette for Expression of GPCR + Reporter

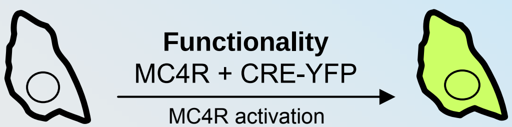


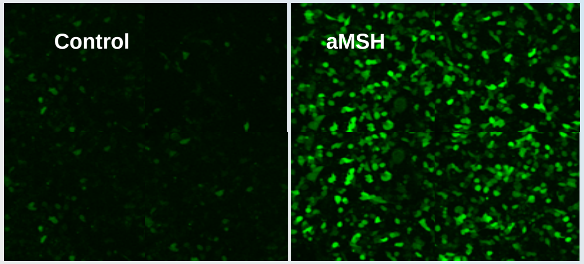
Receptor

Single vector:
Stable Expression
HEK-293 Celline

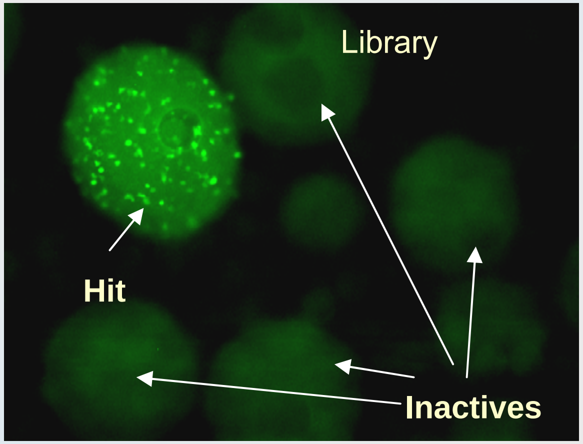
YFP
or
DS-Red





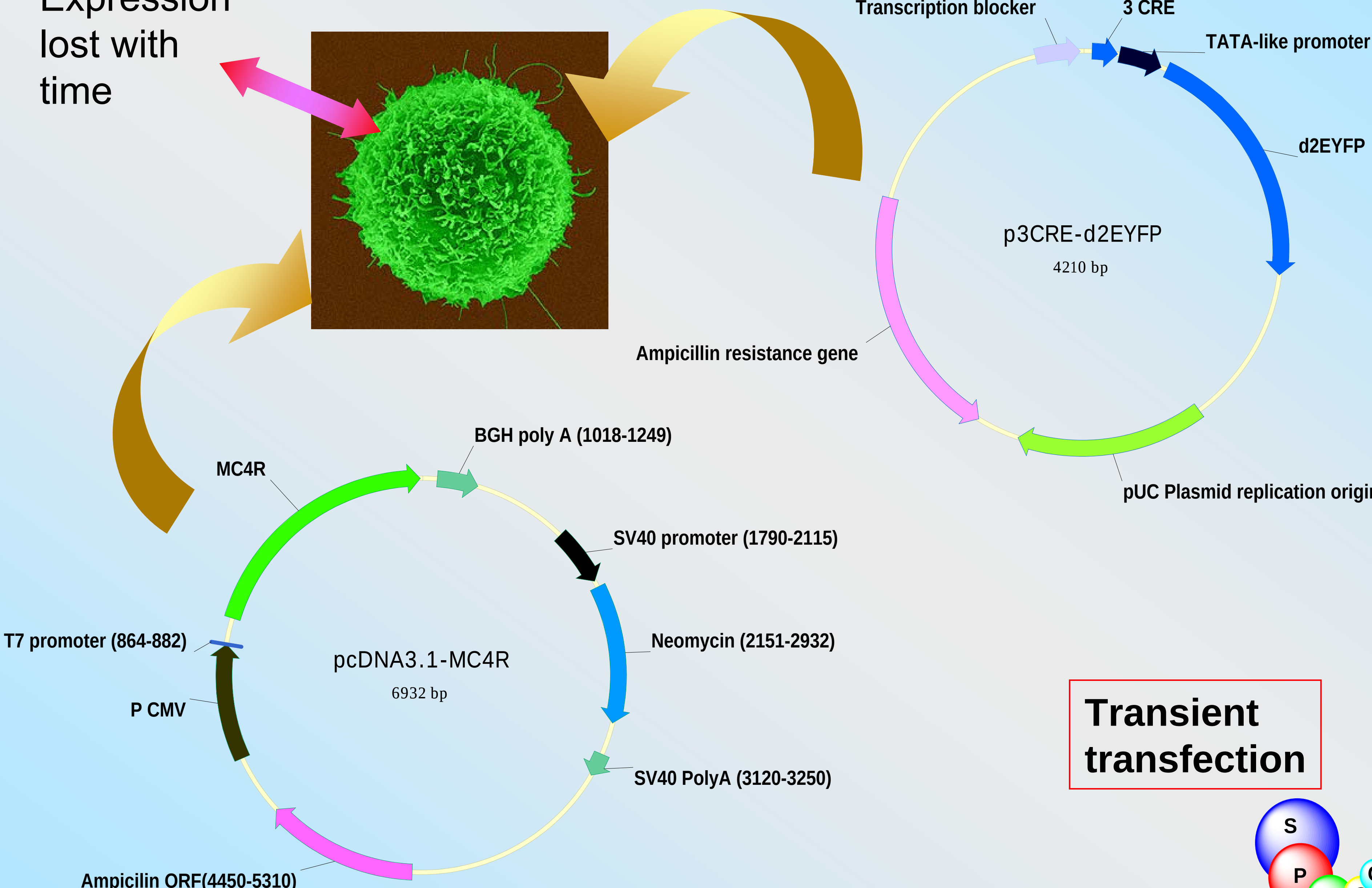


Primary screen



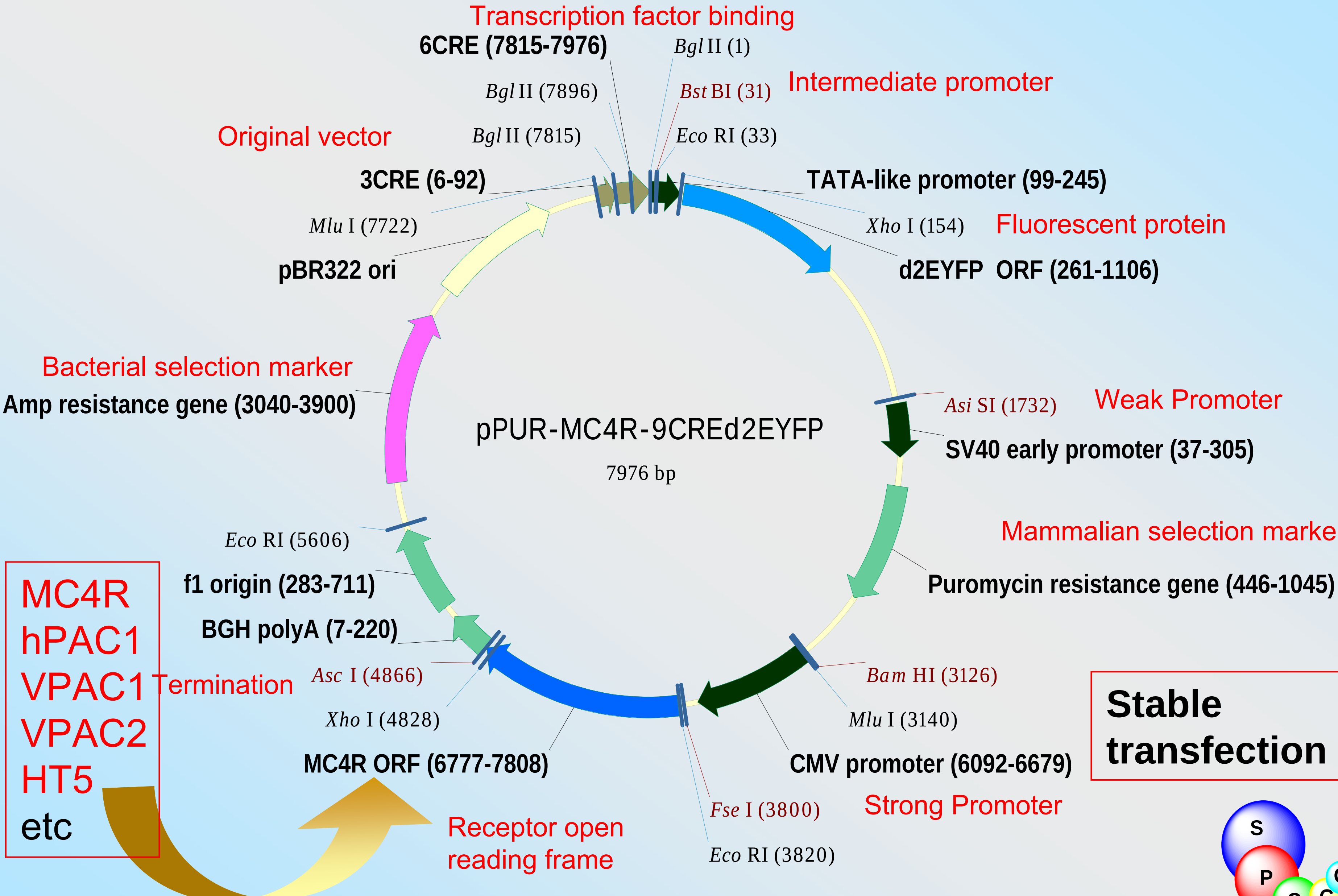
Establishing stable cellular functional - and specificity assays

Expression lost with time



Transient transfection

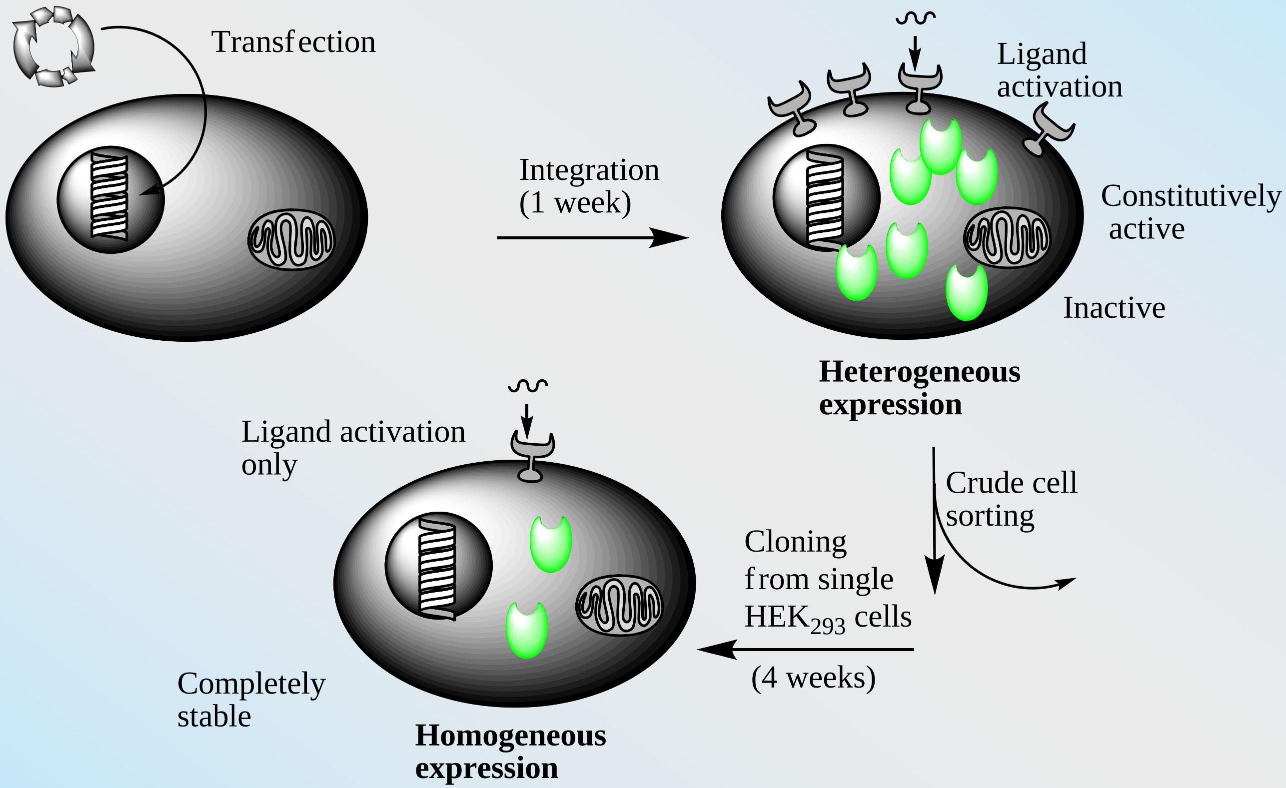
PEGA-Cell adhesion peptide: negative control

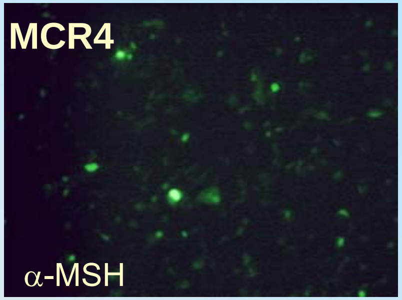


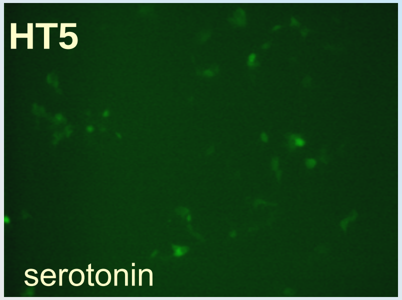
MC4R
hPAC1
VPAC1
VPAC2
HT5
etc

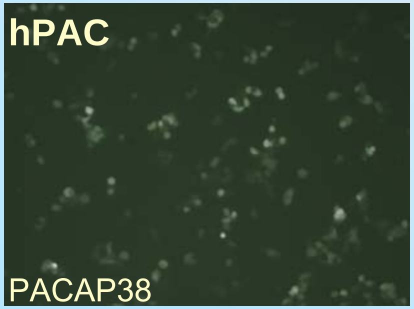
Stable transfection

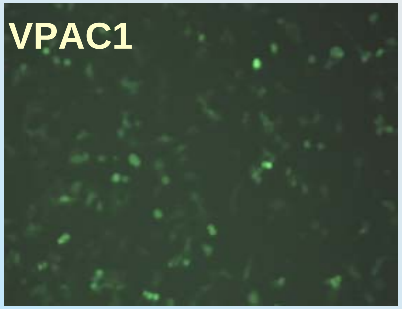
The single vector construct and cloning of PAC1, VPAC1 and VPAC2

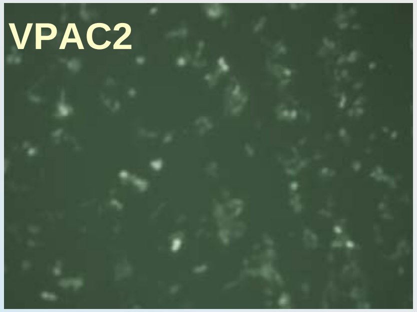










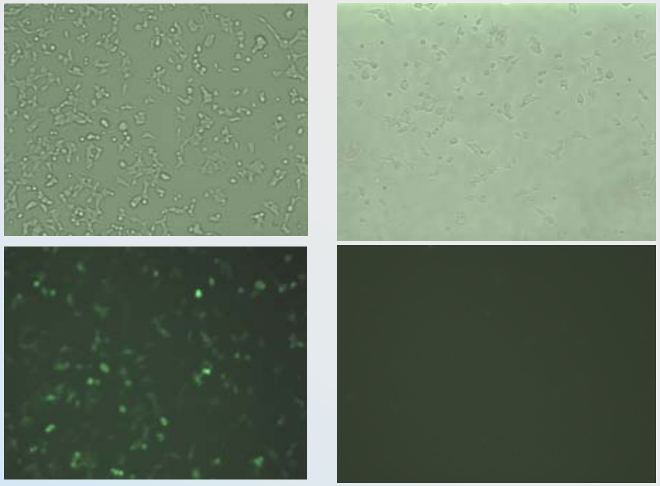


Cloning is essential

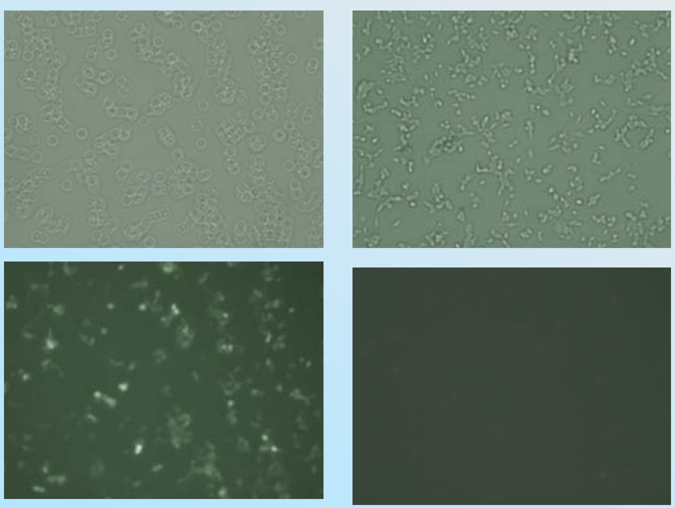
The single vector and cloning of hPAC, VPAC1 and 2

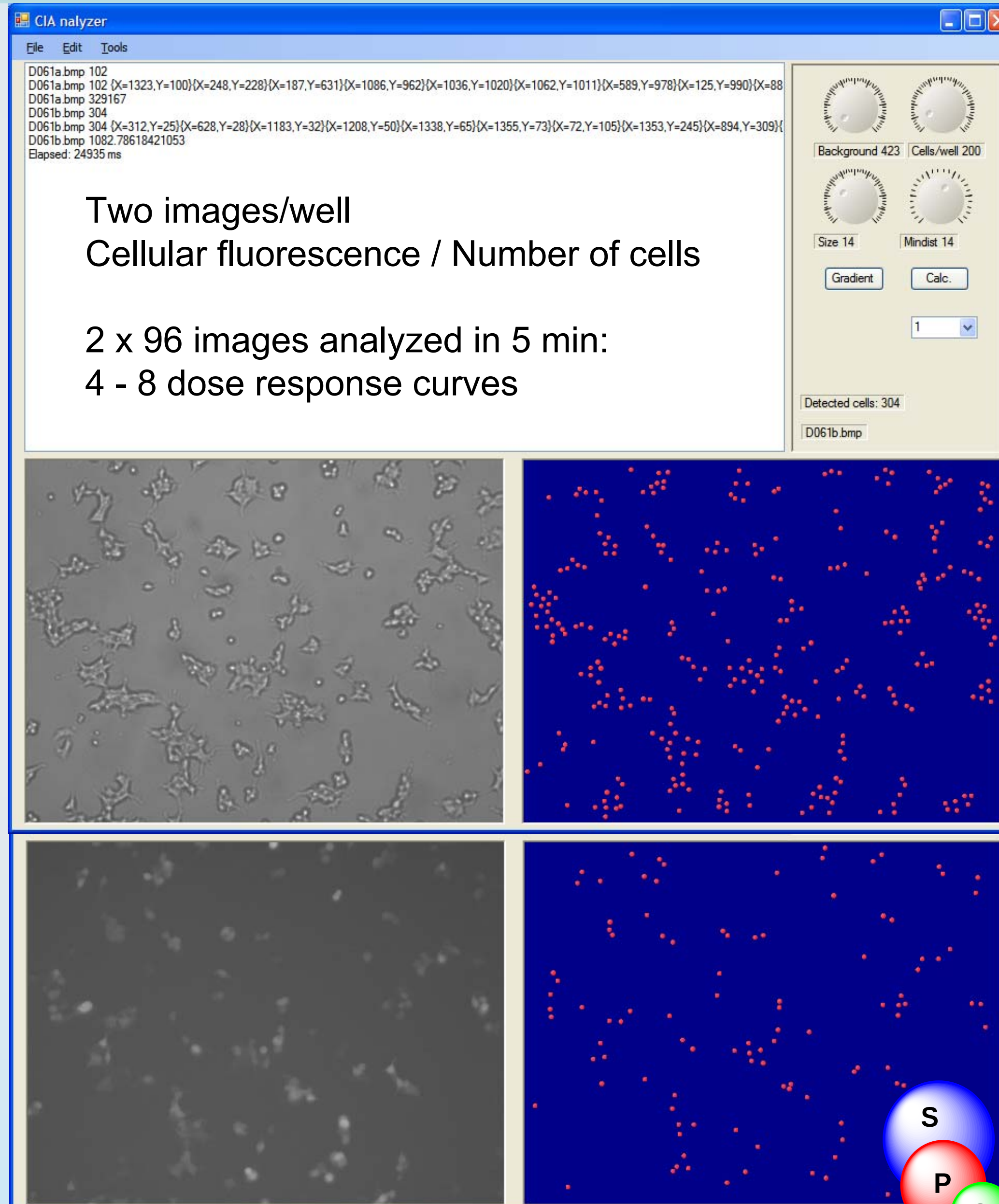
Cloned

VPAC1 / Pacap38 VPAC1 / control



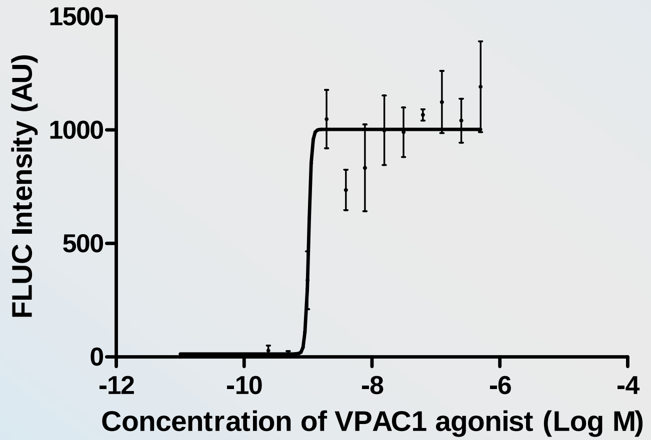
VPAC2 / Pacap38 VPAC2 / control



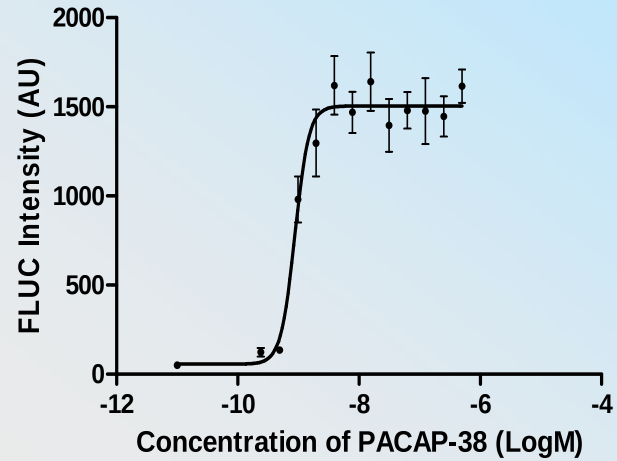


VPAC1 and 2 cloned. PACAP38 agonist assay

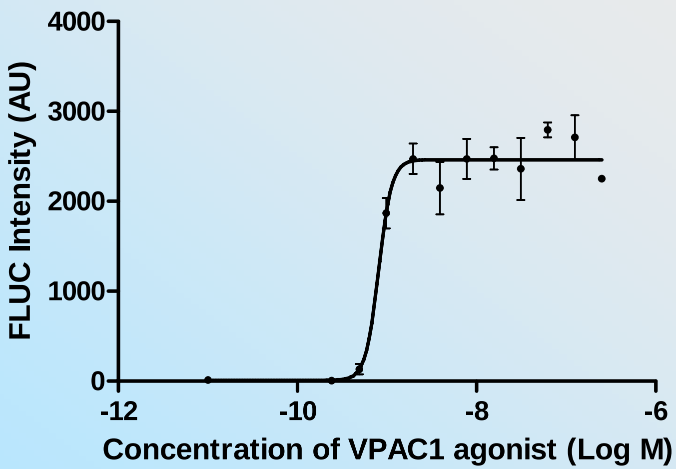
VPAC1 agonist assay on VPAC1 receptor (day1)
EC50=1.0 nM



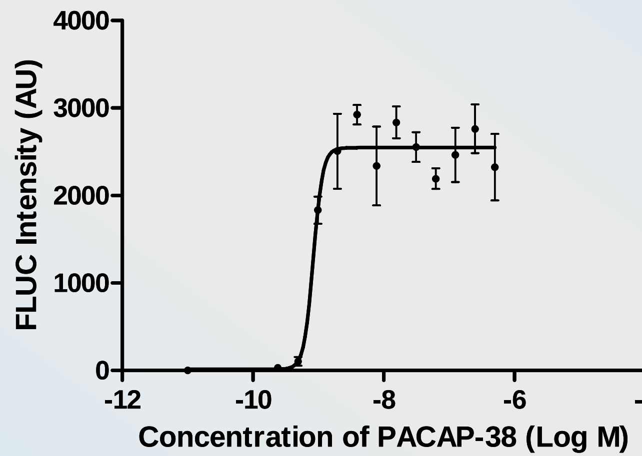
PACAP-38 assay on VPAC2 receptor (day 1)
EC50=0.88 nM



VPAC1 agonist assay on VPAC receptor (day 2)
EC50=0.81 nM

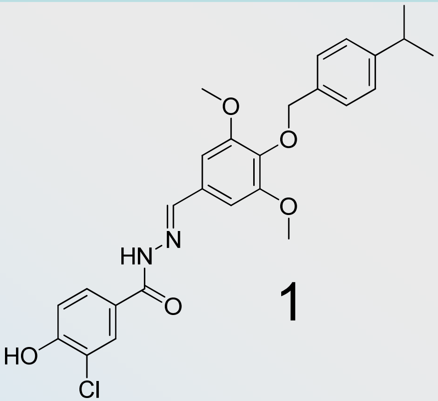


PACAP-38 assay on VPAC1 receptor (day 2)
EC50=0.84 nM

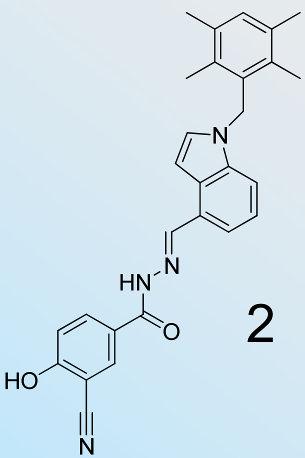


PACAP: Pituitary adenylate cyclase-activating peptide

VPAC1 and 2 cloned. Antagonist assay, 20 nM PACAP38, 24 h



1



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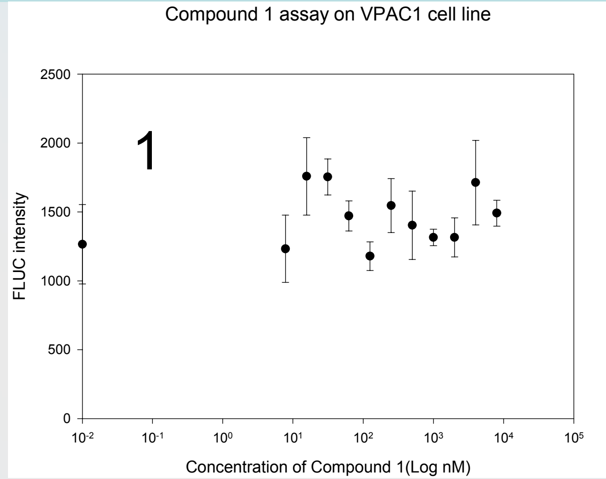
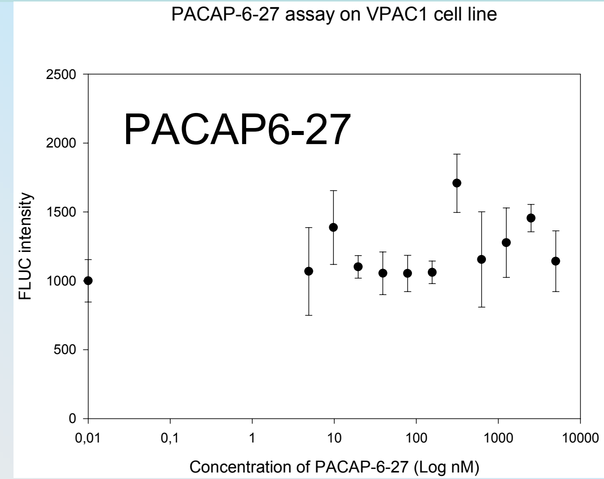
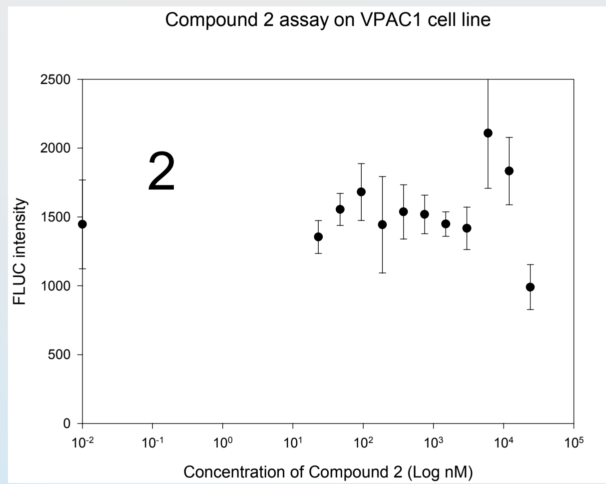
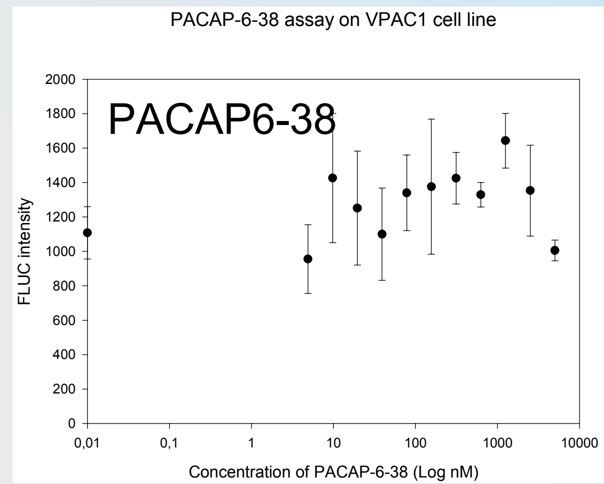








