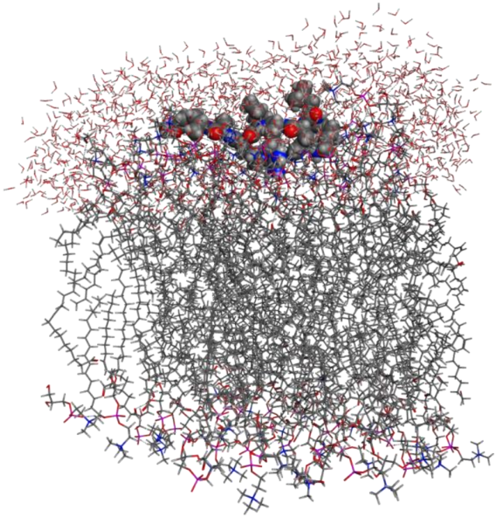
Intramolecular N-AcylIminium Cascade reactions

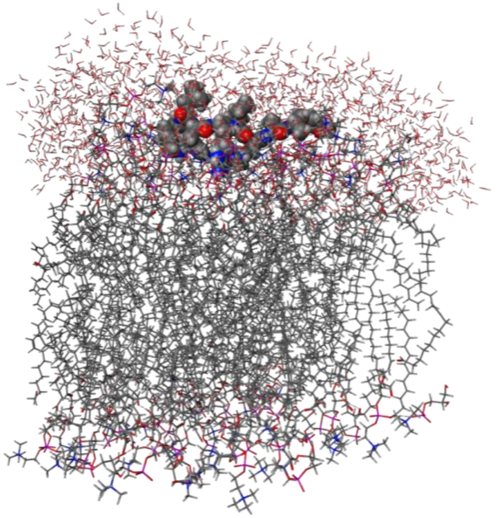
INAIC

Click reactions on solid support

For CombiChem and GPCR's



CECB
Nano-Science Center
Copenhagen University
Denmark



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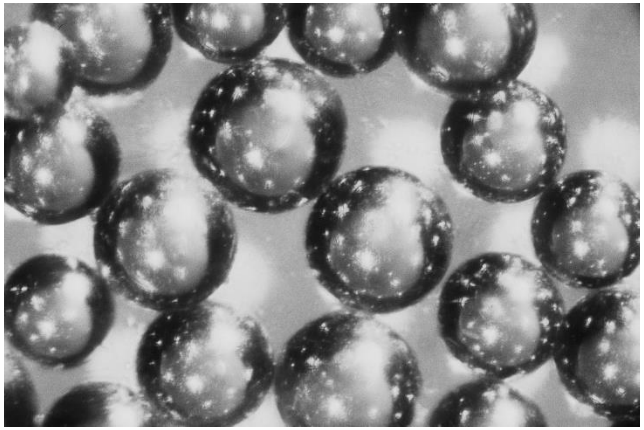
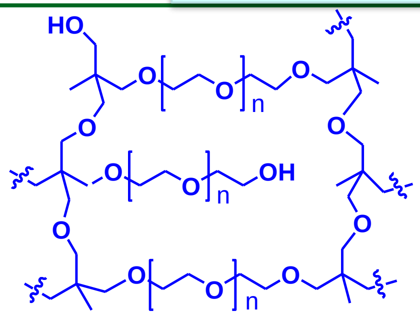
Solid phase combinatorial chemistry

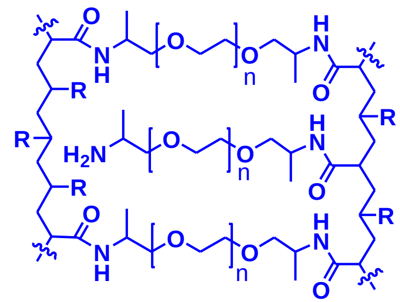
Quantitative on Resin Enzymatic Transformation:

Subtilisin/PEGA
Trypsin/PEGA
Chymotrypsin/PEGA
Pepsin/PEGA
Papain/PEGA
MMP9/PEGA4000 or 6000
MMP12/PEGA
Cruzipain/PEGA
Leishmania-CP/PEGA
Fucosyl transferase/PEGA4000
b-1,4-Galactosyltransf/PEGA
Human and Yeast
Prot. Disulf. Isom./PEGA4000





SPOCC



PEGA

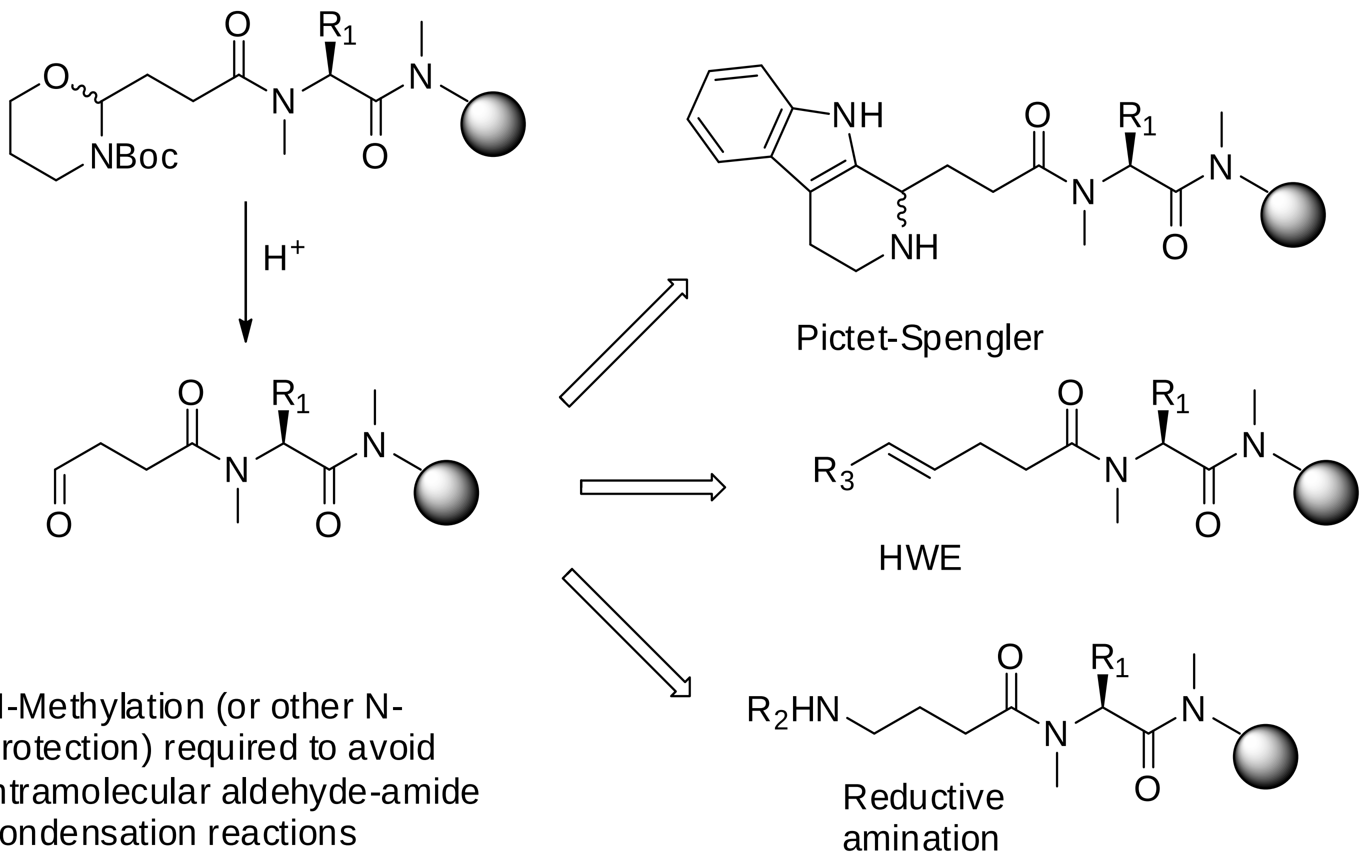
compatible with synthesis and biology

Unique platform for combinatorial synthesis and iterative selection assays in biology.

Quantitative on Resin Chemical Transformations:

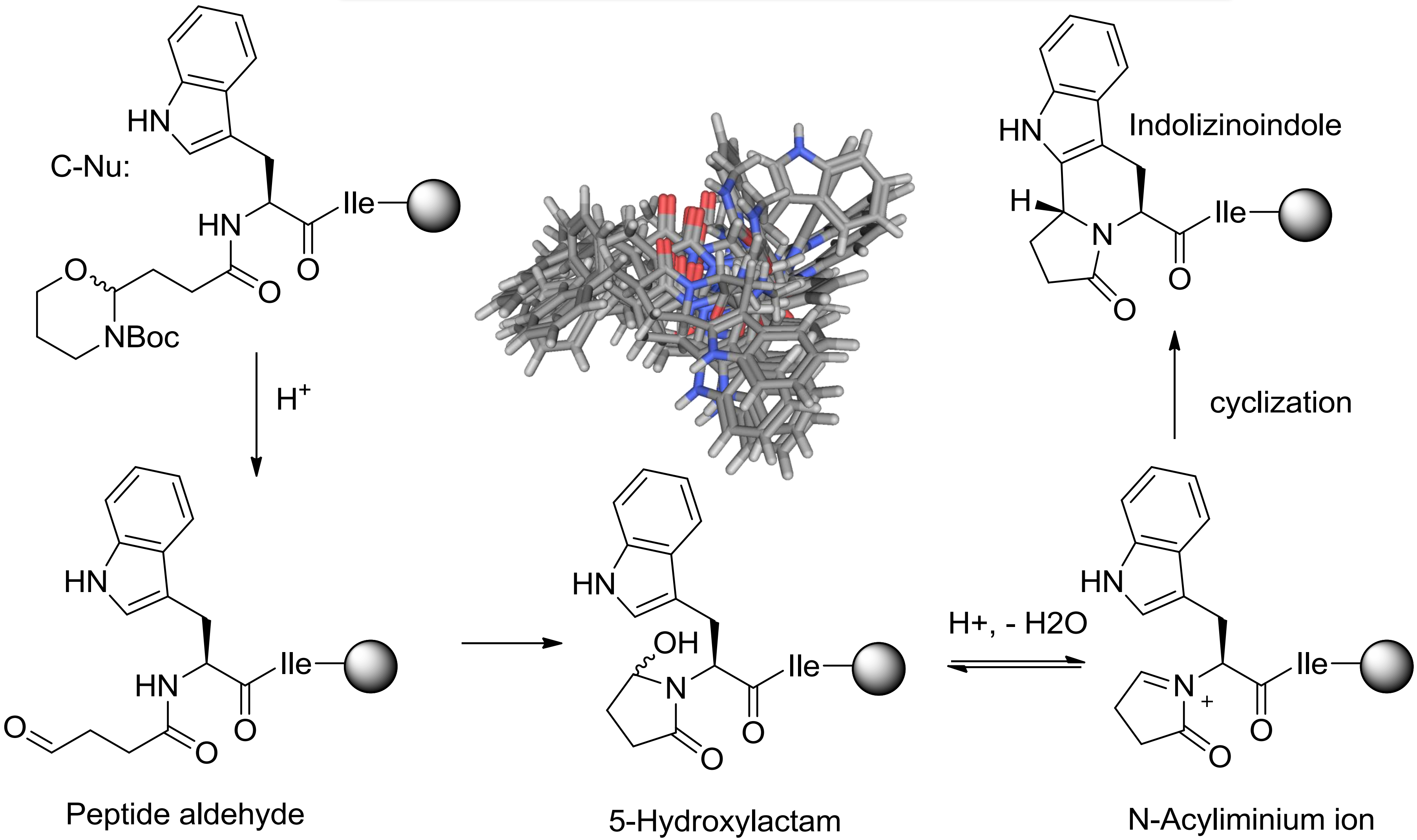
Diels-Alder
Aldol
Nitroaldol
Glycosylation
Transaminations
Metathesis
Wittig-reactions
Redox-reactions
Dihydroxylation-oxidations
WHE-reactions
C-Allylations
Phosphorylations
Sulfatations
Silylations
CuAAC-reactions
***N*-Acyl iminium ion reactions**
***N*-Carbamyl iminium reactions**
Carbene chemistry
Phosphine chemistry
Palladation
Catalytic C-C bond formations
Sonogashira
Suzuki

Unexpected observation……………………..New reaction



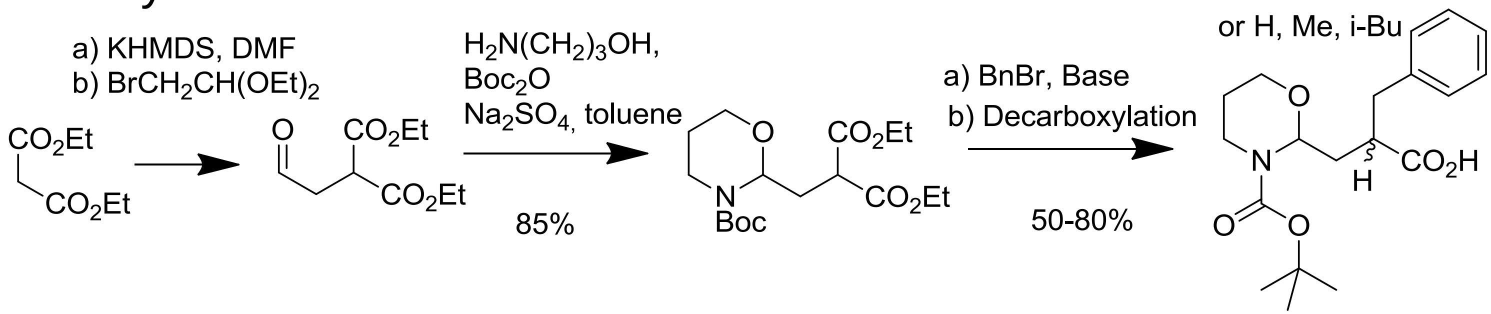
N-Methylation (or other N-protection) required to avoid intramolecular aldehyde-amide condensation reactions

*I*ntramolecular ***N*-*A***cyl ***I***minium ***C***ascade Reactions

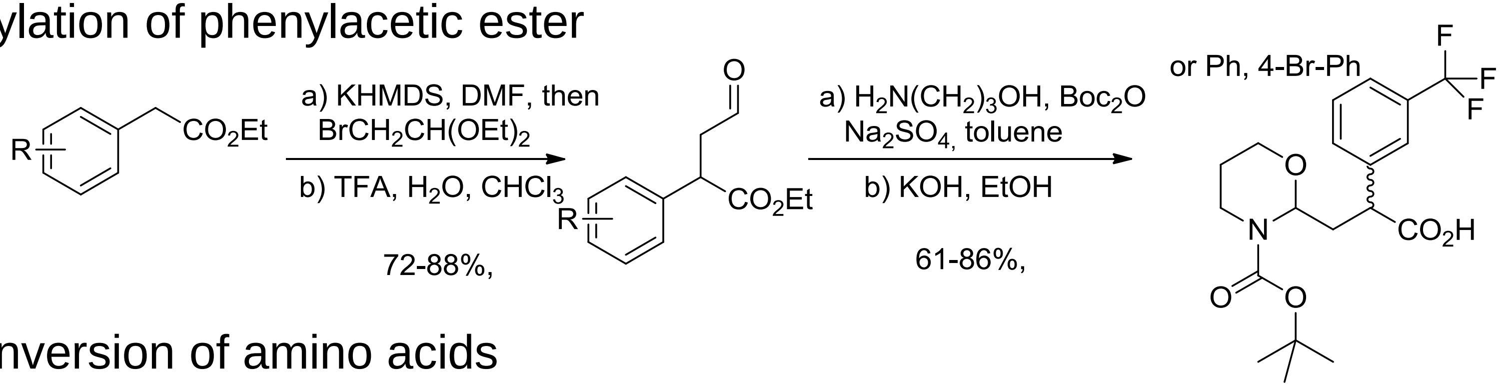


INAIC reaction: Building Blocks

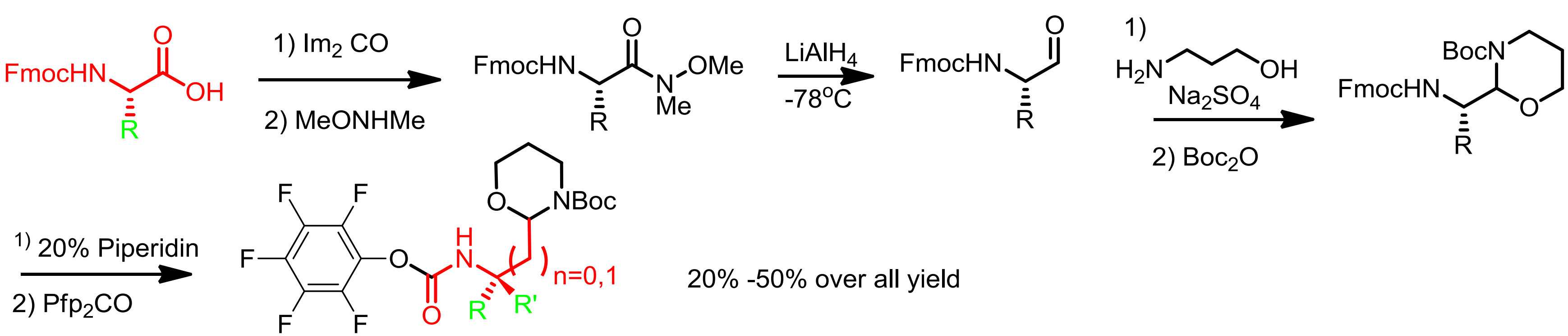
Double alkylation of malonic ester



Alkylation of phenylacetic ester

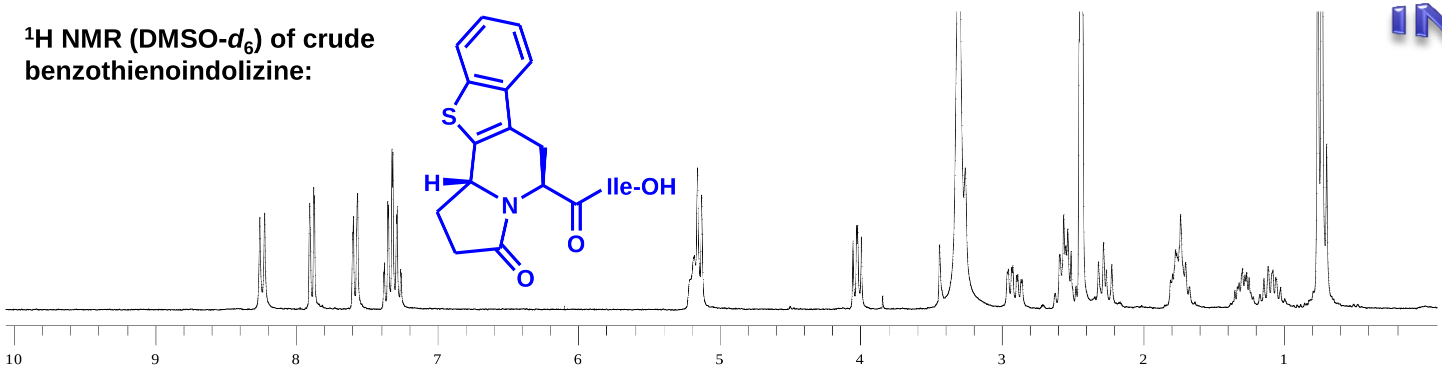


Conversion of amino acids

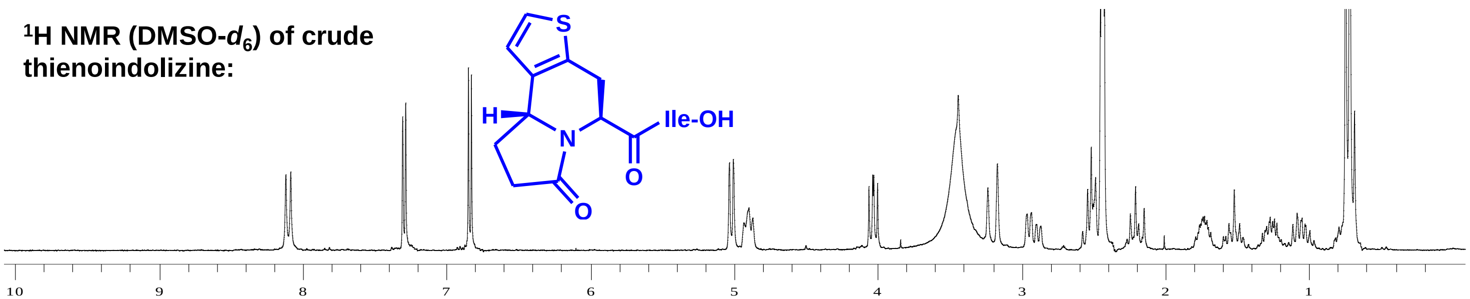


Quantitative Chemical Transformation: Intramolecular “Click” reaction

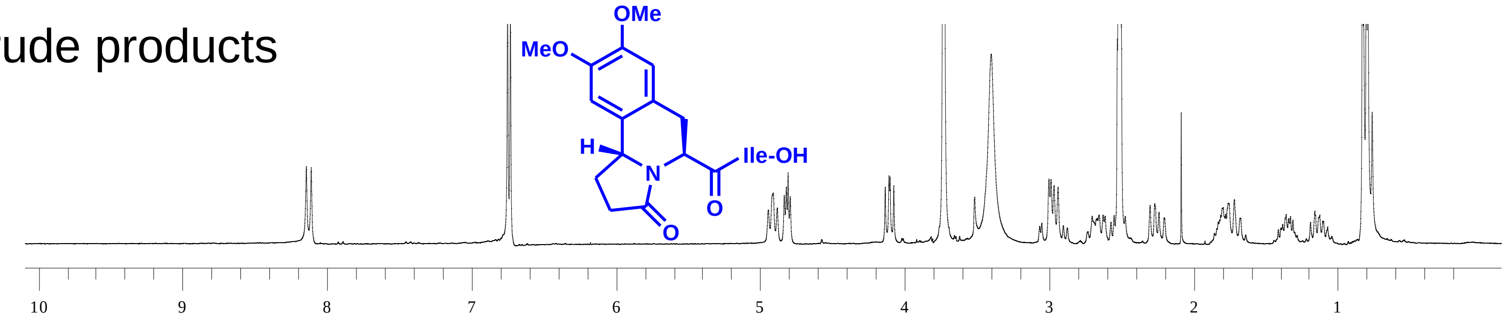
^{1}H NMR (DMSO-d_6) of crude benzothienoindolizine:



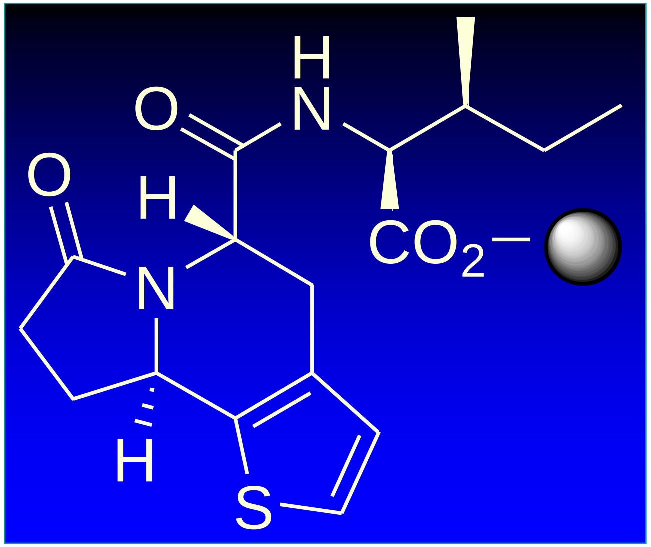
^{1}H NMR (DMSO-d_6) of crude thienoindolizine:

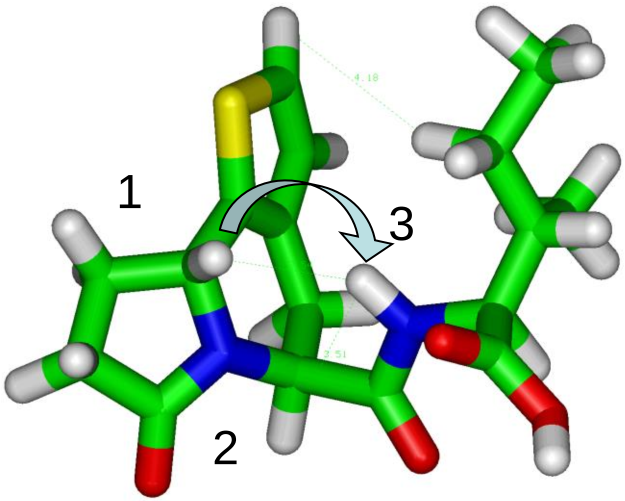


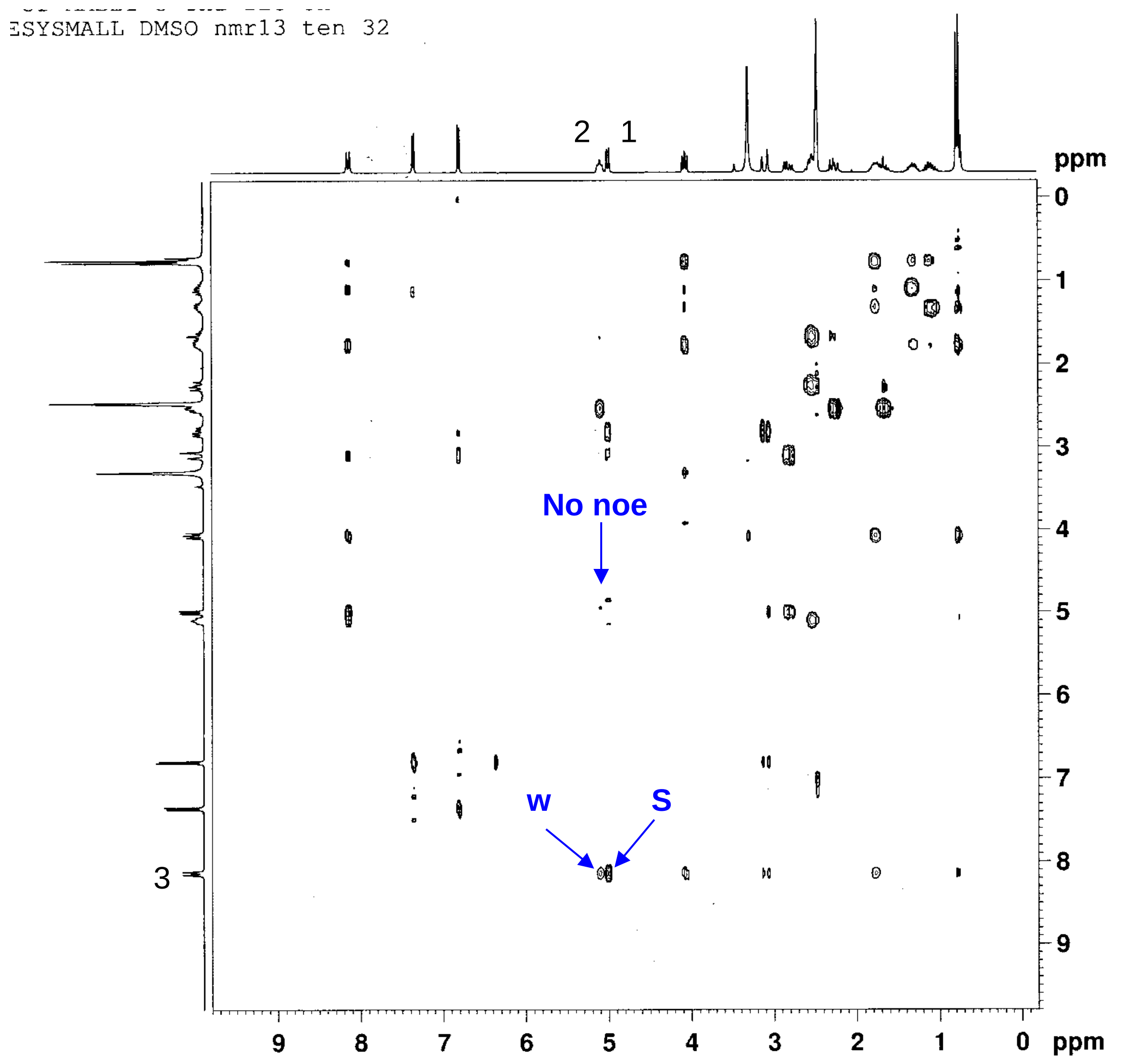
Crude products



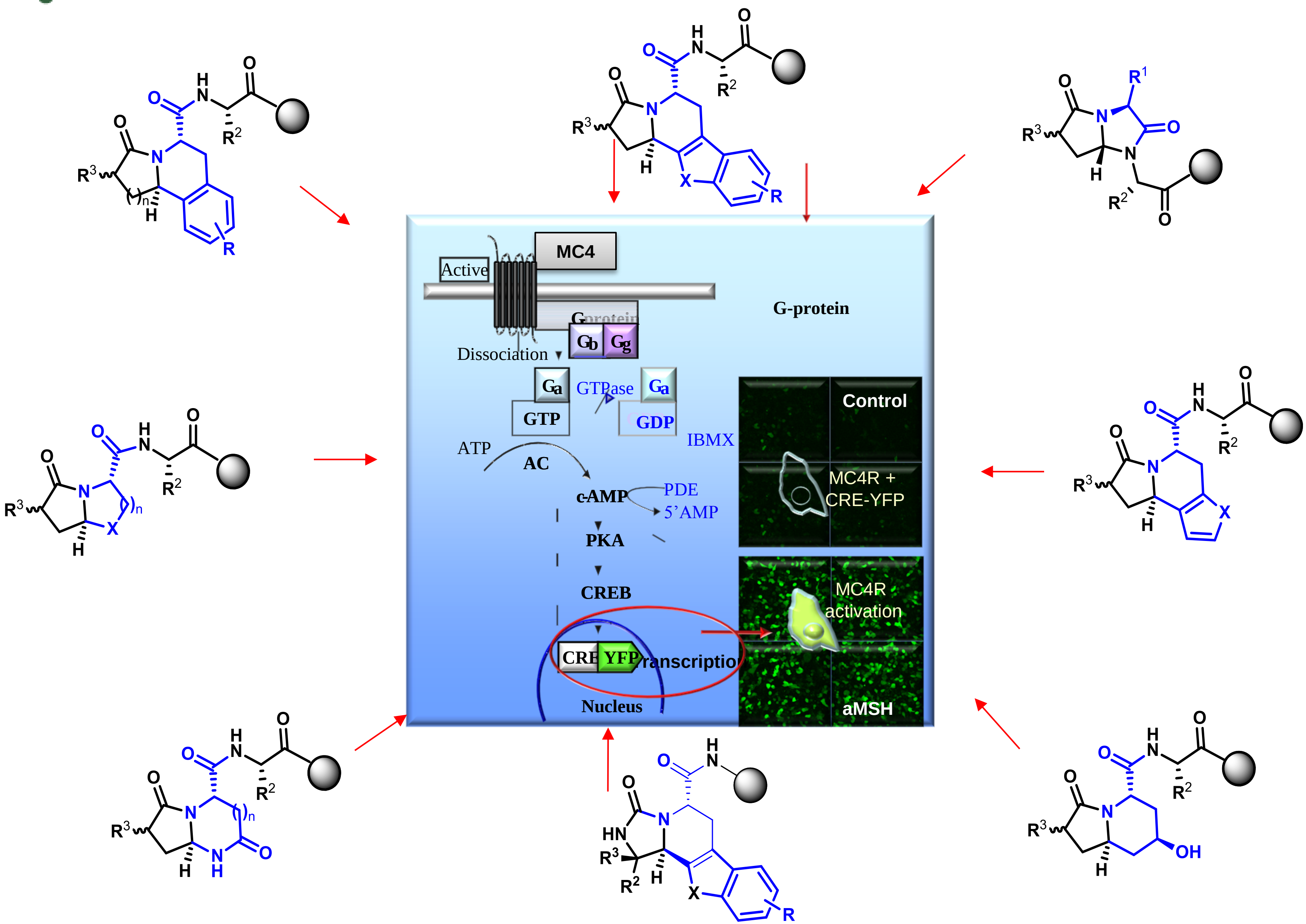
Stereo-selectivity of the INAIC reaction



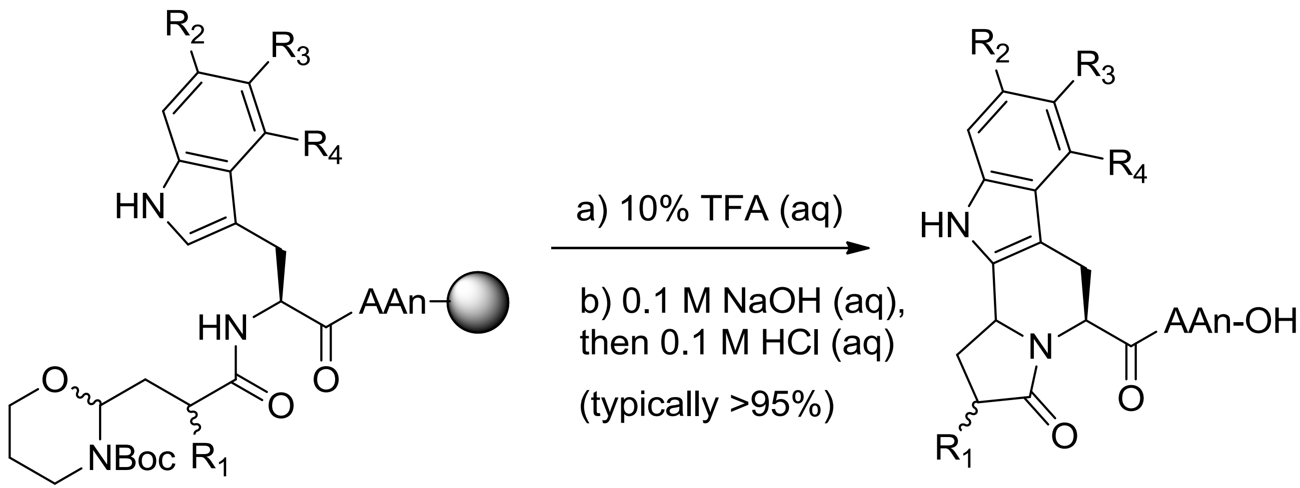


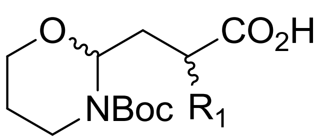


INAIC - Scaffolds for GPCR screening



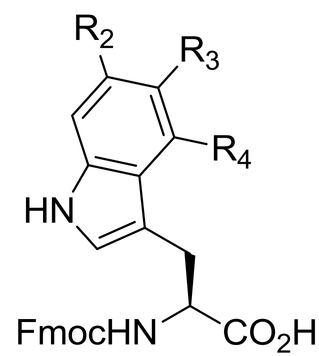
Indoles in INAIC reactions





Substituted masked aldehyde building blocks

R1 =
H,
Me,
HO-CH2,
i-Bu,
Bn,
Ph,
4-Br-Ph,
3-CF3-Ph



Substituted Fmoc-Trp-OH derivatives

(R2, R3, R4) =
(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),

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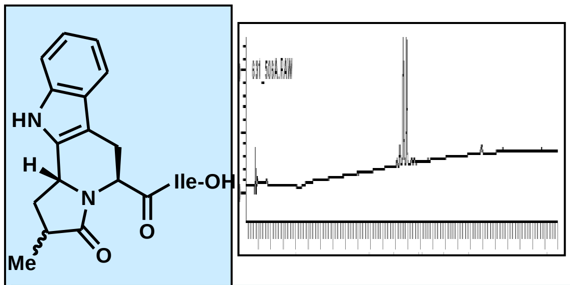
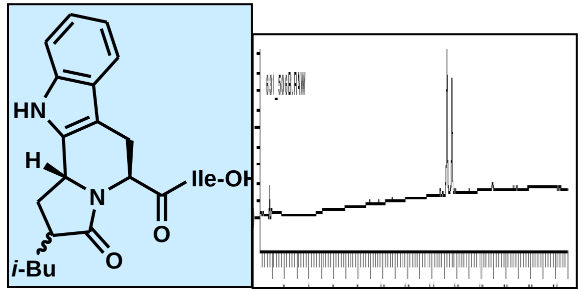
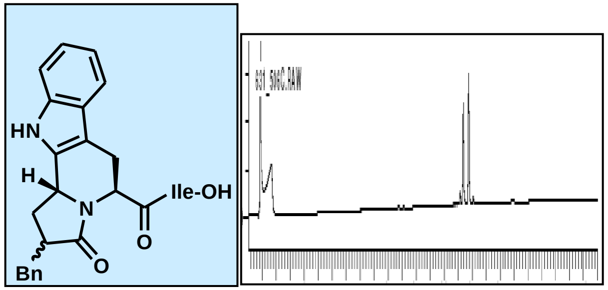
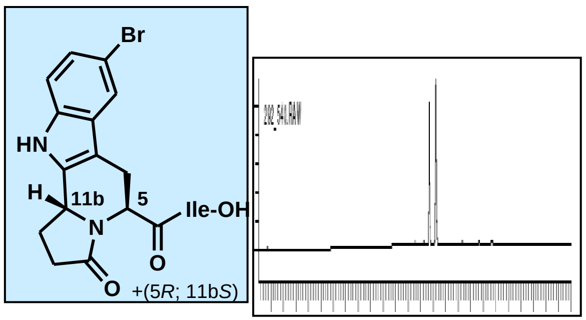
Scaffold diversity: INAIC Reaction

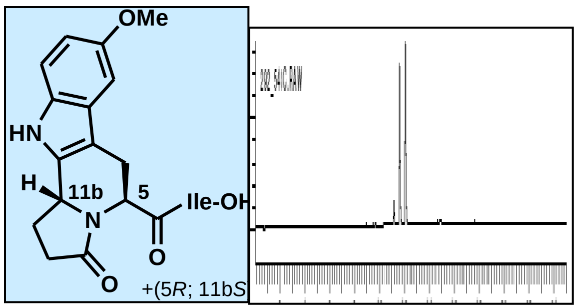


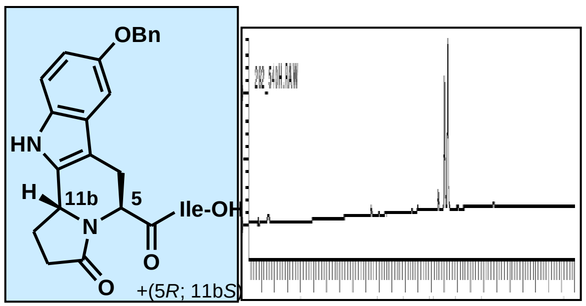


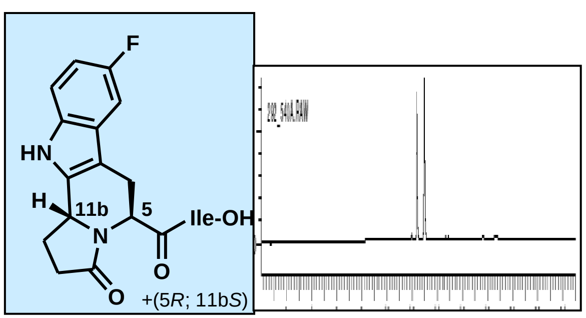


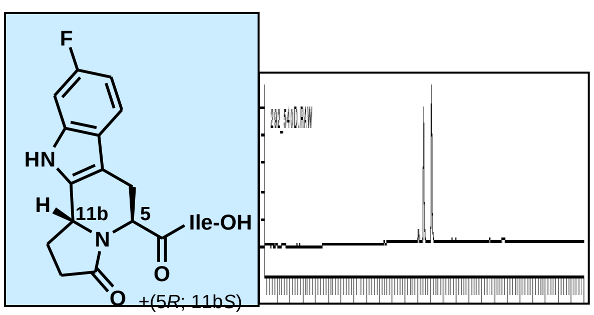
HPLC's of crude products; purity >95%; *dr* = 1:1

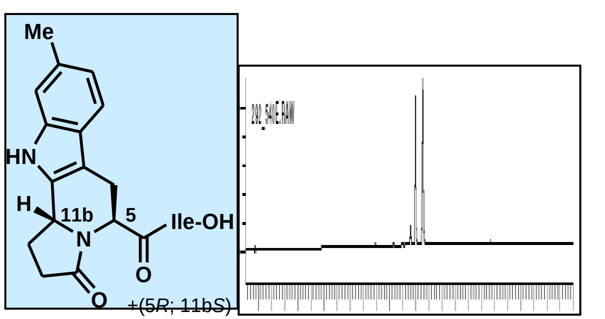




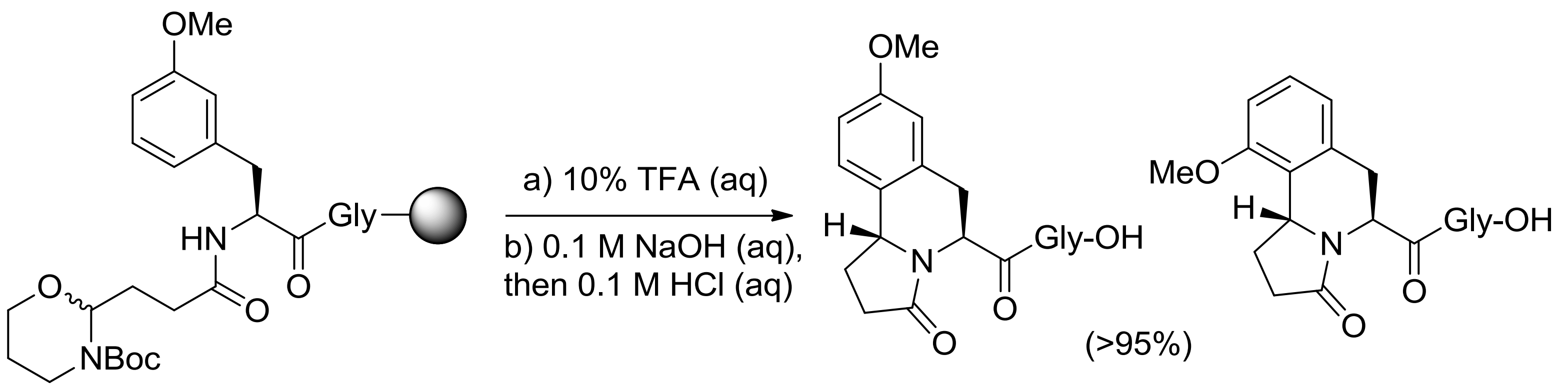


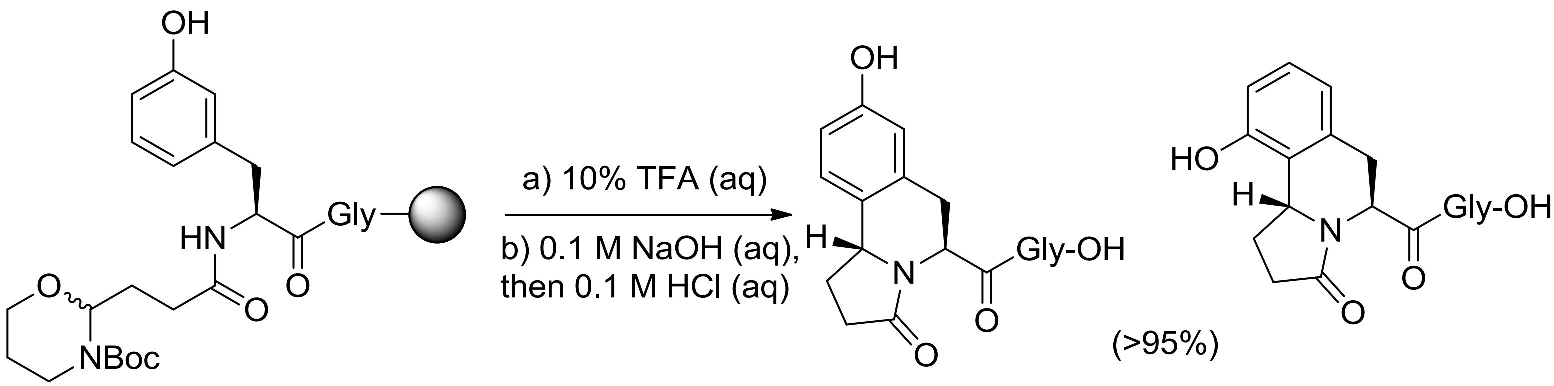


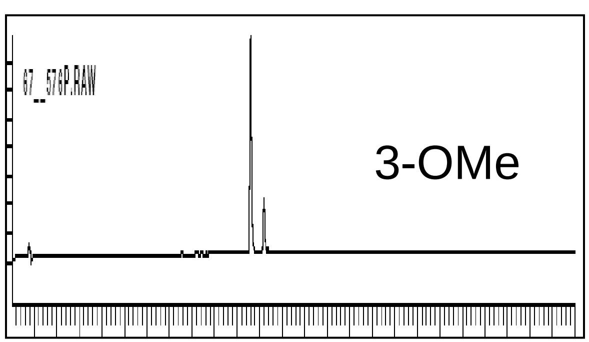


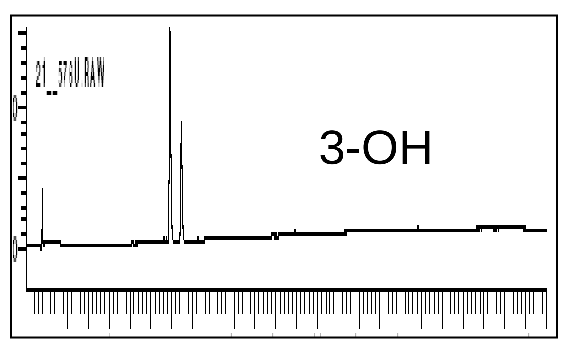


INIAC: Regioselectivity

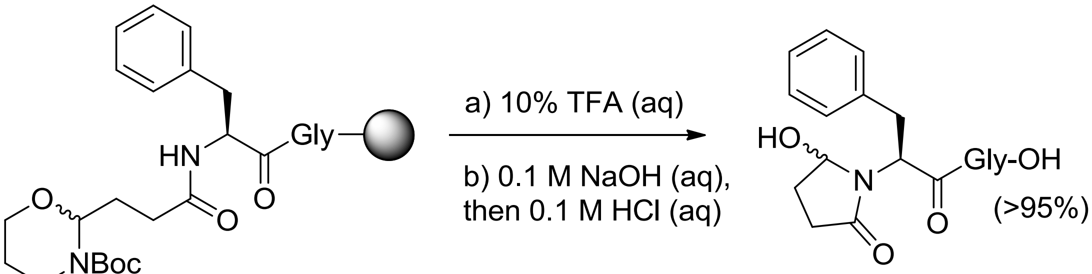


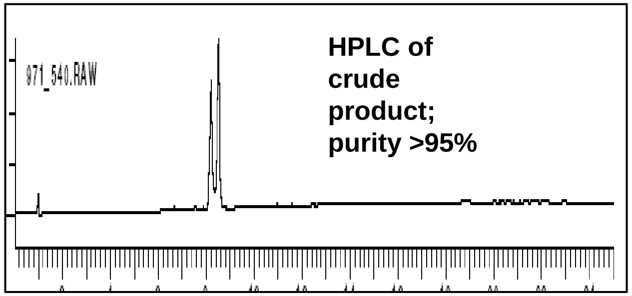


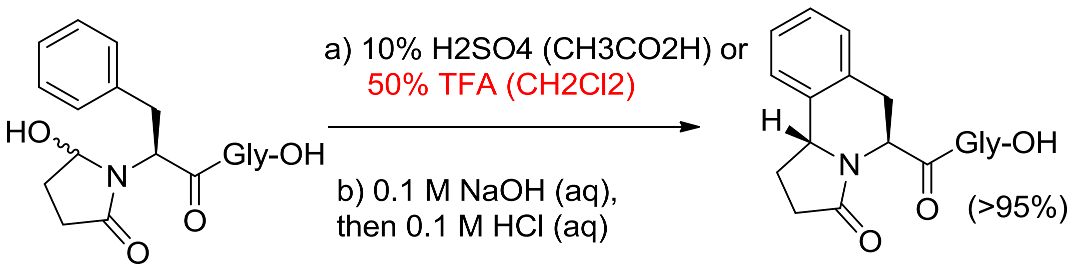


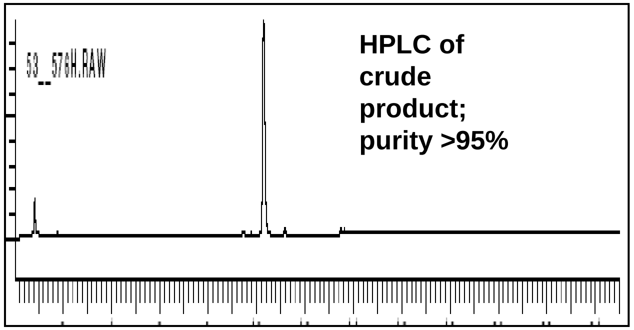


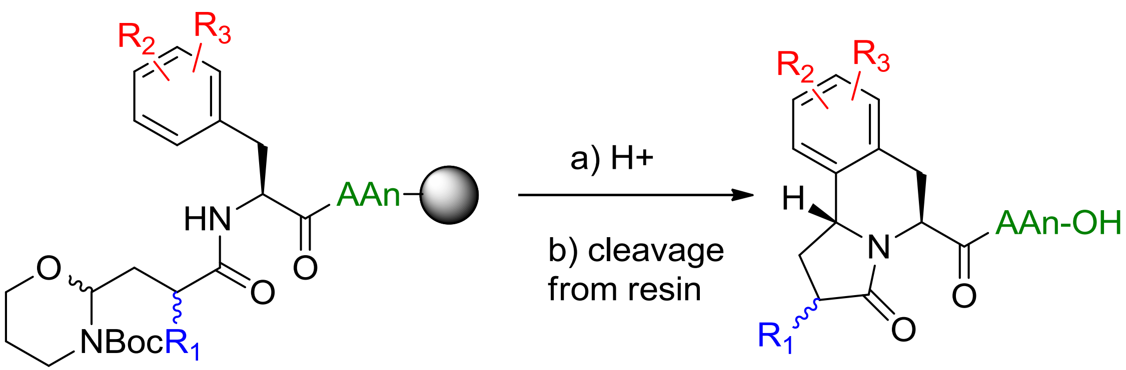
INAIC: Non-activated nucleophiles











Pyrroloisoquinoline

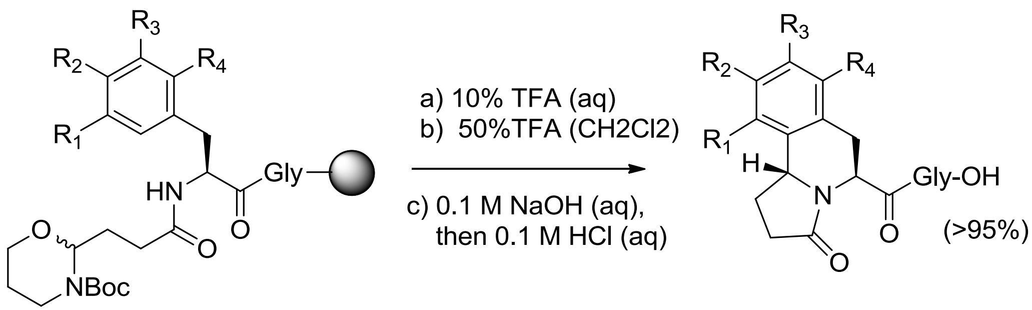
Building blocks :

Fmoc-amino acids AA

(R2,R3)-Substituted Fmoc-Phe-OH derivatives

R1-Substituted masked aldehyde building blocks

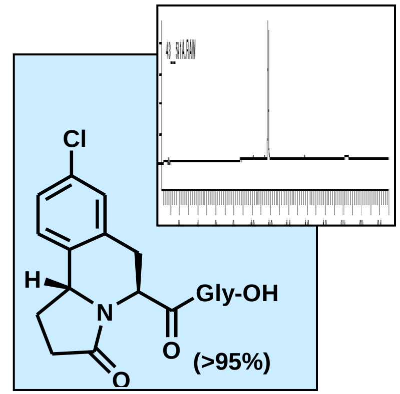
Scaffold diversity: Deactivated nucleophiles

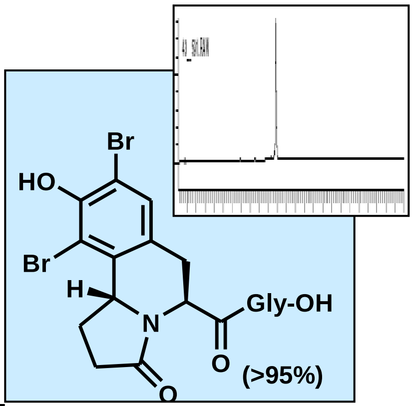


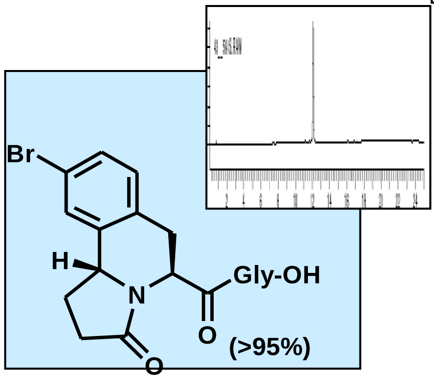
Entry	R^1	R^2	R^3	R^4
1	H	OH	H	H
2	H	OMe	H	H
3	H	Ph	H	H
4	H	Me	H	H
5	H	H	Me	H
6	H	H	H	Me
7	H	Br	H	H
8	H	I	H	H
9	H	Cl	H	H
10	H	H	Cl	H
11	H	Cl	Cl	H
12	Br	OH	Br	H

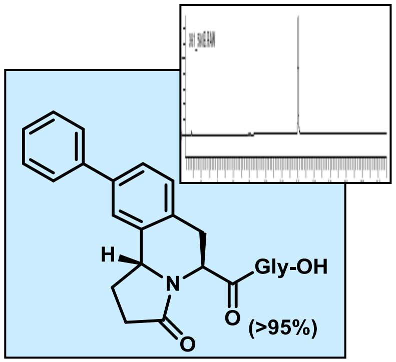
Incomplete cyclizations when R is -NH_2, -N_3, -CF_3, -NO_2, -CN