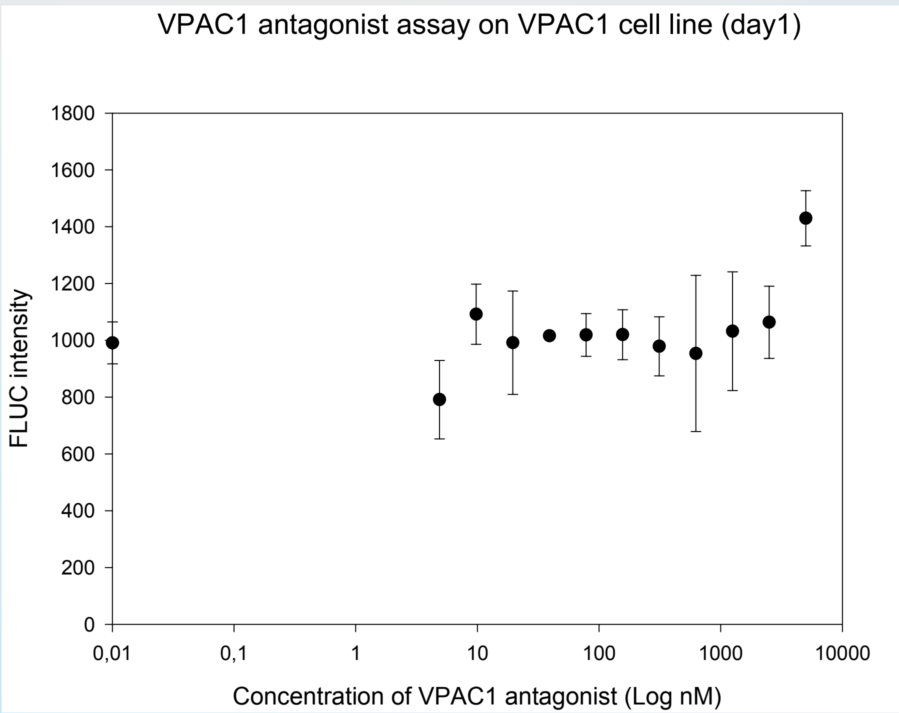
↓6 ... 27↓

PACAP38: HSGDIFTDSYSRYRKQMAVKKYLAAVLGKRYKQRVKNK-NH_2

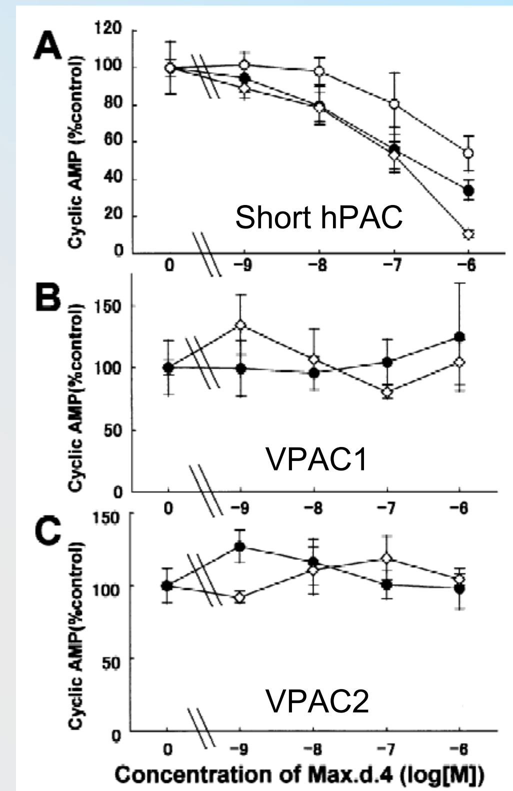
VIP: HSDAVFTDNYTRLRKQMAVKKYLNSILN-NH_2

VPAC1Ag HSDAVFTNSYRKVLKRLSARKLLQDIL-NH_2

VPAC1 and 2 cloned. Antagonist assay, 20 nM PACAP38, 16 h



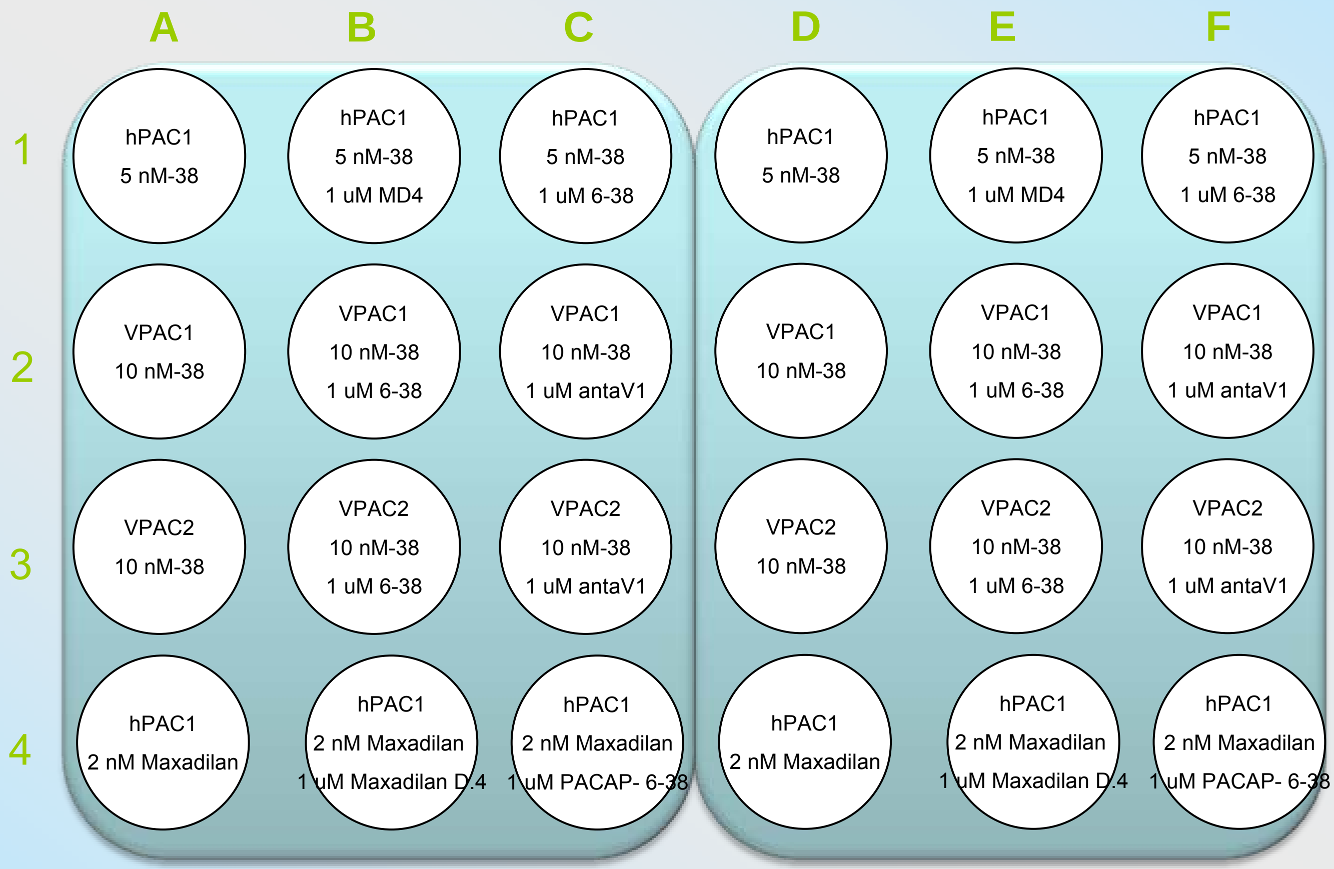
VPAC1Ag HSDAVFTNSYRKVLKRLSARKLLQDIL-NH_2
VPAC1Ant



CHO cells
I. Tatsuno et al. Brain Research 889 (2001) 138-148

The single vector construct with with and without antagonists

PACAP-38 stimulation of Hek293 cells. Antagonist addtion before or after agonist addition.



Maxadilan a vascodilatory peptide from sandfly saliva

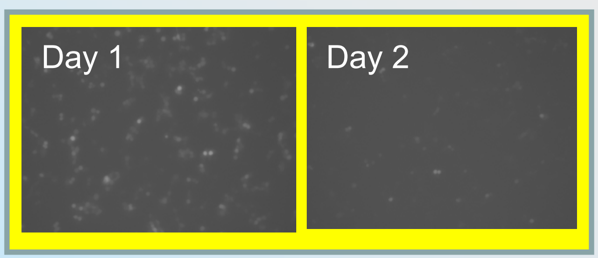
Maxadilan: CDATCQFRKAIDDCQKQAHHSNVLQTSVQTTATFTSMDTSQLPGNSVFKECMKQKKKEFKA

Maxadilan D4: CDATCQFRKAIDDCQKQAHHSNV-----------------------------------PGNSVFKECMKQKKKEFKAGK

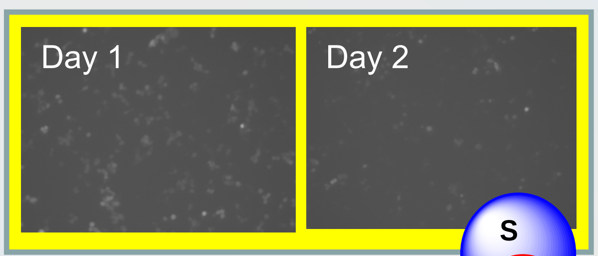
The cell’s-on-bead assay with single vector construct

Receptor	Agonist	Conc nM	Antagonist	Conc nM	Activity	Activity
					24 h	48 h
hPAC1	Pacap38	5			9	5
	Pacap38	5	Maxadilan D4	1000	10	4
	Pacap38	5	Pacap6-38	1000	10	5
hPAC1	Maxadilan	2			10	6
	Maxadilan	2	Maxadilan D4	1000	10	2
	Maxadilan	2	Pacap6-38	1000	9	3
VPAC1	Pacap38	10			10	5
	Pacap38	10	Pacap6-38	1000	9	5
	Pacap38	10	VPAC1-antag.	1000	10	5
VPAC2	Pacap38	10			9	5
	Pacap38	10	Pacap6-38	1000	10	6
	Pacap38	10	VPAC1-antag.	1000	10	7

Maxadilan

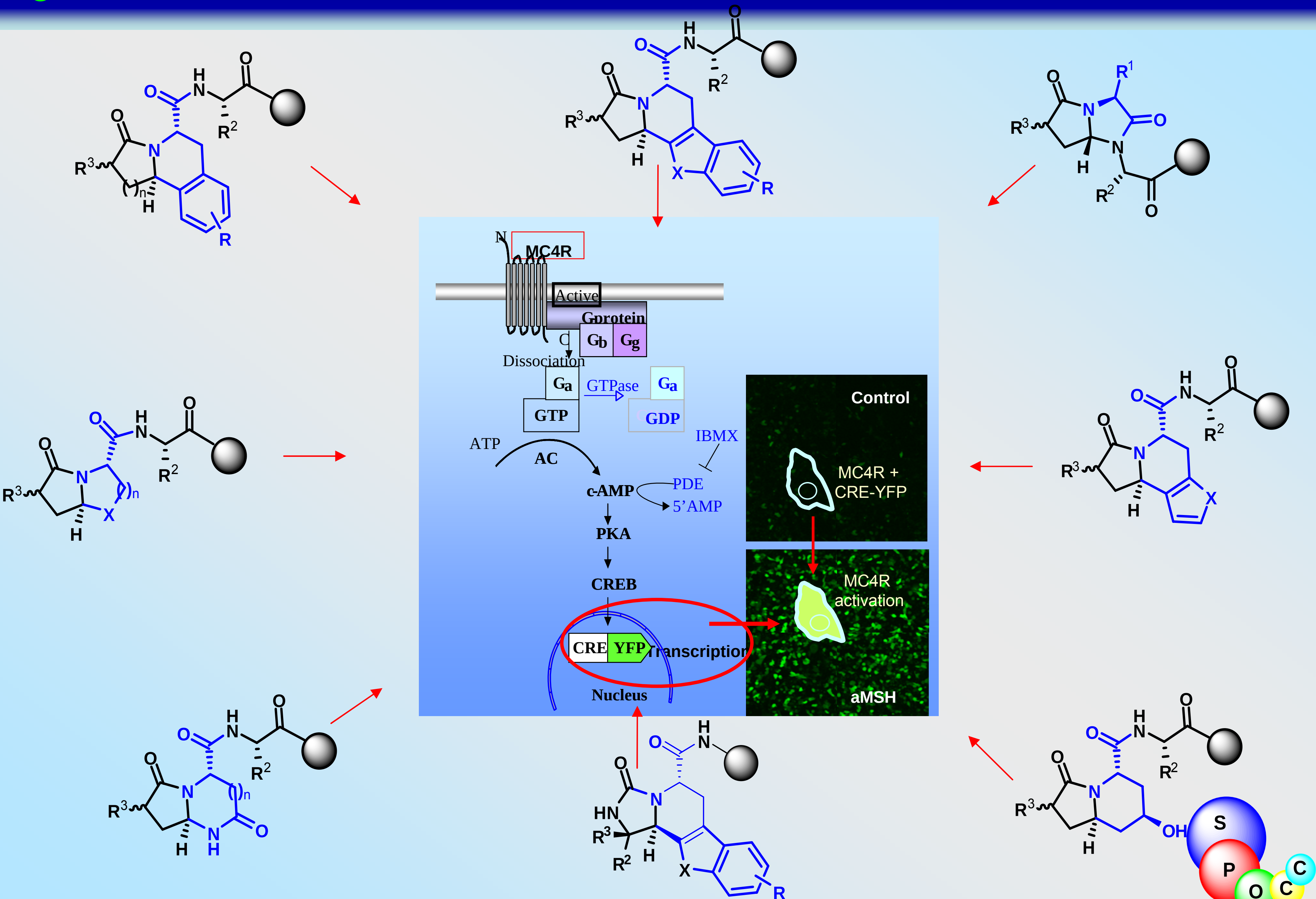


Maxadilan/ Maxadilan D4

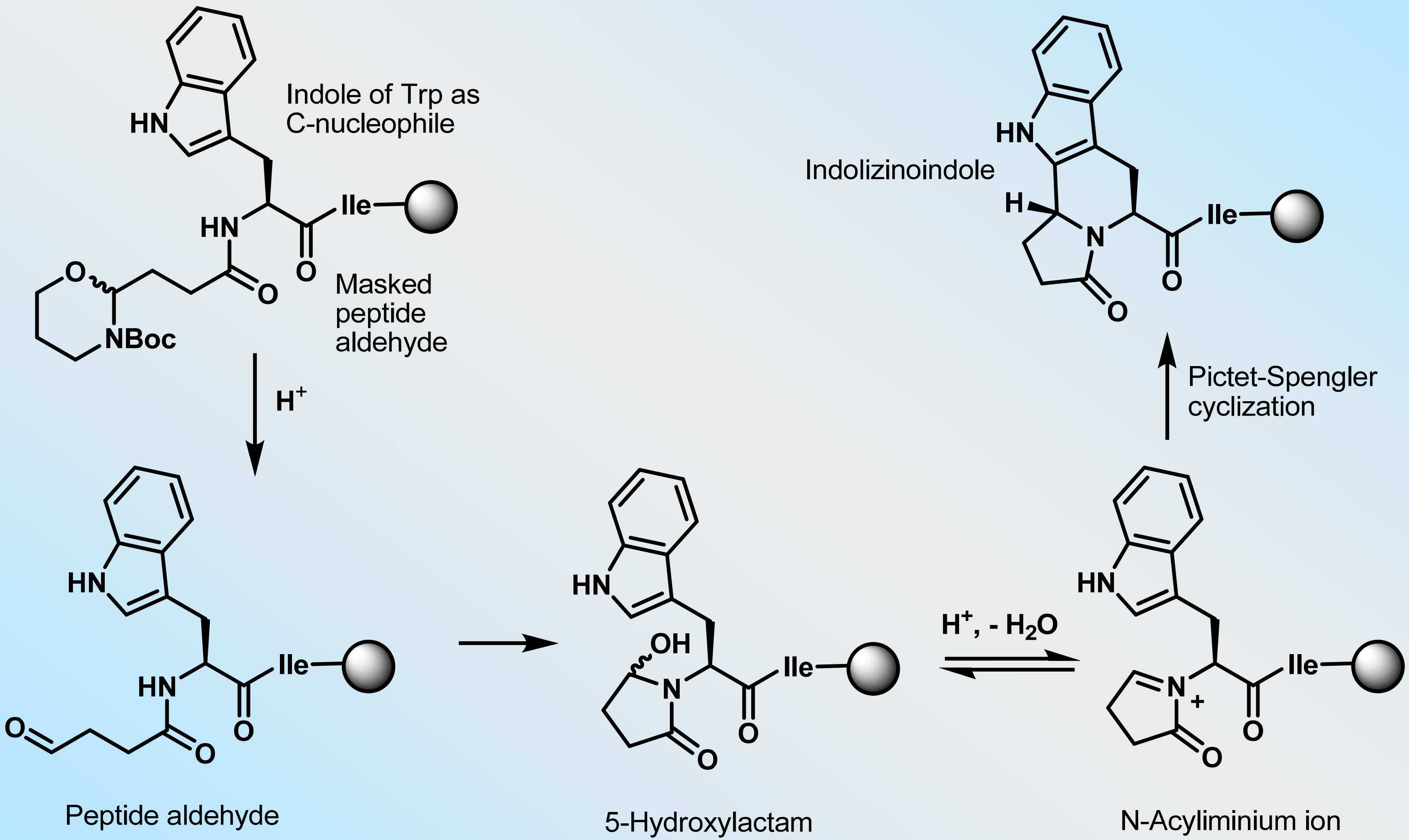


Maxadilan/ Pacap38

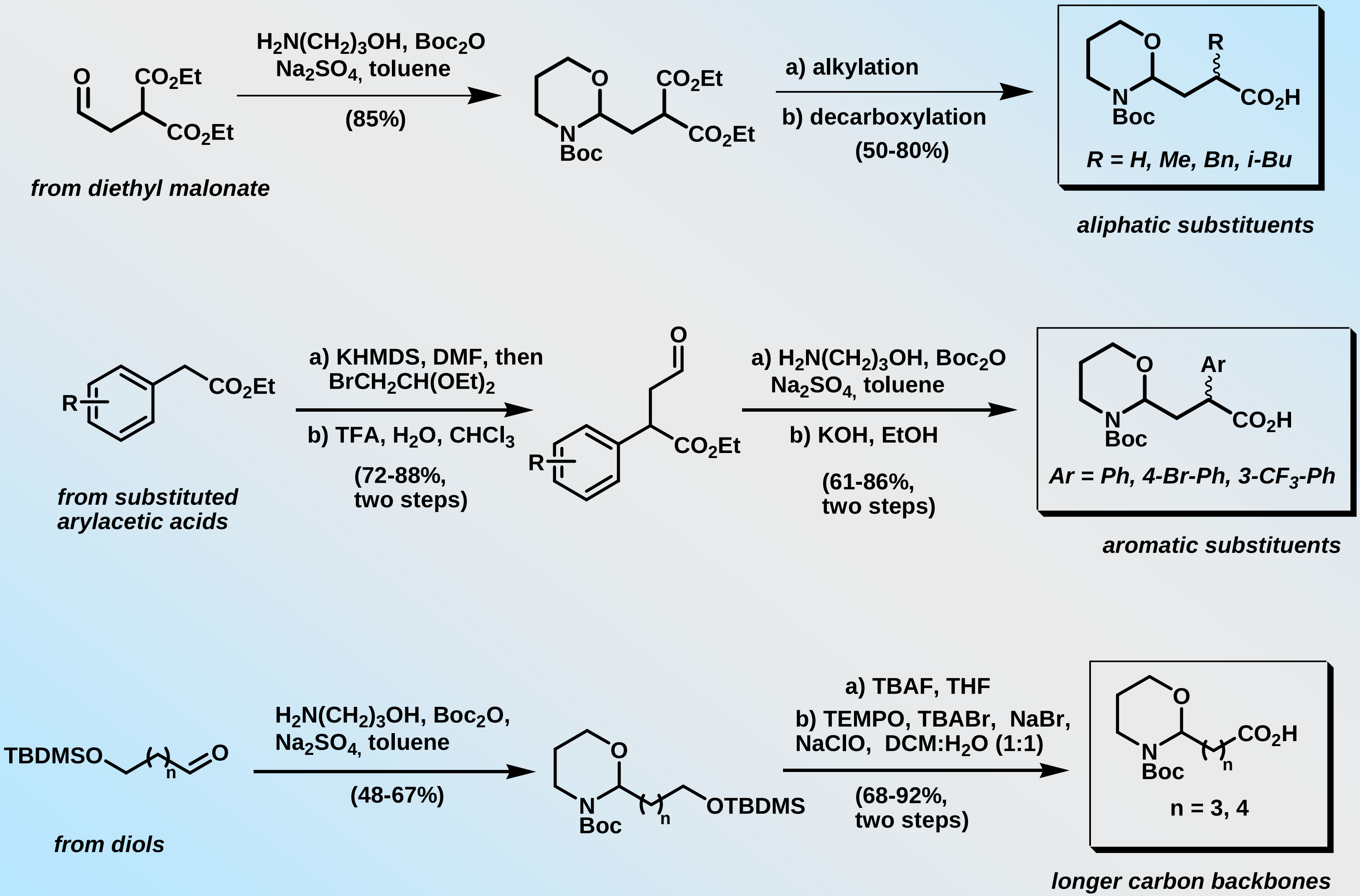
Scaffolds by N-acyliminium Cascade Chemistry



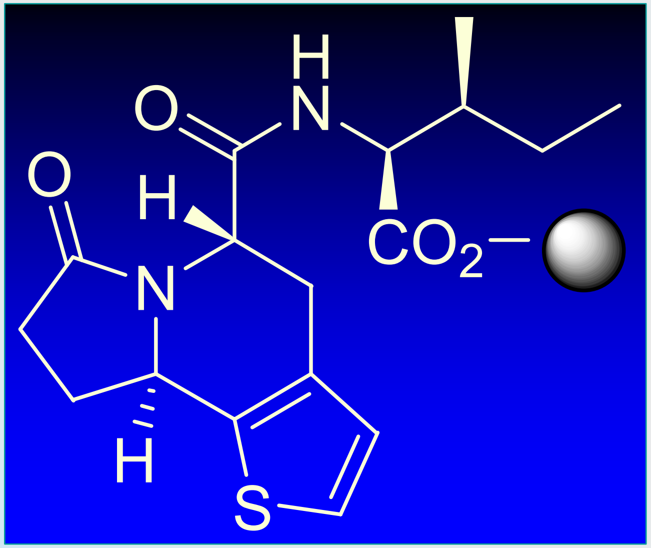
The Intramolecular *N*-Acyliminium "Pictet-Spengler" Reaction

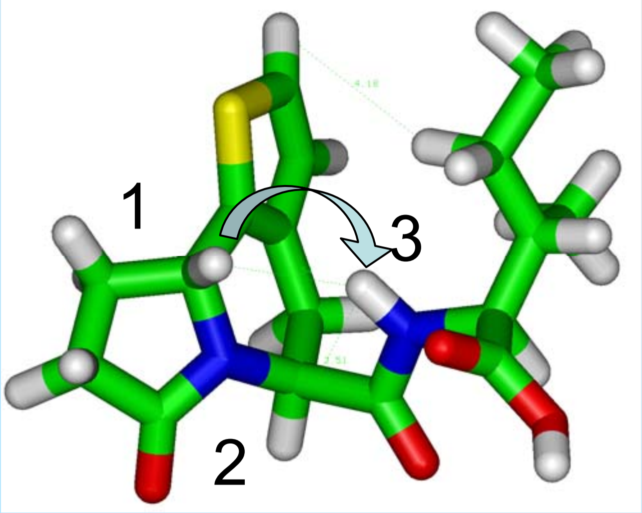


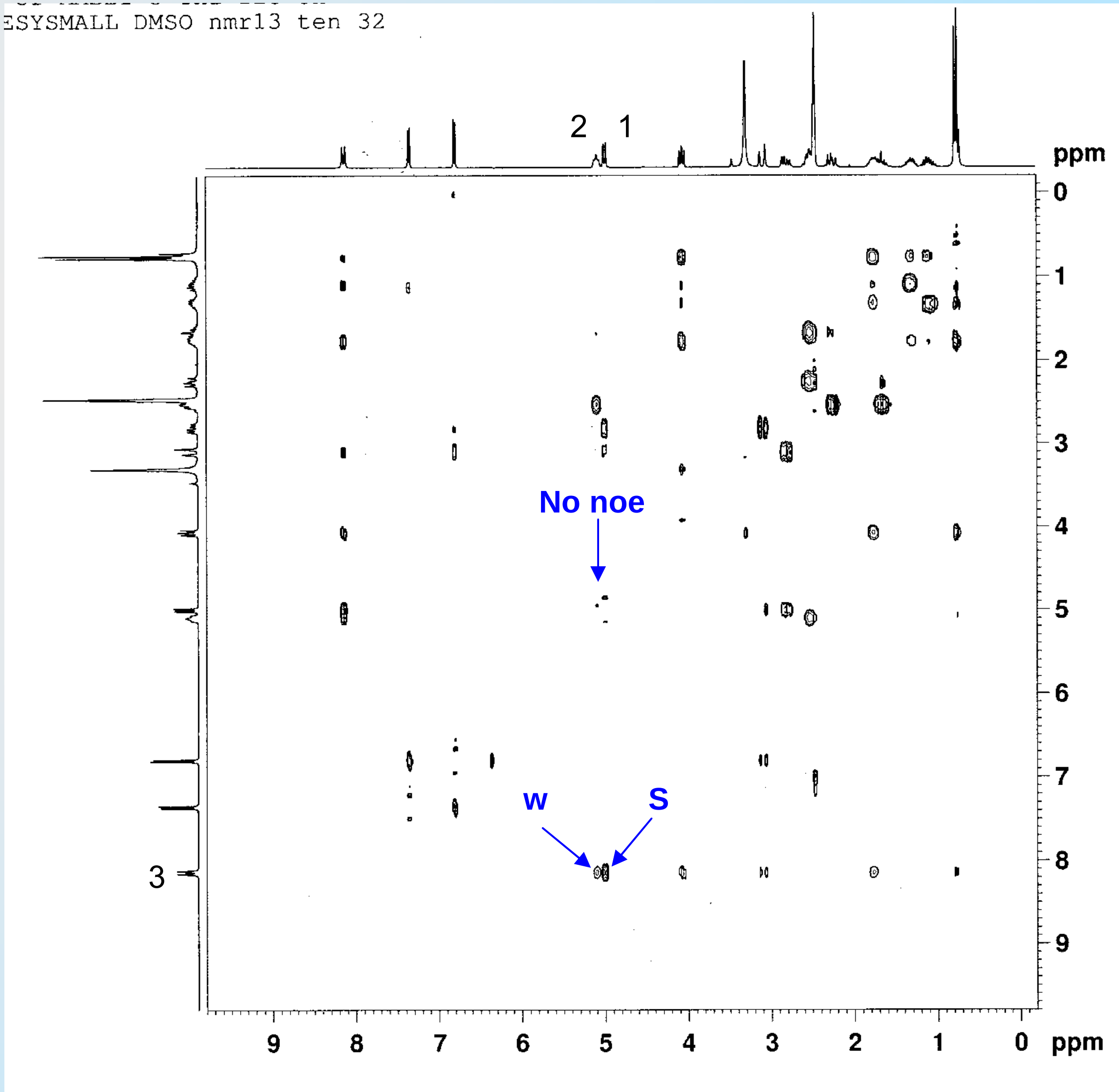
Scaffold diversity: Building Blocks



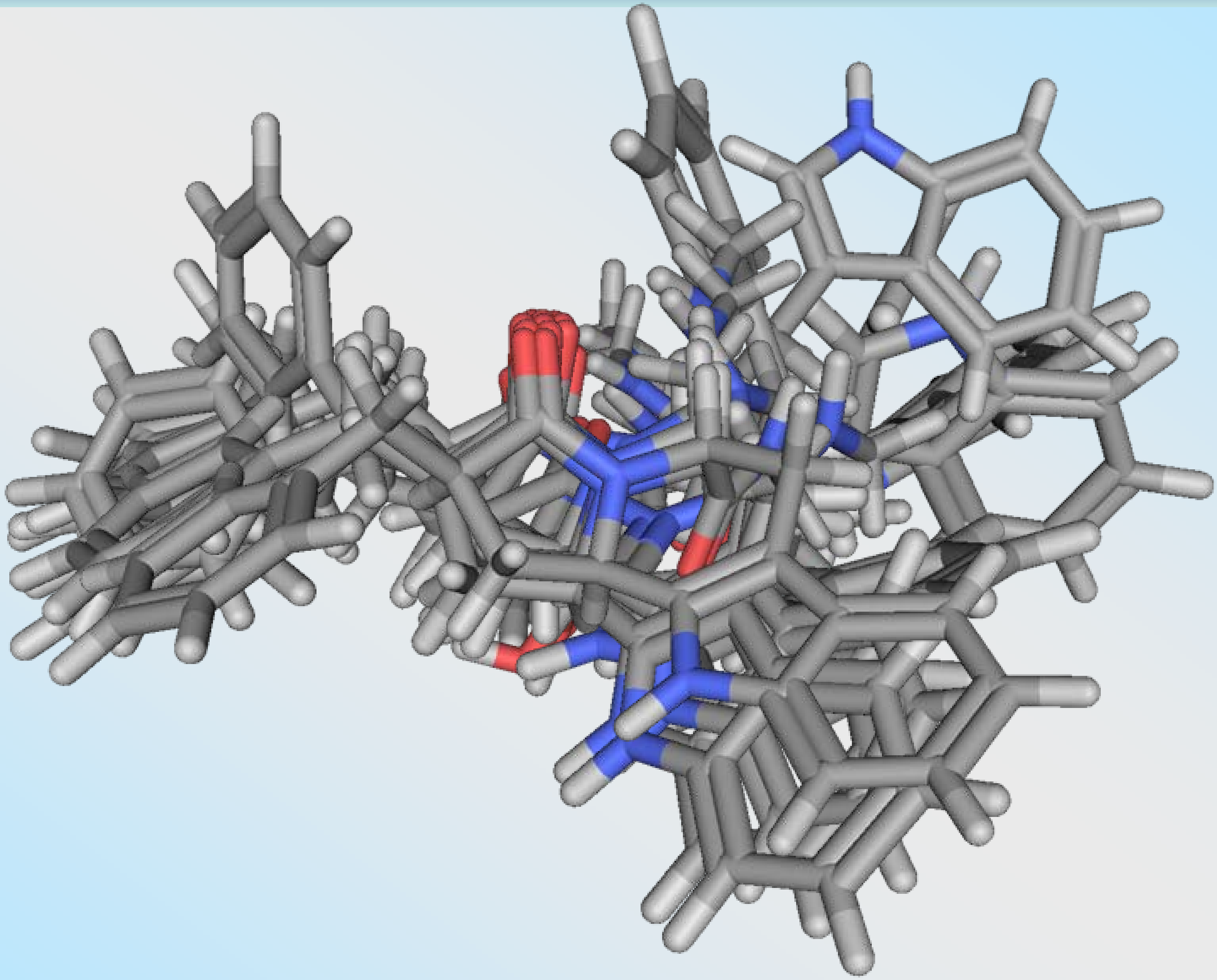
Stereo-selectivity of the intramolecular cascade reaction