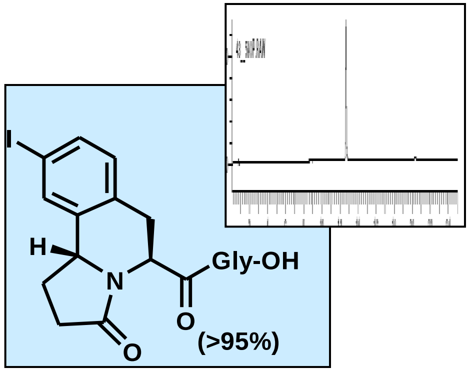
Two step cyclization

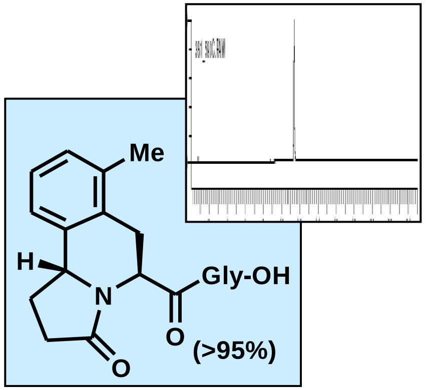


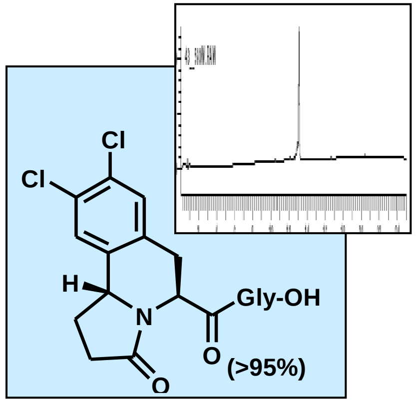




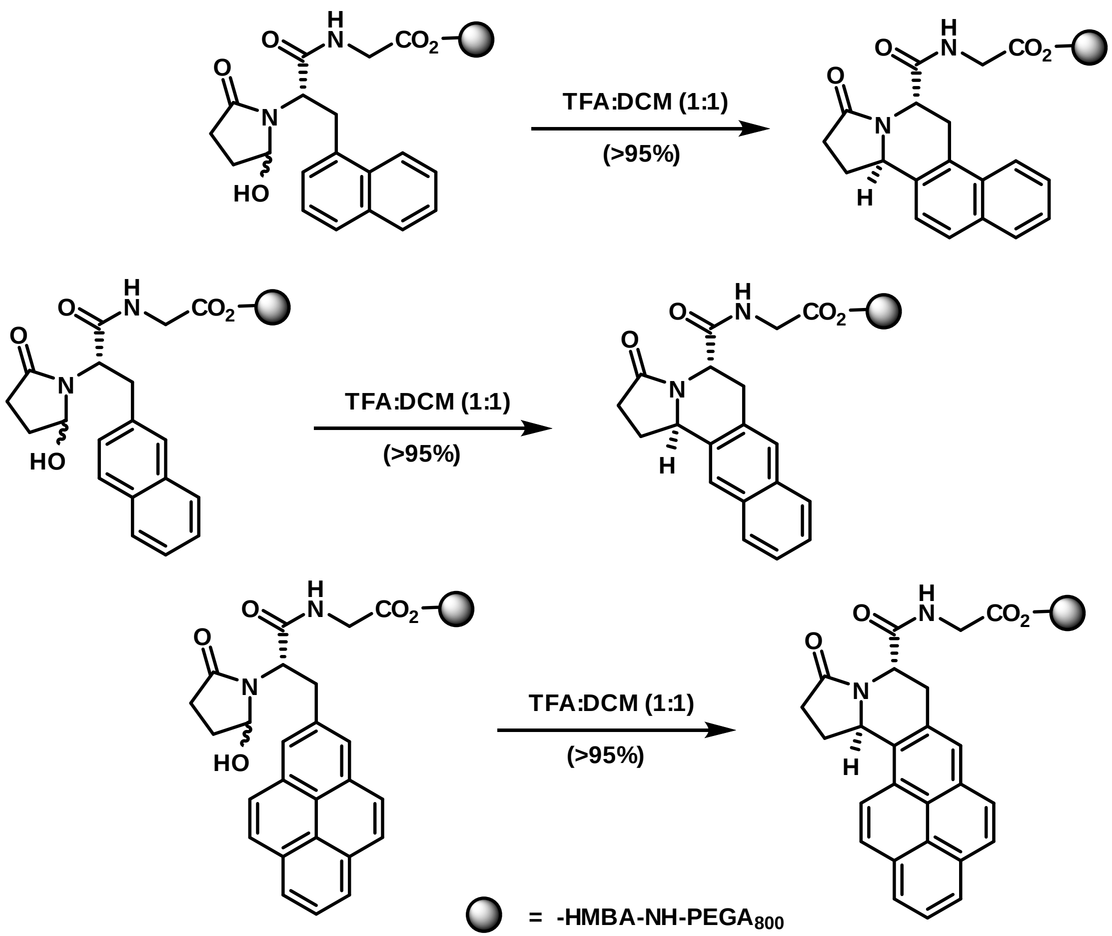


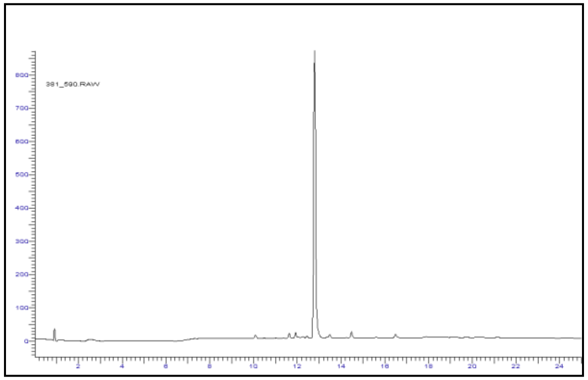


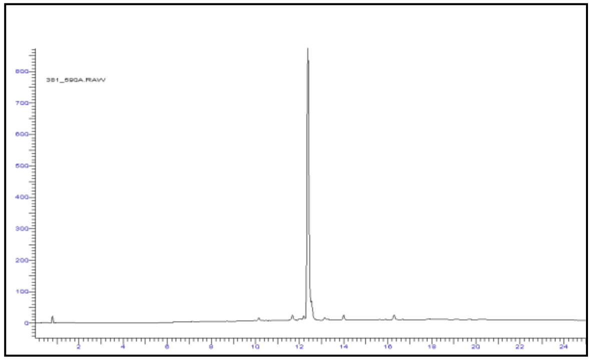


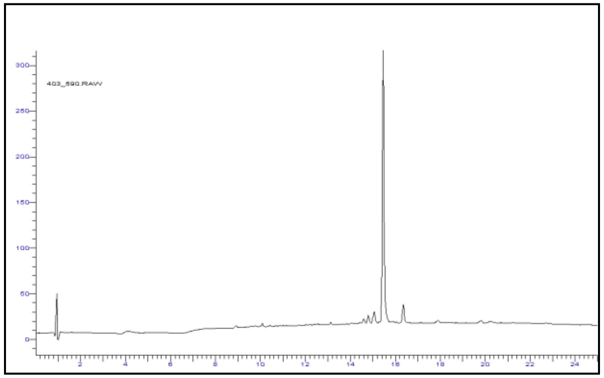


Scaffold diversity: Fused Aromatic Ring-systems

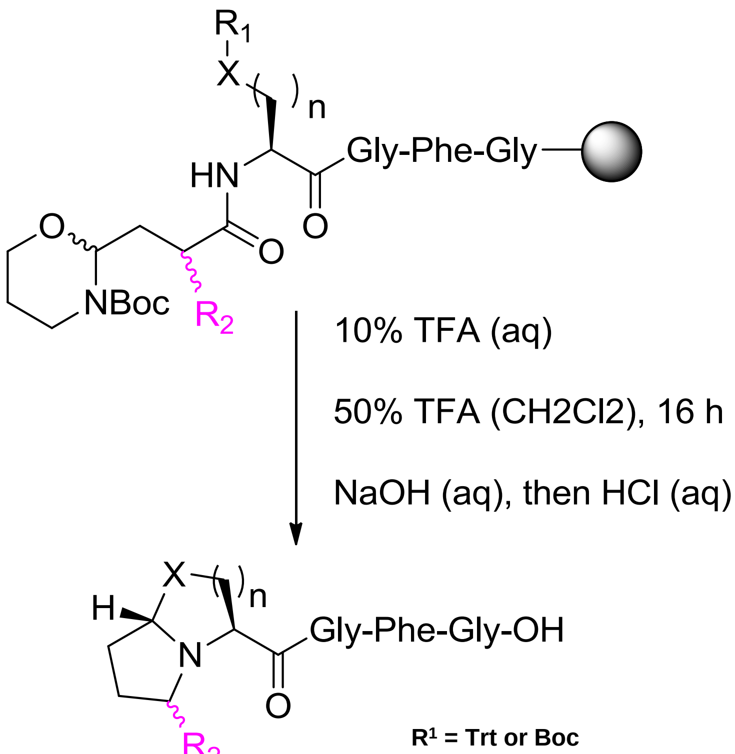






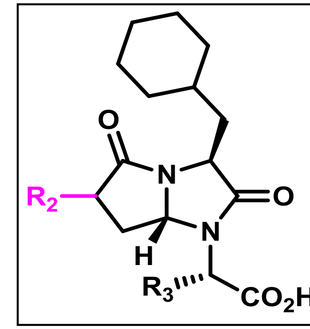


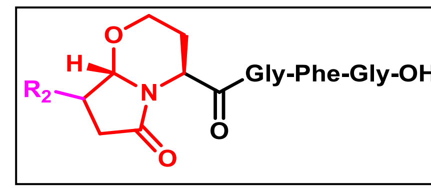
INAIC: Heteroatom nucleophiles

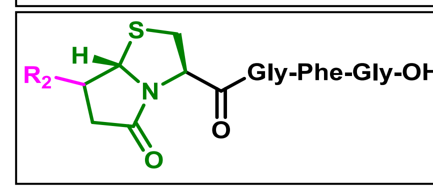


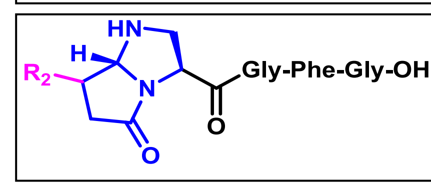
R^1 = Trt or Boc

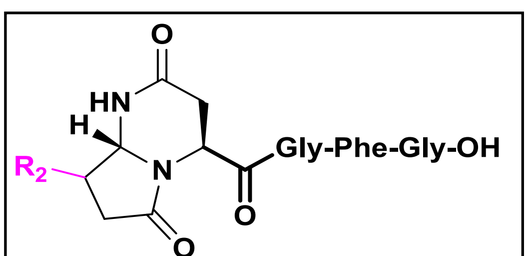
Entry	X	n	R^2	Purity (%)
1	O	1	H	complex mixture
2	O	2	H	>95
3	O	2	*i*-Bu	>95
4	O	2	Bn	>95
5	S	1	H	91
6	S	1	*i*-Bu	94
7	S	1	Bn	>95
8	NH	1	H	>95
9	NH	1	*i*-Bu	91
10	NH	1	Bn	91
11	NH	2	H	>95
12	NH	3	H	>95
13	NH	4	H	complex mixture

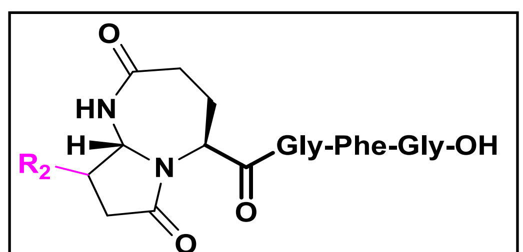


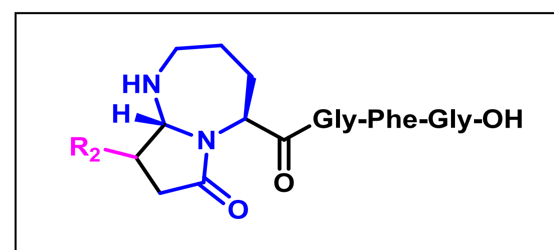




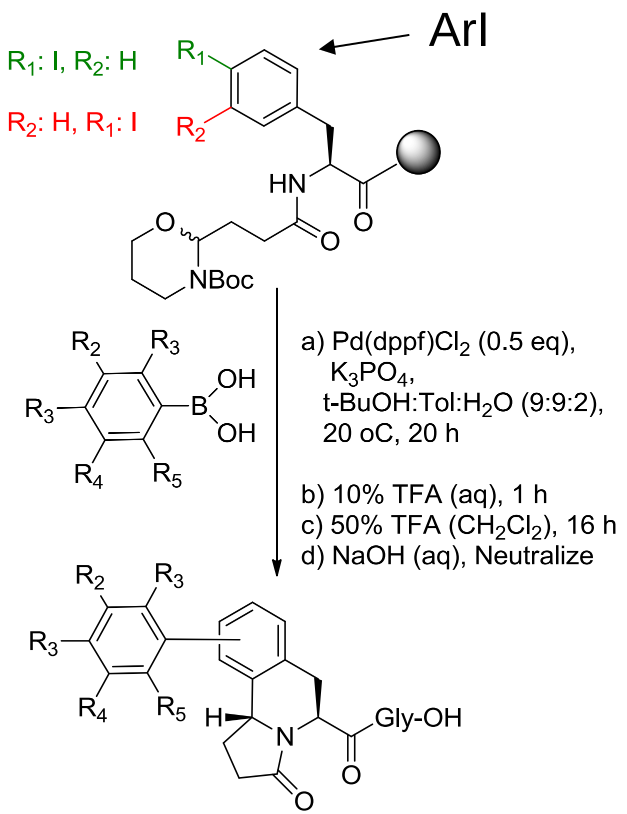






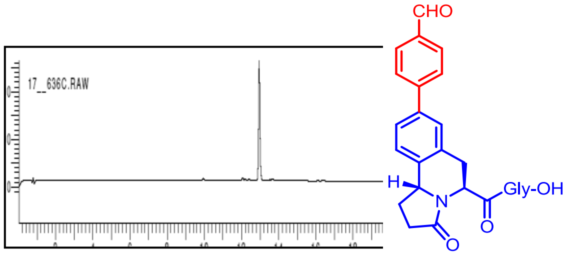


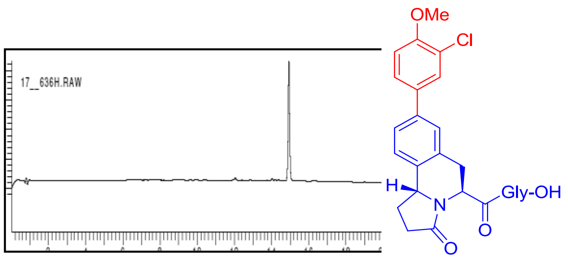
Suzuki prior to INAIC reactions

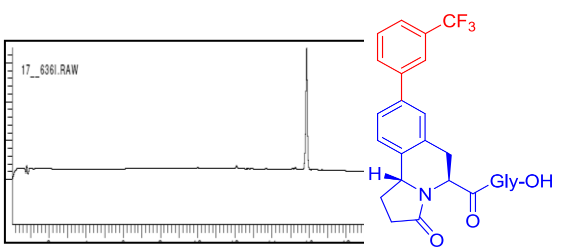


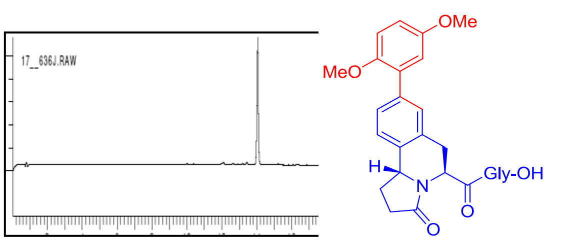
Entry	Ar	Product, Purity (%)
1	Ph	IIIa, >95; IVa, >95
2	4-Me-Ph	IIIb, >95; IVb, >95
3	4-(CHO)-Ph	IIIc, >95; IVc, >95
4	2-MeO-Ph	IIId, >95; IVd, >95
5	4-BuO-Ph	IIIe, 89; IVe, >95
6	4-MeS-Ph	IIIf, 85; IVc, 90
7	4-MeO-Ph	IIIg, >95; IVg, >95
8	4-MeO-3-Cl-Ph	IIIh, >95; IVh, >95
9	3-CF_3-Ph	IIIi, >95; IVi, >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

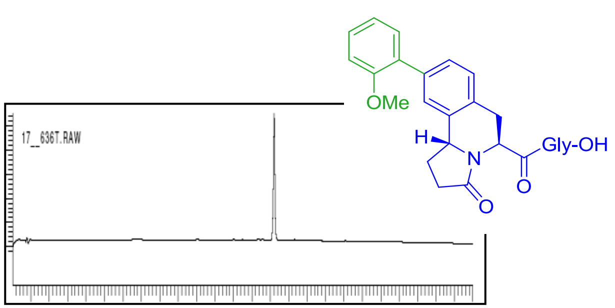
HPLC's of crude product; purity >95%

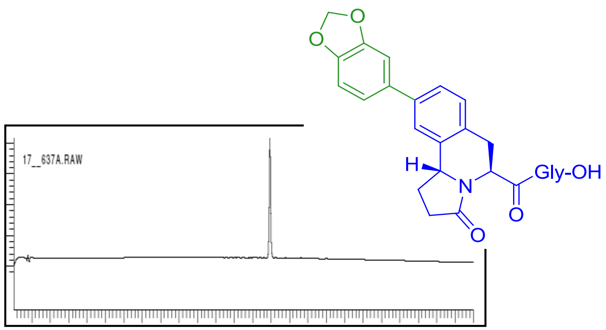






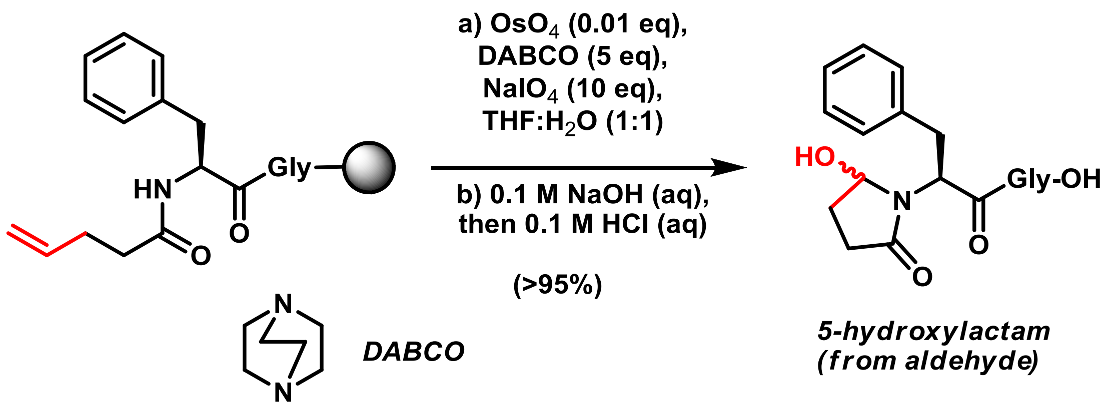




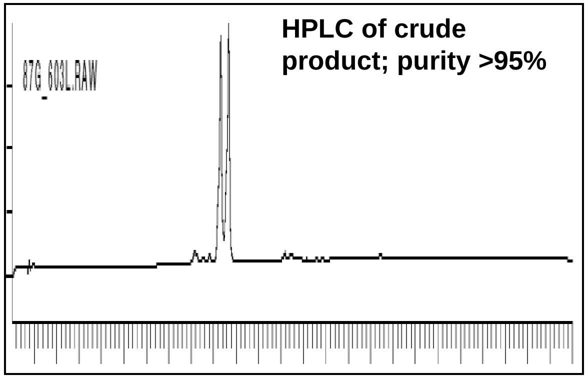


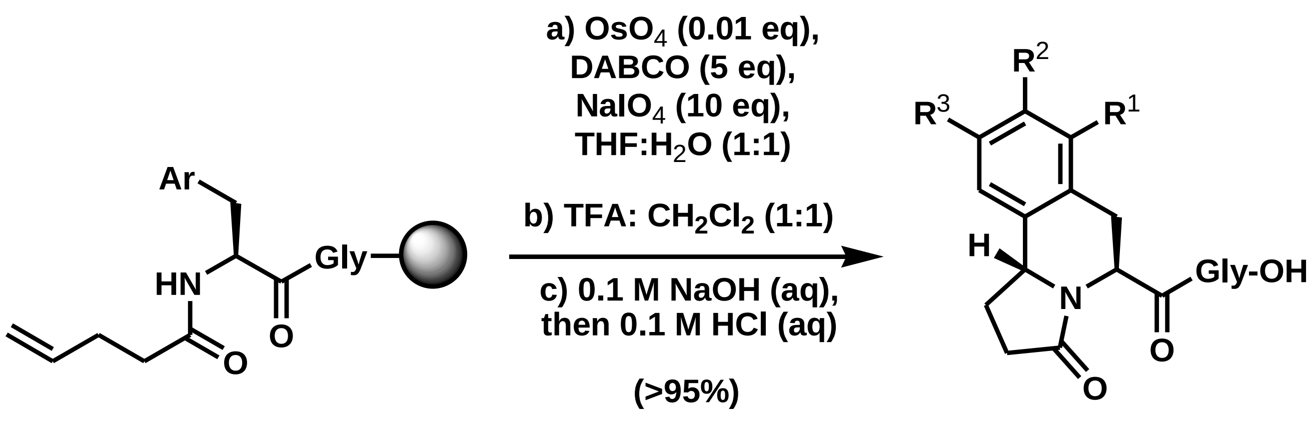
dppf: 1,1′-Bis(diphenylphosphino)ferrocene

Scaffold diversity: Building Blocks



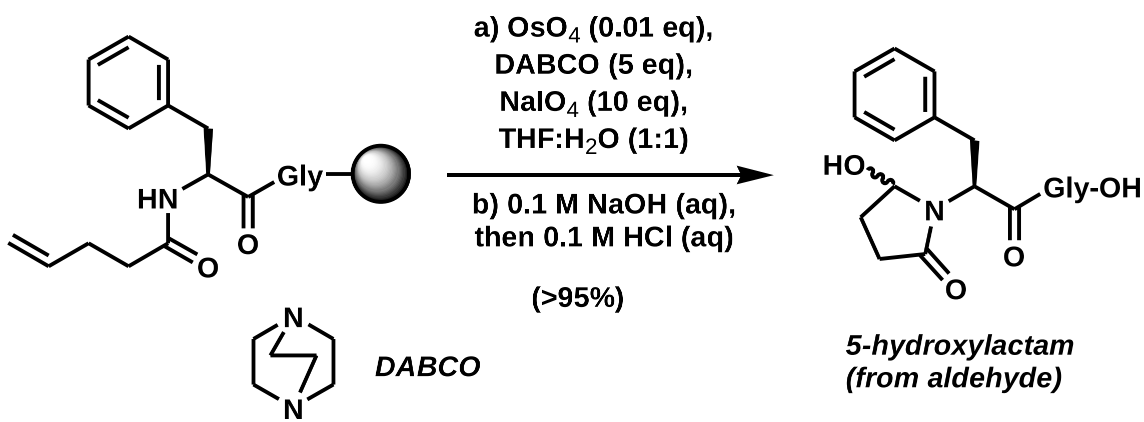
5-hydroxylactam (from aldehyde)



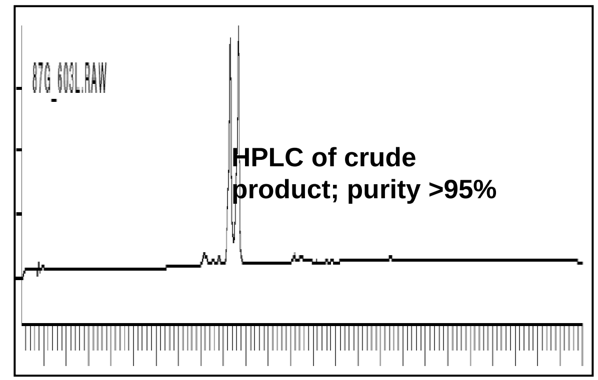


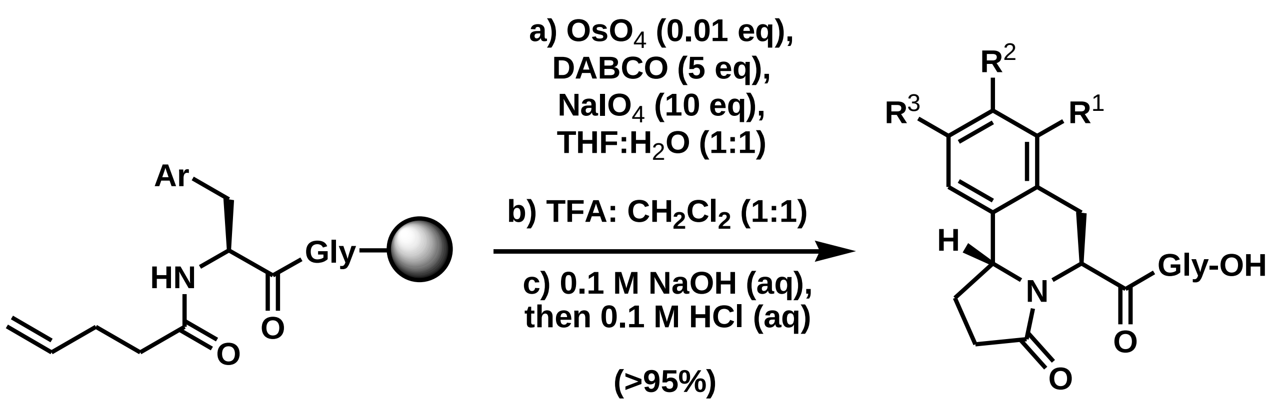
8 examples

Alkenes as aldehyde source



5-hydroxylactam (from aldehyde)