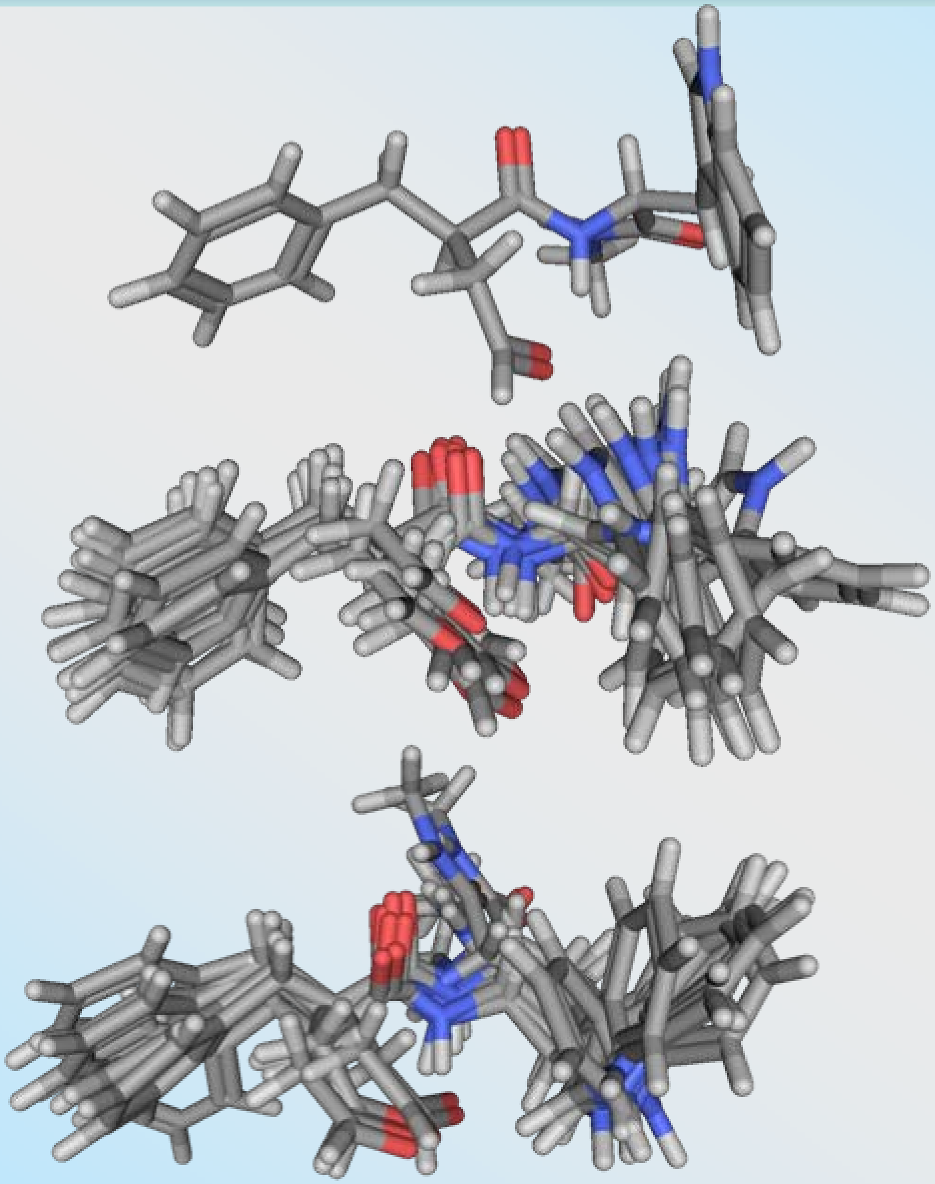


The Aldehyde/Amide Mediated Intramolecular "Click" Reaction



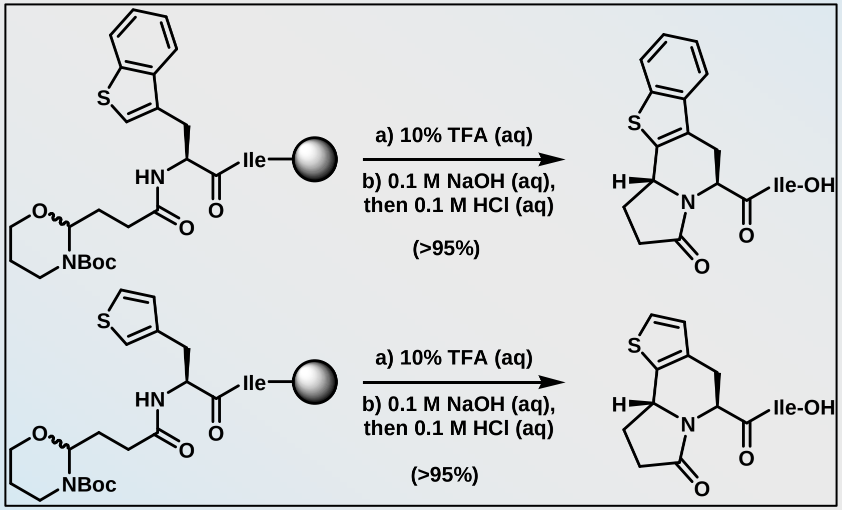
A new highly stereoselective cascade reaction

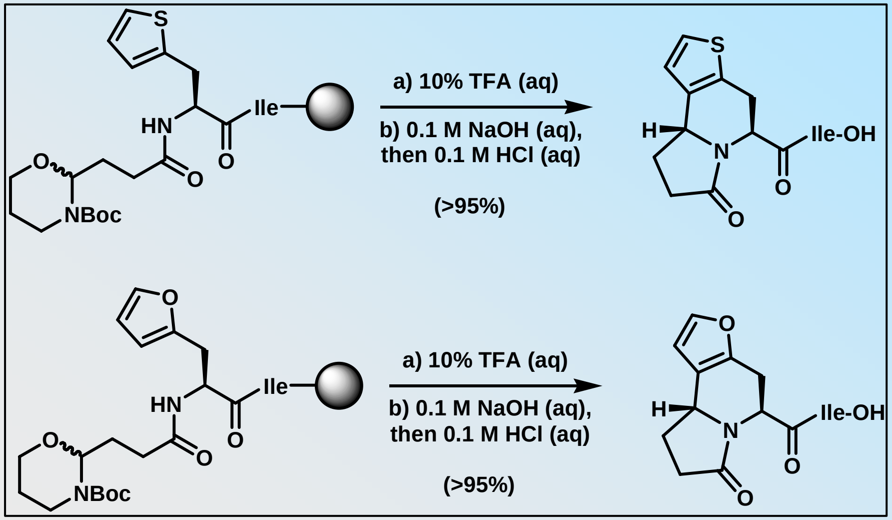
The Aldehyde/Amide Mediated Intramolecular Meldal Reaction



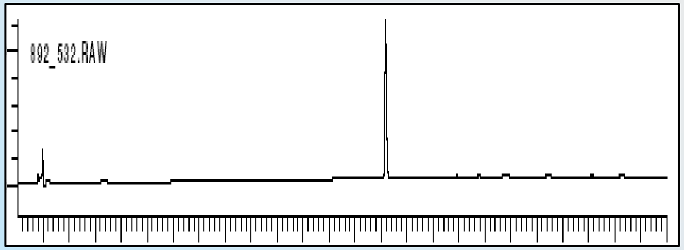
Free precursor
aldehyde
MD-simulation

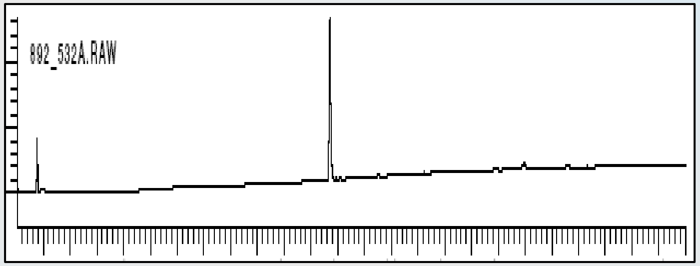
Thiophenes, Benzothiophene and Furane

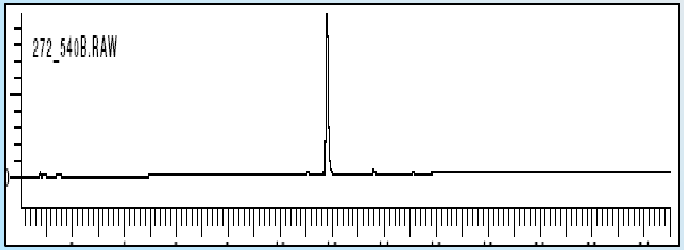


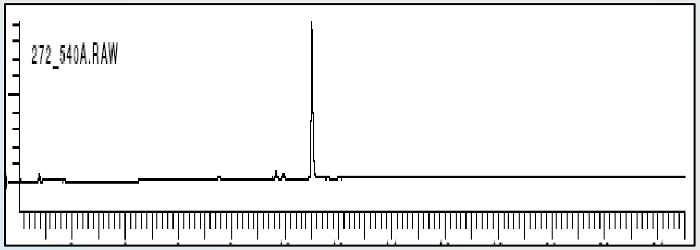


HPLC crude products

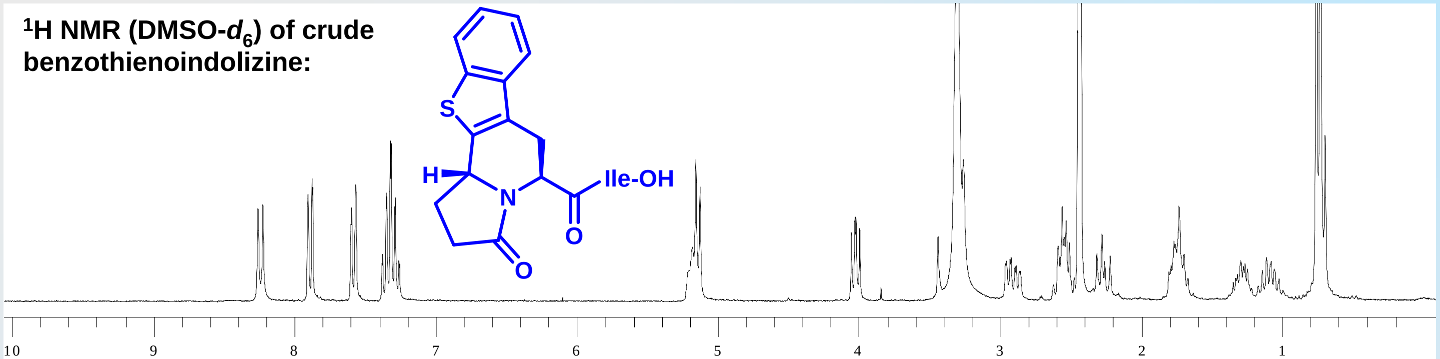


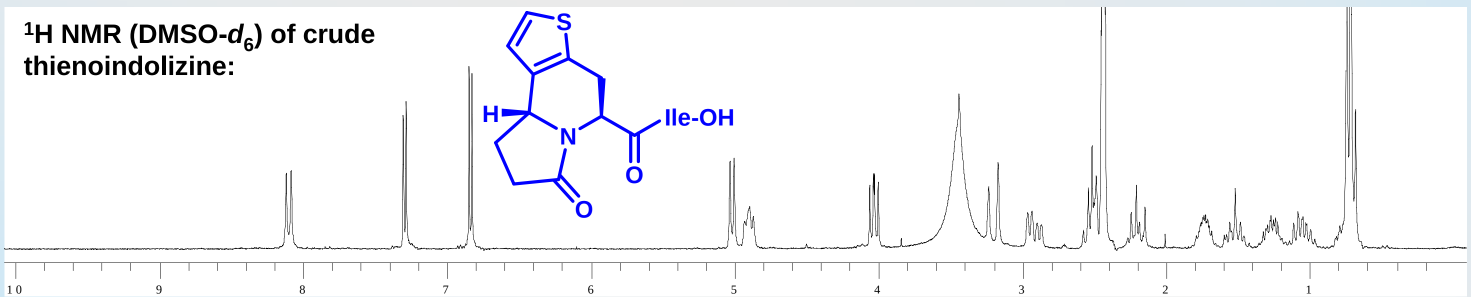


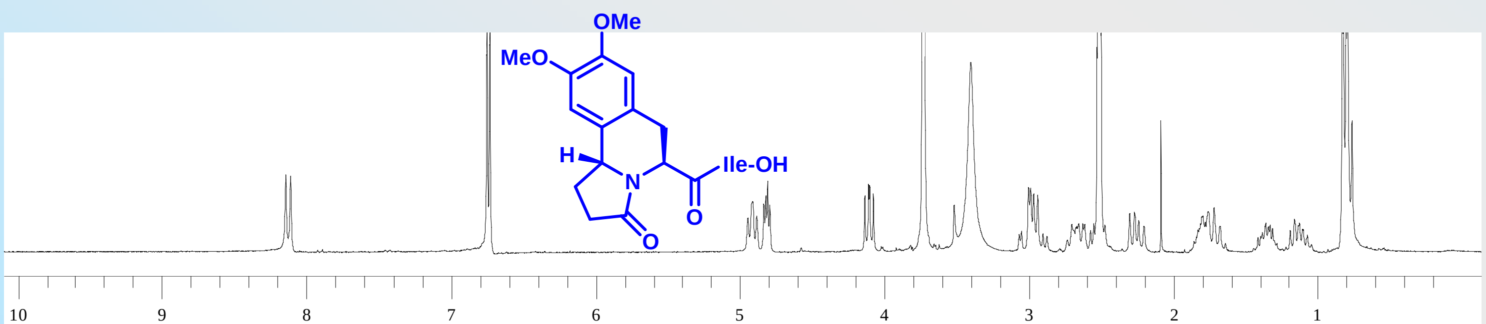




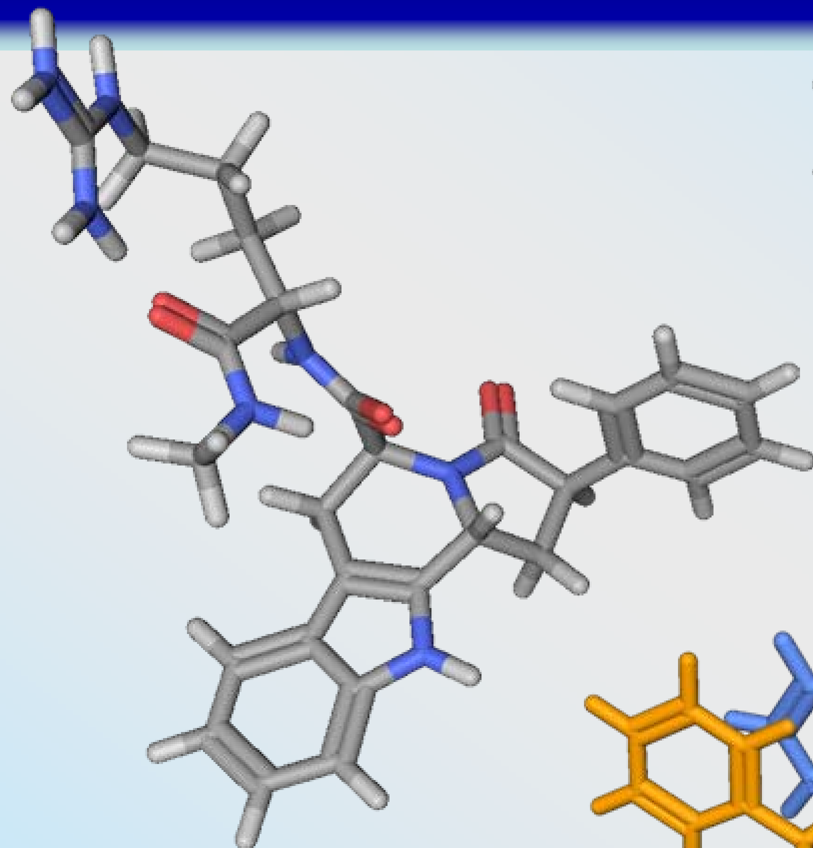
Quantitative Chemical Transformation: Intramolecular “Click”

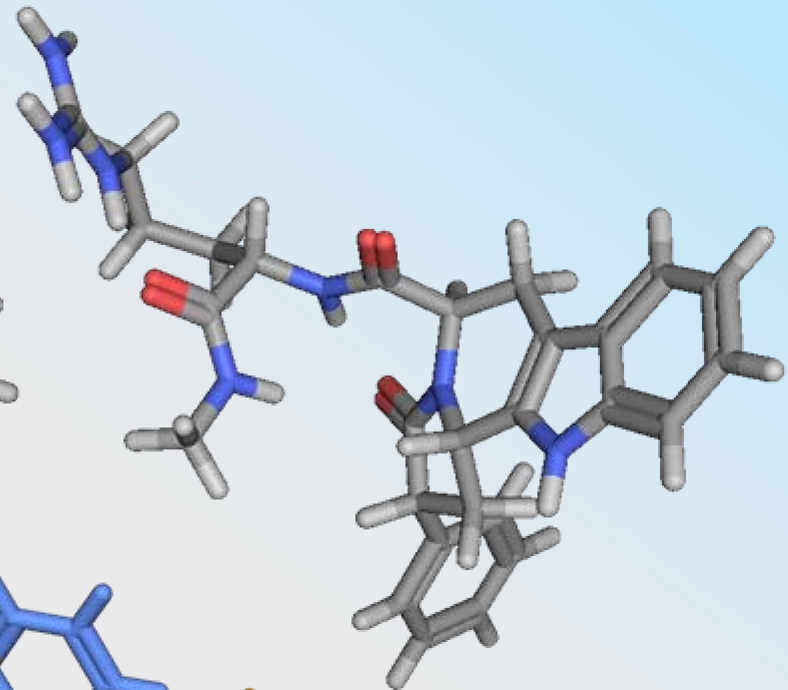




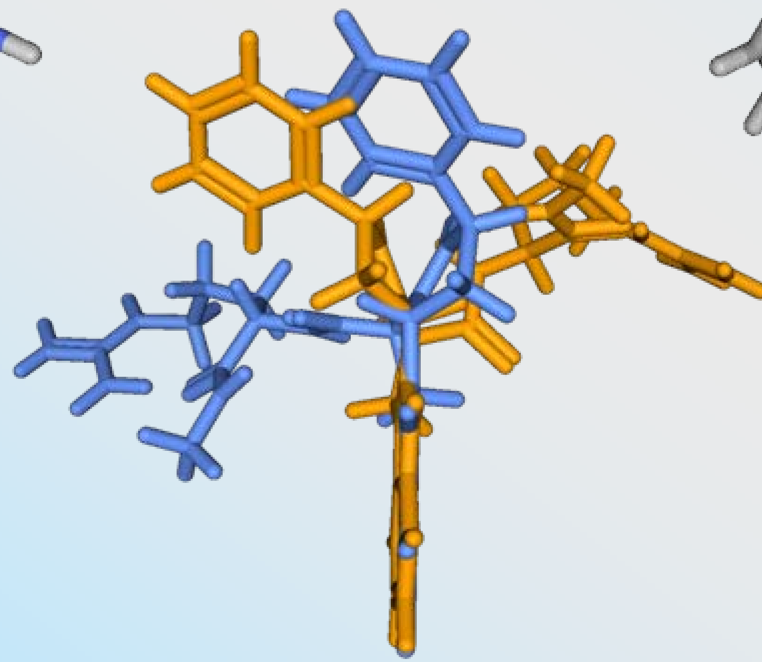


Changing from L to D-Trp



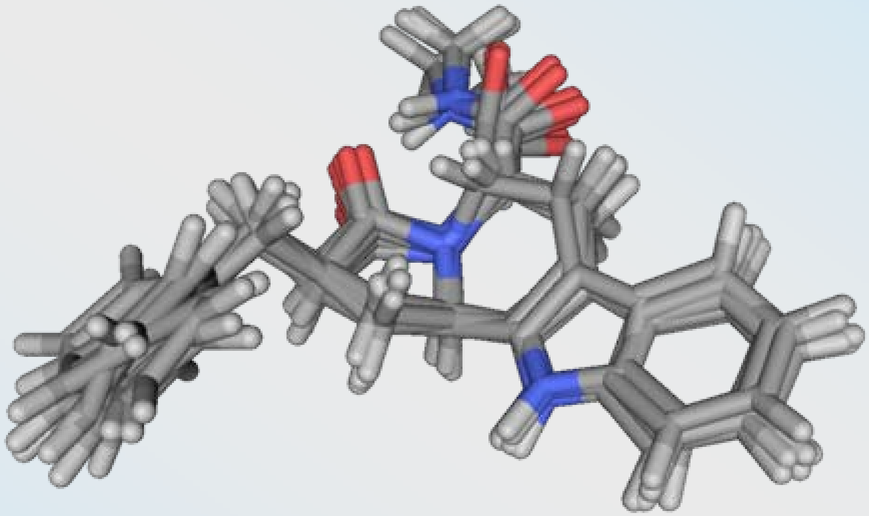


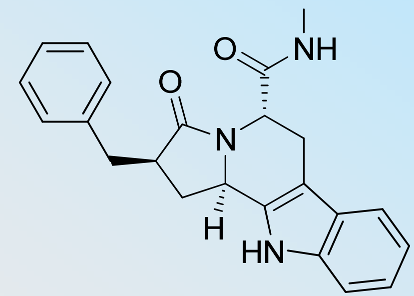
L-Trp (Blue)

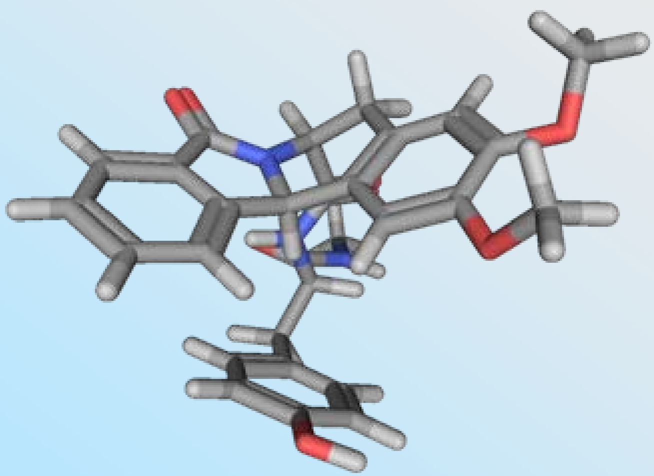


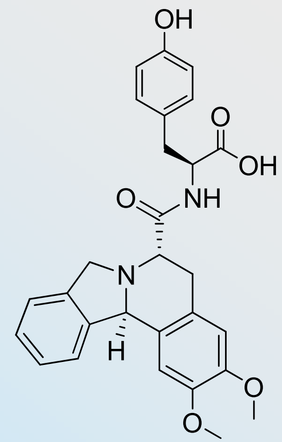
D-Trp (Orange)

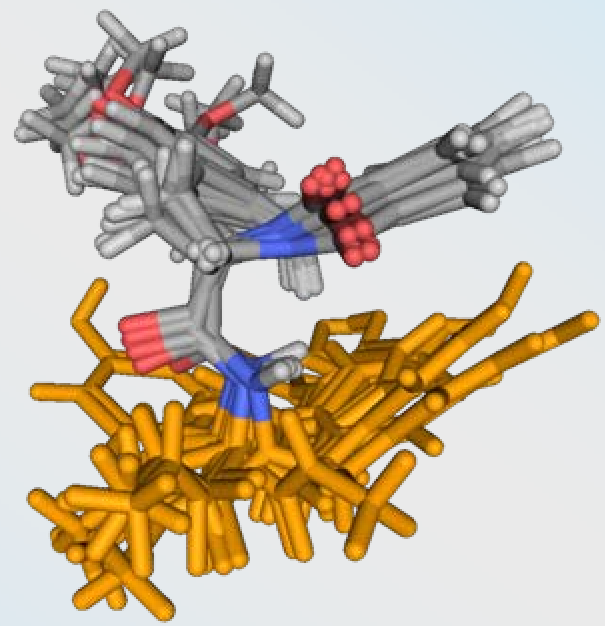
The aldehyde/amide ”click”-end-product is rigid



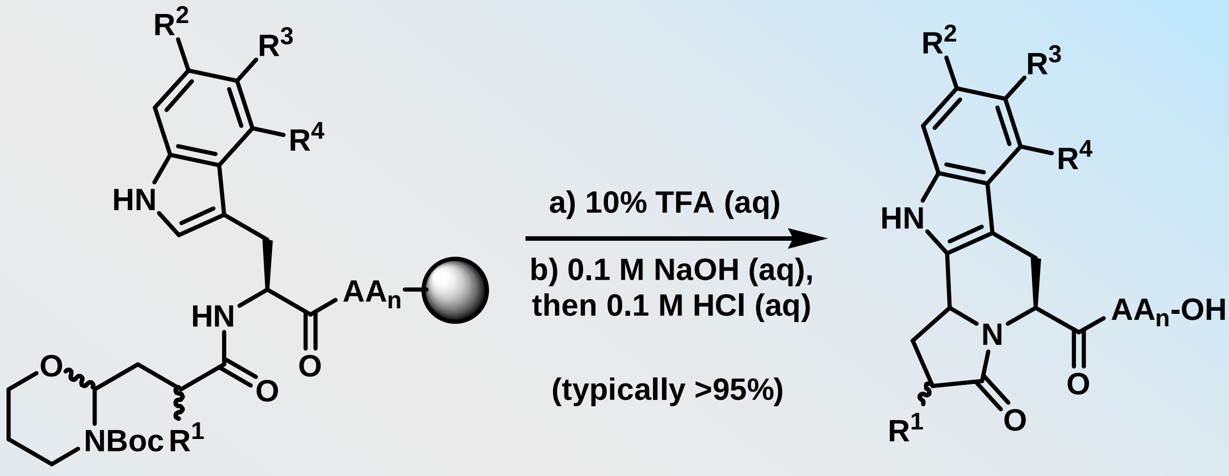


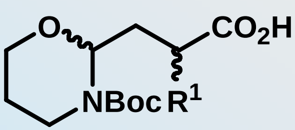




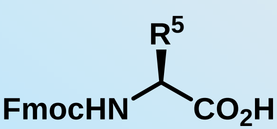


Indoles in the Intramolecular *N*-Acyliminium Pictet-Spengler Reaction



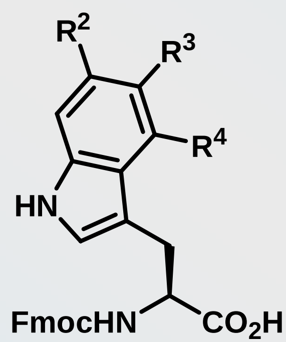


Substituted masked aldehyde building blocks



Fmoc-amino acids (commercially available)

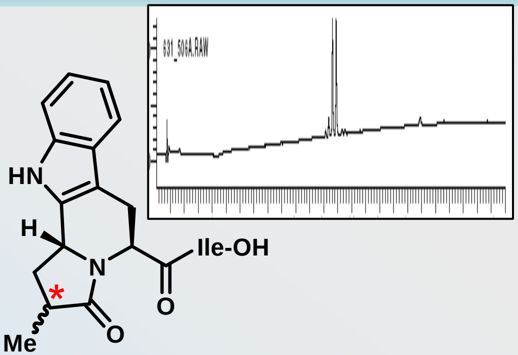
R^1 =
H,
Me,
HO-CH_2,
i-Bu,
Bn,
Ph,
4-Br-Ph,
3-CF_3-Ph

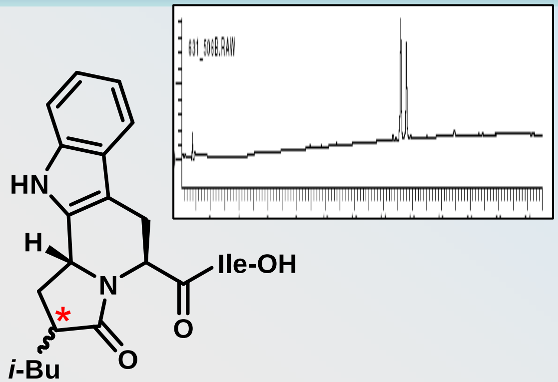


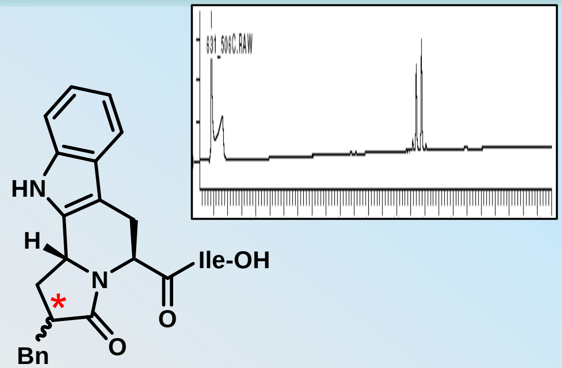
Substituted Fmoc-Trp-OH derivatives

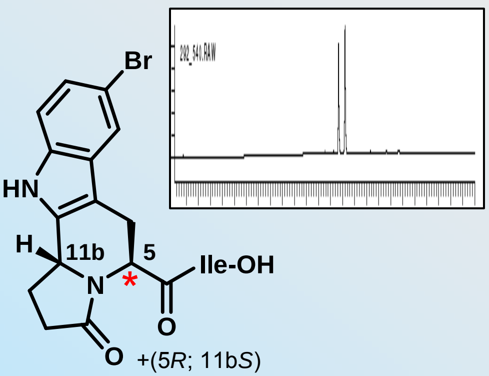
(R^2, R^3, R^4) =
(H, H, H),
(H, Br, H),
(F, H, H),
(H, F, H),
(H, H, Me),
(H, Me, H),
(Me, H, H),
(H, OH, H),
(H, MeO, H),
(H, BnO, H),

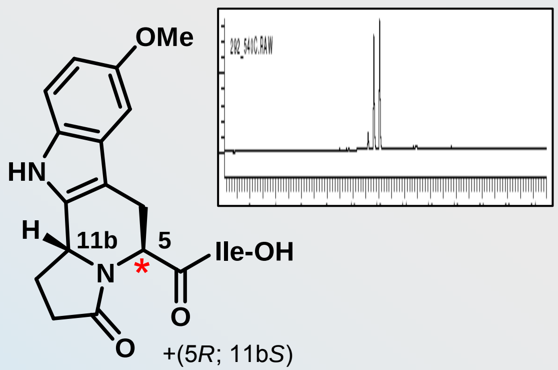
Scaffold diversity: The Intramolecular *N*-Acyliminium Pictet-Spengler Reaction

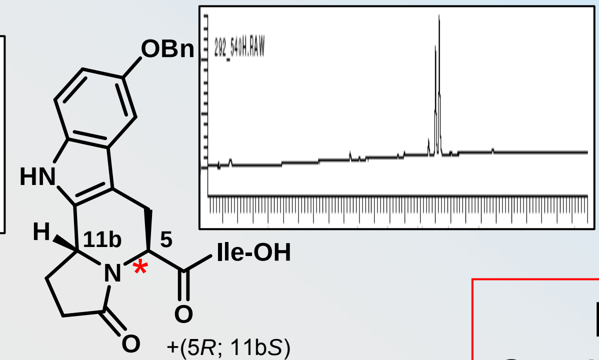




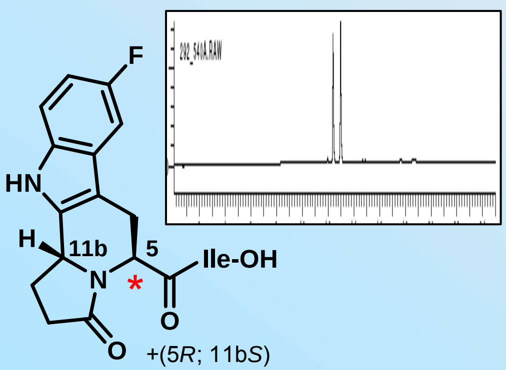


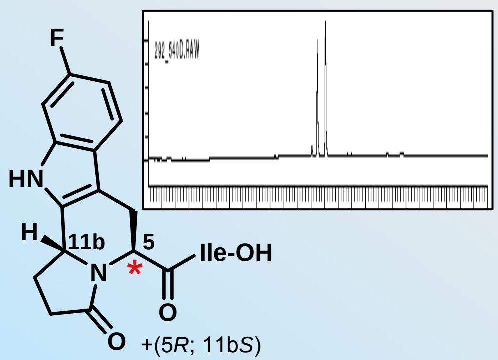


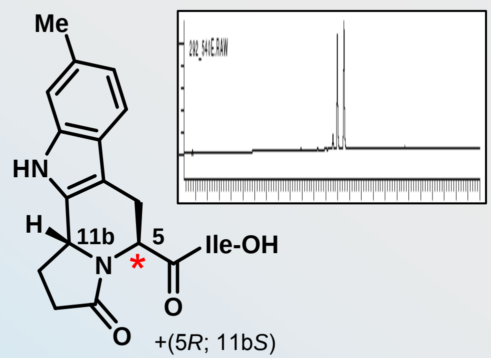




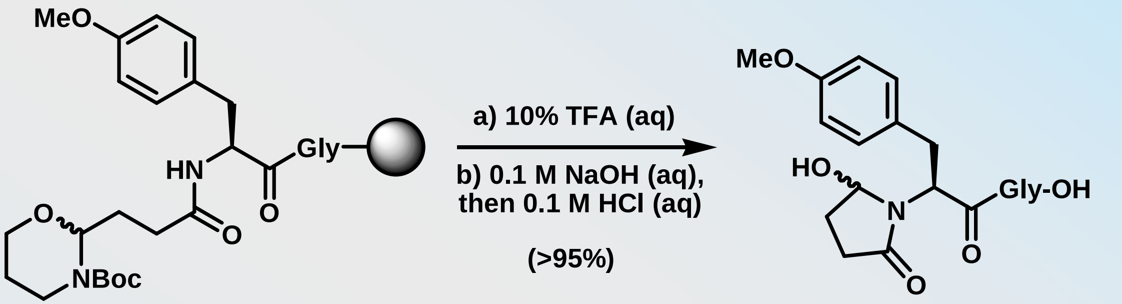
HPLC
Crude products
~95% yield
d,r =1/1

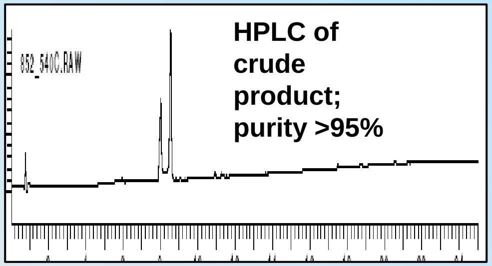


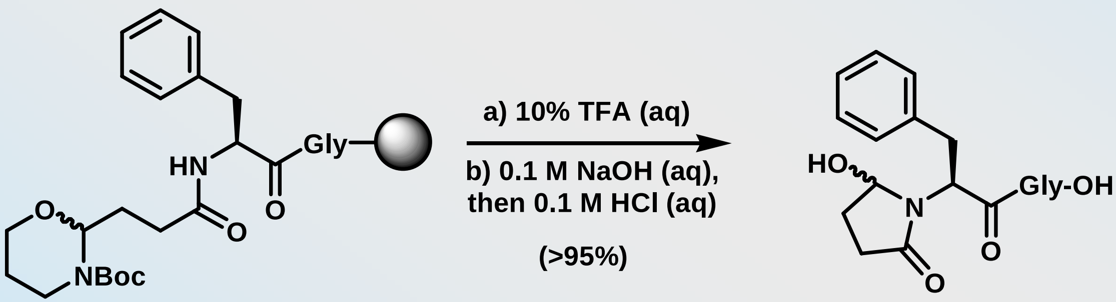


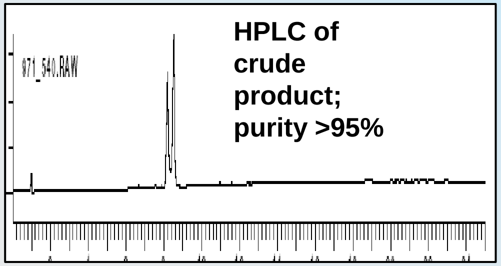


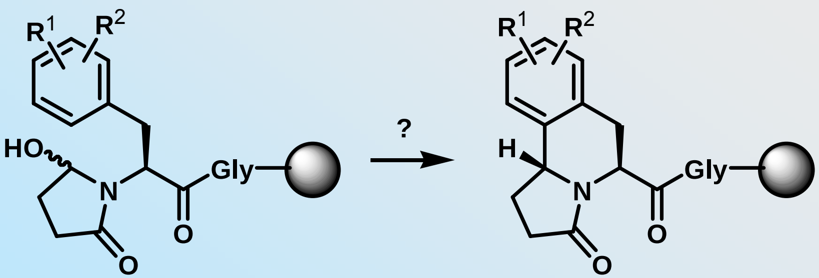
Scaffold diversity: Non-activated nucleophiles











Pyrroloisoquinolines

Lewis or Brønsted acid-mediated reaction

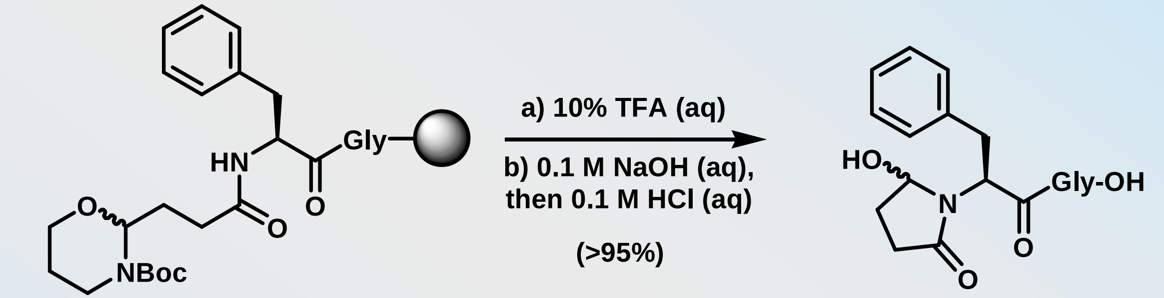
⇩

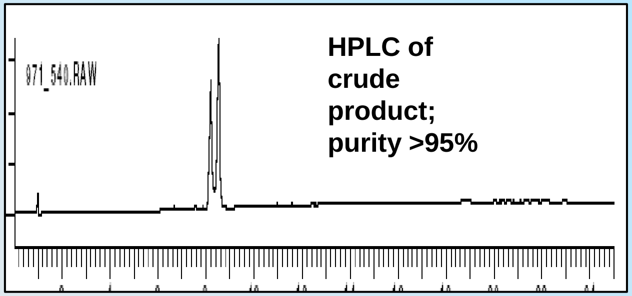
Many pharmacologically relevant Phe-derivatives within the scope of the reaction

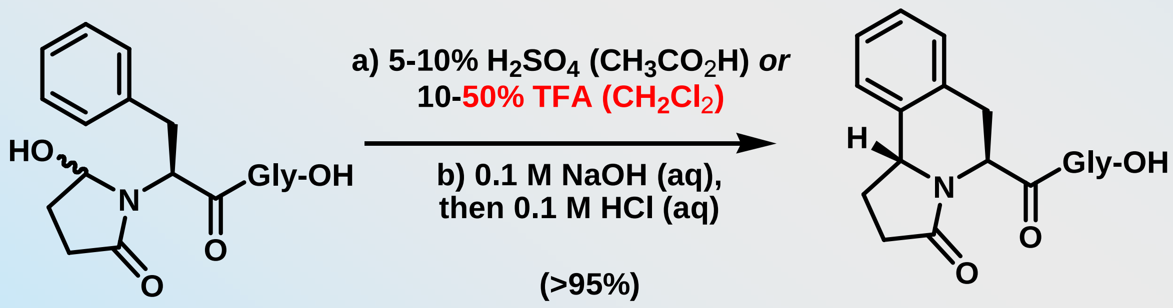
⇩

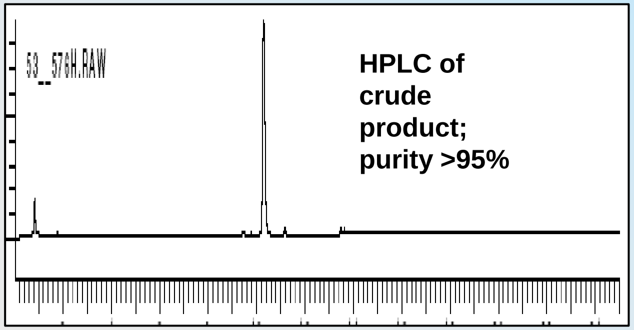
Combinatorial chemistry

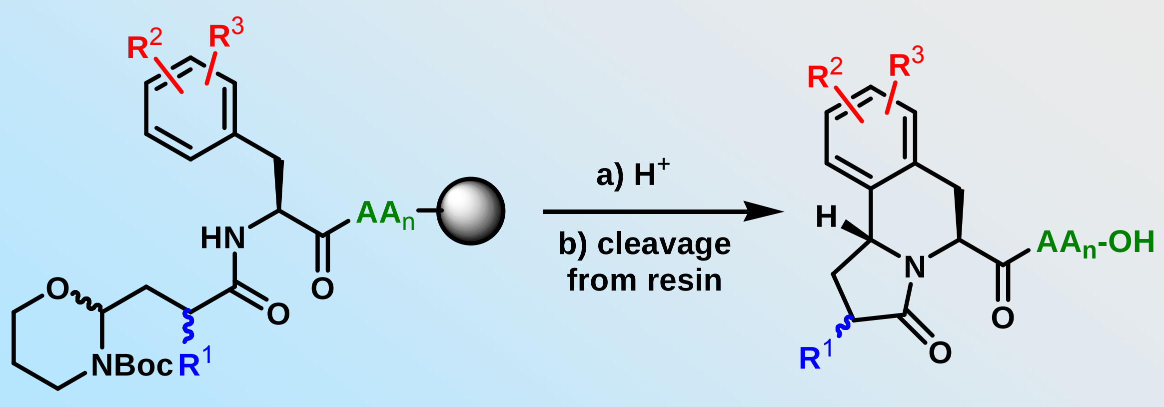
Scaffold diversity: Strong acid











Pyrroloisoquinoline

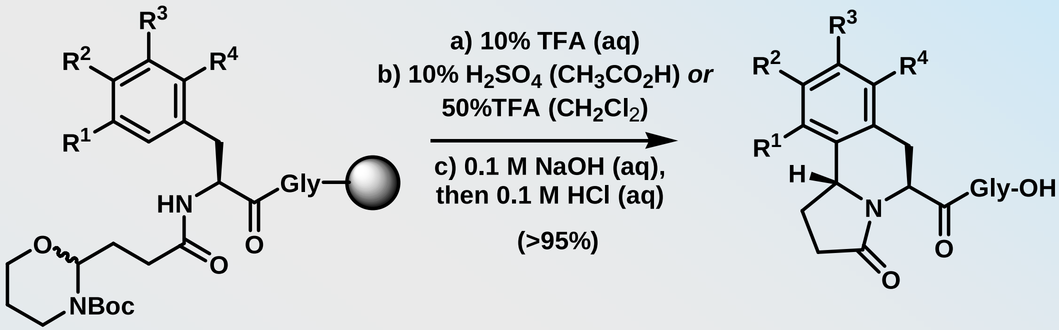
Building blocks required:

Fmoc-amino acids AA (commercially available)

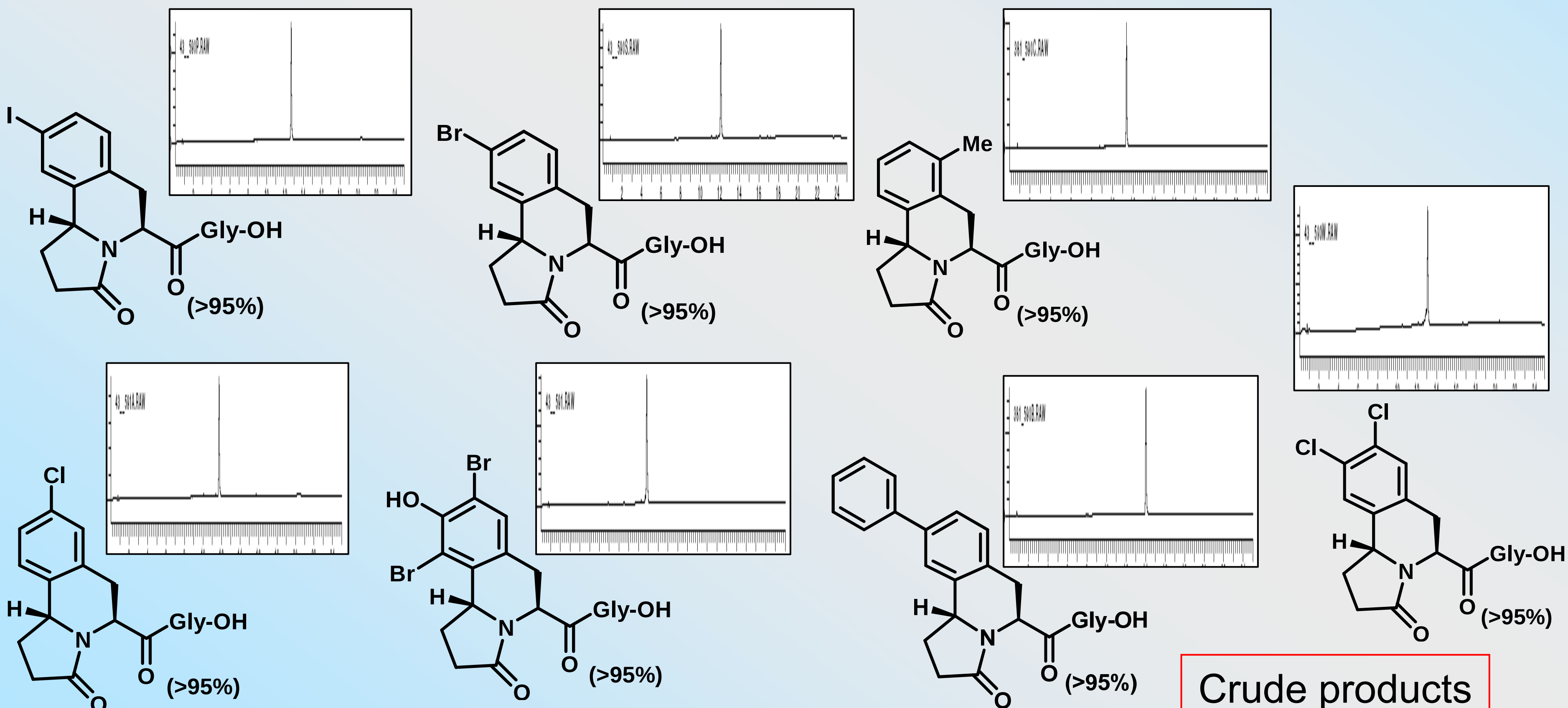
(R^2,R^3)-Substituted Fmoc-Phe-OH derivatives (commercially available)

R^1-Substituted masked aldehyde building blocks (readily availabe)

Scaffold diversity: Deactivated nucleophiles

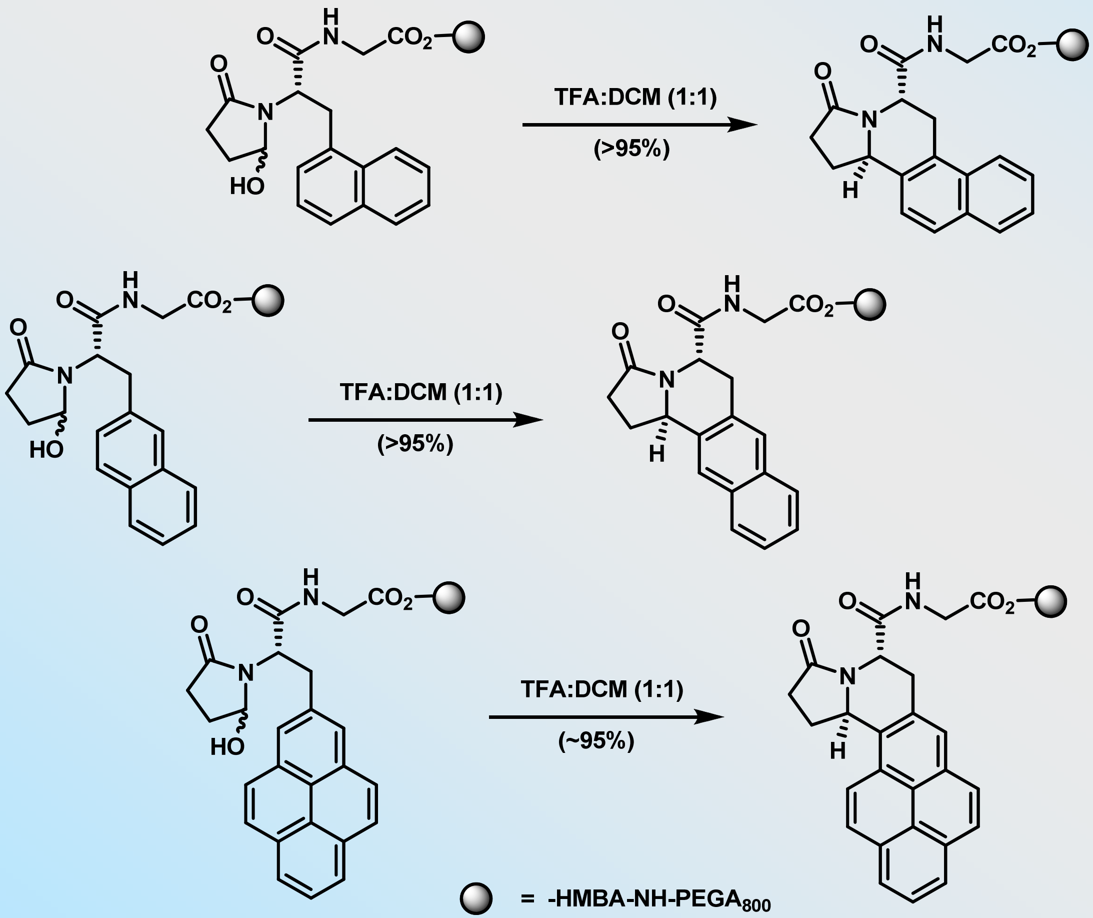


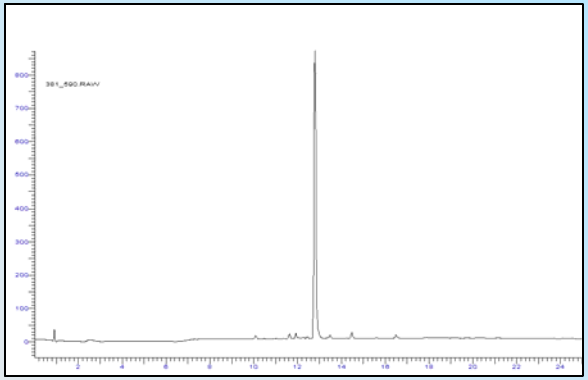
Substituents not compatible with quantitative transformation:
-NH_2, -OH (>1), -N_3, -CF_3, -NO_2, -CN

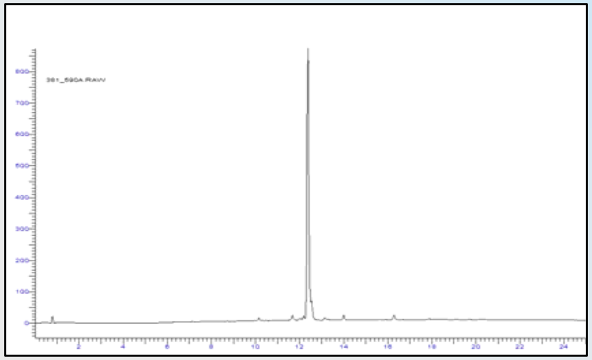


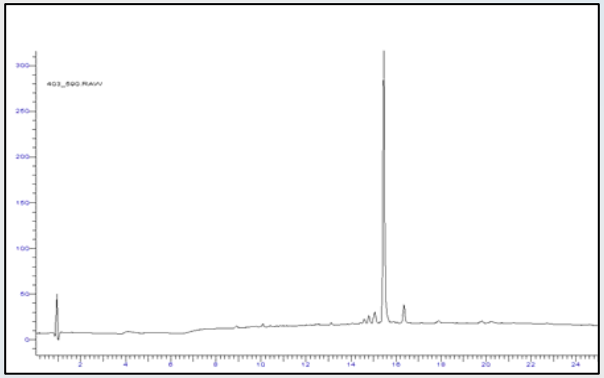
Crude products

Scaffold diversity: Fused Aromatic Ring-systems

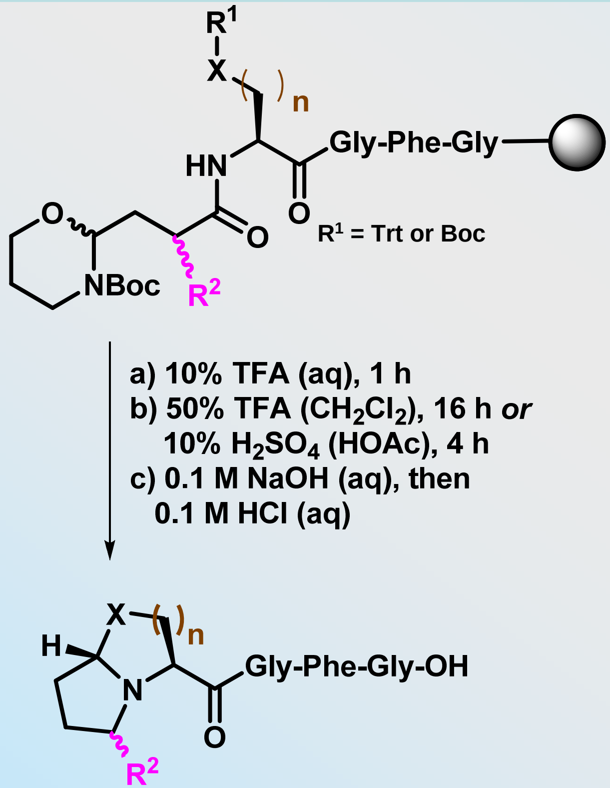




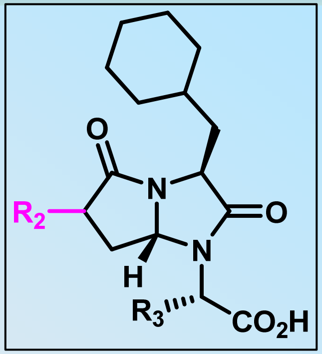


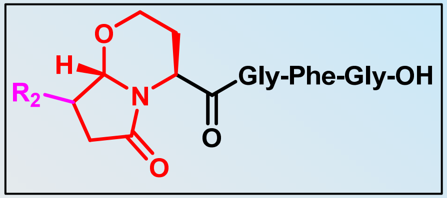


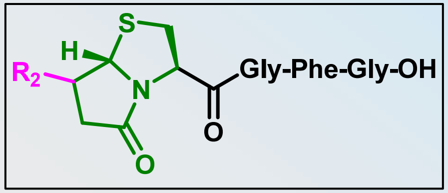
Scaffold diversity: Heteroatom nucleophiles