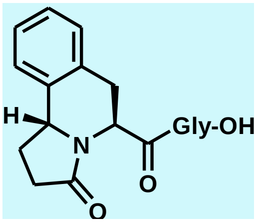


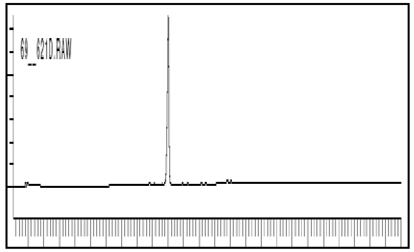


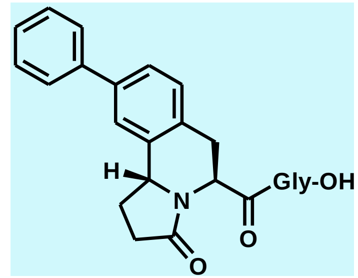
8 examples

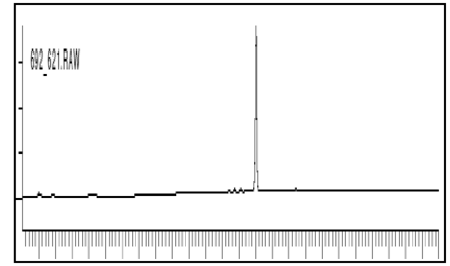
Precursors: Alkene oxidation

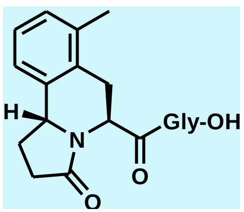
HPLCs of crude product; purity >95%

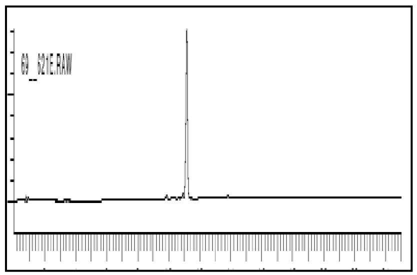


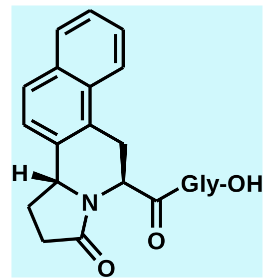


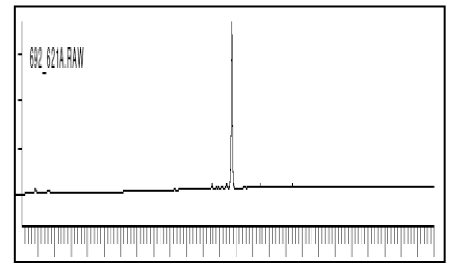


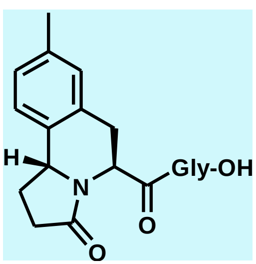


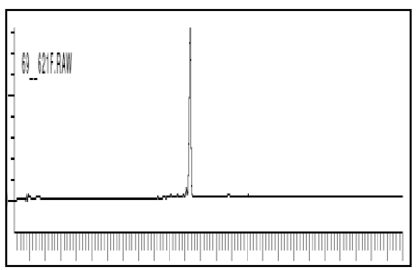


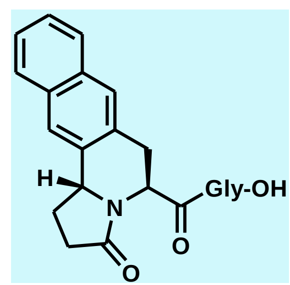


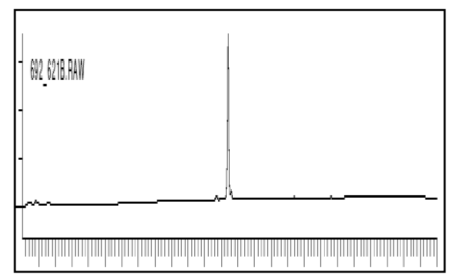


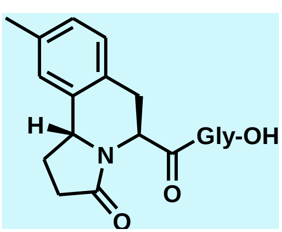


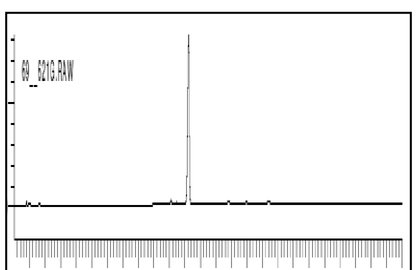


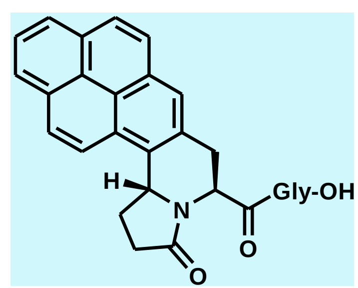


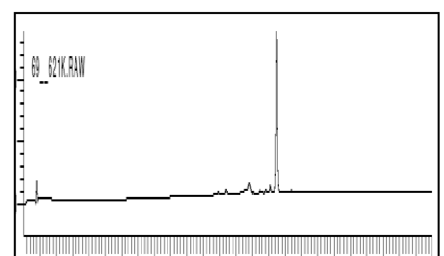






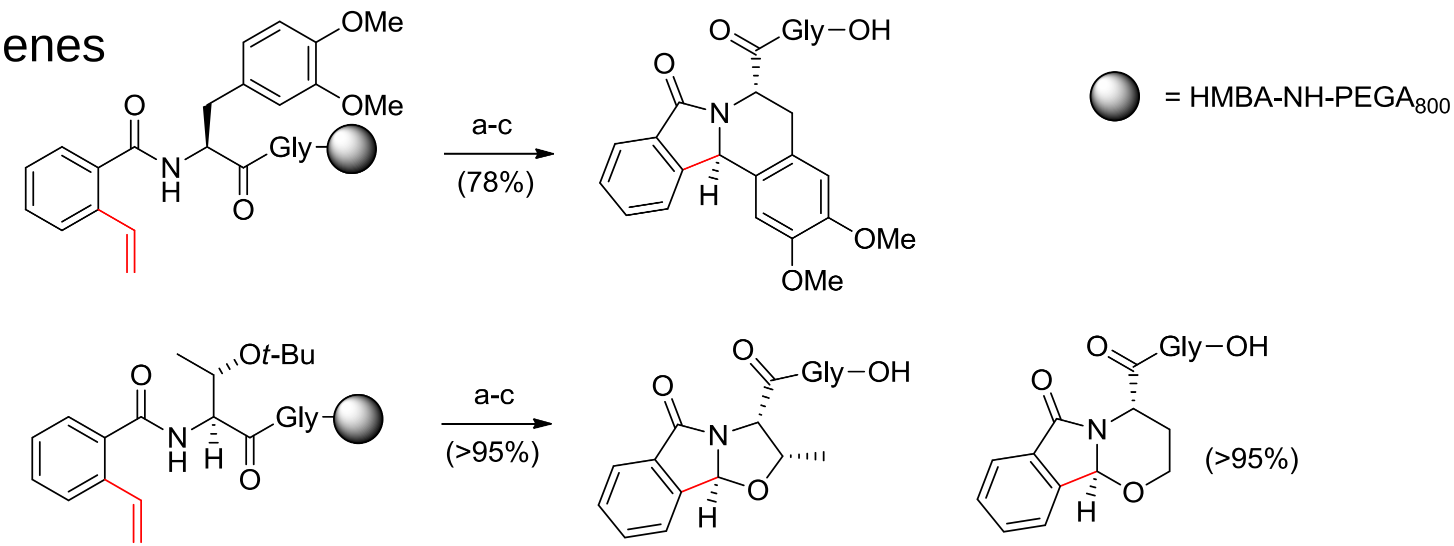




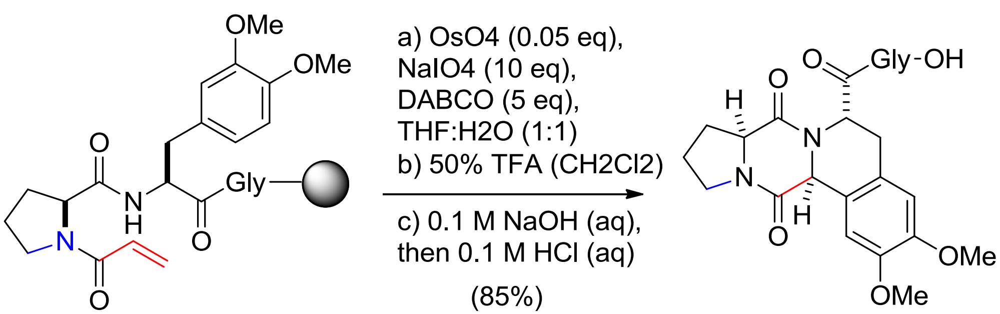


Vinylic precursors for INAIC reactions

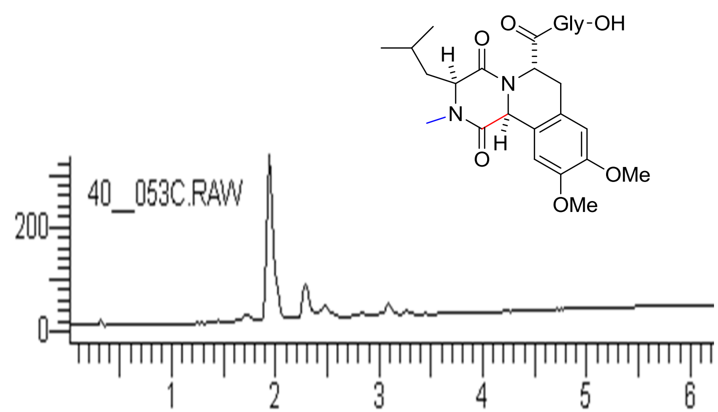
Vinylbenzenes



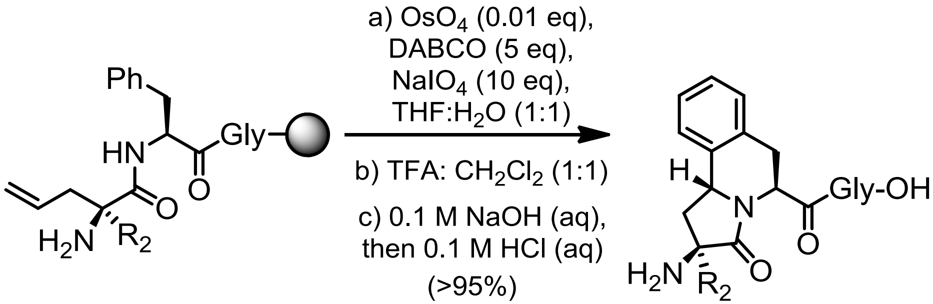
Acrylamides



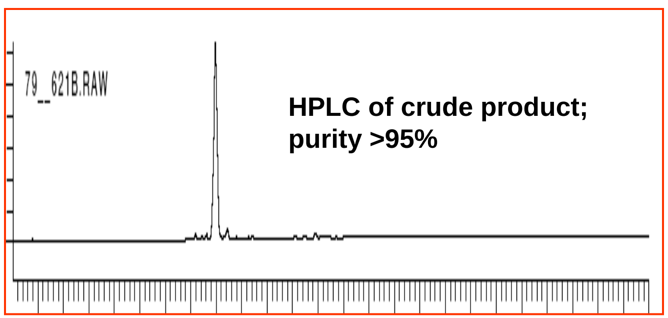
DKP's

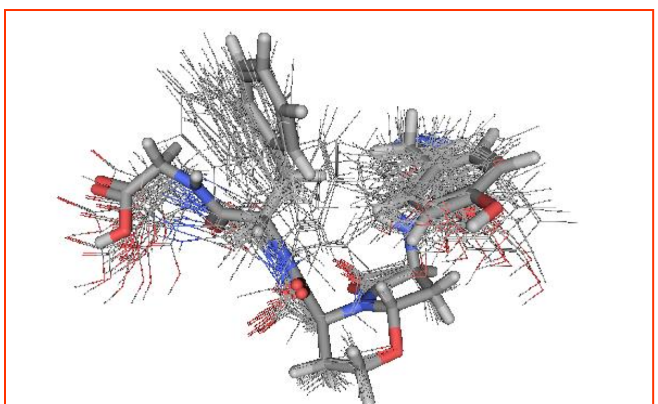


Scaffolds in peptides

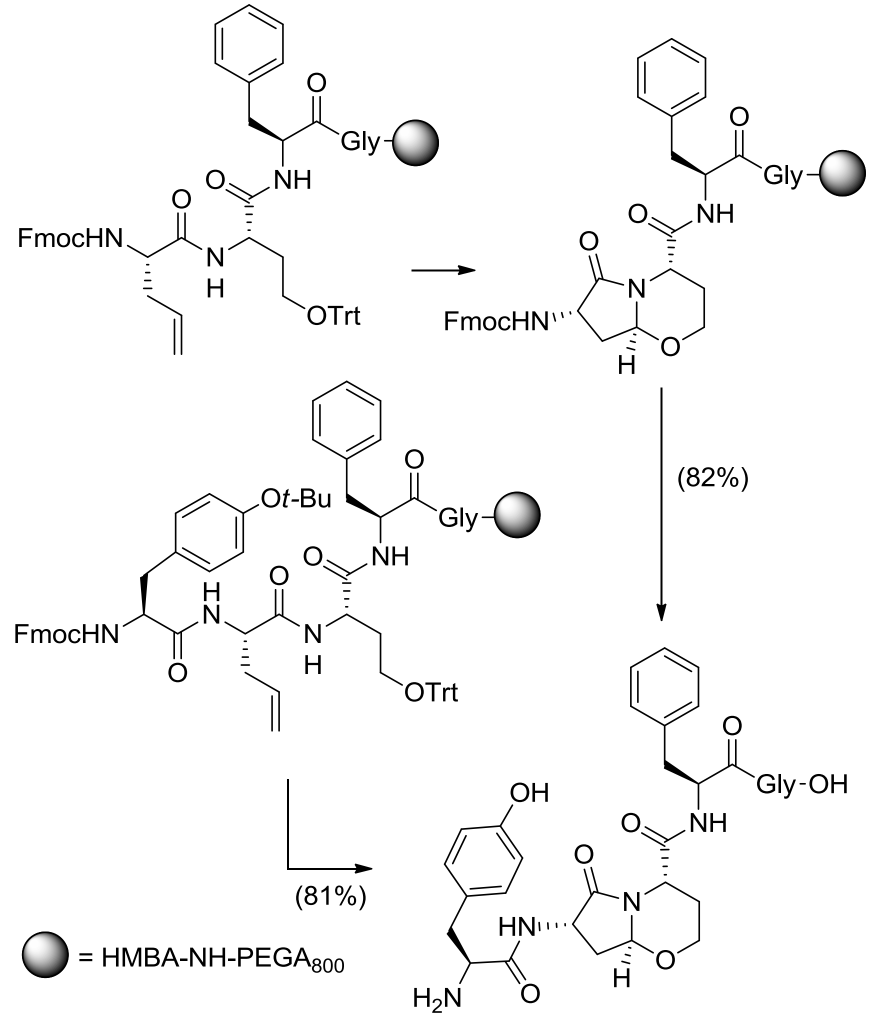


Allylglycine → Internal peptide cyclization

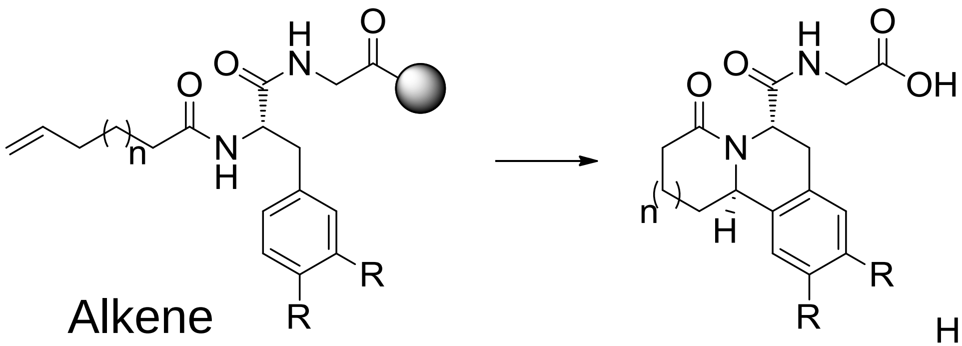




Opioid μ receptor agonist?

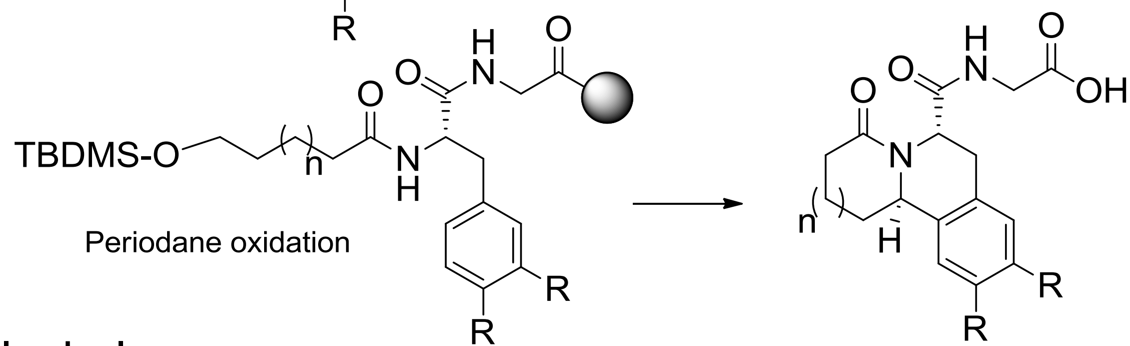


Precursor aldehydes & ringsize

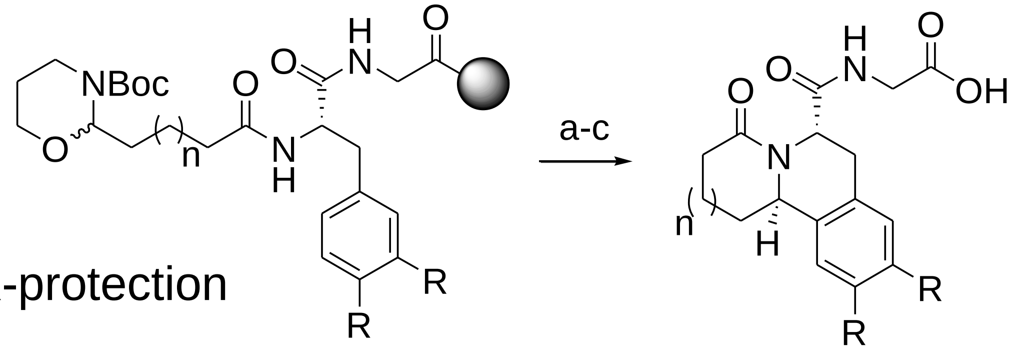


Alkene

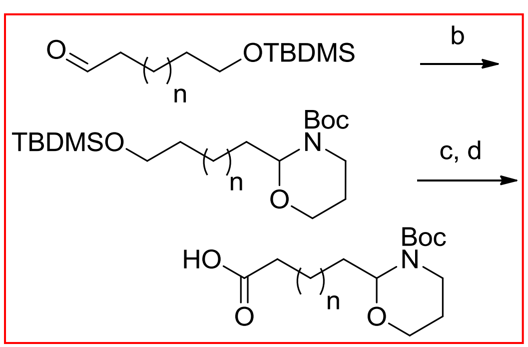
How does precursors affect the yield and purity



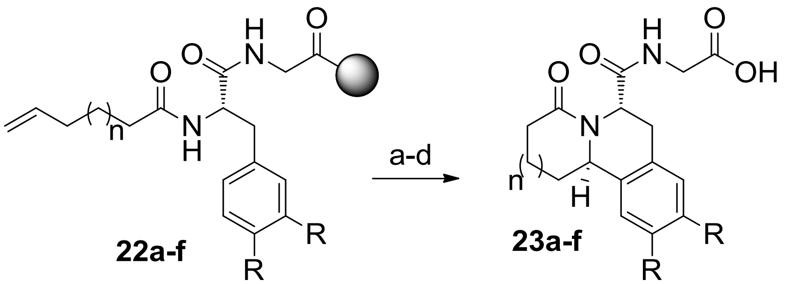
Alcohol



BOX-protection

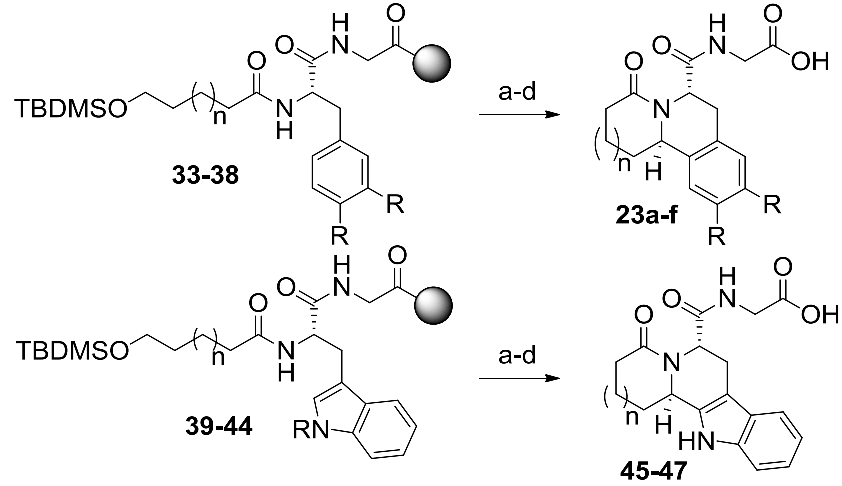


Scaffold diversity INAIC Reaction



Entry	Alkene	R	n	Product[b], purity (%)
1	**22a**	H	0	**23a**, >95
2	**22b**	H	1	**23b**, 76
3	**22c**	H	2	**23c**, 0
4	**22d**	OMe	0	**23d**, >95
5	**22e**	OMe	1	**23e**, 66
6	**22f**	OMe	2	**23f**, 0

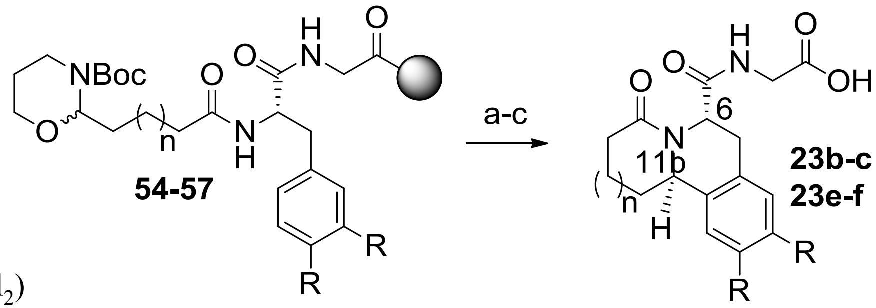
(a) OsO4/NaIO4/DABCO, THF:H2O (1:1);
(b) 10% TFA (aq);
(c) 50% TFA (CH2Cl2);
(d) 0.1 M NaOH (aq).



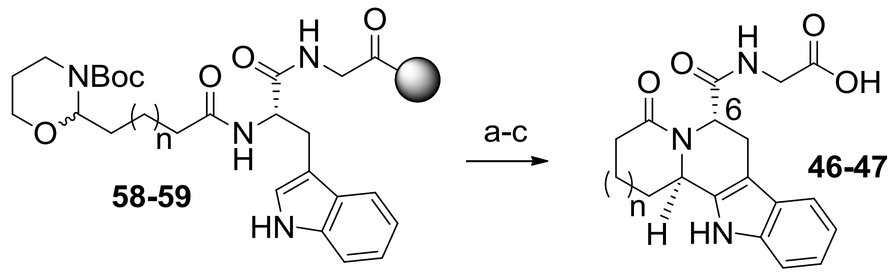
Entry	Silylether	R	n	Product, purity (%)
1	**33**	H	0	**23a**, 86
2	**34**	H	1	**23b**, 43
3	**35**	H	2	**23c**, 0
4	**36**	OMe	0	**23d**, 89
5	**37**	OMe	1	**23e**, 47
6	**38**	OMe	2	**23f**, 0
7	**39/42**	H/Boc	0	**45**, 93
8	**40/43**	H/Boc	1	**46**, 0[b]
9	**41/44**	H/Boc	2	**47**, 0[b]

(a) TBAF, AcOH, THF
(b) Dess-Martin periodinane
(c) 10% TFA (aq)
(d) 50% TFA (CH_2Cl_2)
(e) (e) 0.1 M NaOH

INAIC reactions

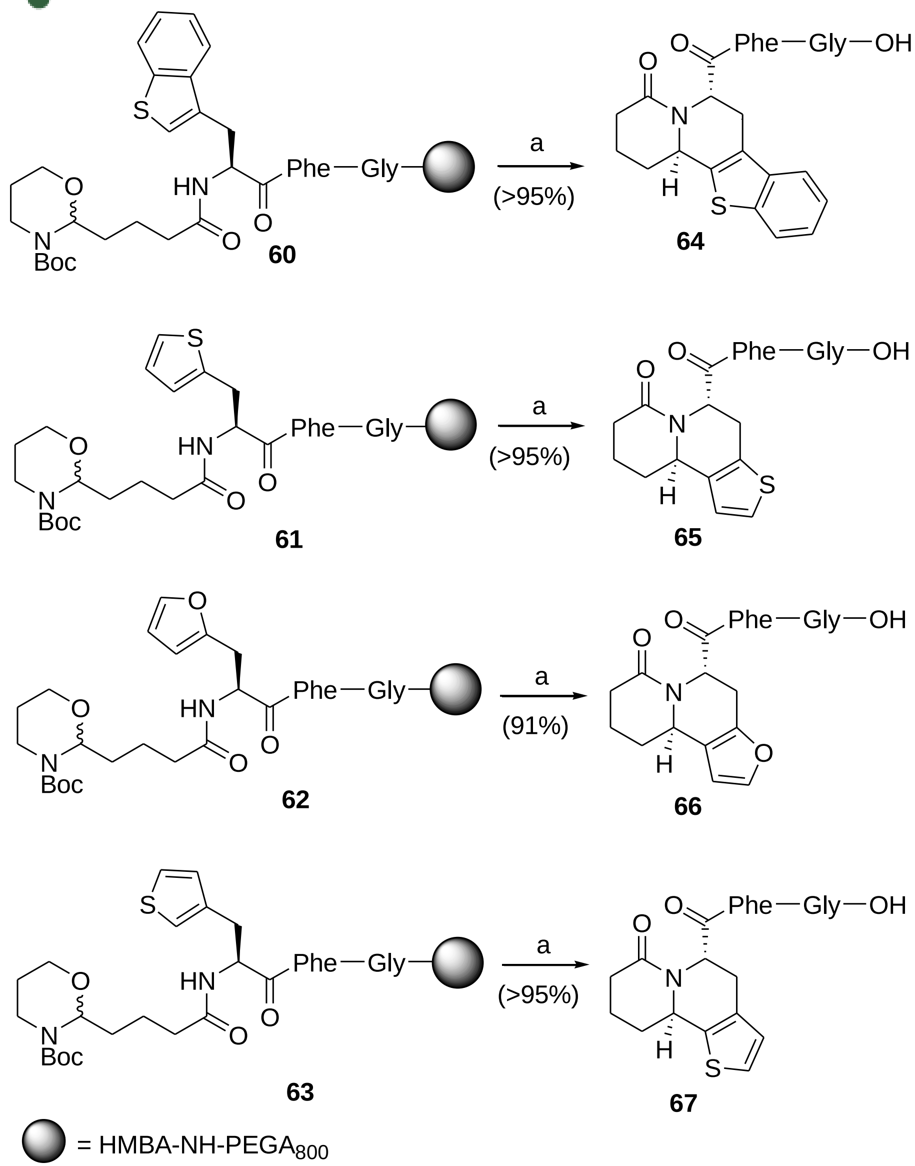


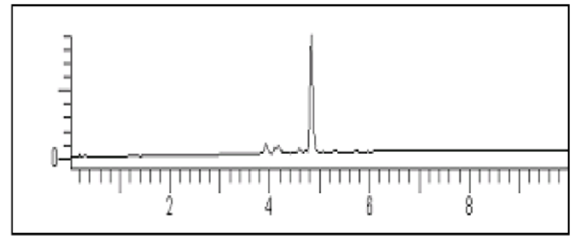
(a) 10% TFA (aq);
(b) 50% TFA (CH_2Cl_2)
(c) 0.1 M NaOH (aq).