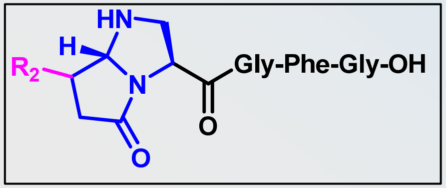


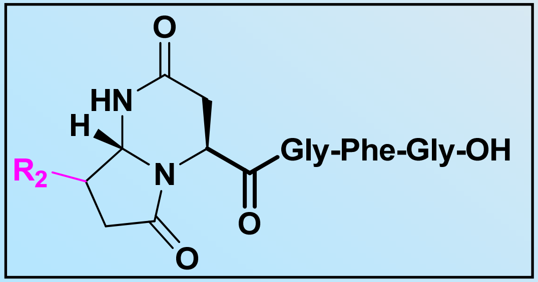
Entry	X	n	R^2	Purity (%)
1	O	1	H	complex mixture
2	O	2	H	>95
3	O	2	*i*-Bu	>95
4	O	2	Bn	>95
5	S	1	H	91
6	S	1	*i*-Bu	94
7	S	1	Bn	>95
8	NH	1	H	>95
9	NH	1	*i*-Bu	91
10	NH	1	Bn	91
11	NH	2	H	>95
12	NH	3	H	>95
13	NH	4	H	complex mixture

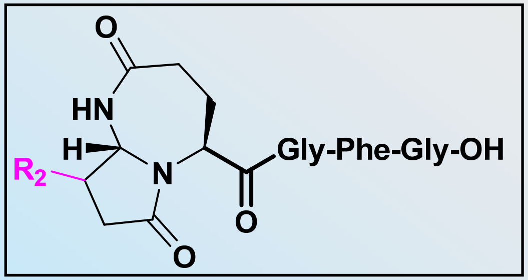


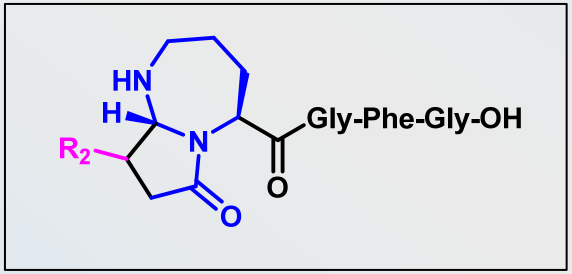






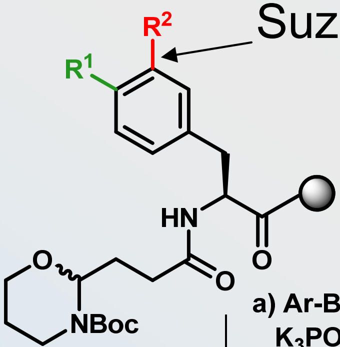






Scaffold diversity: Suzuki reactions

Suzuki on aryl iodides



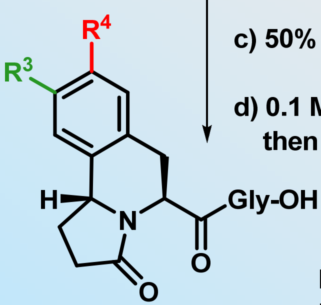
Ia-n (R²=I, R¹=H);
IIa-n (R²=H, R¹=I)

a) $Ar-B(OH)_2$ (10 eq),
K_3PO_4 (10 eq),
$Pd(dppf)Cl_2$ (0.5 eq),
t-BuOH:toluene:H_2O (9:9:2),
20 °C, 20 h

b) 10% TFA (aq), 1 h

c) 50% TFA (CH_2Cl_2), 16 h

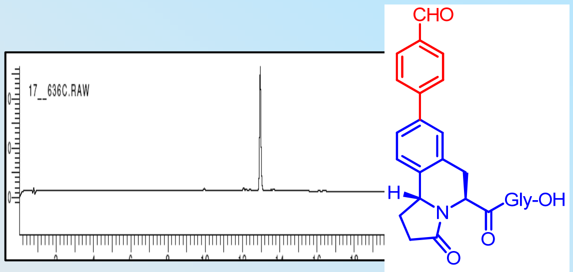
d) 0.1 M NaOH (aq),
then 0.1 M HCl (aq)

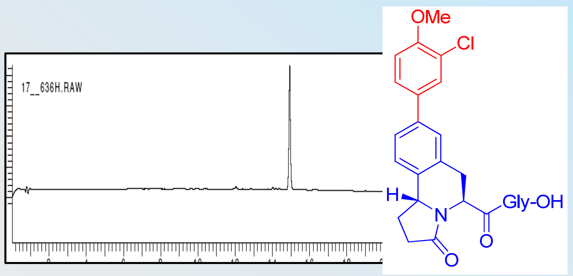


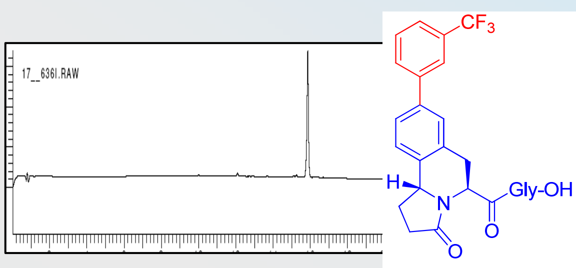
IIIa-n (R^4=Ar, R^3=H);
IVa-n (R^4=H, R^3=Ar)

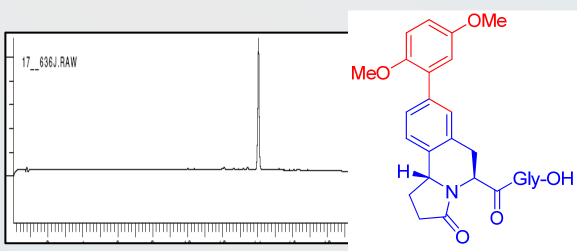
Entry	Ar	Product, Purity (%)
1	Ph	IIIa, >95; IVa, >95
2	4-Me-Ph	IIIb, >95; IVb, >95
3	4-(CHO)-Ph	IIIc, >95; IVc, >95
4	2-MeO-Ph	IIId, >95; IVd, >95
5	4-BuO-Ph	IIIe, 89; IVe, >95
6	4-MeS-Ph	IIIf, 85; IVc, 90
7	4-MeO-Ph	IIIg, >95; IVg, >95
8	4-MeO-3-Cl-Ph	IIIh, >95; IVh, >95
9	3-CF_3-Ph	IIIi, >95; IVi, >95
10	3,5-$(MeO)_2$-Ph	IIIj, >95; IVj, >95
11	4-Cl-Ph	IIIk, >95; IVk, >95
12	3,4-(OCH_2O)-Ph	IIIl, >95; IVl, >95
13	3-NO_2-Ph	IIIm, >95; IVm, >95
14	3-(CHO)-4-MeO-Ph	IIIn, >95; IVn, >95

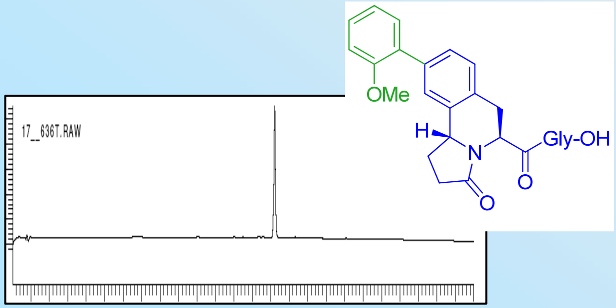
HPLC's of crude product; purity >95%

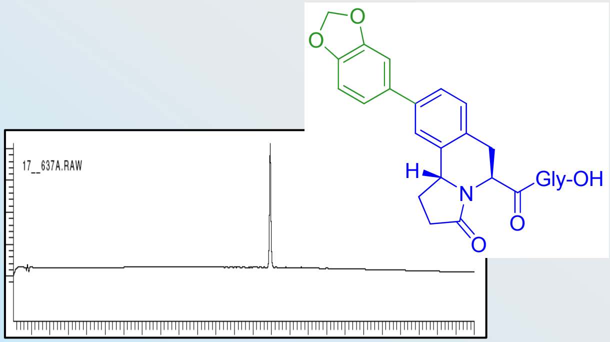






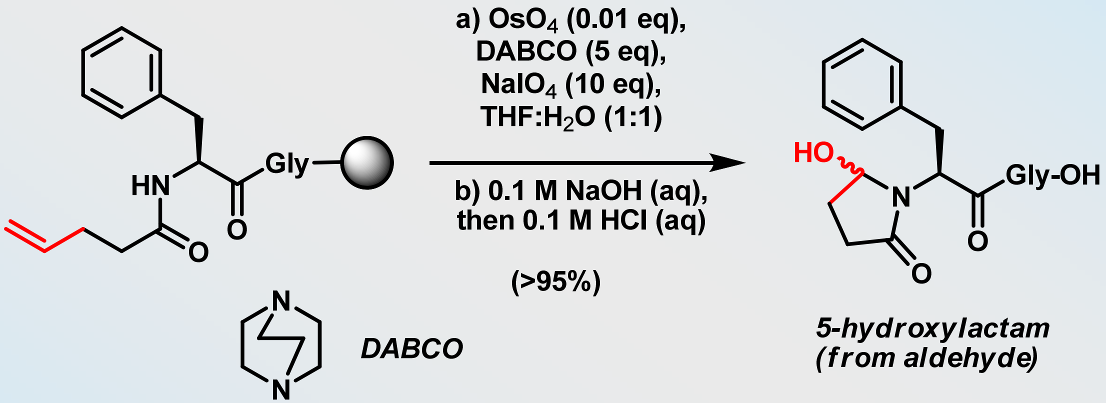


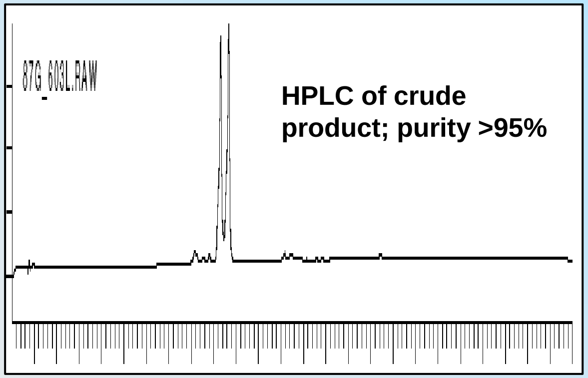


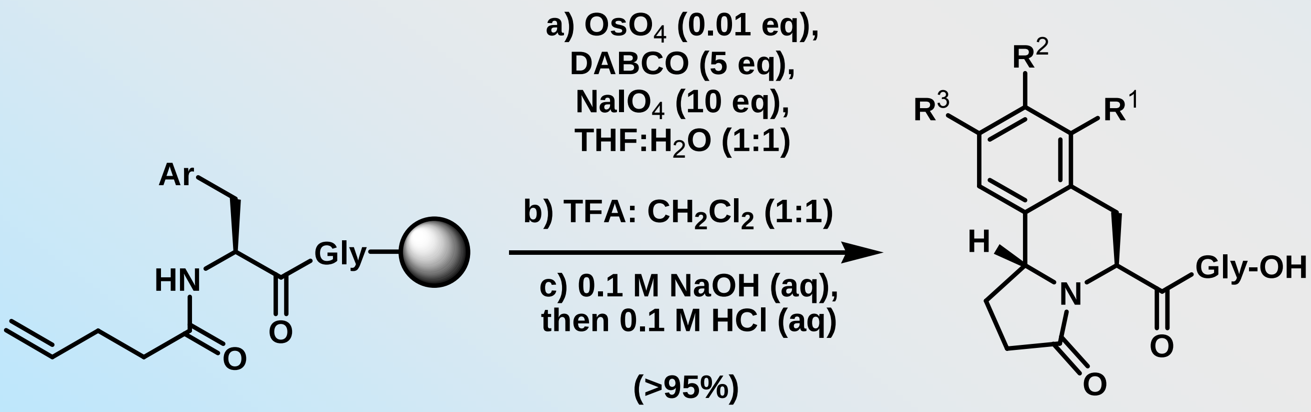


dppf: 1,1′-Bis(diphenylphosphino)ferrocene

Scaffold diversity: Alkenes as the aldehyde source

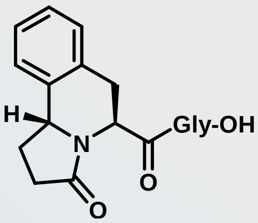


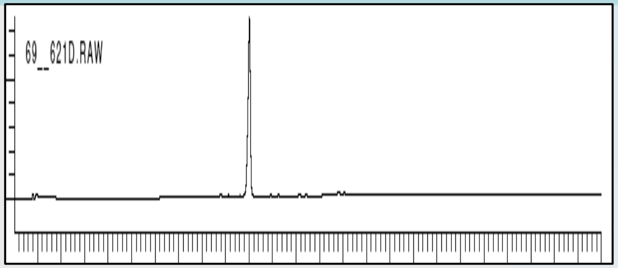


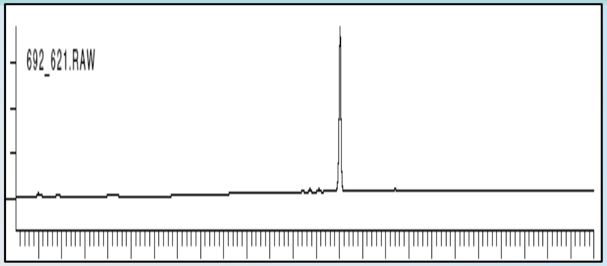


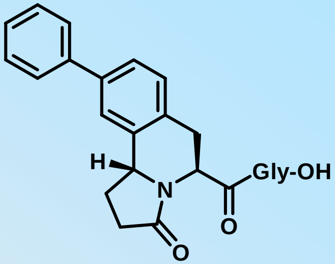
8 examples

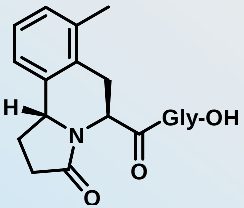
Aldehyde Precursor: Alkene Oxidation

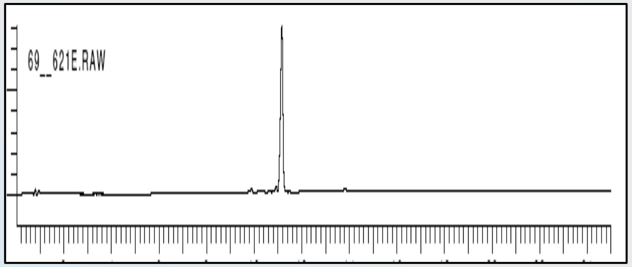


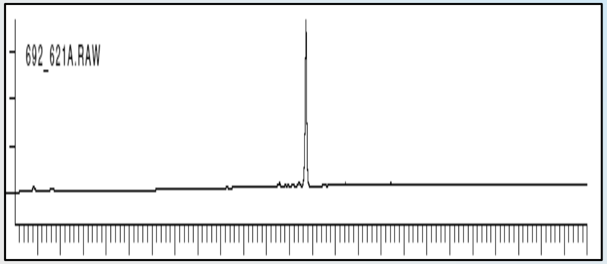


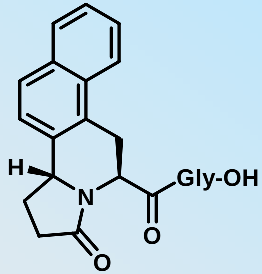


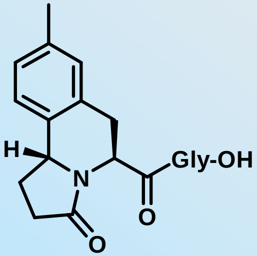


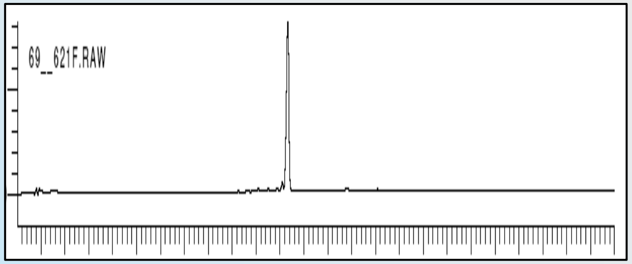


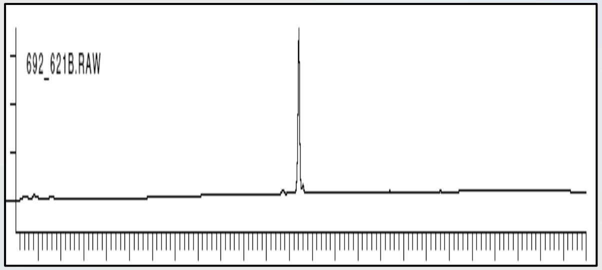


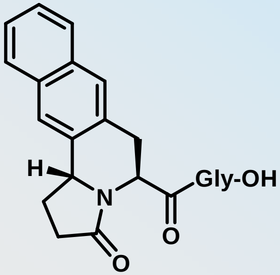


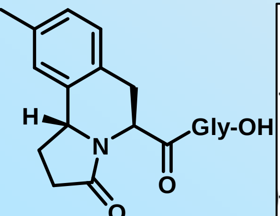


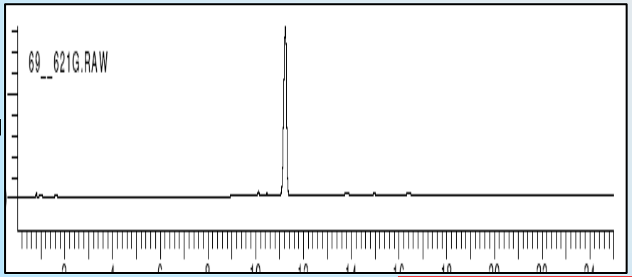


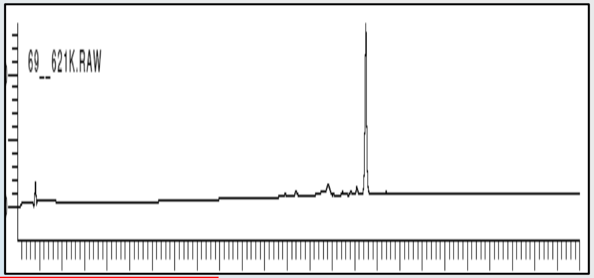


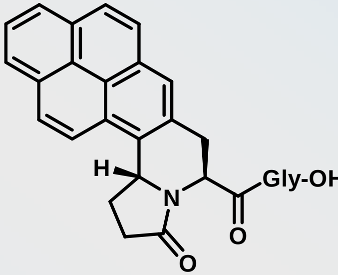








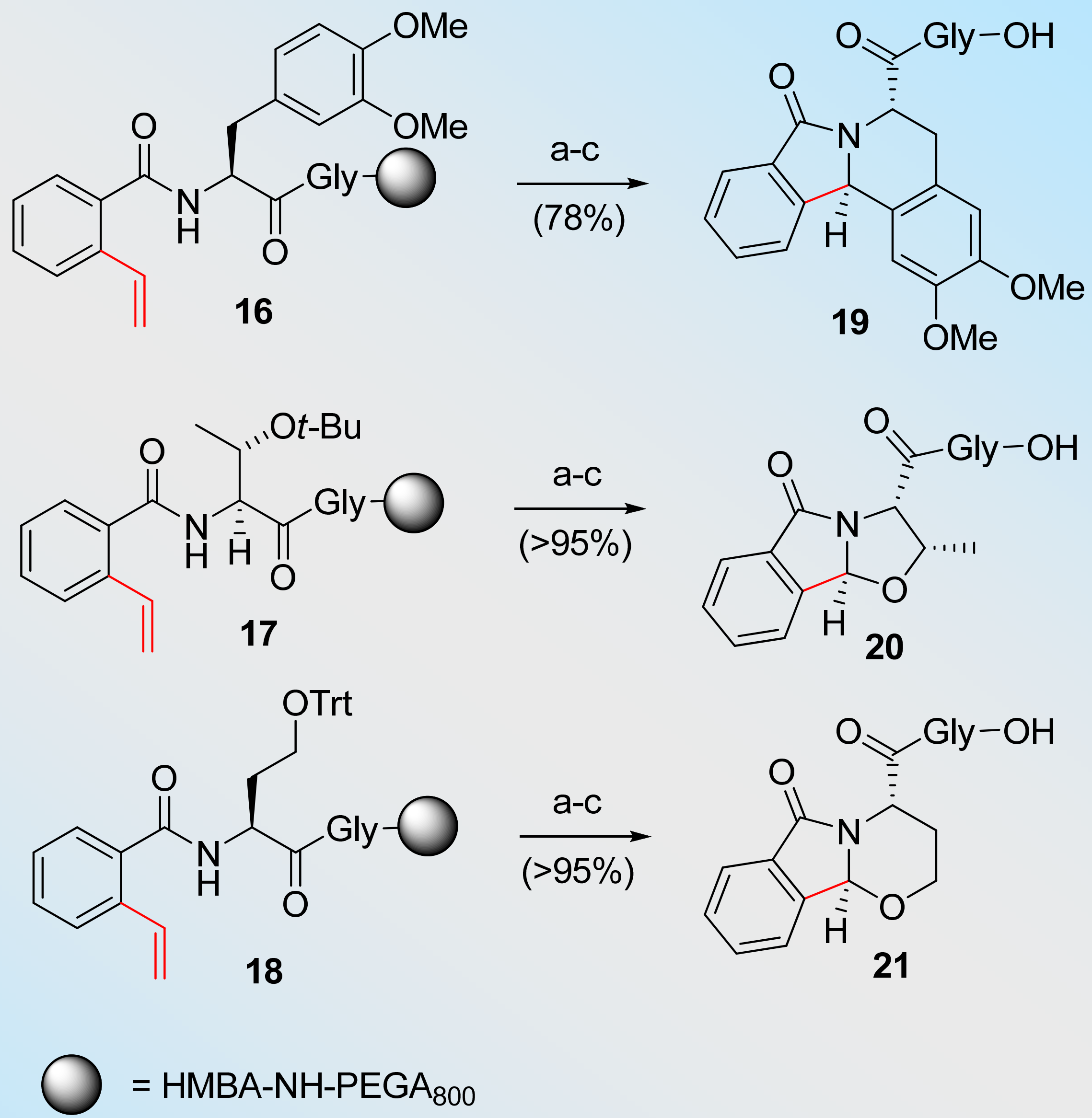




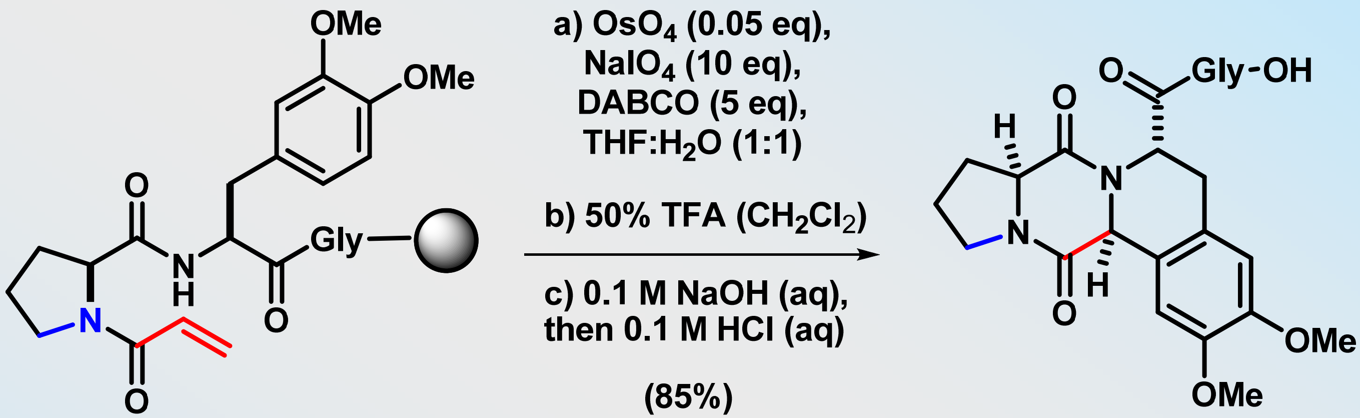
HPLC of crude product; purity >95%

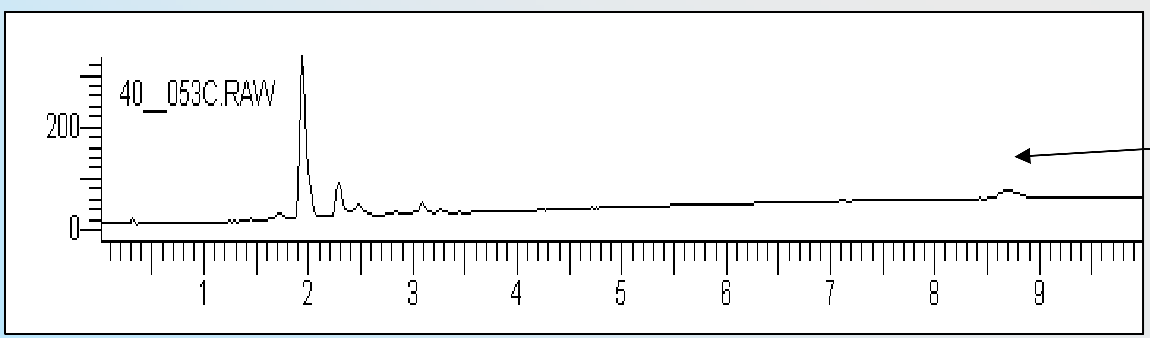
Aldrhyde Precursors: 2-Vinyl Benzamides

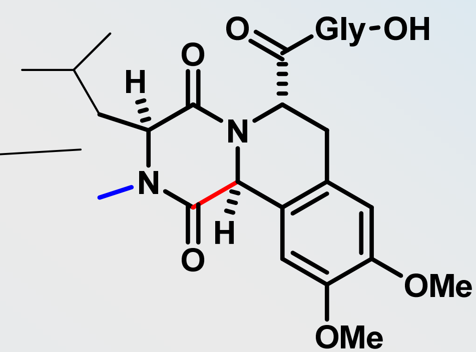
2-Vinyl benzamides



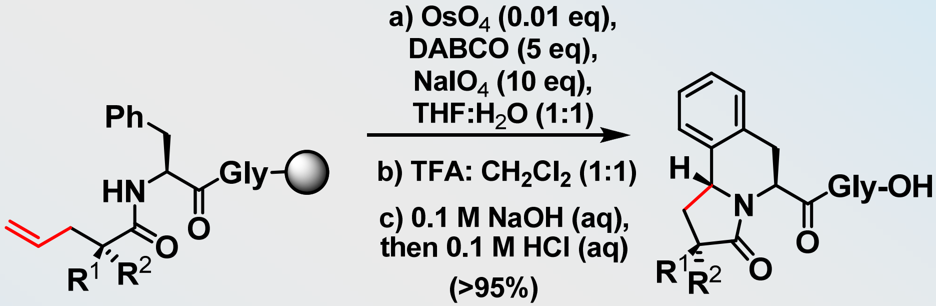
Scaffold diversity: Diketopiperazines



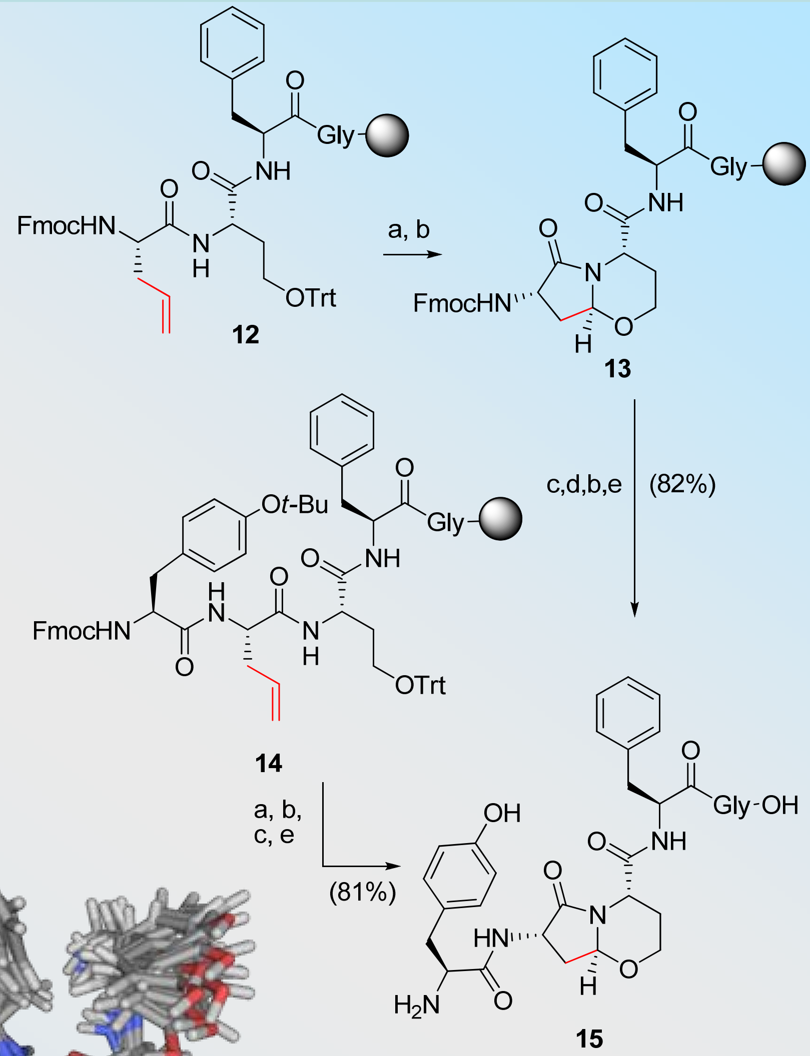


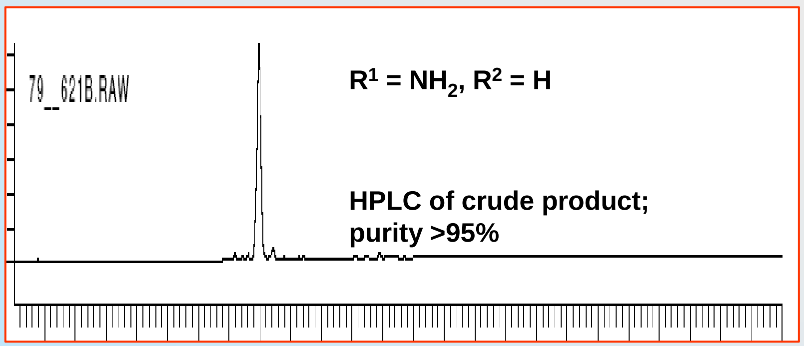


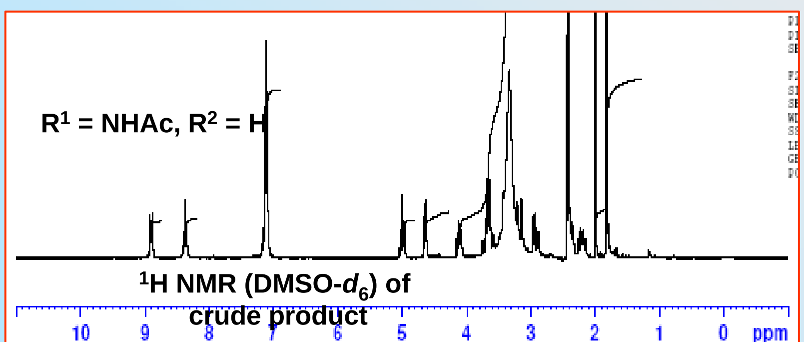
Scaffolds in peptides

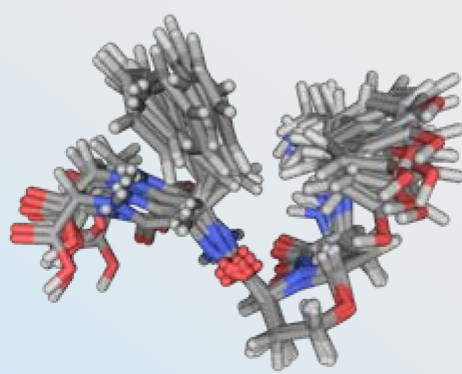


Allylglycine provides pyrroloisoquinoline scaffolds within peptides





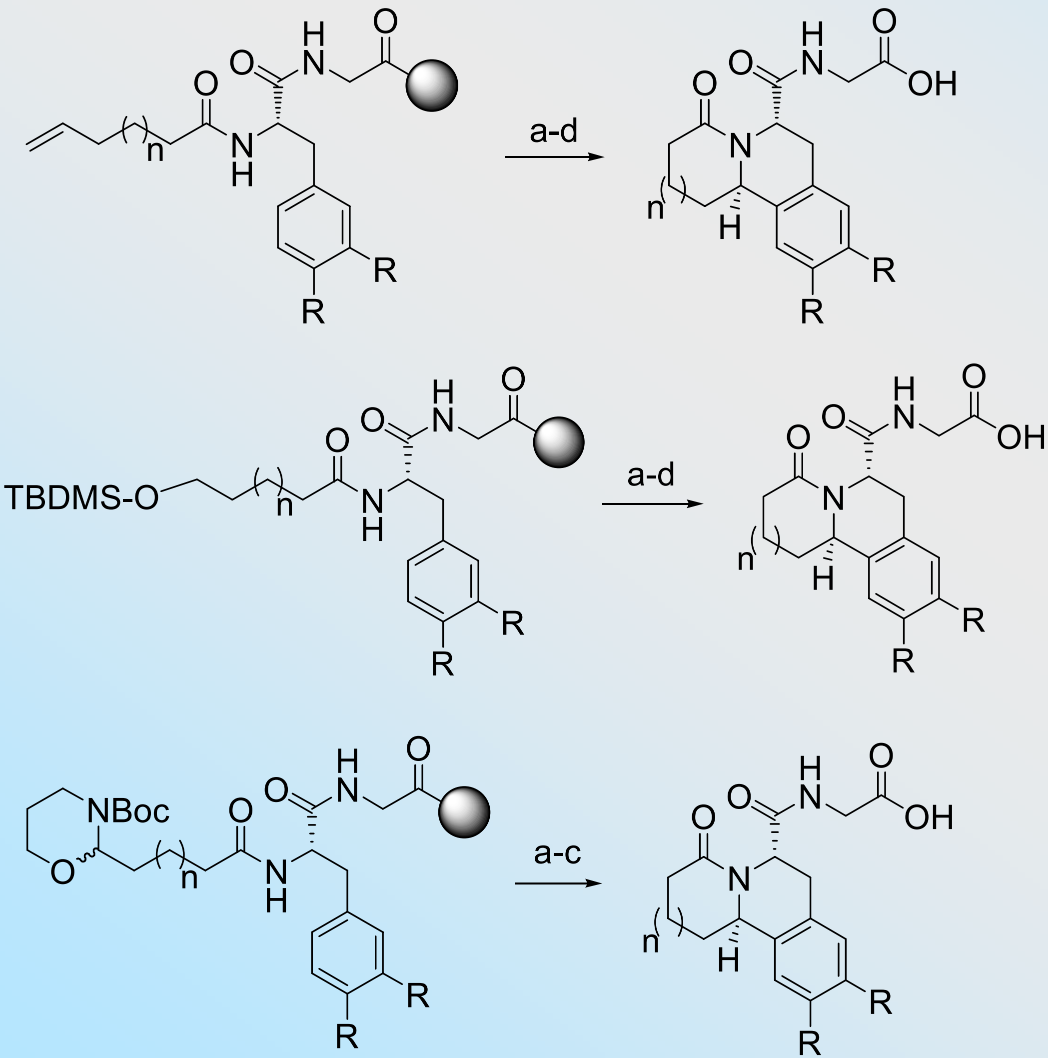




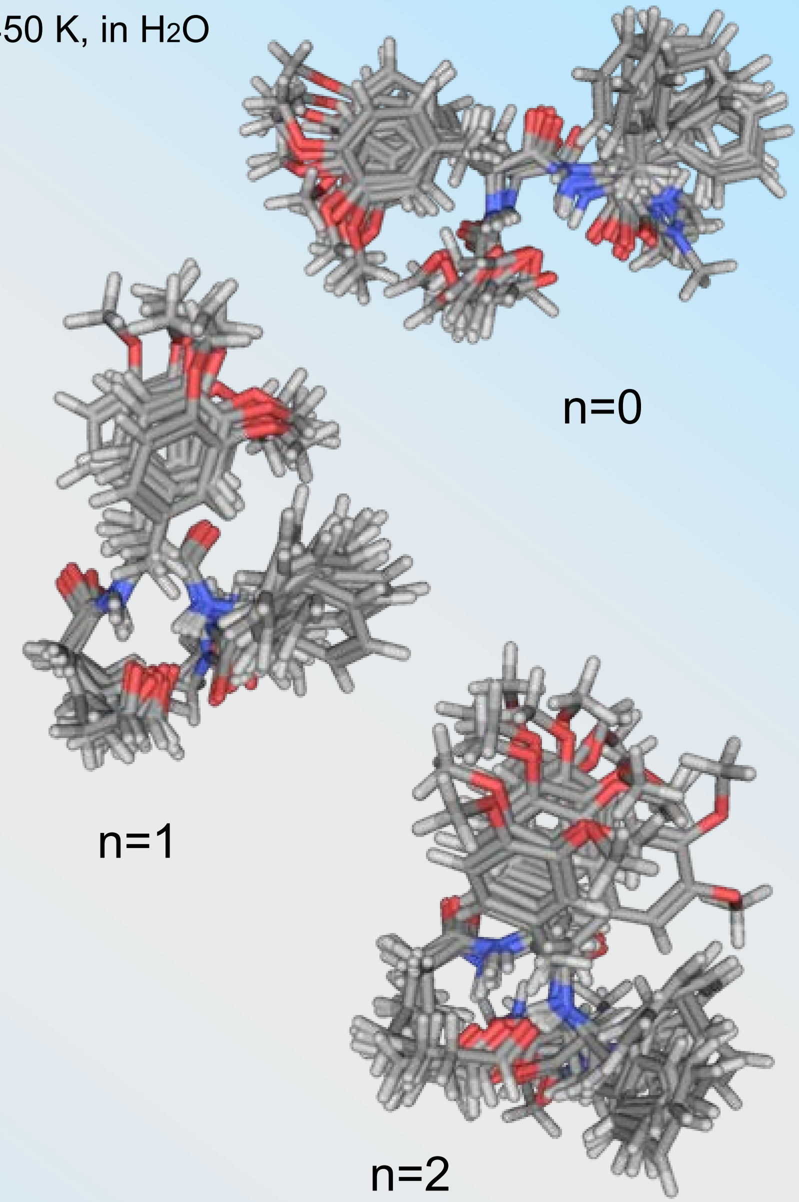
δ-Selective Opioid Receptor Ligand
Gu et al. Org. Lett. 2004, 6, 3285–3288:
Selective opioid receptor binding.

Scaffold diversity The Intramolecular *N*-Acyliminium Pictet-Spengler Reaction

3 aldehyde precursors

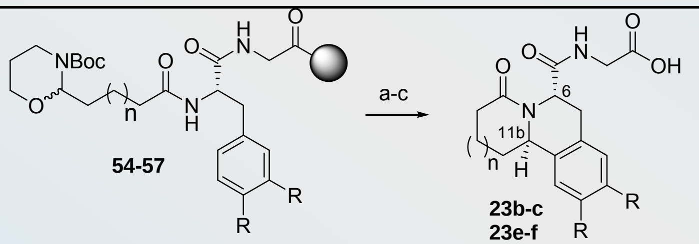


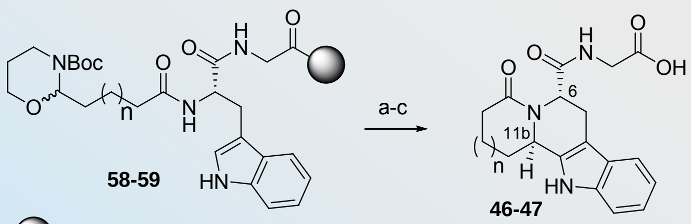
MD, 450 K, in H_2O



Scaffold diversity and Ring-size: The influence of aldehyde precursor

Box-protected Aldehydes





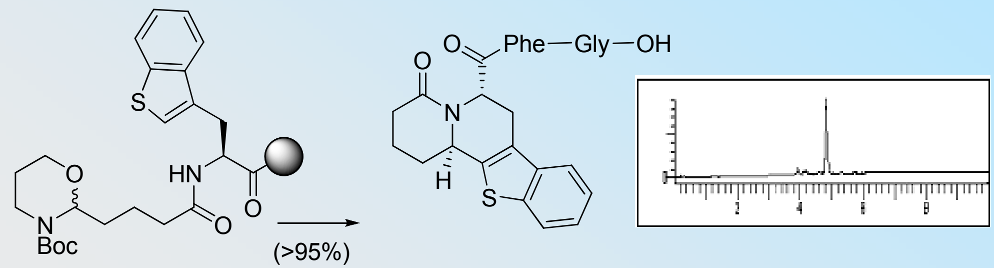
= HMBA-NH-PEGA$_{800}$

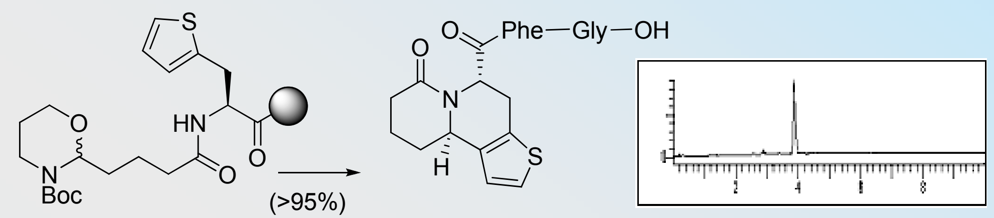
Entry	*N,O*-acetal	R	n	Product[c], purity (%)
0	-	H	0	**23a,** >95
1	**54**	H	1	**23b**, >95
2	**55**	H	2	**23c**, 0
3	**56**	OMe	1	**23e**, >95
4	**57**	OMe	2	**23f**, 0
5	**58**	Trp	1	**46**, Decomp
6	**59**	Trp	2	**47**, Decomp

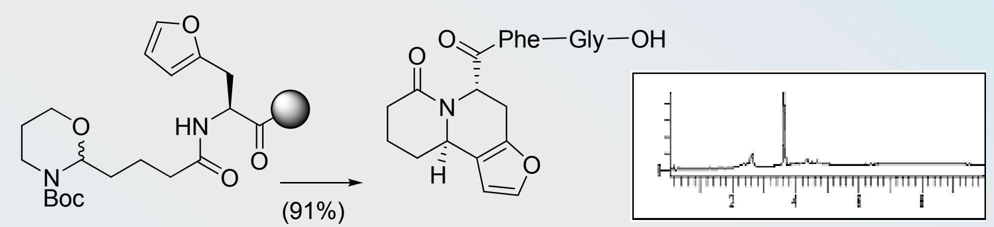
(a) 10% TFA (aq);
(b) 50% TFA (CH_2Cl_2)
(c) 0.1 M NaOH (aq).

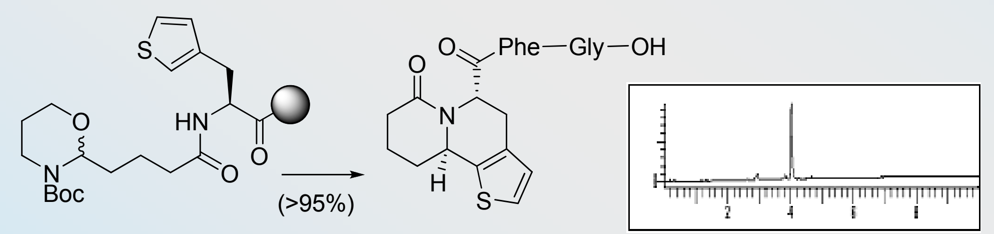
Box>Alkene>Alkohol

6,6-rings, C-nucleophiles





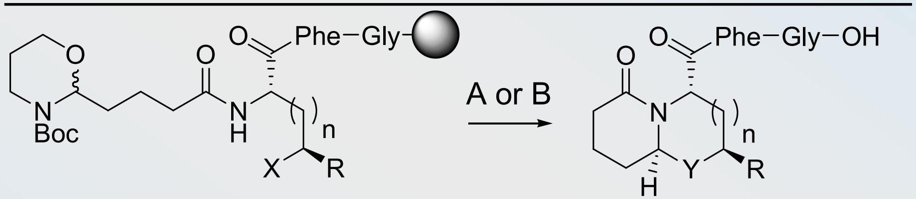




= -Phe-Gly-HMBA-NH-PEGA$_{800}$

Scaffold diversity

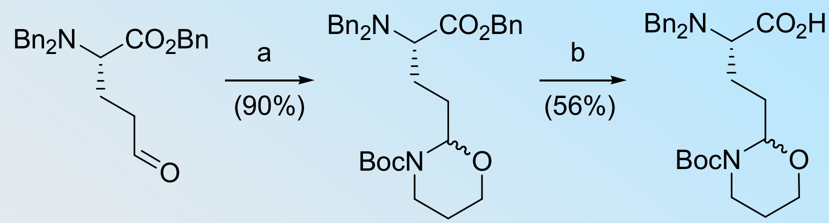
The Intramolecular *N*-Acyliminium Pictet-Spengler Reaction

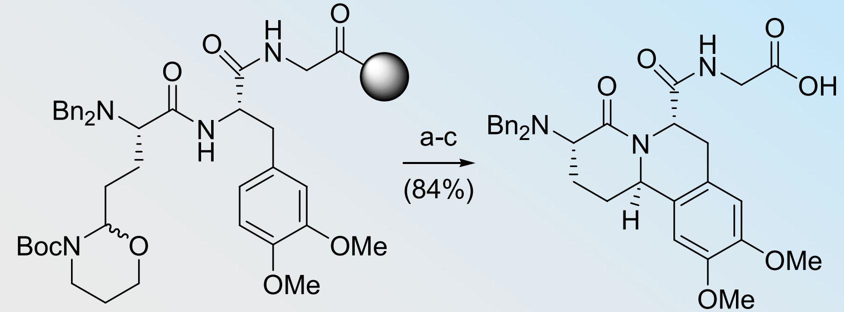


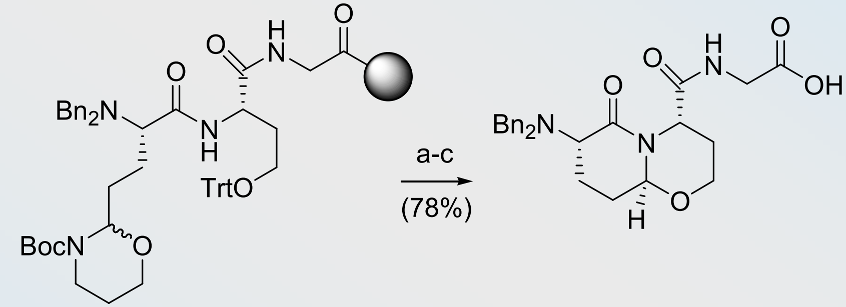
Entry	*N*,*O*-acetal	X	R	Y	n	Reaction conditions[a]	Product, purity (%)
1	**68**	O*t*-Bu	H	O	0	B	**75**, >95
2	**69**	O*t*-Bu	Me	O	0	A or B	**76**, >95
3	**70**	OTrt	H	O	1	A or B	**77**, >95
4	**71**	NHBoc	H	NBoc	0	A	**78**, >95
5	**72**	NHBoc	H	NBoc	1	A	**79**, 86
6	**73**	NHBoc	H	NBoc/ NH	2	A/B	**80**, 0
7	**74**	STrt	H	S	0	A or B	**81**, >95

Conditions A:
- 10% TFA (aq)
- 0.1 M NaOH (aq).

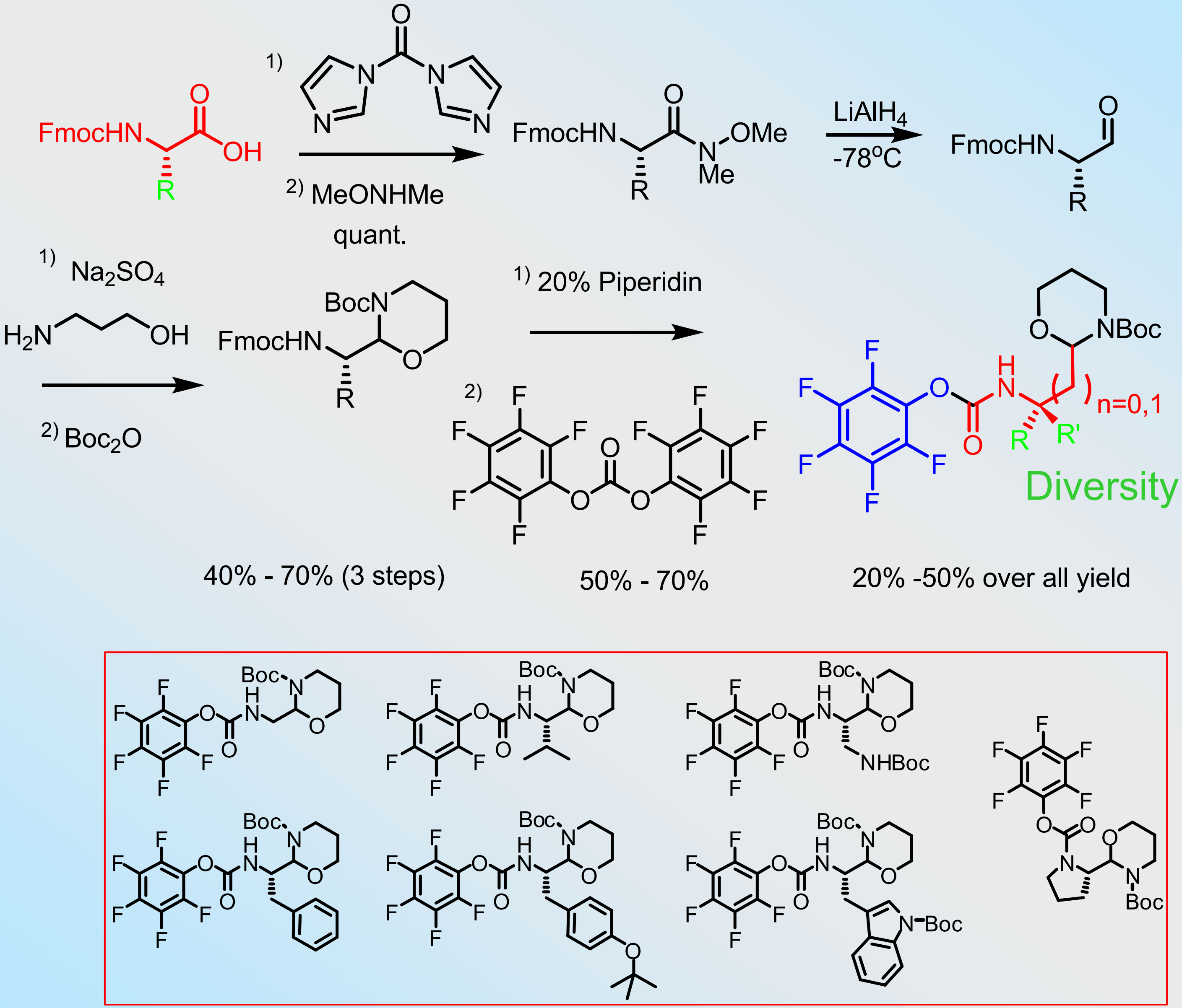
Conditions B:
- 10% TFA (aq)
- 50% TFA (CH_2Cl_2)
- 0.1 M NaOH (aq)



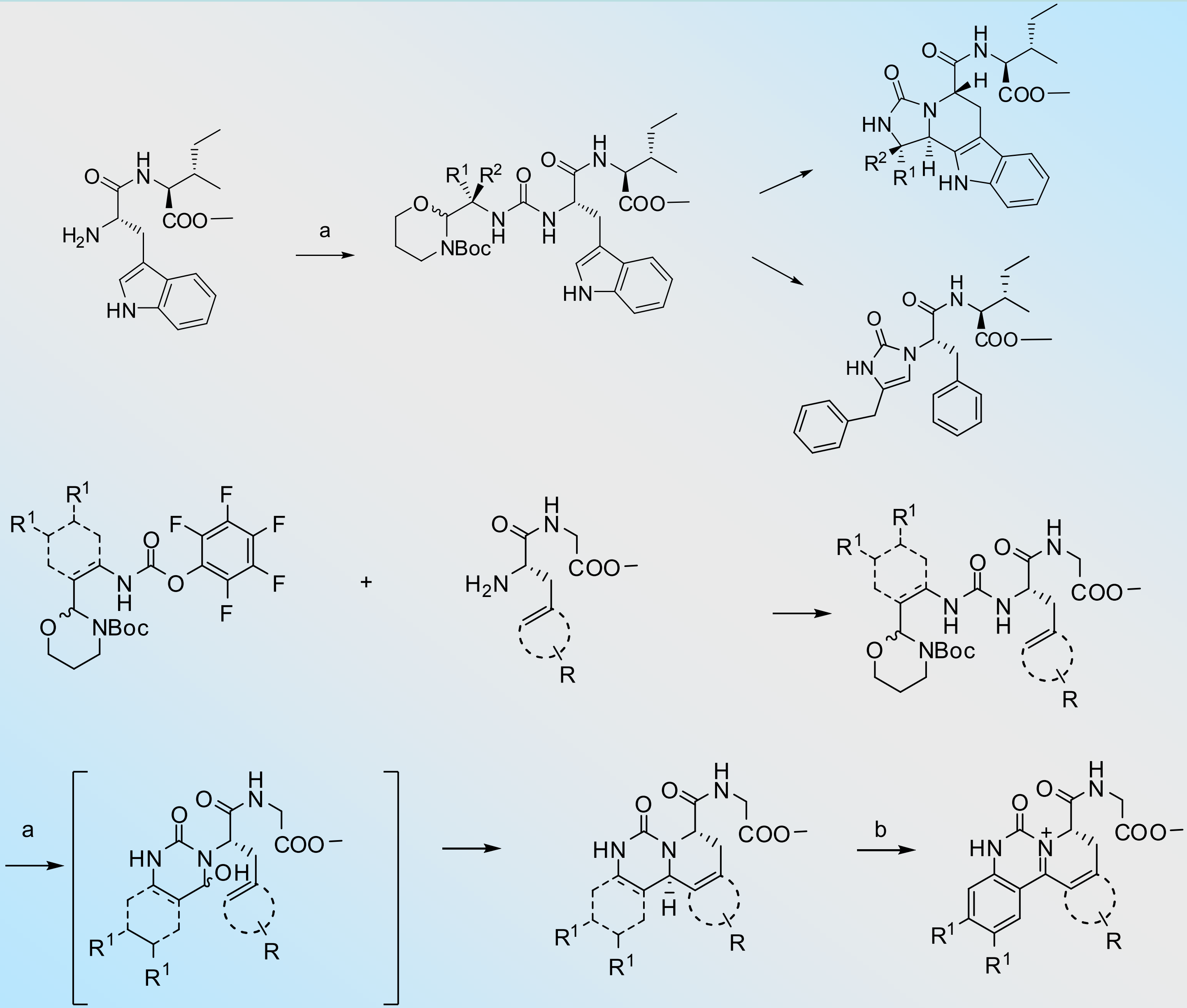




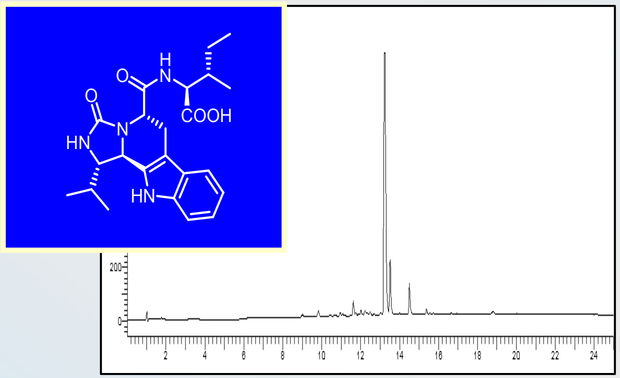
α(or β)-Amino acids as a source of diversity