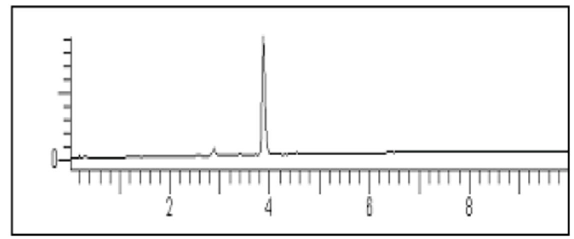


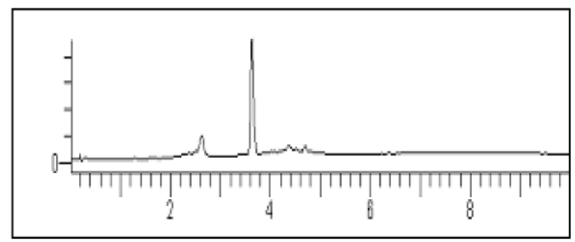
Entry	*N*,*O*-acetal	R	n	Product[c], purity (%)
1	**54**	H	1	**23b**, >95
2	**55**	H	2	**23c**, 0
3	**56**	OMe	1	**23e**, >95
4	**57**	OMe	2	**23f**, 0
5	**58**	-	1	**46**, 0[d]
6	**59**	-	2	**47**, 0[d]

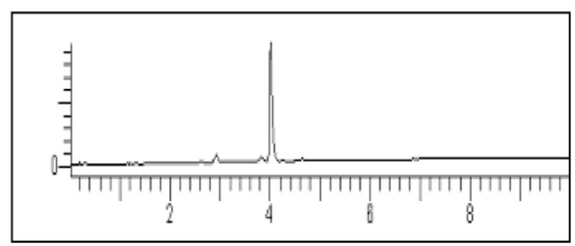
6,6-membered ring systems



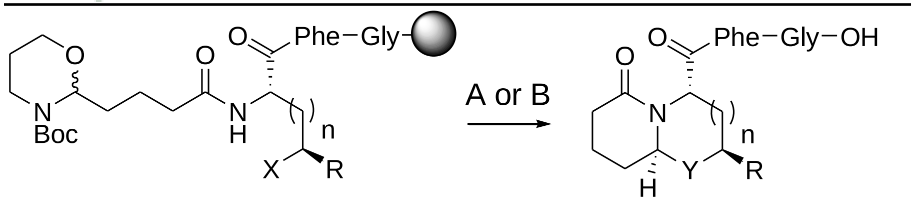




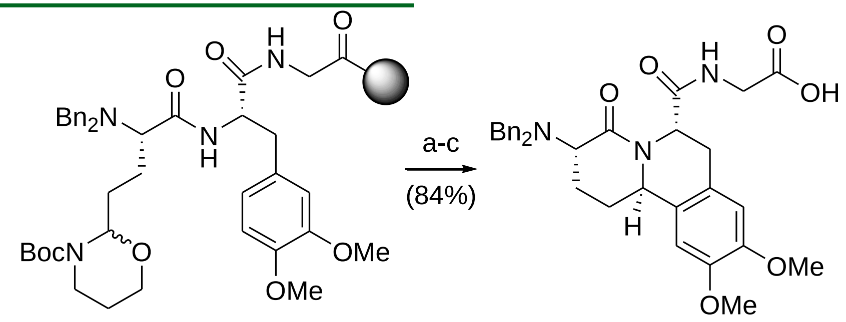


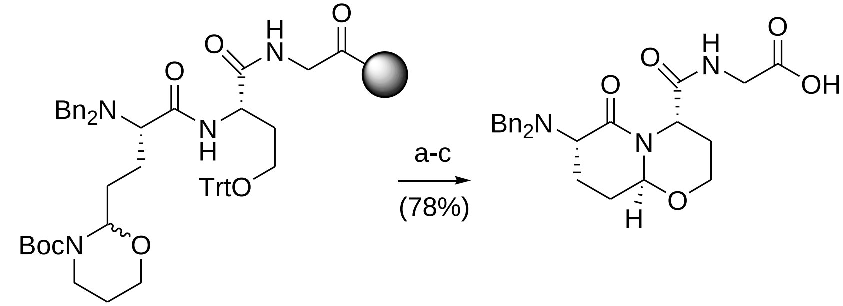


Heteronucleophiles in 6,6 rings



Entry	X	R	Y	n	Reaction conditions[a]	Product, purity (%)
1	O*t*-Bu	H	O	0	B	>95
2	O*t*-Bu	Me	O	0	A or B	>95
3	OTrt	H	O	1	A or B	>95
4	NHBoc	H	NBoc	0	A	>95
5	NHBoc	H	NBoc	1	A	86
6	NHBoc	H	NBoc/NH	2	A/B	0
7	STrt	H	S	0	A or B	>95



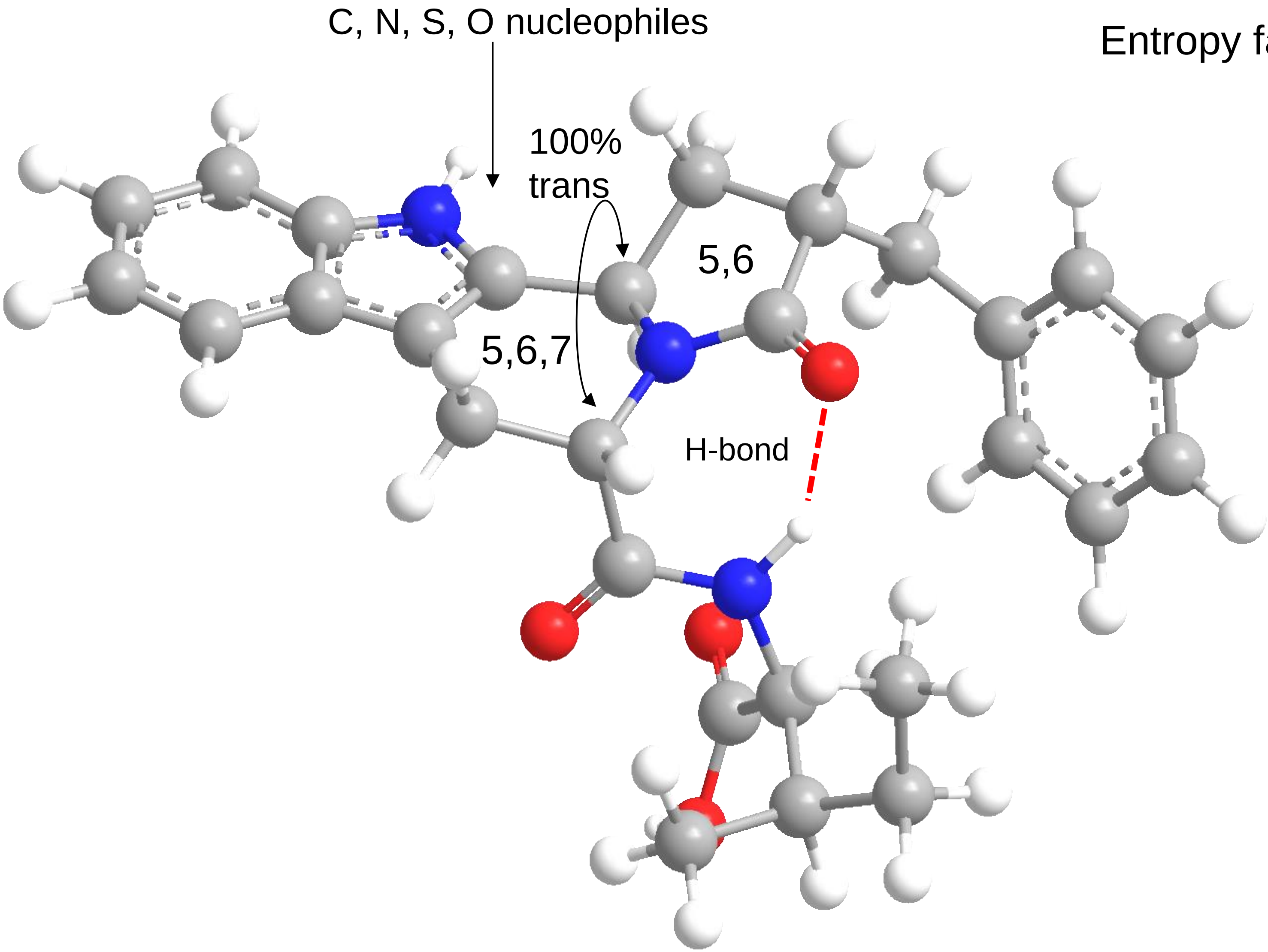


Conditions A:
- 10% TFA (aq)
- 0.1 M NaOH (aq).

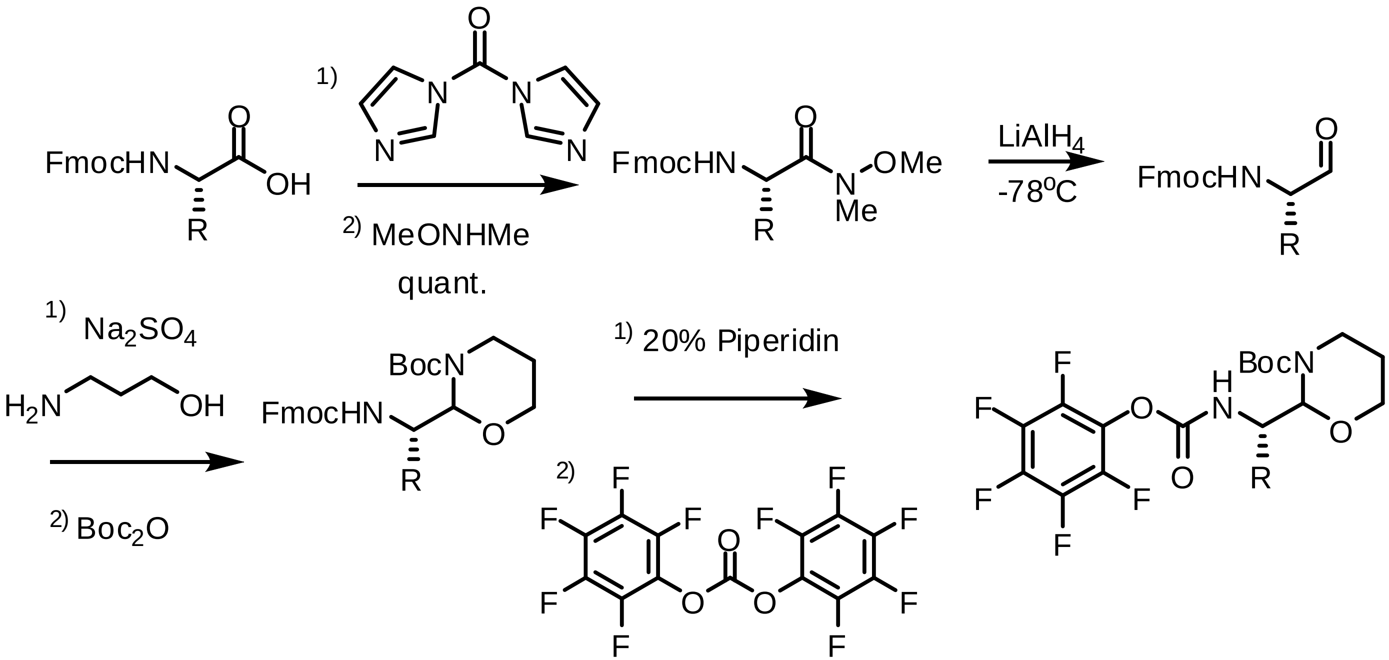
Conditions B:
- 10% TFA (aq)
- 50% TFA (CH_2Cl_2)
- 0.1 M NaOH (aq)

INAIC reaction scope

Entropy facilitated



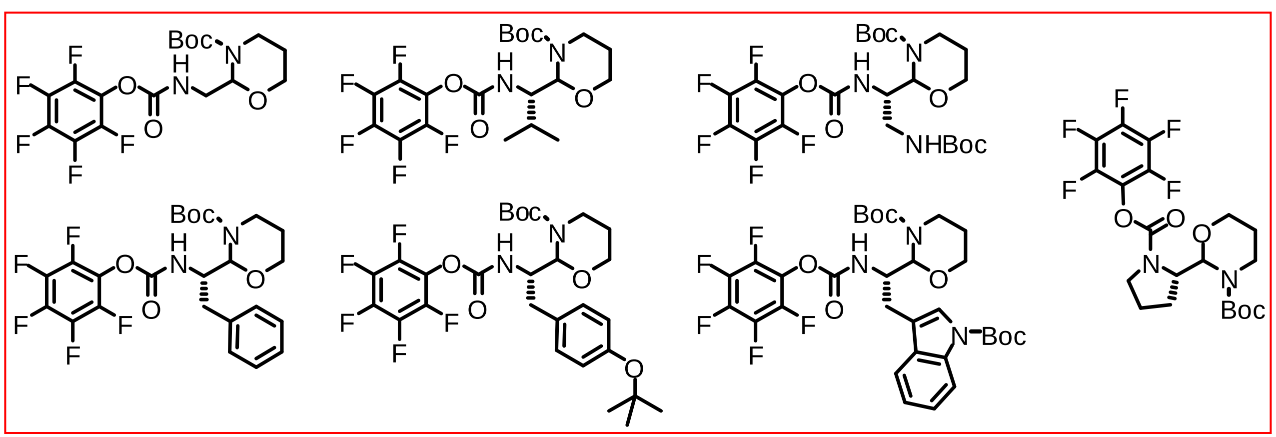
α-Amino acids as a source of diversity



40% - 70% (3 steps)

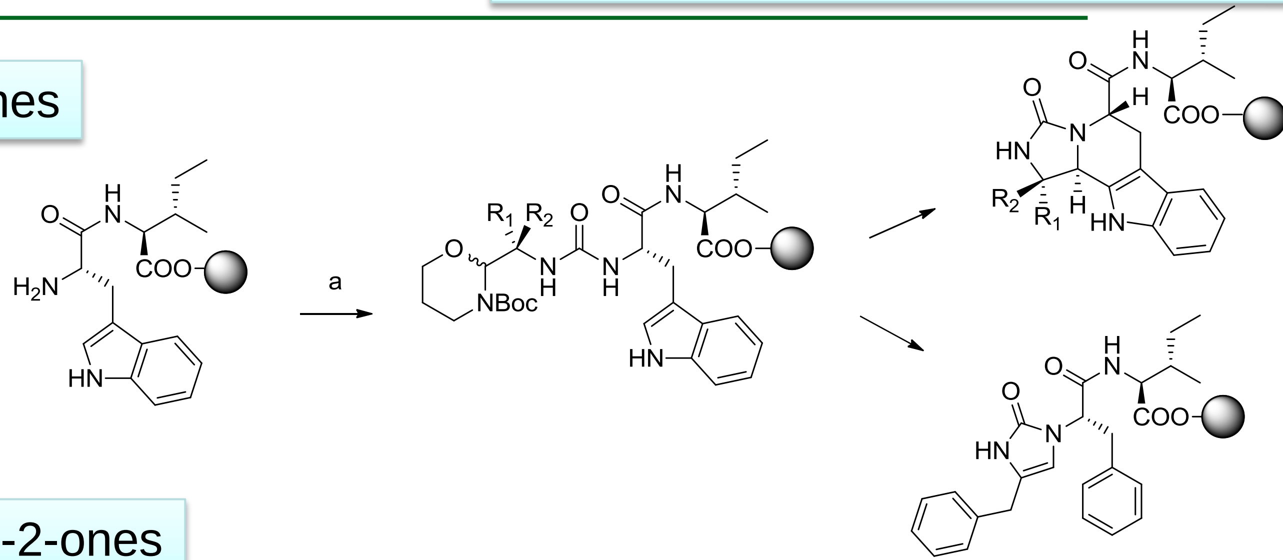
50% - 70%

20% -50% over all yield

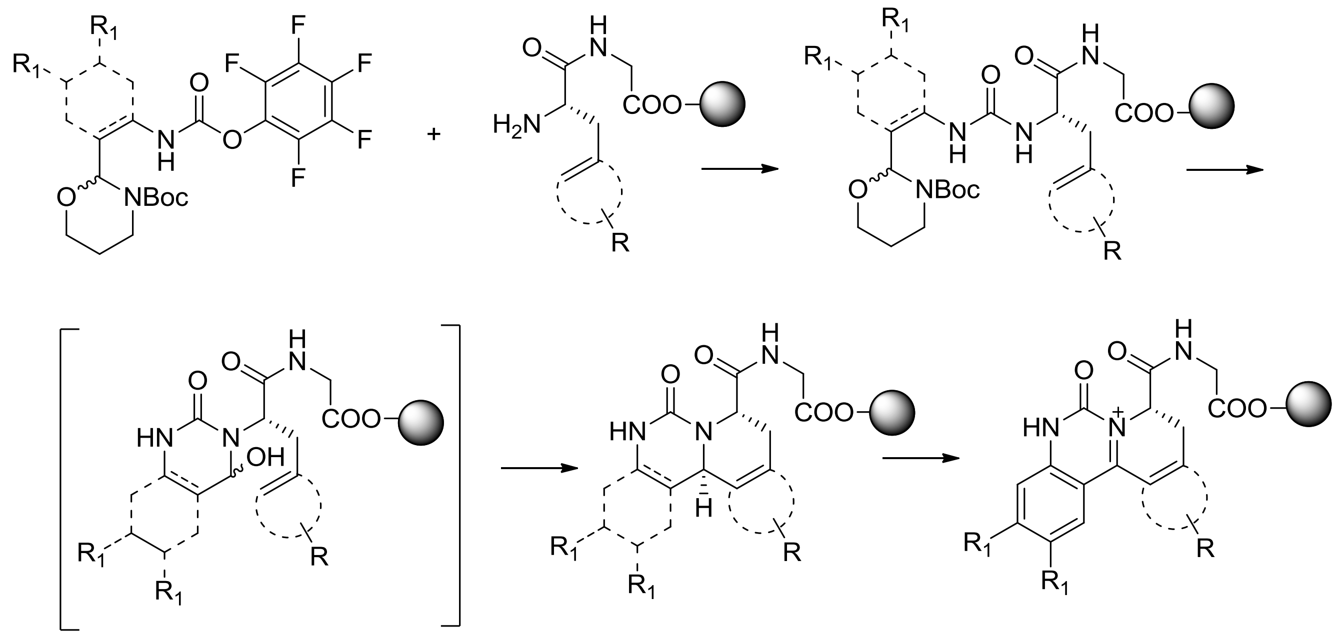


INCIC: N-carbamoylinimium reactions

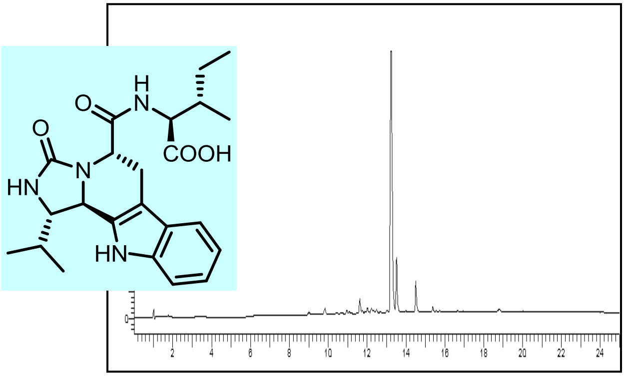
Imidazolinones

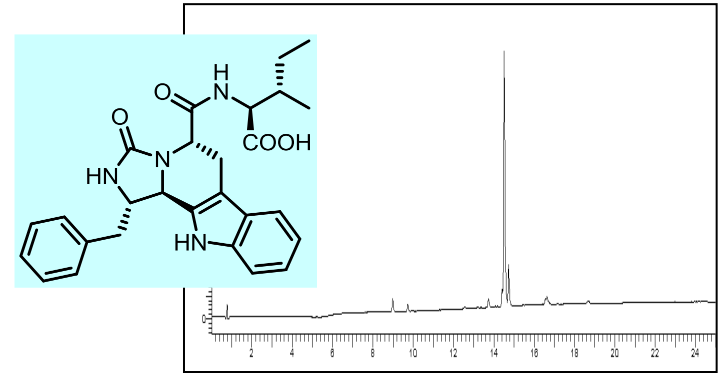


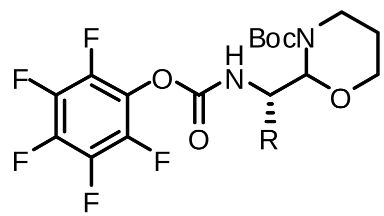
1,3 piperazin-2-ones



INCIC: Fused β-carbolino-imidazolinones

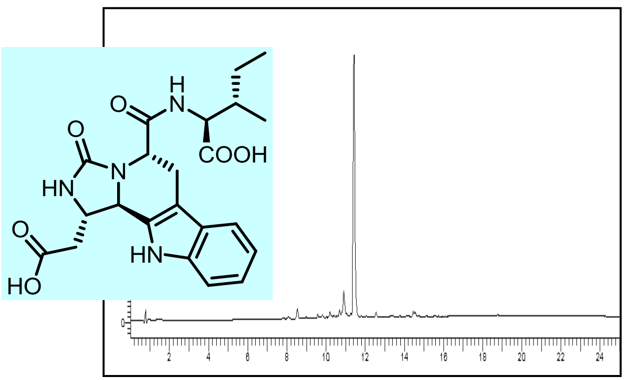


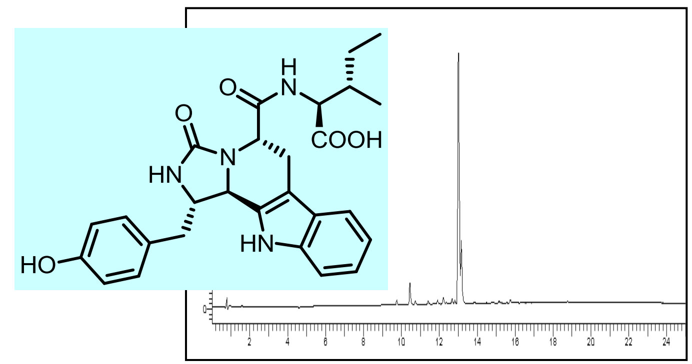


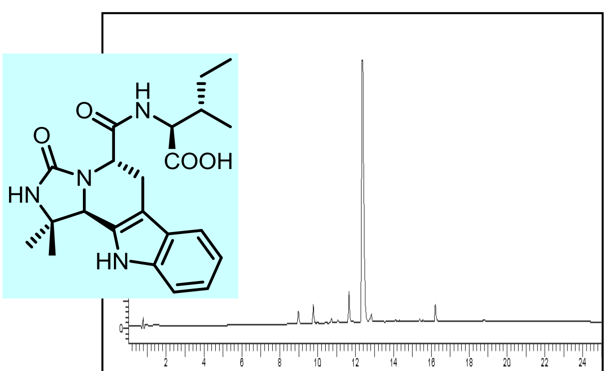


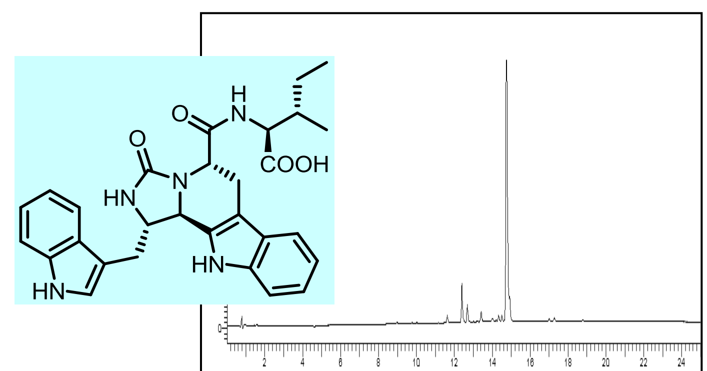
Diastereo-selectivity: >10

Purity > 92%

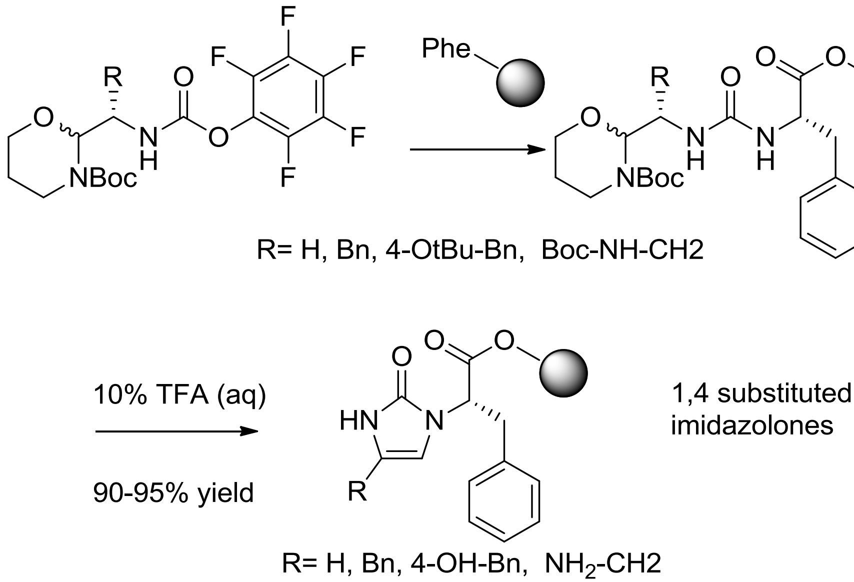






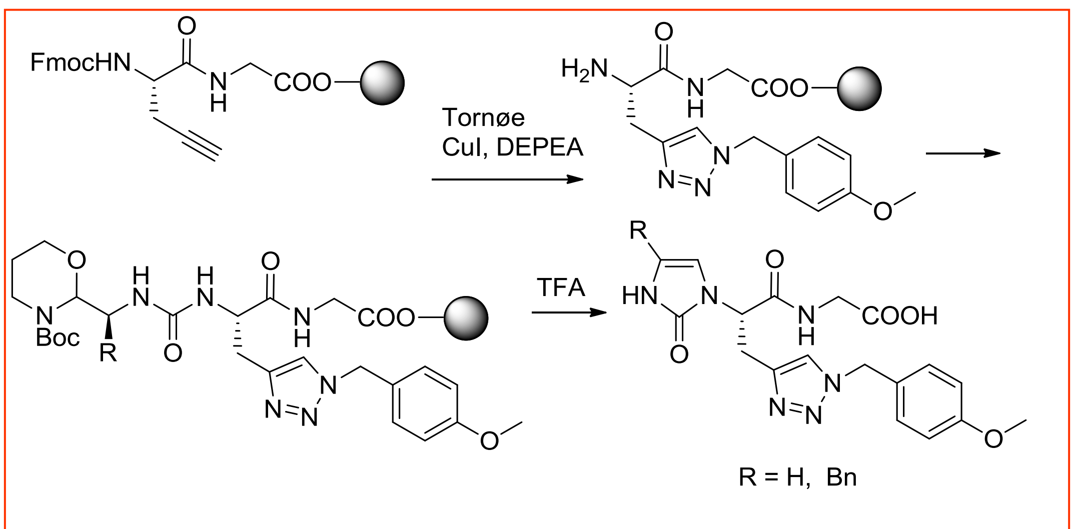


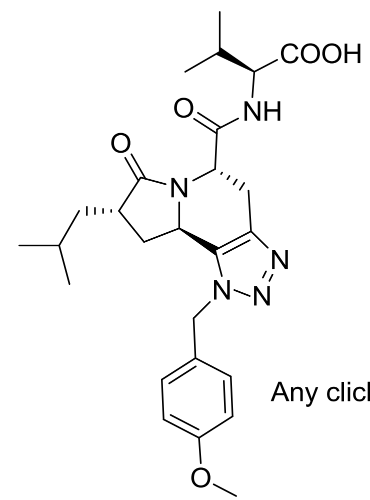
N-Carbamyliminium ions in imidazolone synthesis



INCIC: Less reactive nucleophiles give imidazolones

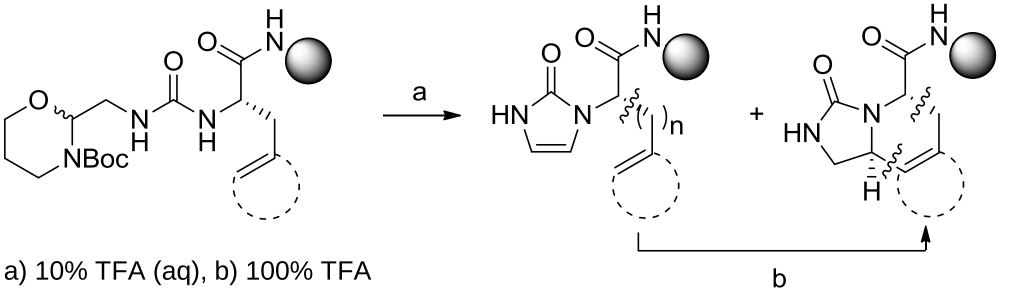
INAIC: Less reactive nucleophiles give cascade reactions

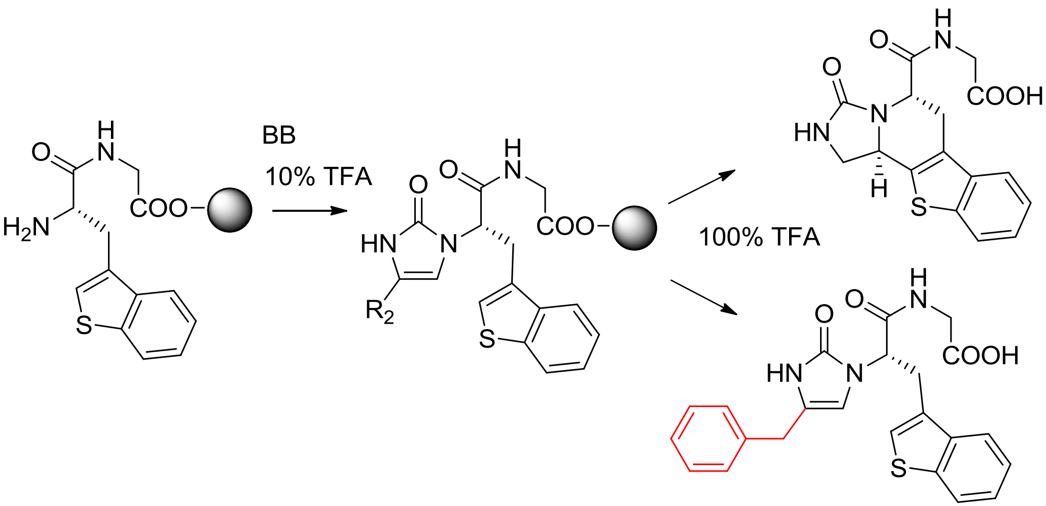


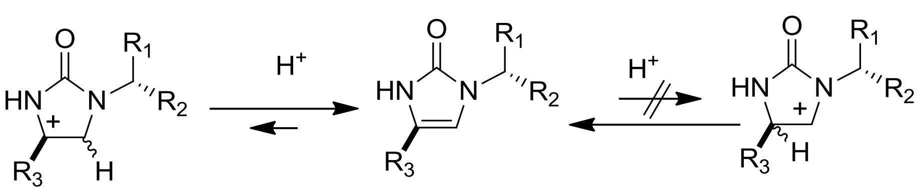


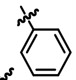
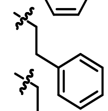
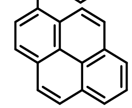
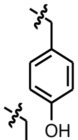
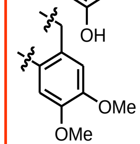
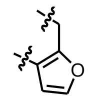
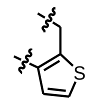
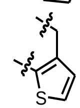
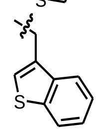
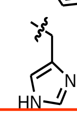
Any clickable function

Imidazolones as electrophiles

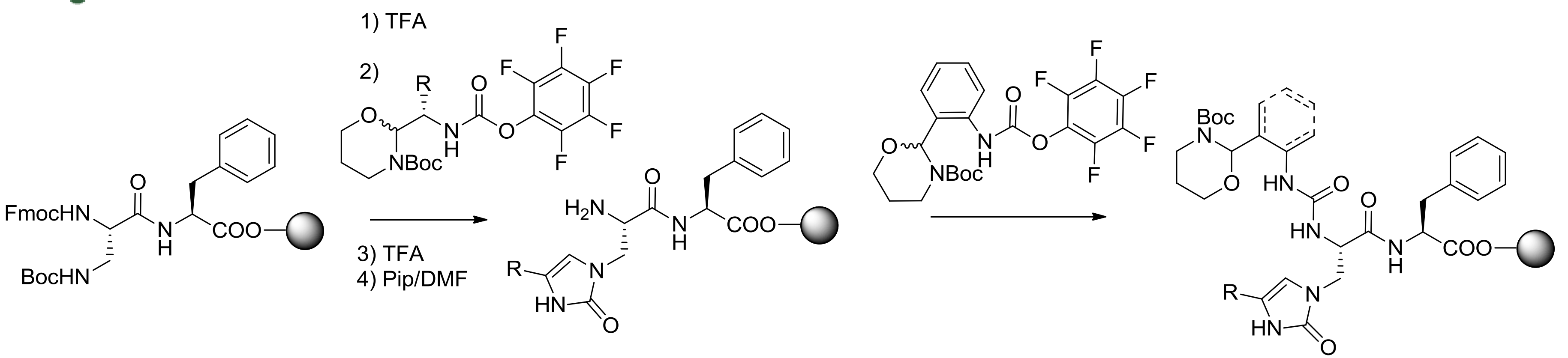


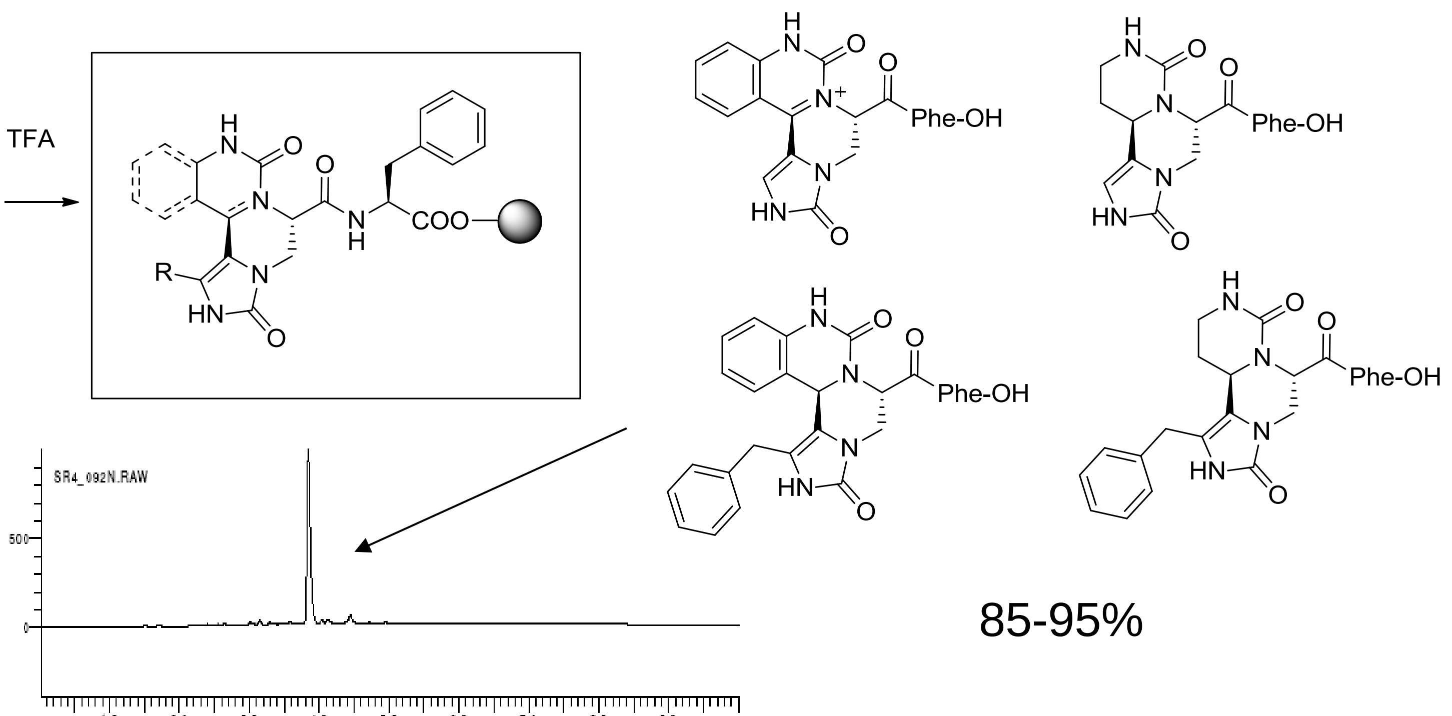




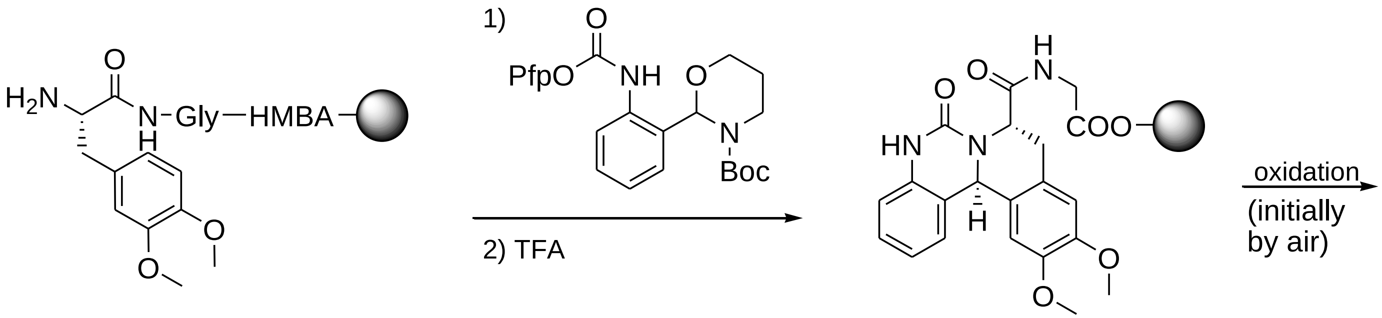
product	10% TFA(aq) **6/7** (purity)	100% TFA **6/7** (purity)
	100 / 0 (98%)	100 / 0 (98%)
	100 / 0 (99%)	100 / 0 (99%)
	100 / 0 (99%)	100 / 0 (99%)
	-	100 / 0 (99%)
	93 / 7 (98%)	0 / 100 (97%)
	100 / 0 (96%)	-
	100 / 0 (95%)	0 / 100 (98%)
	66 / 34 (92%)	0 / 100 (97%)
	100 / 0 (95%)	0 / 100 (96%)
	100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

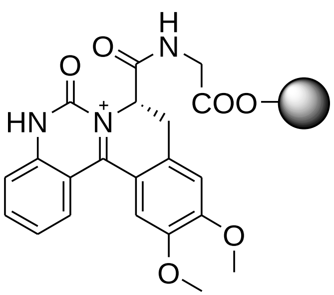
Imidazolones as nucleophiles

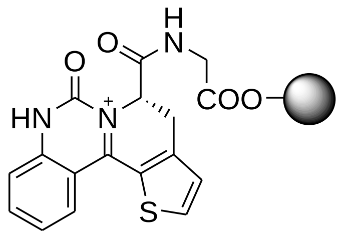


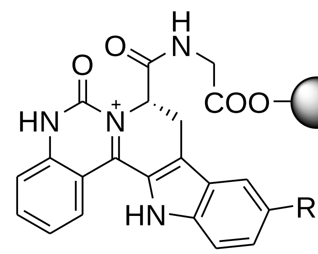


Novel fluorescent compounds by INAIC

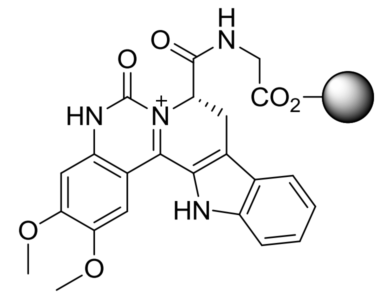


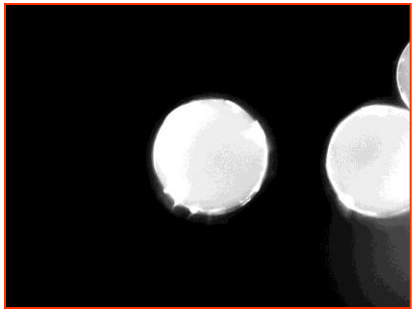


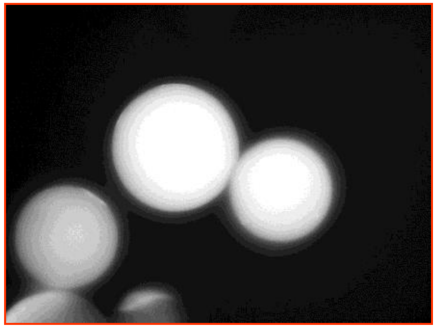




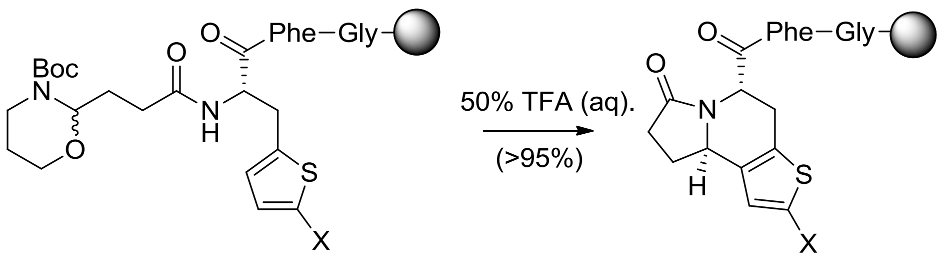
R=H, F, Br



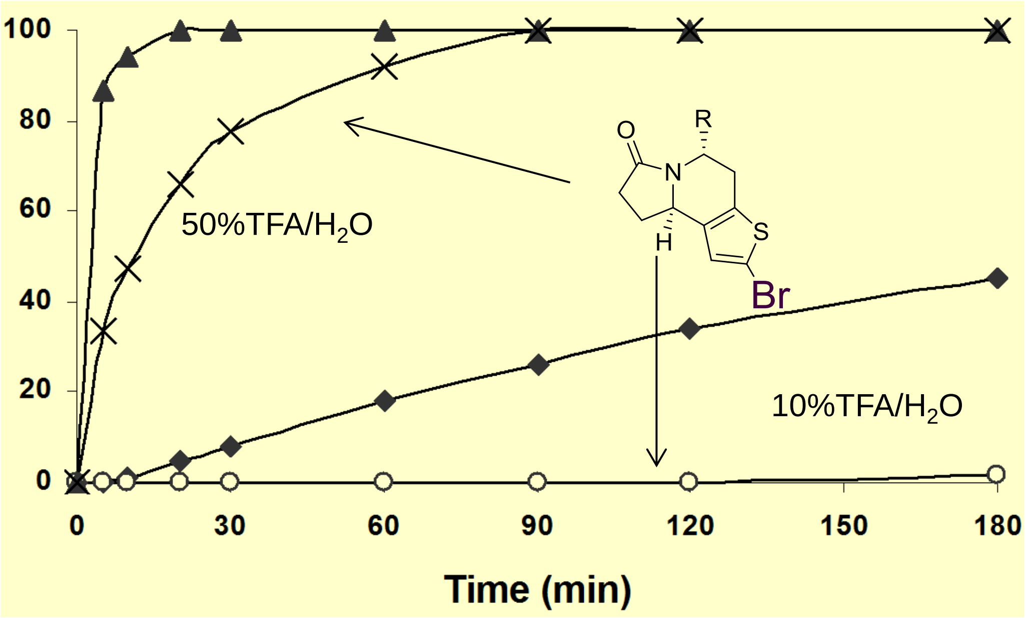


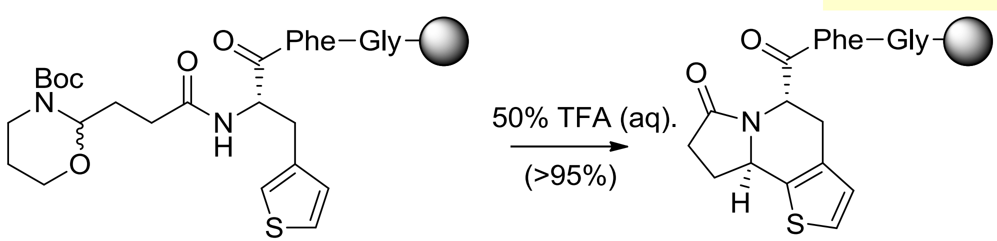


Kinetics of the INIAC reaction



X = H or Br





Reactivity !!
Mono-bromination ???

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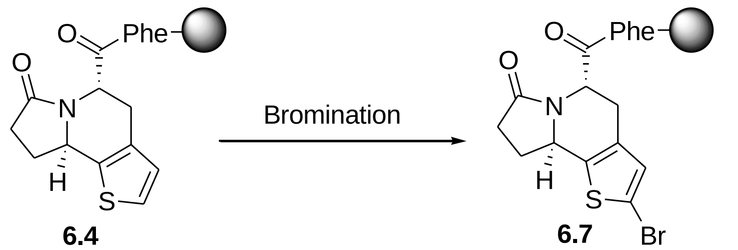
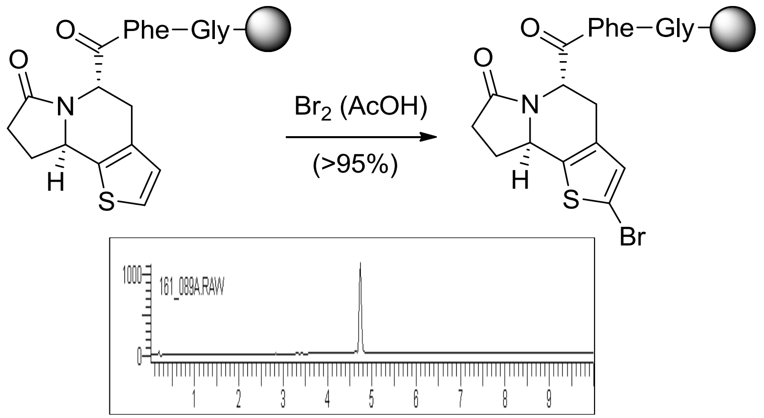
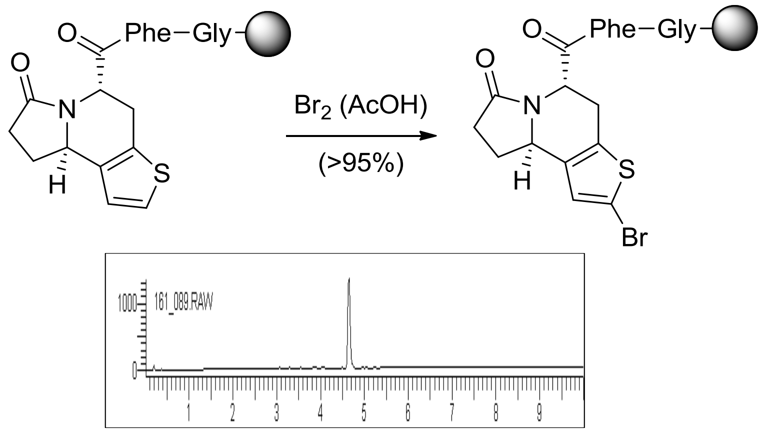
INIAC: Bromination on solid phase



Br	solvent	equiv.	reaction time (min)	purity (%)
NBS	DMF	4	30	39
NBS	DMF	12	30	28
NBS	AcOH	2	45	59
NBS	AcOH:$CHCl_3$	2	45	70
NBS	MeCN	2	45	65
NBS	CH_2Cl_2	2	45	79
NBS	AcOH: CH_2Cl_2	2	90	71
NBS	AcOH: CH_2Cl_2	3	90	52
Br_2	**AcOH**	**2**	**90**	**>95**
Br_2	$CHCl_3$	2	90	45[b]
Br_2	DMF	2	90	26[b]

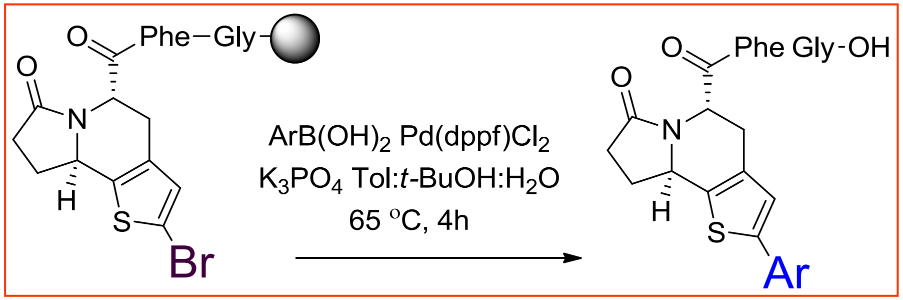
[b] Incomplete conversion of substrate

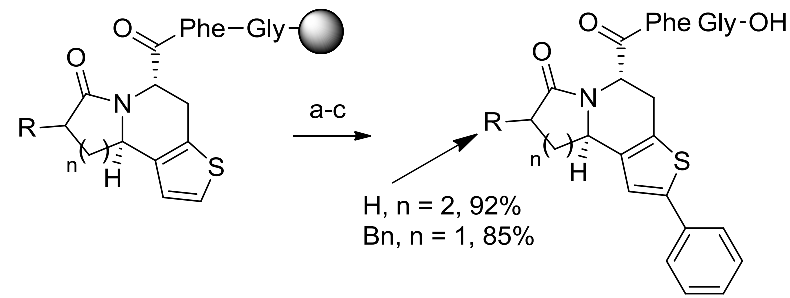


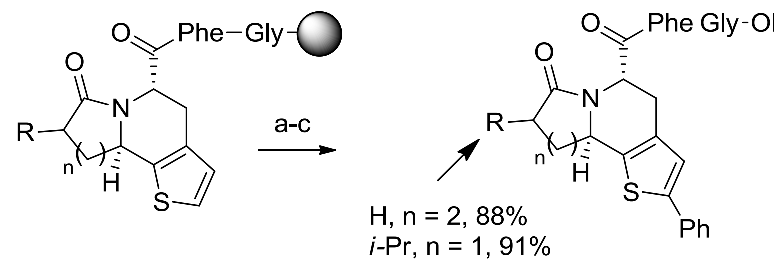


INAIC: Suzuki reactions

on preformed scaffolds from 3-thienyl-Ala





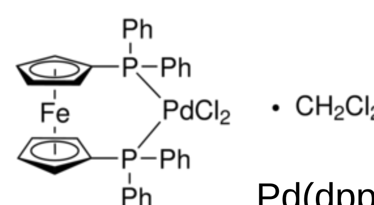


$ArB(OH)_2$	Purity (%)		
3-NH_2-$C_6H_4B(OH)_2$	92	3-Cl-4-OMe- $C_6H_3B(OH)_2$	87
3-OH-$C_6H_4B(OH)_2$	95	4-CHO-$C_6H_4B(OH)_2$	75
2-CF_3-$C_6H_4B(OH)_2$	87	$C_6F_5B(OH)_2$	0
3-CF_3-$C_6H_4B(OH)_2$	92	3-NO_2-$C_6H_4B(OH)_2$	88
4-CO-Ph-$C_6H_4B(OH)_2$	90	4-OBu-$C_6H_4B(OH)_2$	82
3,4-(OCH_2CH_2O)-$C_6H_3B(OH)_2$	86	3,4-$(OMe)_2$-$C_6H_4B(OH)_2$	85
(2-OMe)pyrimidine-5-$B(OH)_2$	65	3,5-$(OMe)_2$-$C_6H_4B(OH)_2$	92
(2-OMe)pyridine-5-$B(OH)_2$	81	4-CF_3-$C_6H_4B(OH)_2$	86
Benzothiophene-2-$B(OH)_2$	91	3,4-Cl_2-$C_6H_4B(OH)_2$	89
Indole-2-$B(OH)_2$	50	$PhB(OH)_2$	86

a Br_2, AcOH

b $PhB(OH)_2$, K_3PO_4, $Pd(dppf)Cl_2$
Tol:*t*-BuOH:H2O 65 ˚C, 4 h

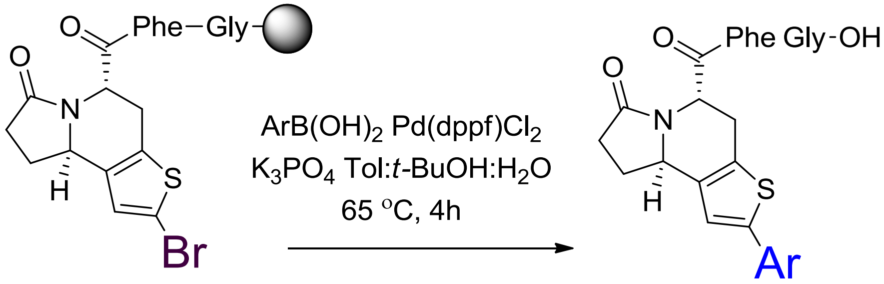
c 0.1 M NaOH (aq)