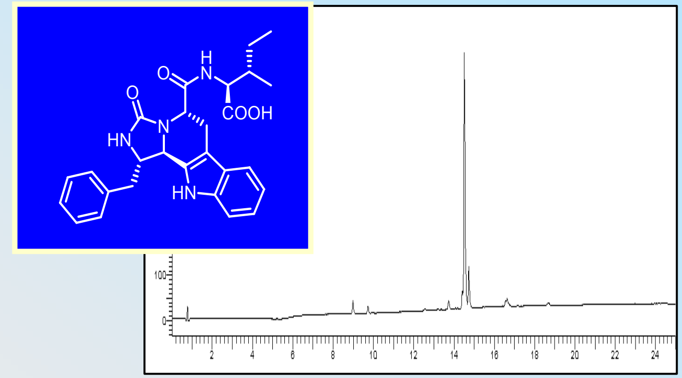


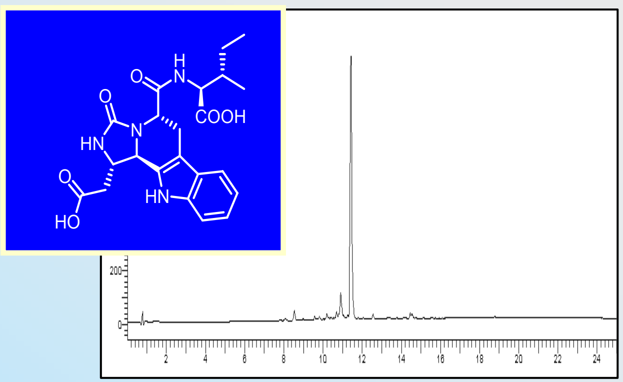
Imidazolinones and 1,3 piperazin-2-ones

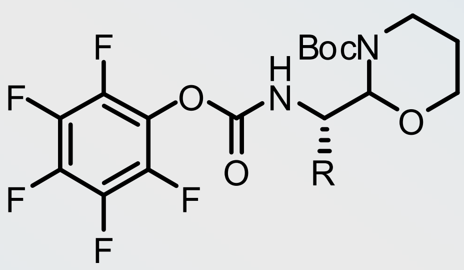


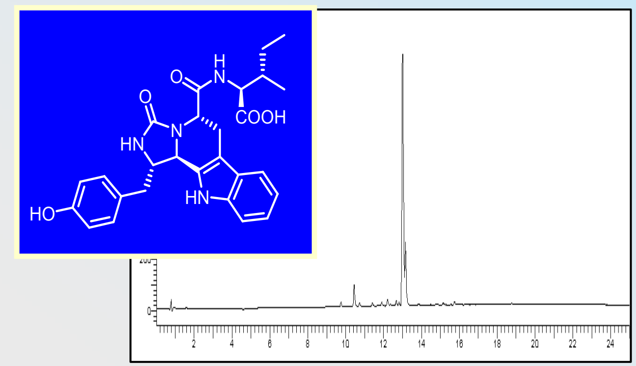
Fused β-carbolino imidazolinones

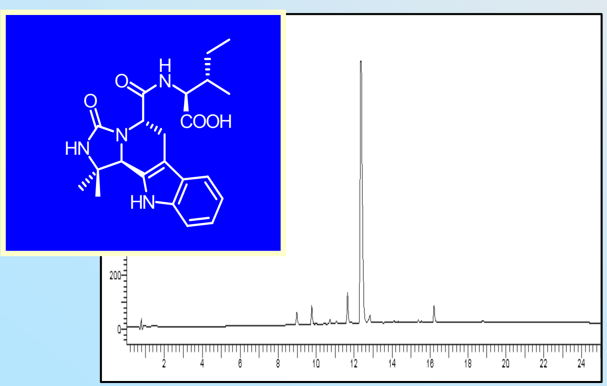


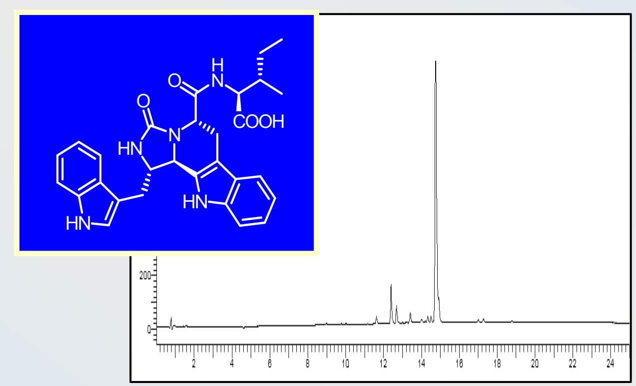








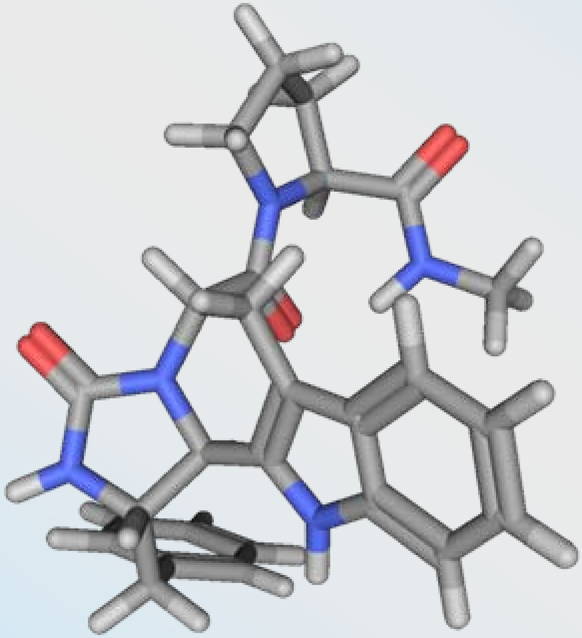




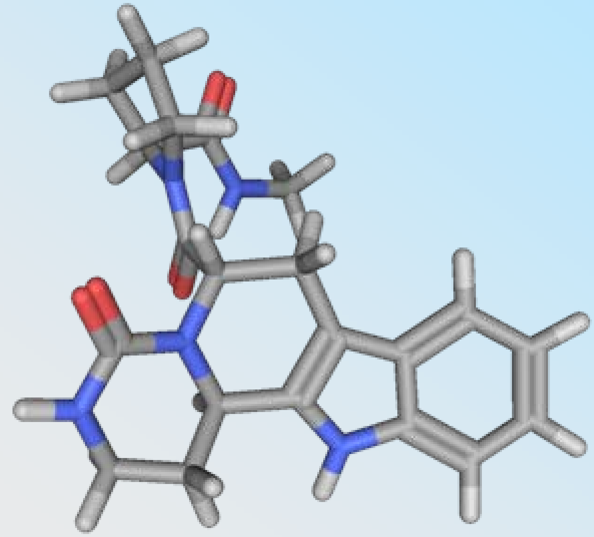
Diastereo-selectivity: >10

Purity > 92%

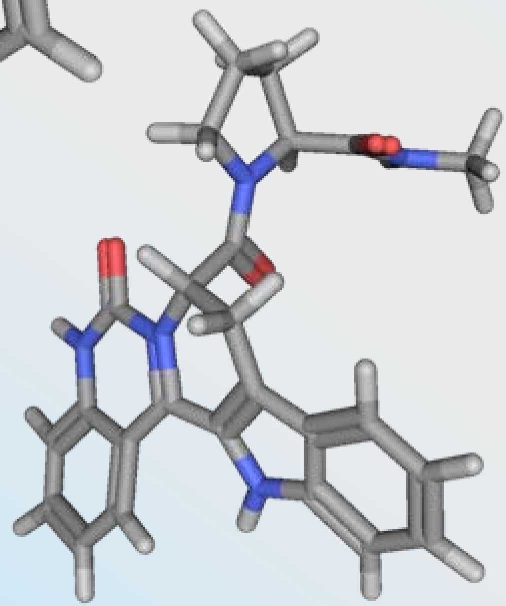
Exploiting Amino Acid Diversity in Carbamyliminium Chemistry



5,6-rings, α-Amino acids

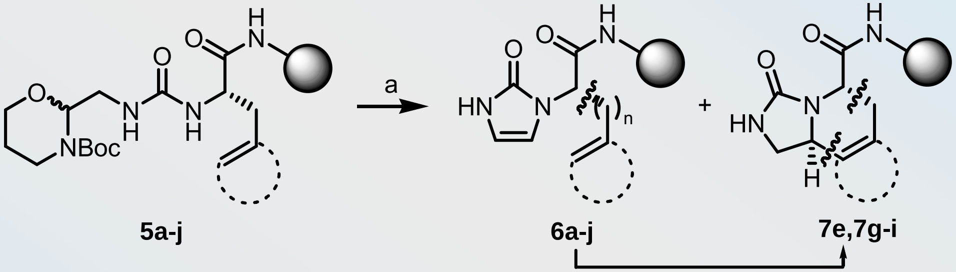


6,6-rings, β-Amino acids

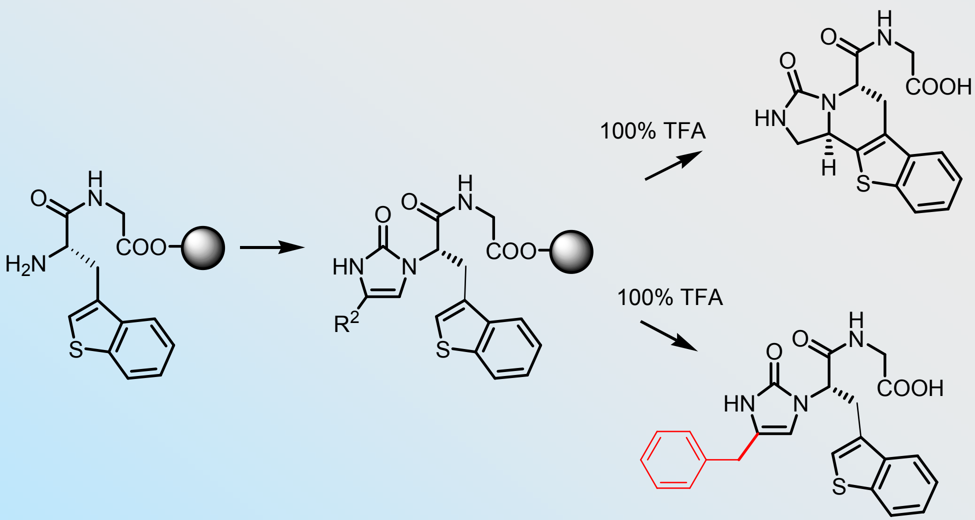


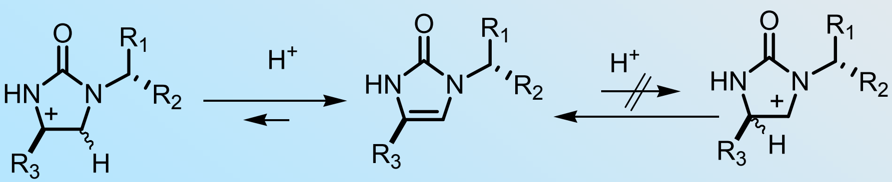
6,6-rings, ABz-precursors

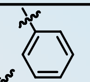
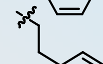
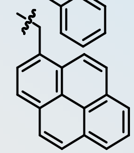
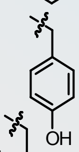
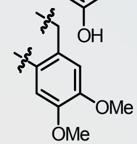
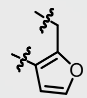
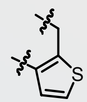
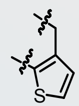
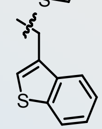
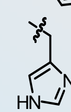
Imidazolones as electrophiles in the Pictet-Spengler Reaction



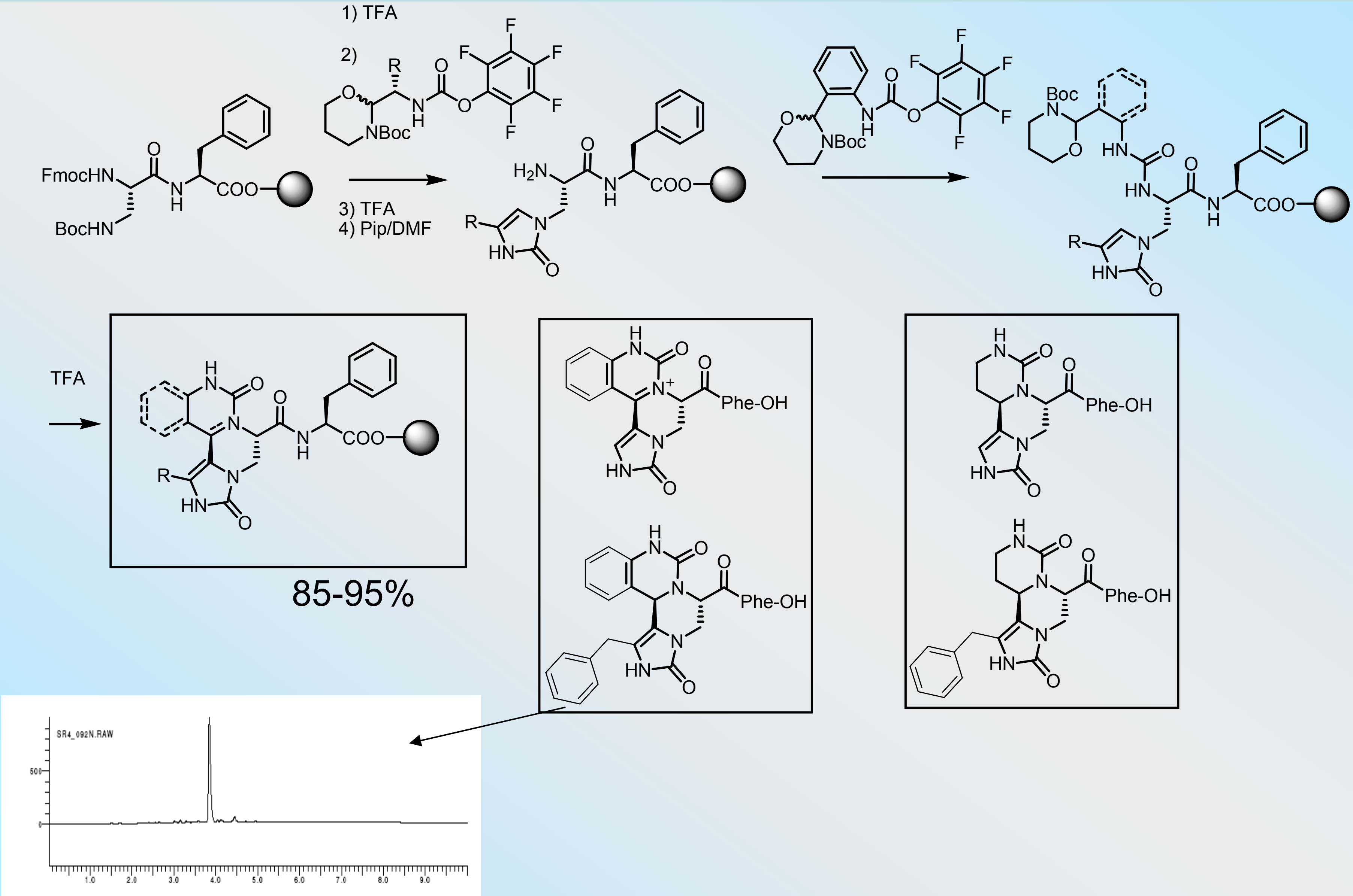
a) 10% TFA (aq), b) 100% TFA



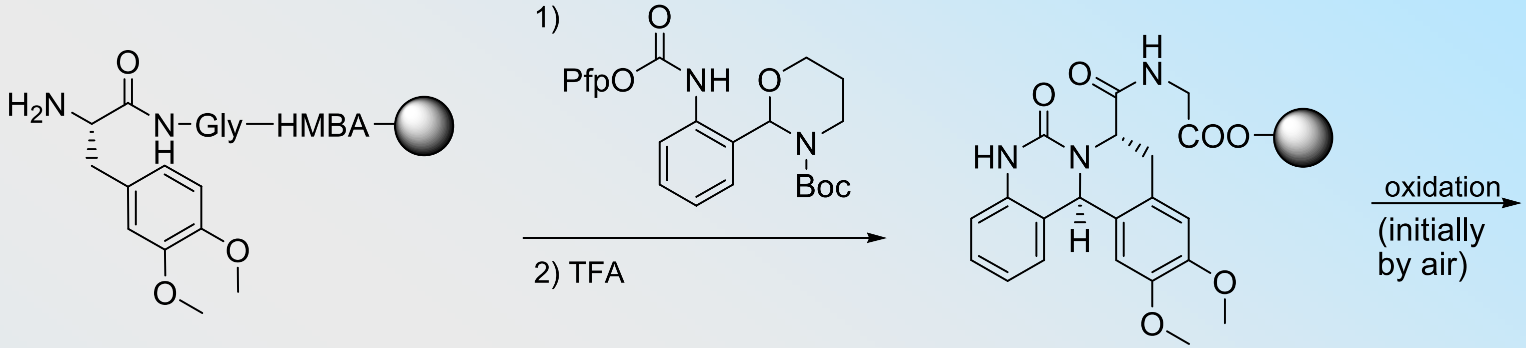


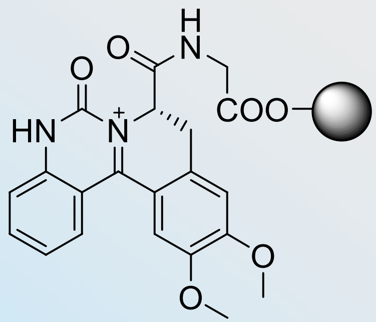
product	10% TFA(aq) 6/7 (purity)	100% TFA 6/7 (purity)
	100 / 0 (98%)	100 / 0 (98%)
	100 / 0 (99%)	100 / 0 (99%)
	100 / 0 (99%)	100 / 0 (99%)
	-	100 / 0 (99%)
	93 / 7 (98%)	0 / 100 (97%)
	100 / 0 (96%)	-
	100 / 0 (95%)	0 / 100 (98%)
	66 / 34 (92%)	0 / 100 (97%)
	100 / 0 (95%)	0 / 100 (96%)
	100 / 0 (99%)	100 / 0 (99%)

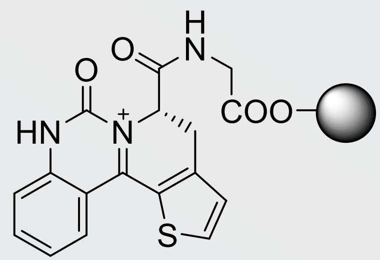
Imidazolones as Nucleophiles in the Pictet-Spengler Reaction

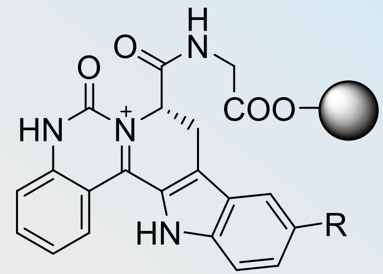


Scaffold diversity: Novel fluorescent compounds

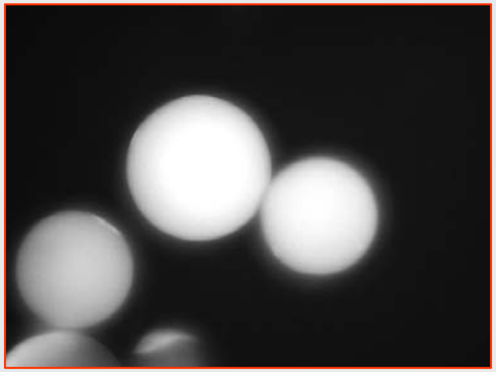








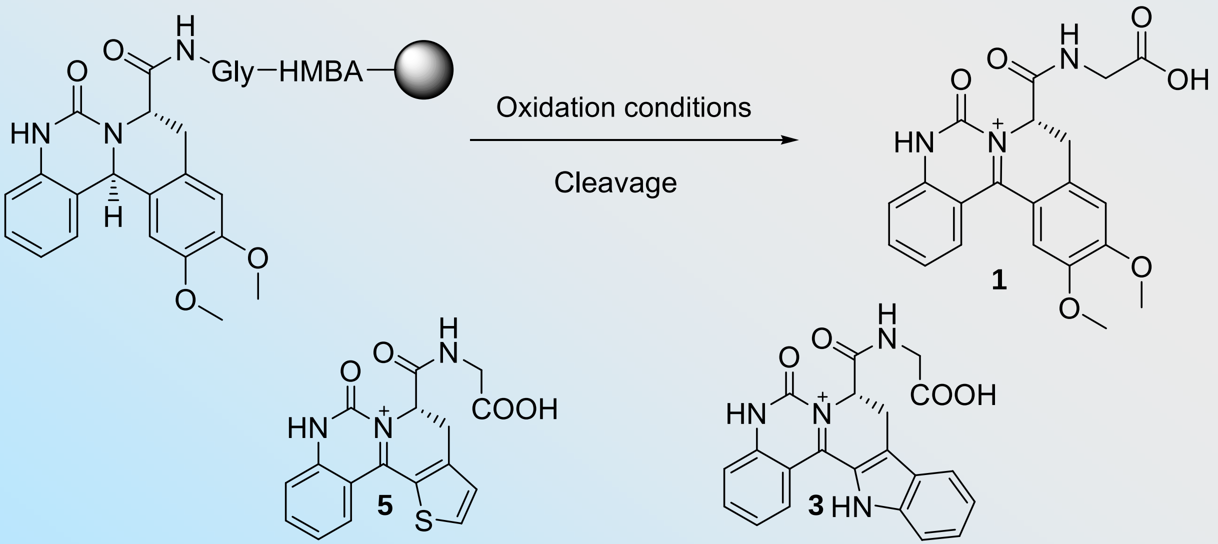
R=H, F, Br



Oxidation of Pictet-Spengler products

Sub.	Solv.	DDQ	Chloranil	BQ
1	DCM	90% (24h)	33% (24h)	0% (24h)
1	5% TFA	100% (2h)	10% (24h)	0% (24h)
3	DCM	0% (decomp)	0% (24h)	0% (24h)
3	5% TFA	100% (2h)	10% (24h)	10% (24h)
5	DCM	0% (decomp)	25% (24h)	0% (24h)
5	5% TFA	100% (2h)	100% (24h)	0% (decomp)

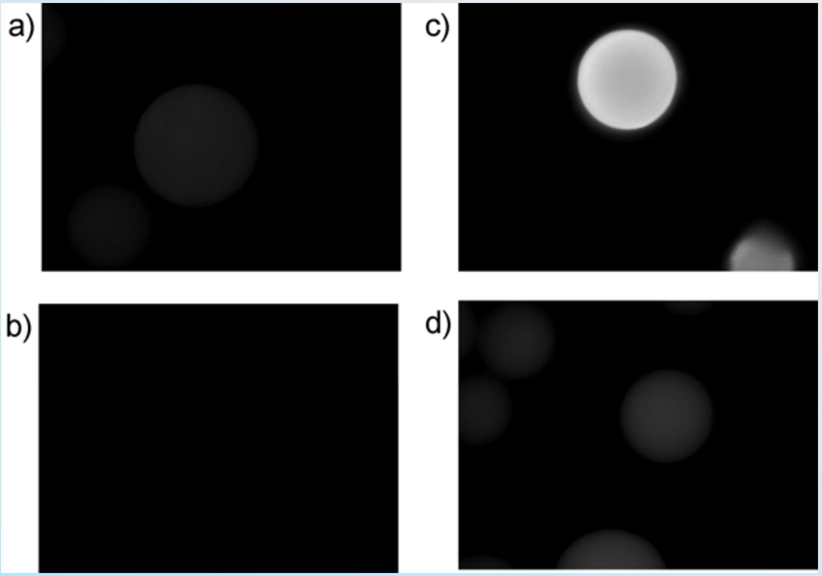
Peroxides: only ~33% yield
Oxygen/TFA: 25%

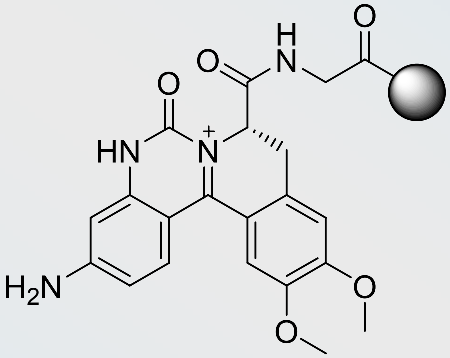


Scaffold diversity

The Intramolecular *N*-Acyliminium Pictet-Spengler Reaction

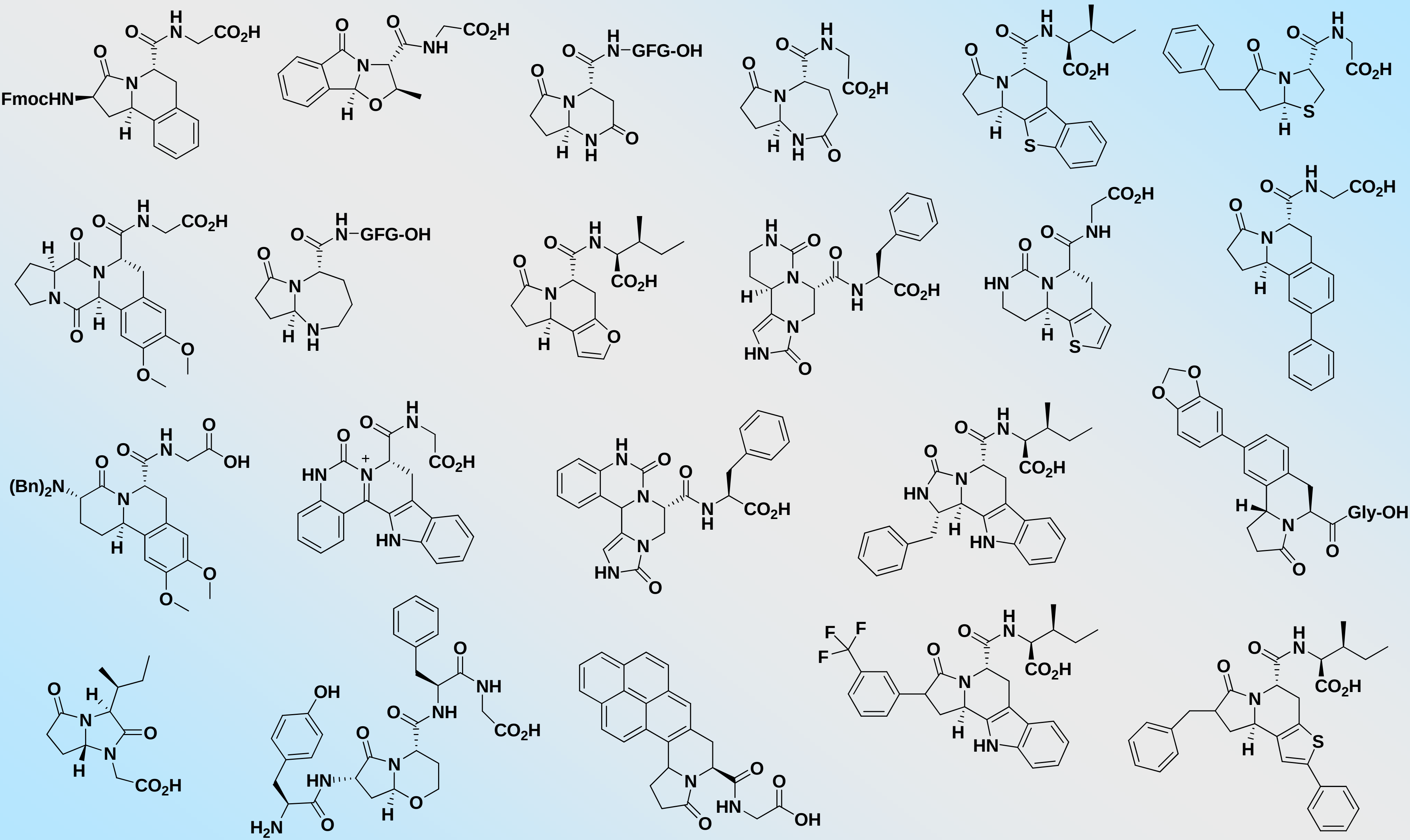
Entry	Solvent	Filter 1 ex480/30 em535/40	Filter 2 ex500/20 em535/30	Filter 3[b] ex540/25 em605/50	Filter 4 ex550/25 em605/70
1	0.1 M HCl	91 ms^{-1}	39 ms^{-1}	1487 ms^{-1}	814 ms^{-1}
2	water	159 ms^{-1}	41 ms^{-1}	605 ms^{-1}	487 ms^{-1}
3	P-buffer[c]	510 ms^{-1}	281 ms^{-1}	113 ms^{-1}	103 ms^{-1}
4	0.1 M NaOH	463 ms^{-1}	87 ms^{-1}	32 ms^{-1}	46 ms^{-1}





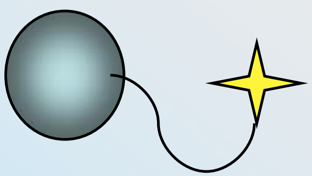
a) 0.1 M HCl,
b) b) water,
c) c) 0.1 M phosphate buffer, pH = 7.2,
d) d) 0.1 M NaOH.

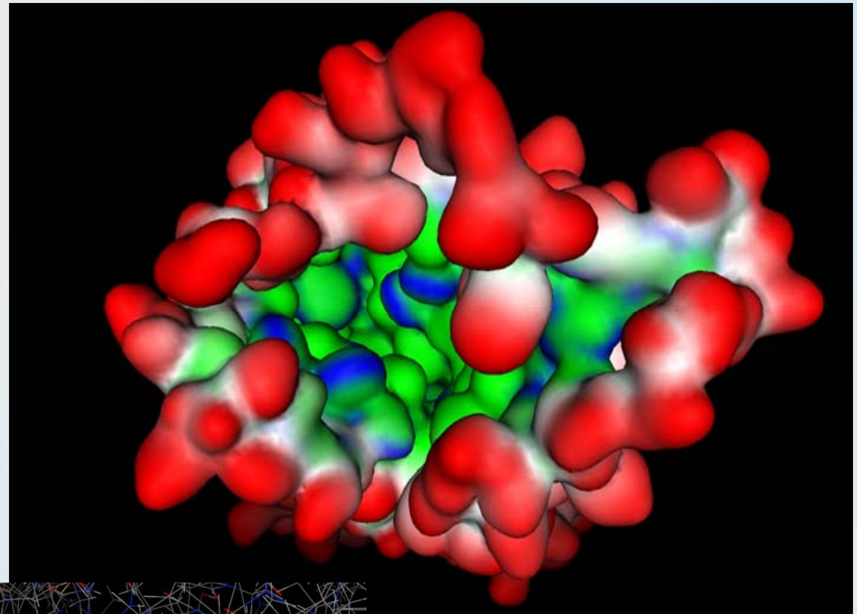
Incredible Scaffold Diversity from a Single "Click" Reaction



Rodopsin based homology model of h-MCR4

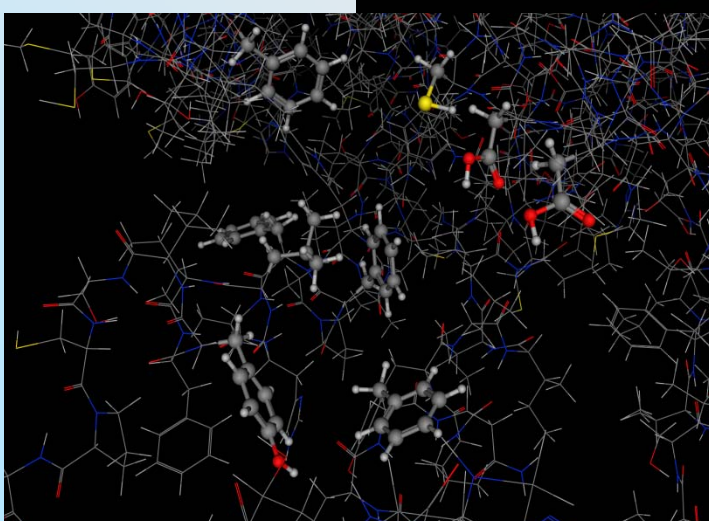
MCR's
NK
CCK
Morphine
etc



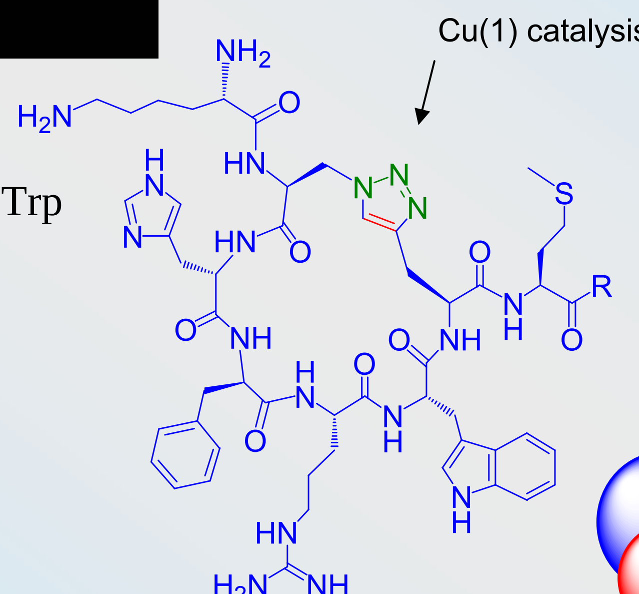


MCR4:

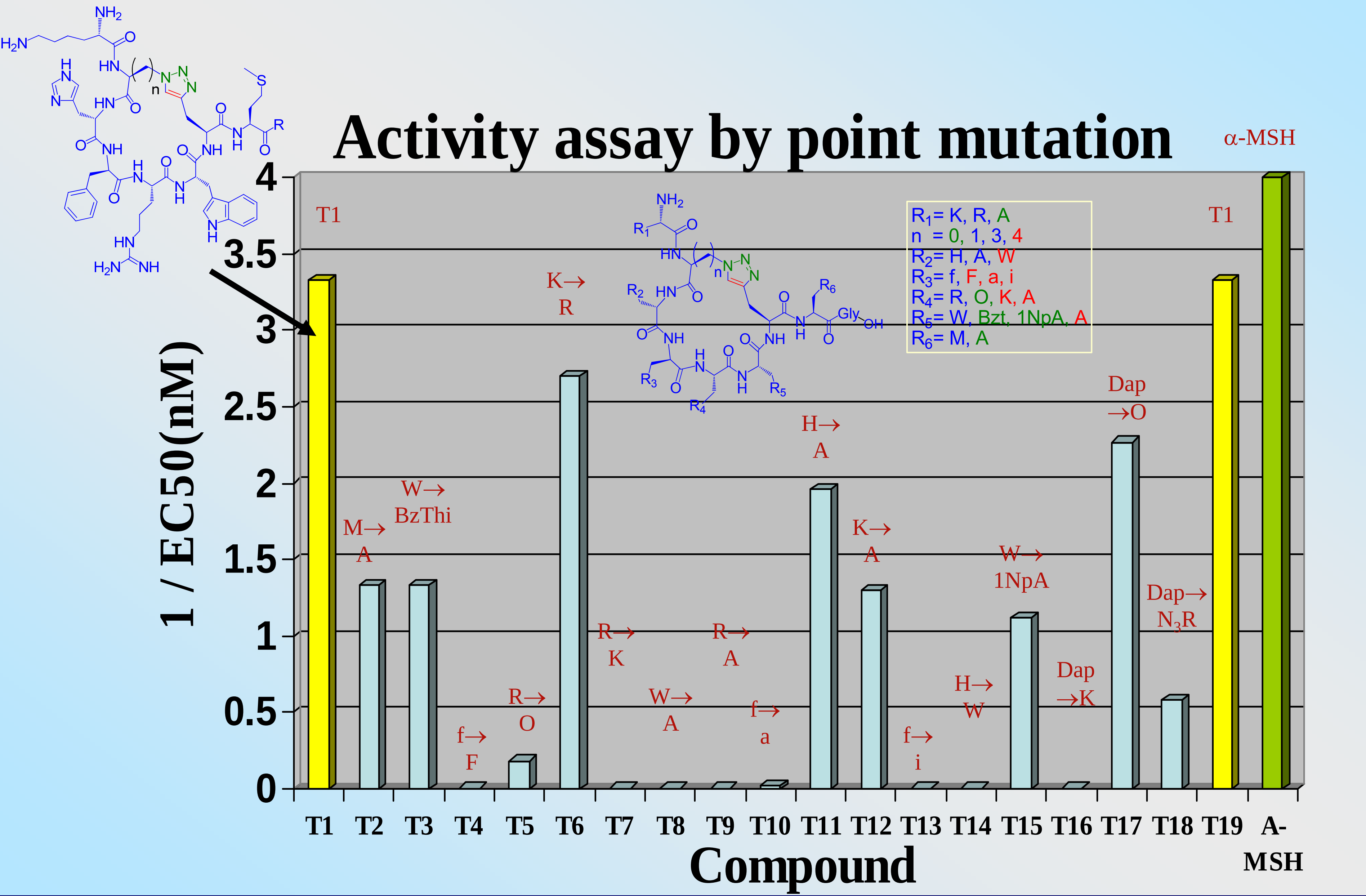
Energy homeostasis
Food intake
Obesity



His-phe-Arg-Trp



Solution Assays of Selected Triazoles Towards MCR4



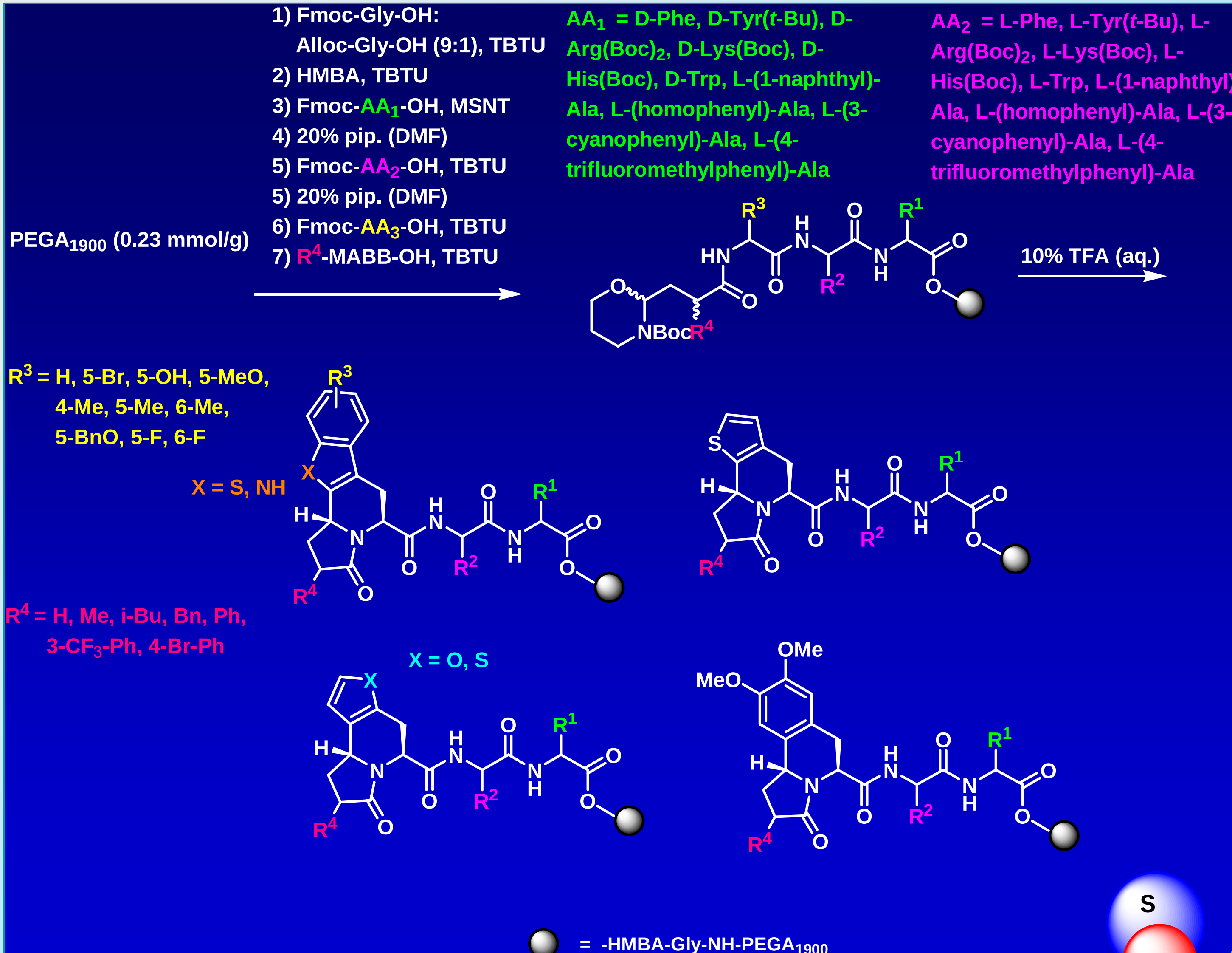
GPCR library by Pictet-Spengler reactions

Synthesis of a 10500-membered library

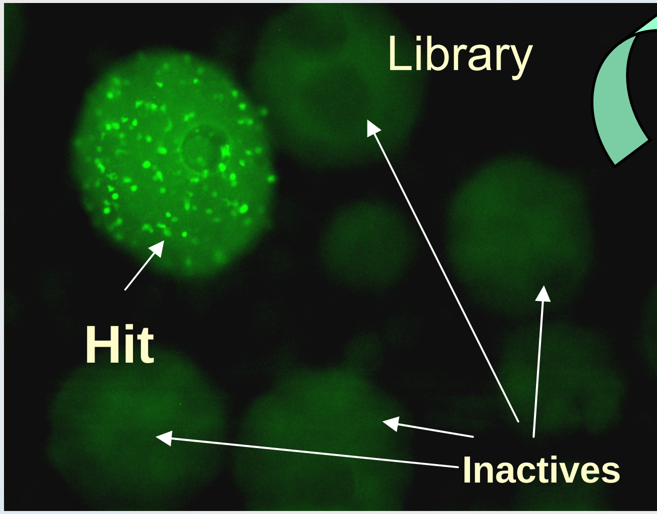
34500-membered library including stereoisomers

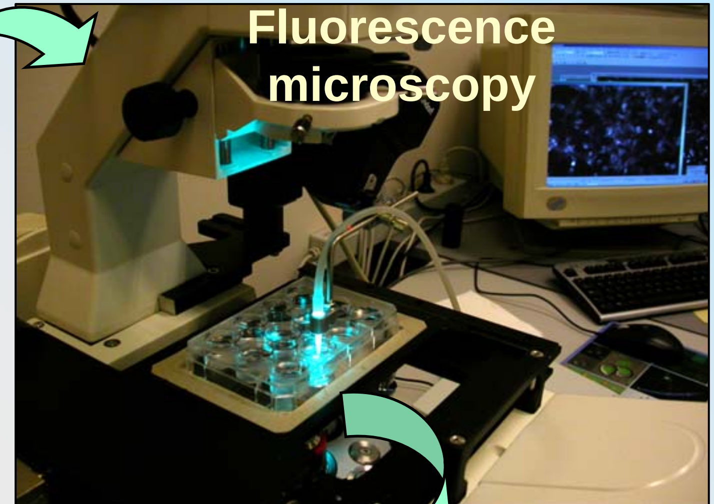
Target: Solid Phase whole cell receptor assay

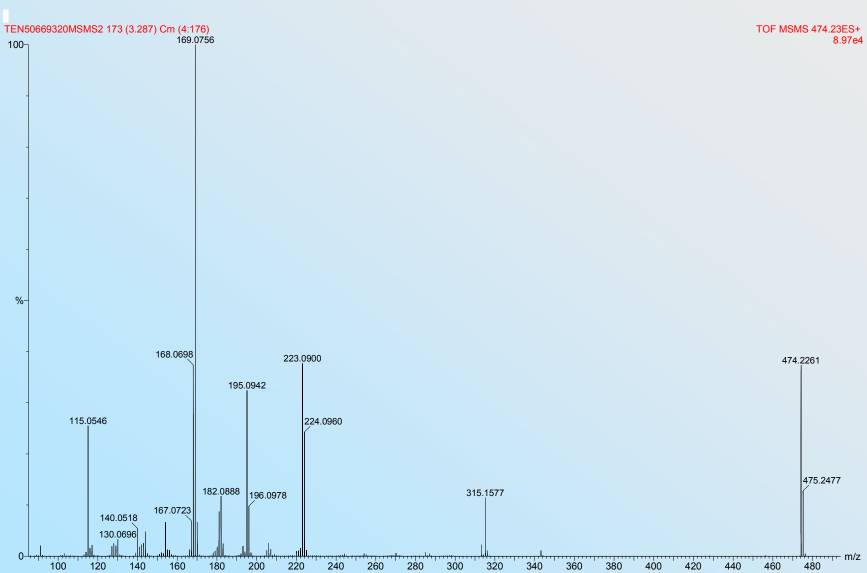
Structure determination by single bead ES MSMS analysis

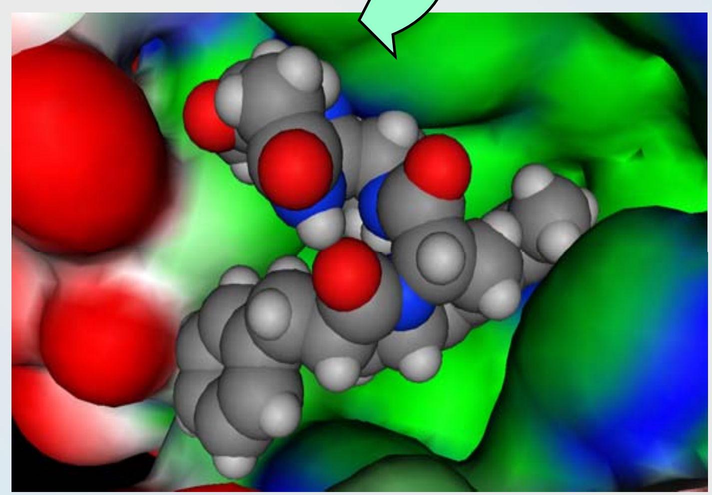


Mammalian cells are screened on library beads for functional activity

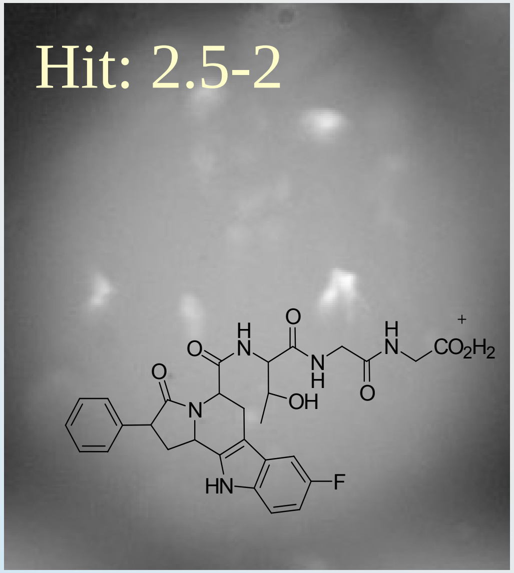


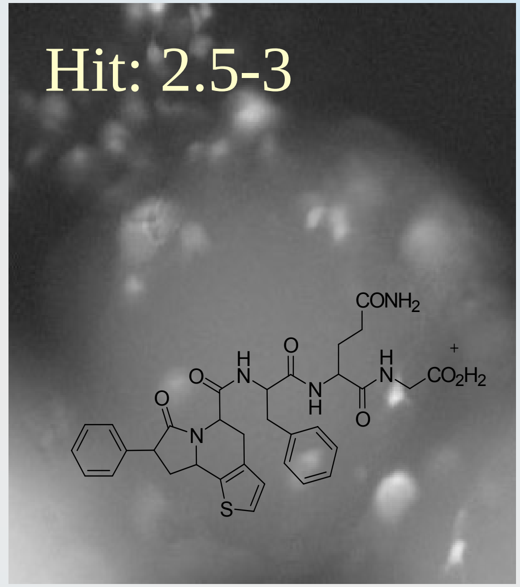


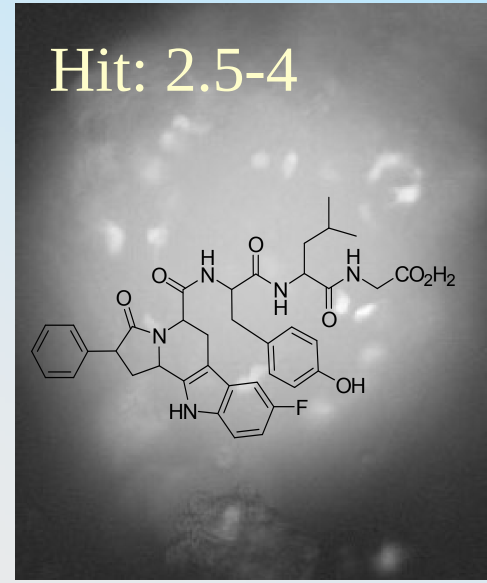


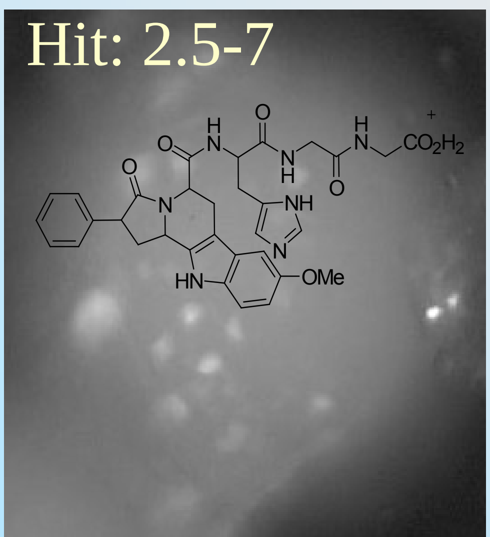


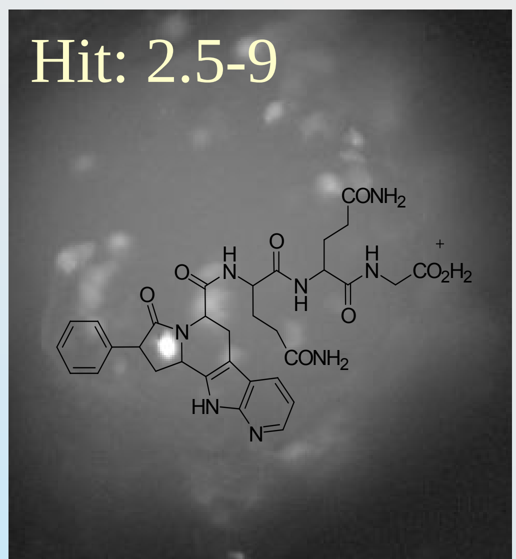
Structures of most active hits

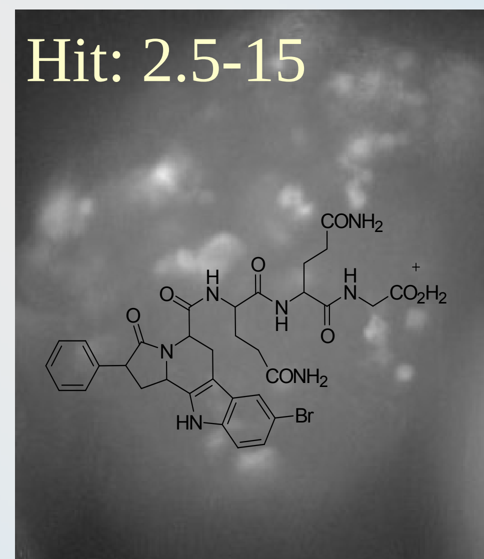




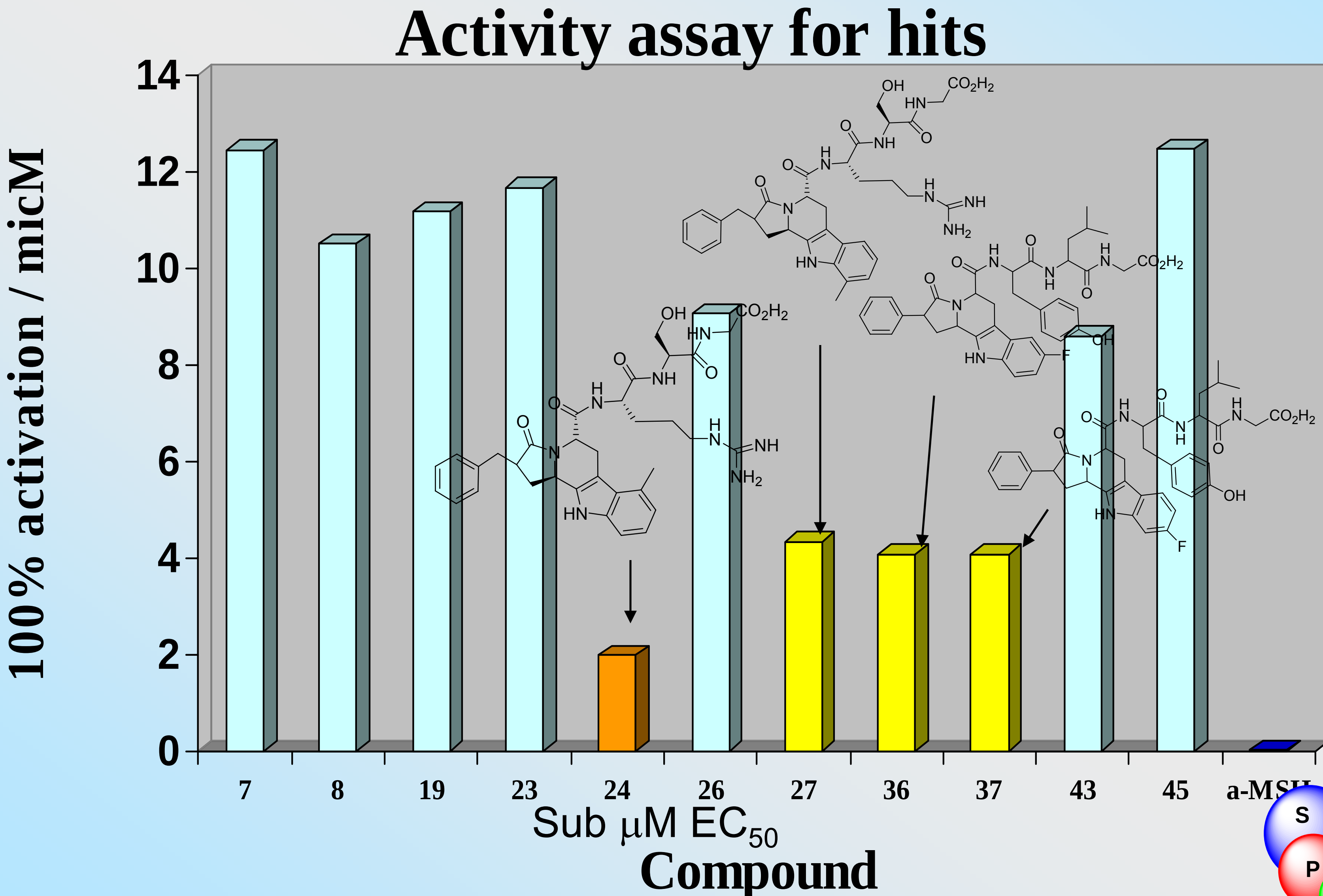








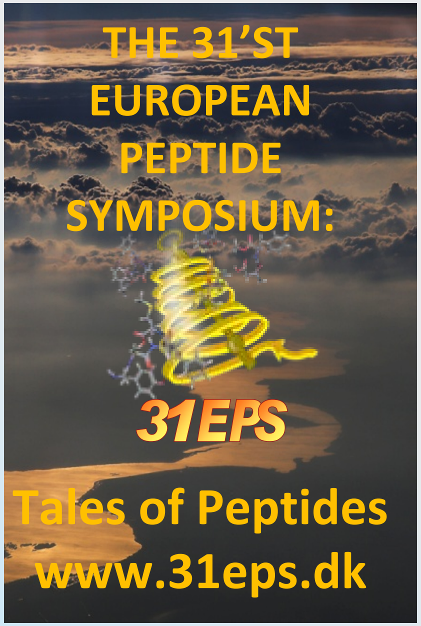
Solution assays of selected PS-hits towards MCR4



Conclusion:

A new plasmid construct for GPCR’s was presented
Stable reporter/GPCR expression was established
Homogeneous cells by cloning
Cellular adhesion to beads established
Intramolecular click reactions for receptor ligand synthesis
Merging peptide diversity with small molecule chemistry
Extremely high scaffold and ligand diversity through one reaction
Screening of GPCR’s on solid support in split mix format.

Welcome to EPS in Copenhagen in 2010



Peptide Biochemistry and Biology
Synthetic Chemistry of Peptides and AAs
Peptidomimetics
Peptide Synthesis and Technology
Peptide Properties
Combinatorial Peptide Chemistry
Macromolecular Peptide Assemblies
Ligation and Chemoselective PepChem
Peptide Materials and Catalysts
Antibacterial- CCP's and Lipo-peptides
Peptide Signalling
Neuro-peptides and Peptide Hormones
Peptide Nanotechnology
Expansion of the Genetic Code
Prodrugs, Targeting and Uptake
Chemical Biology and Proteomics

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