$Pd(dppf)Cl_2$

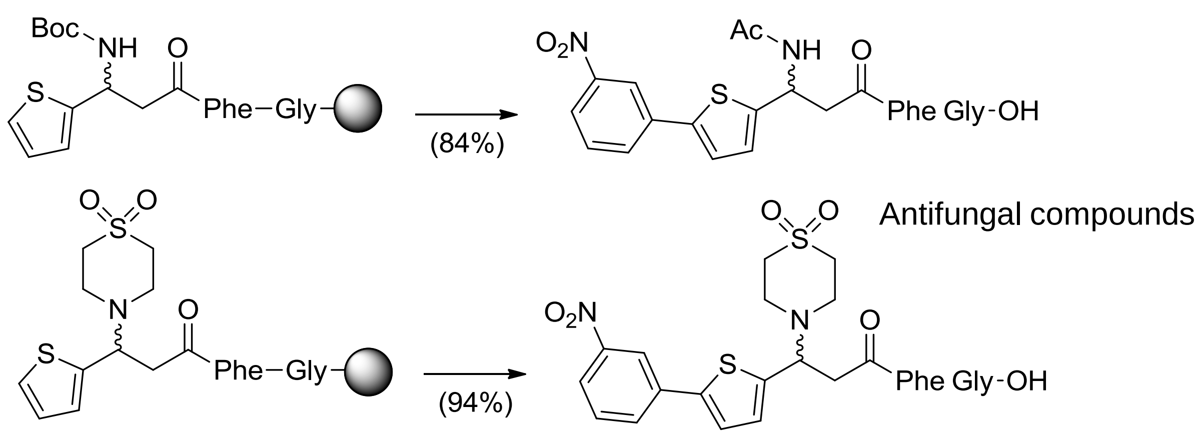
INAIC - Bromination - Suzuki

preformed scaffolds from 2-thienyl-Ala



3 steps overall
60-90 % yield

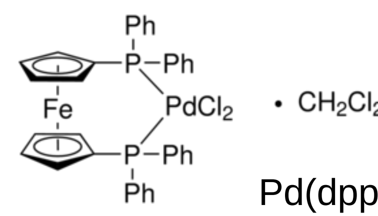
$ArB(OH)_2$	%		
$4\text{-MeO-}C_6H_4B(OH)_2$	85	$2,6\text{-}Me_2\text{-}C_6H_3B(OH)_2$	50
$4\text{-CN-}C_6H_4B(OH)_2$	86	$3\text{-}CO_2H\text{-}C_6H_4B(OH)_2$	69
$3\text{-}NO_2\text{-}C_6H_4B(OH)_2$	87	$3\text{-(CHO)-4-(OMe)-}C_6H_3B(OH)_2$	61
$3\text{-Ph-}C_6H_4B(OH)_2$	86	$C_6F_5B(OH)_2$	0
$4\text{-F-}C_6H_4B(OH)_2$	89	$4\text{-Me-}C_6H_4B(OH)_2$	77
$3,5\text{-}Cl_2\text{-}C_6H_3B(OH)_2$	87	$2\text{-Ac-}C_6H_4B(OH)_2$	68
$Nap\text{-}2\text{-}B(OH)_2$	84	$3,5\text{-(OMe)}_2\text{-}C_6H_3B(OH)_2$	86
$3,4\text{-}(OCH_2O)\text{-}C_6H_3B(OH)_2$	90	$PhB(OH)_2$	75
$4\text{-SMe-}C_6H_4B(OH)_2$	>95	$4\text{-Cl-}C_6H_4B(OH)_2$	85
$4\text{-OPh-}C_6H_4B(OH)_2$	85	$3,5\text{-}Me_2\text{-}C_6H_3B(OH)_2$	86
		$4\text{-}CF_3\text{-}C_6H_4B(OH)_2$	81



a Br_2, AcOH

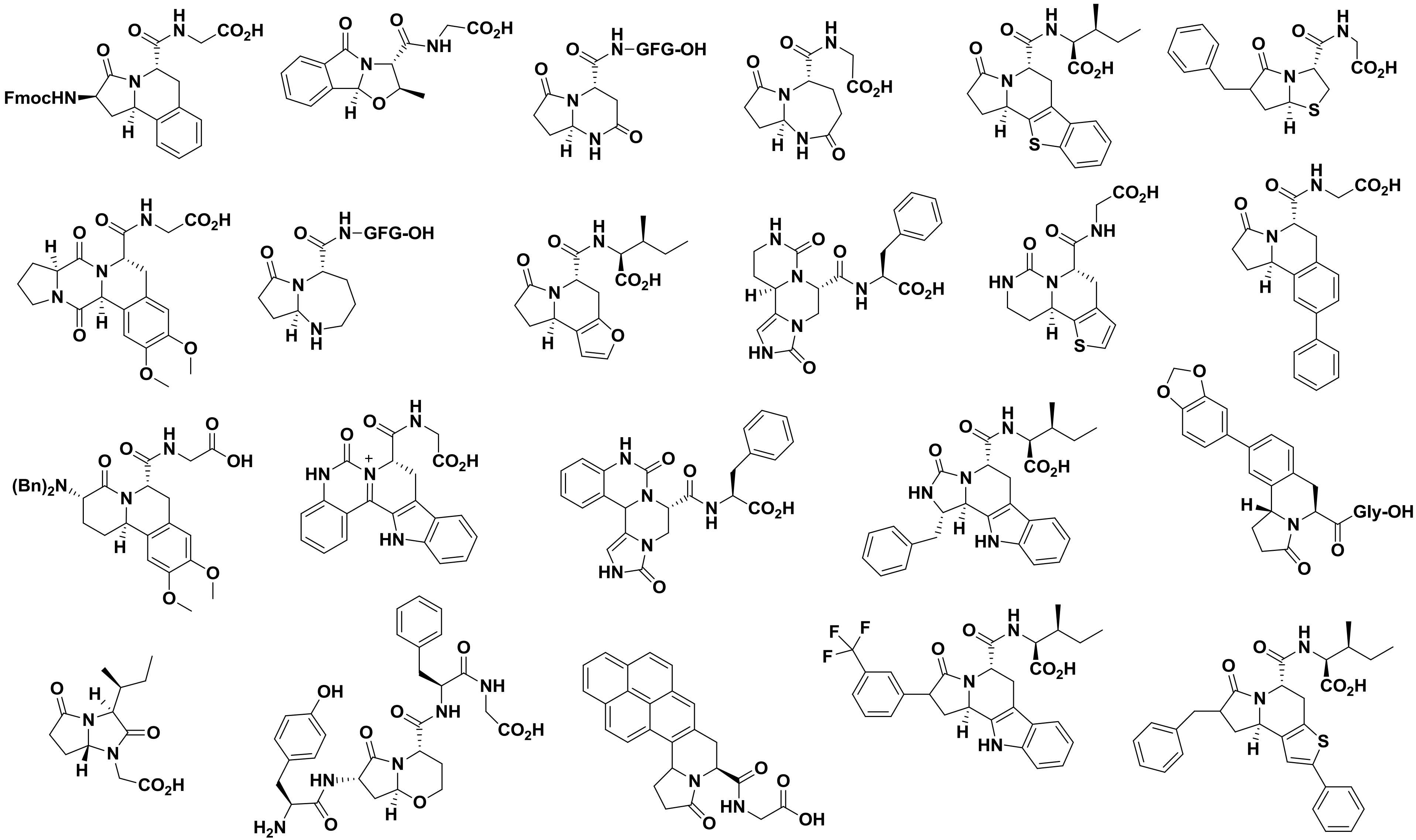
b $PhB(OH)_2$, K_3PO_4, $Pd(dppf)Cl_2$
Tol:*t*-BuOH:H2O 65 ˚C, 4 h

c 0.1 M NaOH (aq)

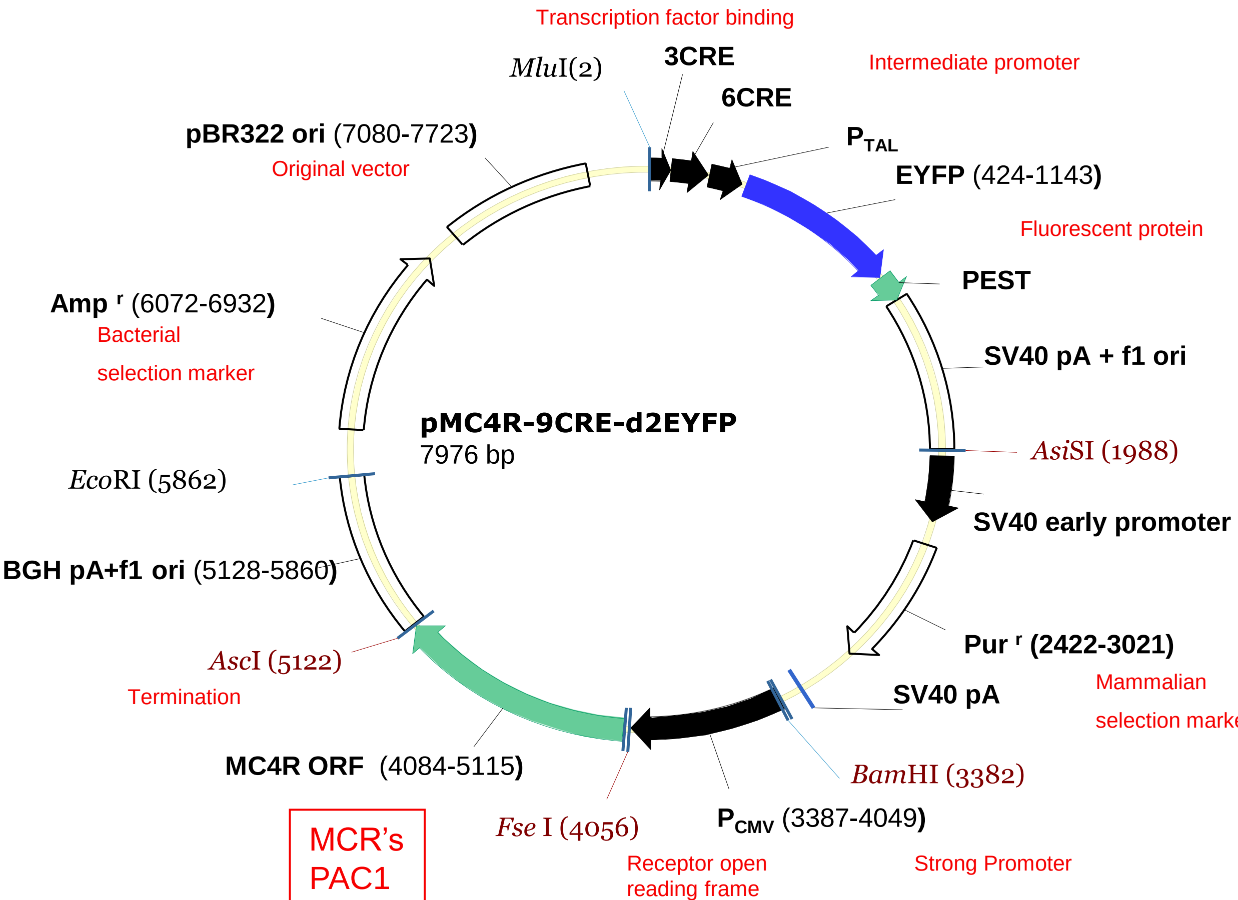


$Pd(dppf)Cl_2$

INAIC reactions: One reaction multiple scaffolds



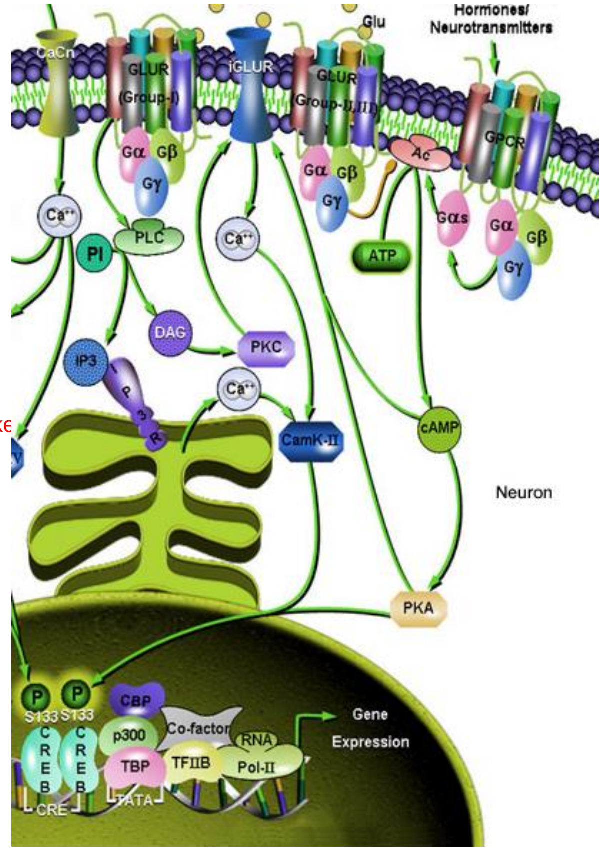
Casette for Expression of GPCR + Reporter



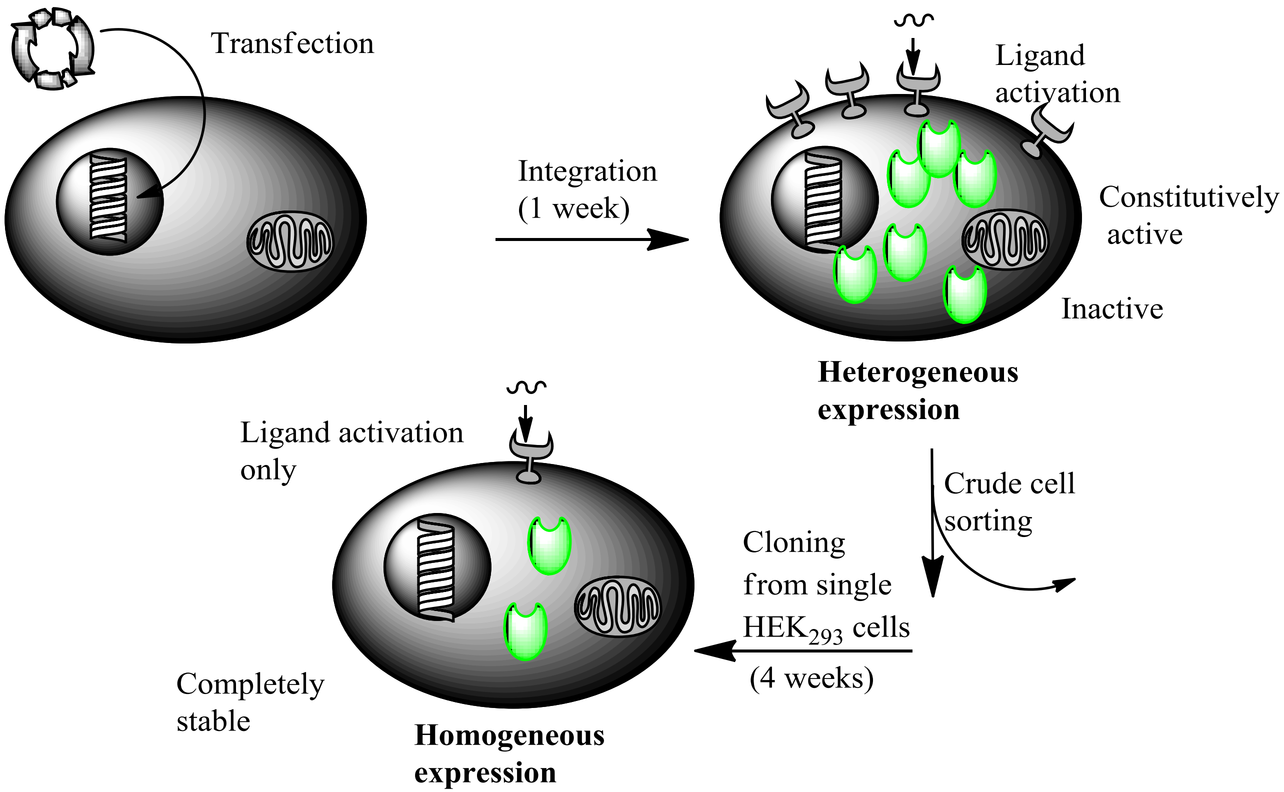
MCR's
PAC1
VPAC
CCK
etc

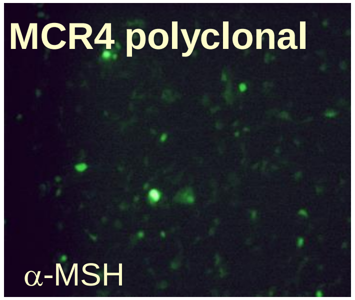
Stable transfection

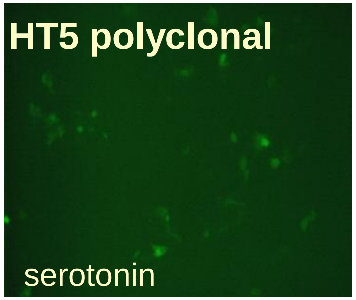
Direct detection of gene expression

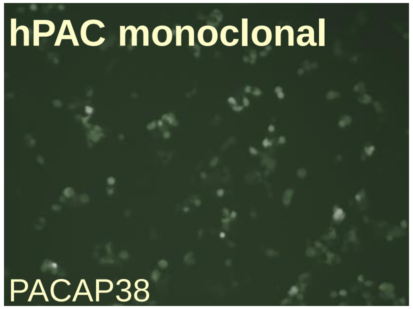


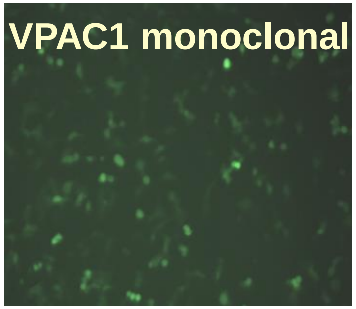
One vector, all GPCRs PAC1, VPAC1 and VPAC2

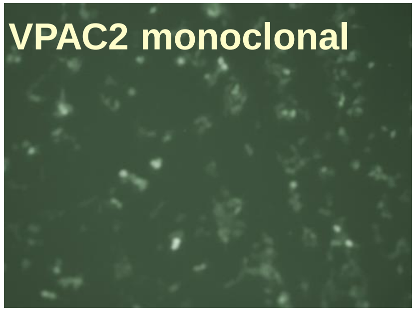






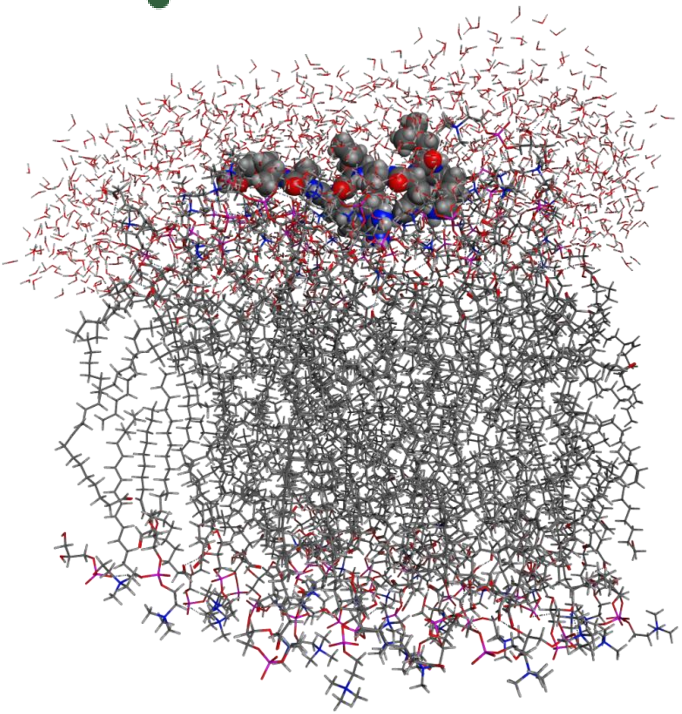


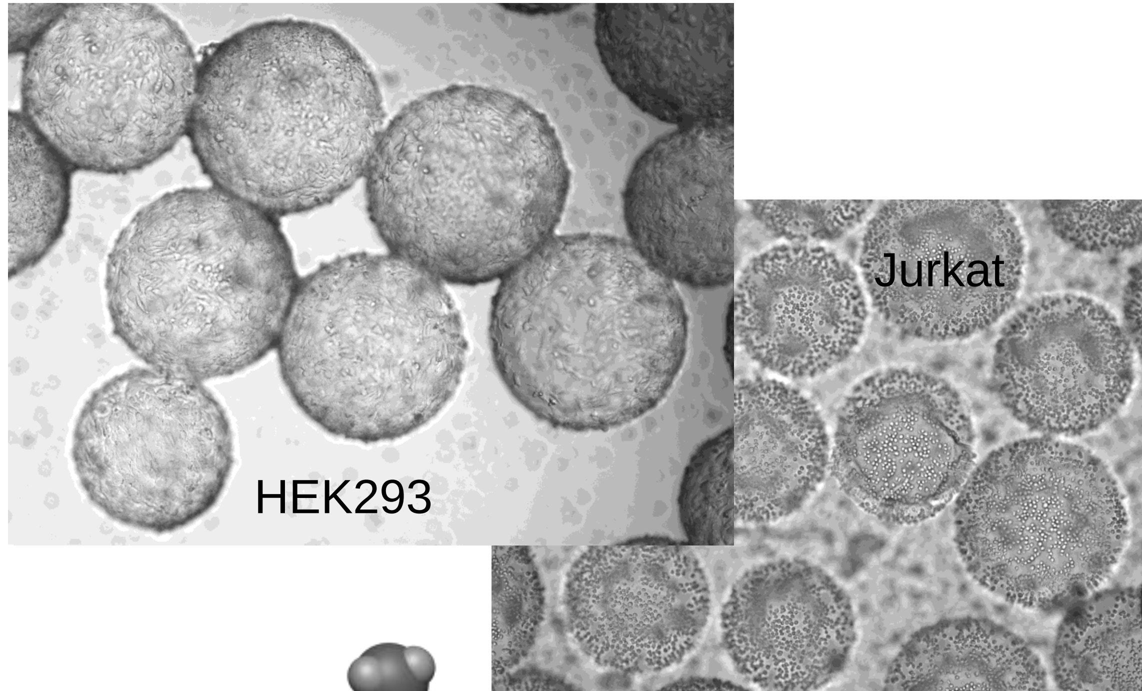


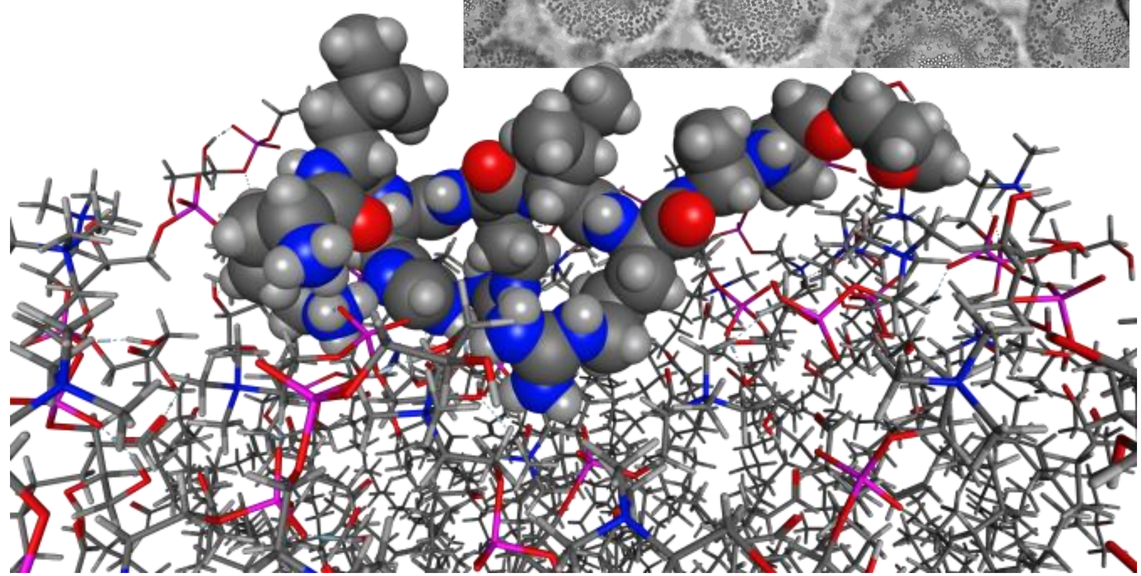


Cloning is essential

Cell adhesion peptides Binding to the lipid

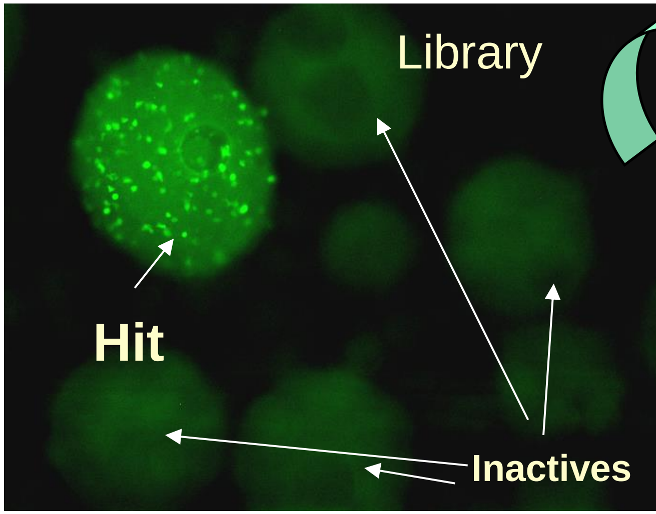


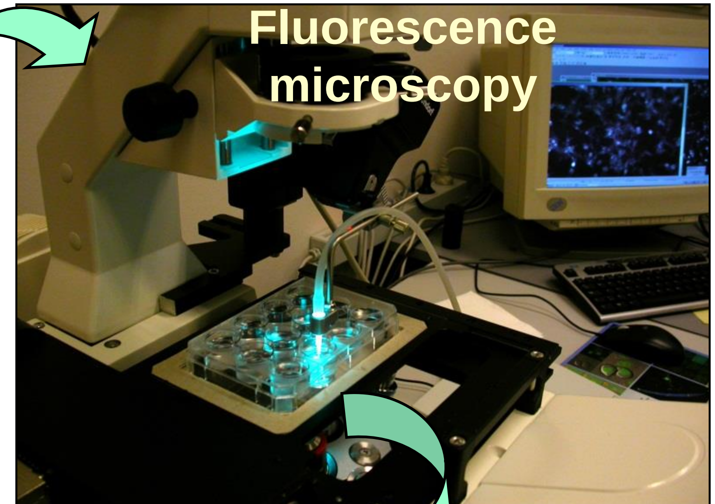


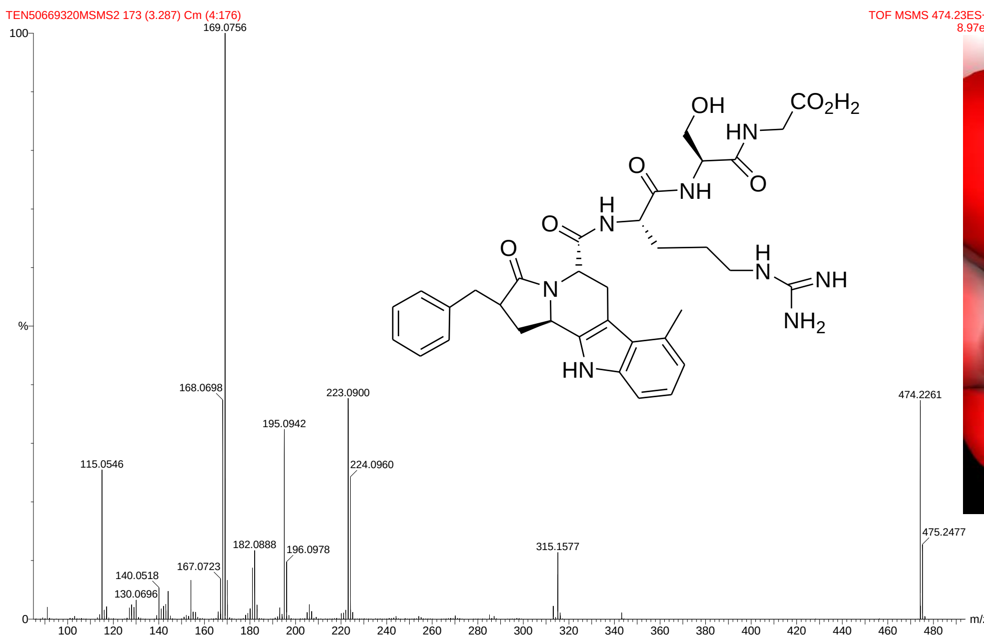


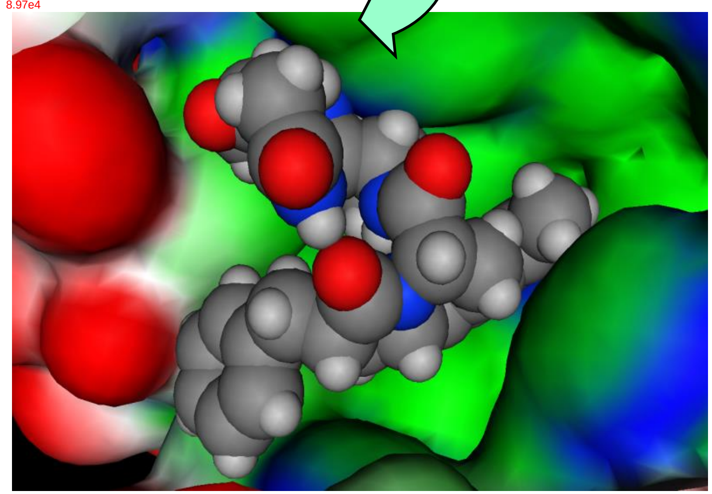
Interaction with phosphatidylcholine layer of the lipid.
Maintaining structure
No cell penetration
No lysis
No adverse effects

Scaffold diversity: Building Blocks









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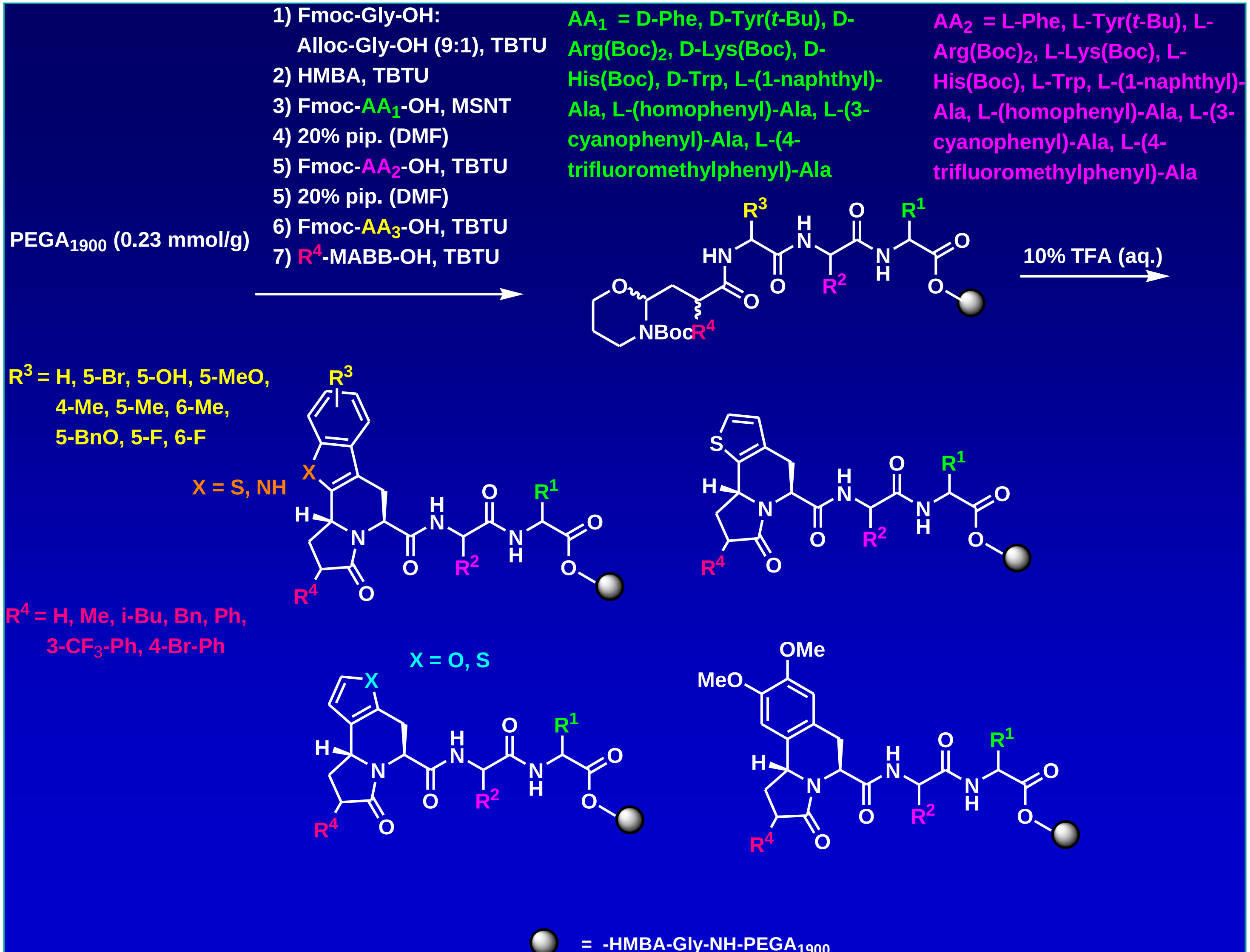
Scaffold diversity: Library

Synthesis of a 10500-membered library

34500-membered library including stereoisomers

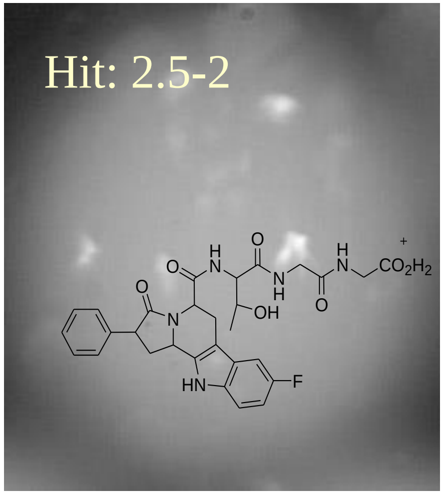
Target: Solid Phase whole cell receptor assay

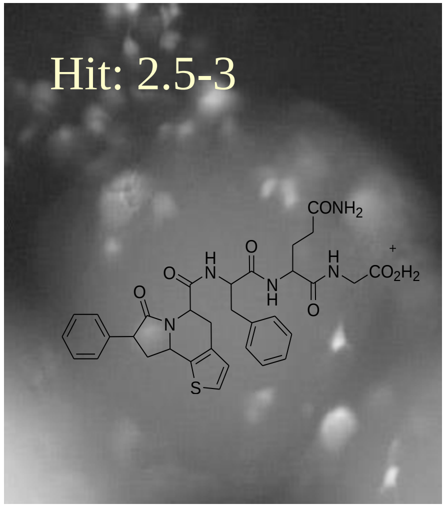


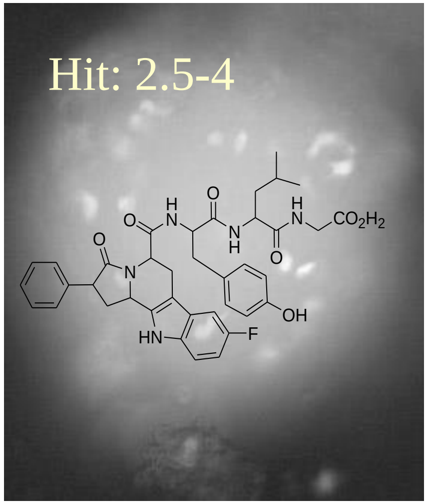
Structure determination by single bead ES MSMS analysis

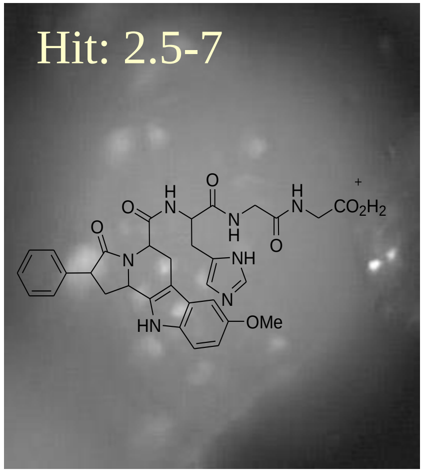
Screening INAIC library

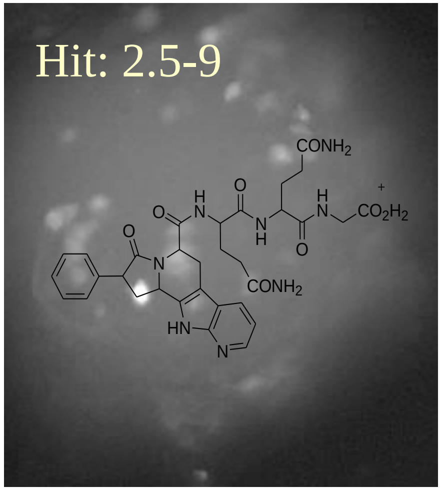
MC4R

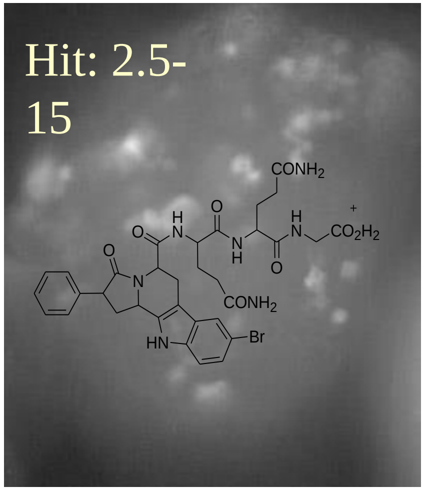






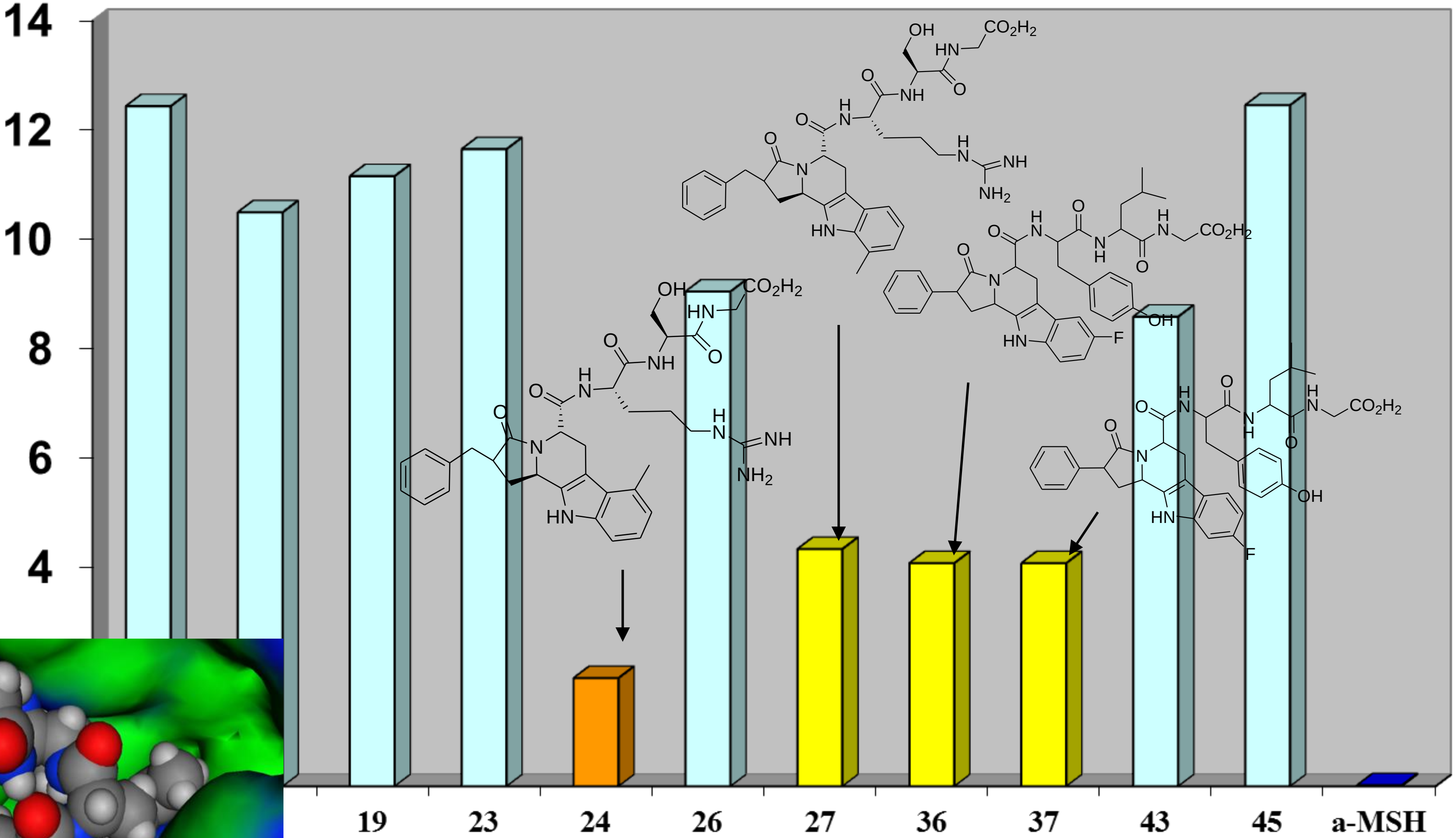


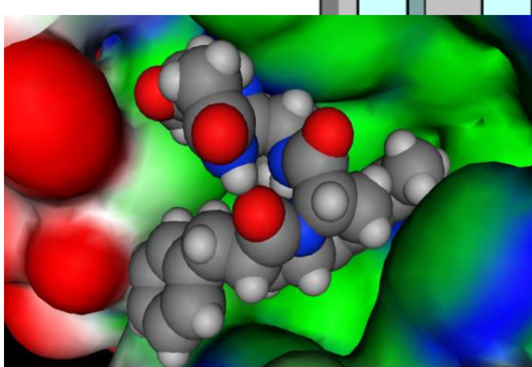




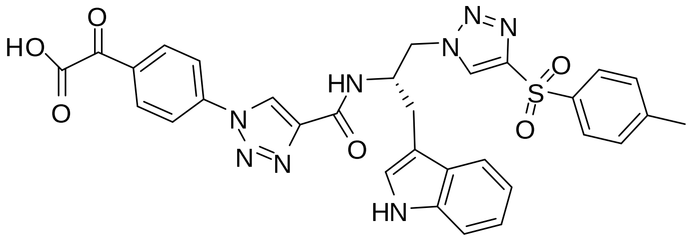
Assay results

Activity assay for hits

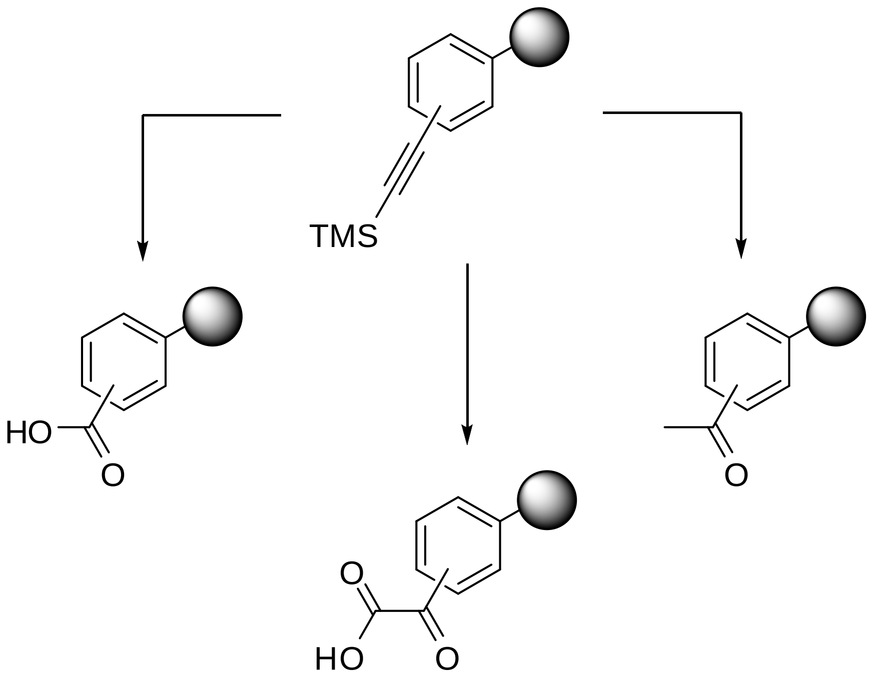


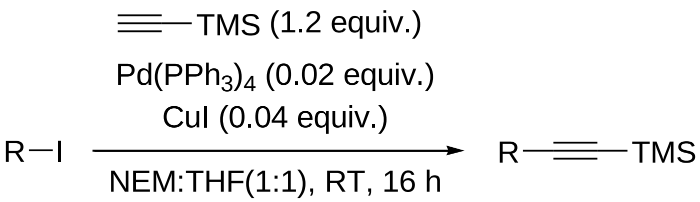


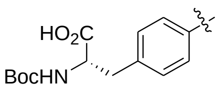
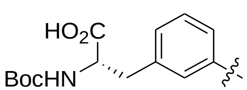
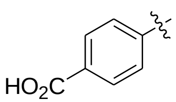
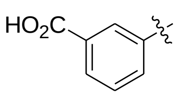
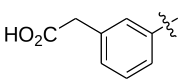
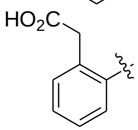
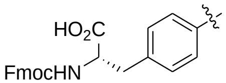
Oxidation of aromatic acetylenes



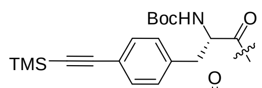
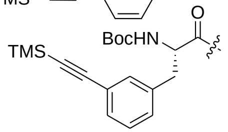
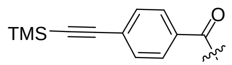
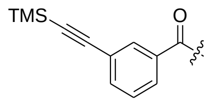
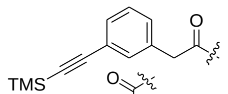
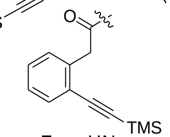
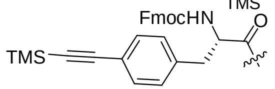
Potent selective tyrosine phosphatase inhibitor



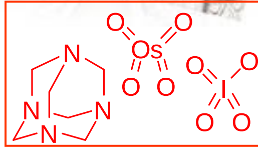


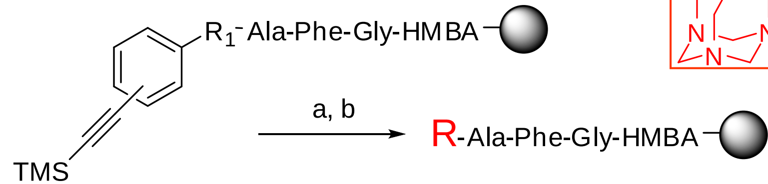
R	product, yield (%)
	89
	92
	>95
	94
	>95
	87
	>95

Diversity from aromatic acetylenes

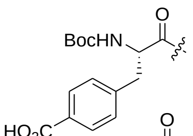
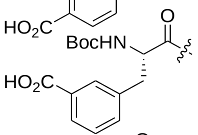
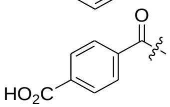
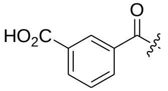
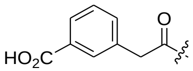
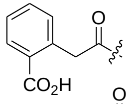
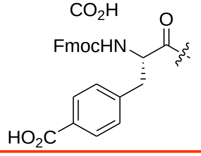
R	Purity (%)
	>95
	>95
	>95
	>95
	>95
	>95
	>95

(a) HMBA, TBTU, NEM, DMF;
(b) Fmoc-Gly-OH, MSNT, 1-methylimidazole, CH2Cl2;
(c) 20% pip. in DMF & TBTU /Fmoc-Aa-OH or acetylene BB
(d) 0.1 M NaOH (aq)

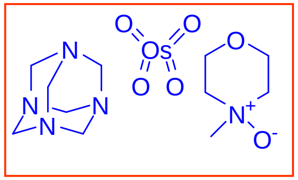


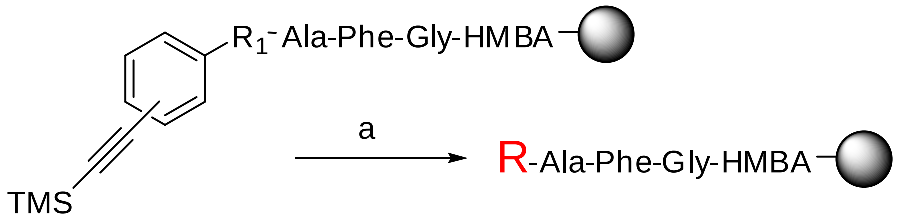


a) TBAF, THF
b) OsO4, NaIO4, HMTA, THF:H2O (1:1)

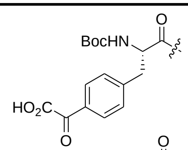
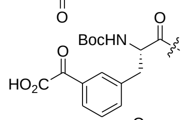
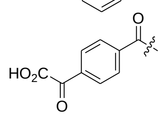
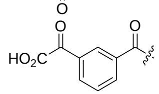
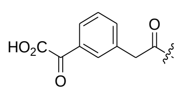
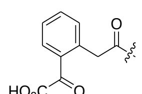
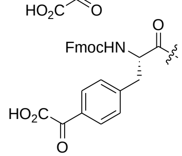
R	product, purity (%)[b]
	>95
	>95
	>95
	>95
	>95
	>95
	78

Diversity from aromatic acetylenes

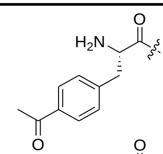
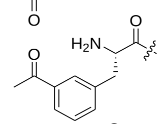
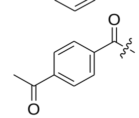
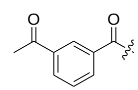
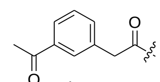
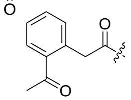
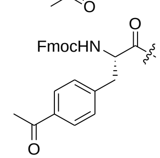




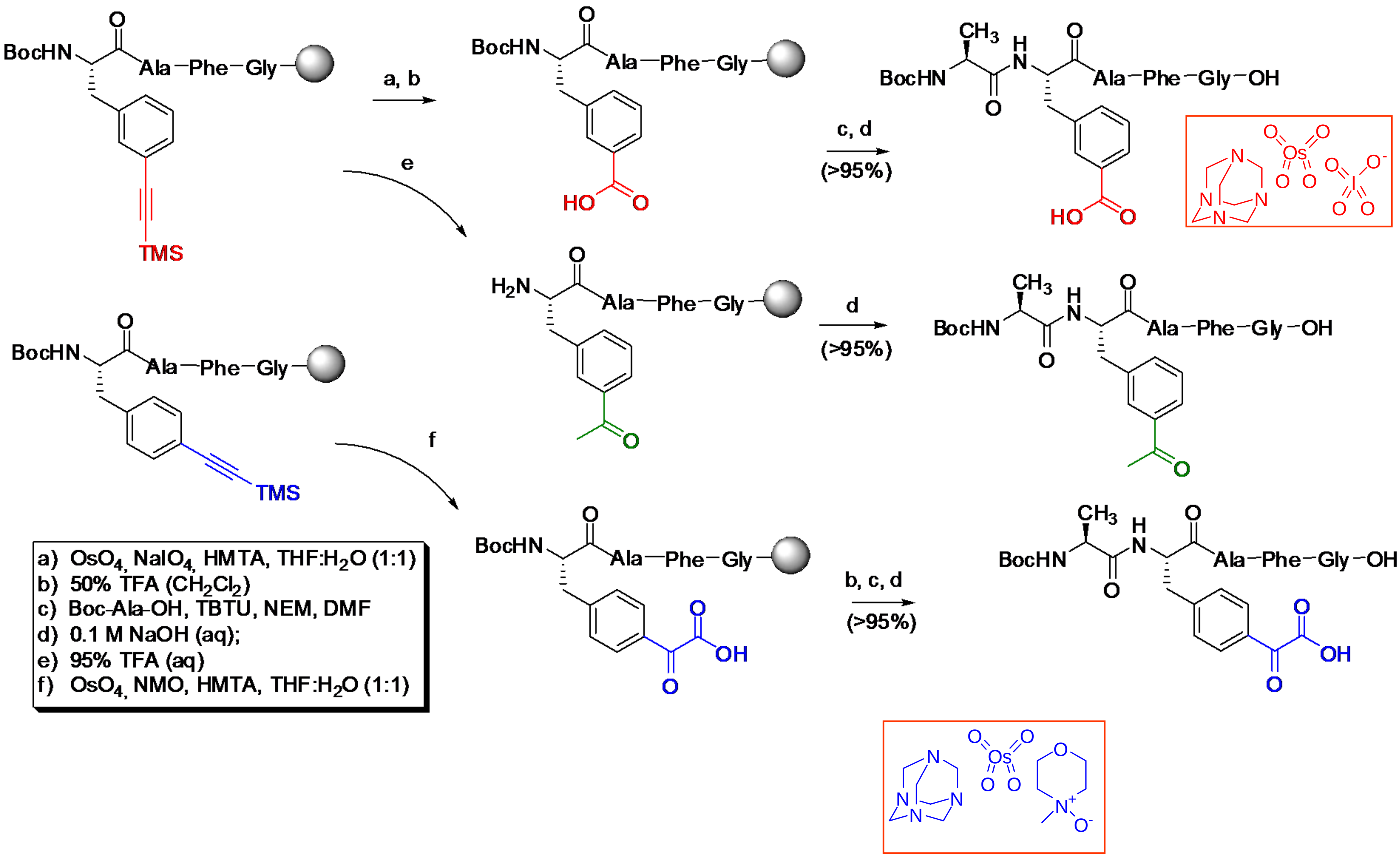
a) OsO4, NMO, HMTA, THF:H2O (1:1).

R	product, purity (%)
	>95
	>95
	73
	90
	92
	0
	92

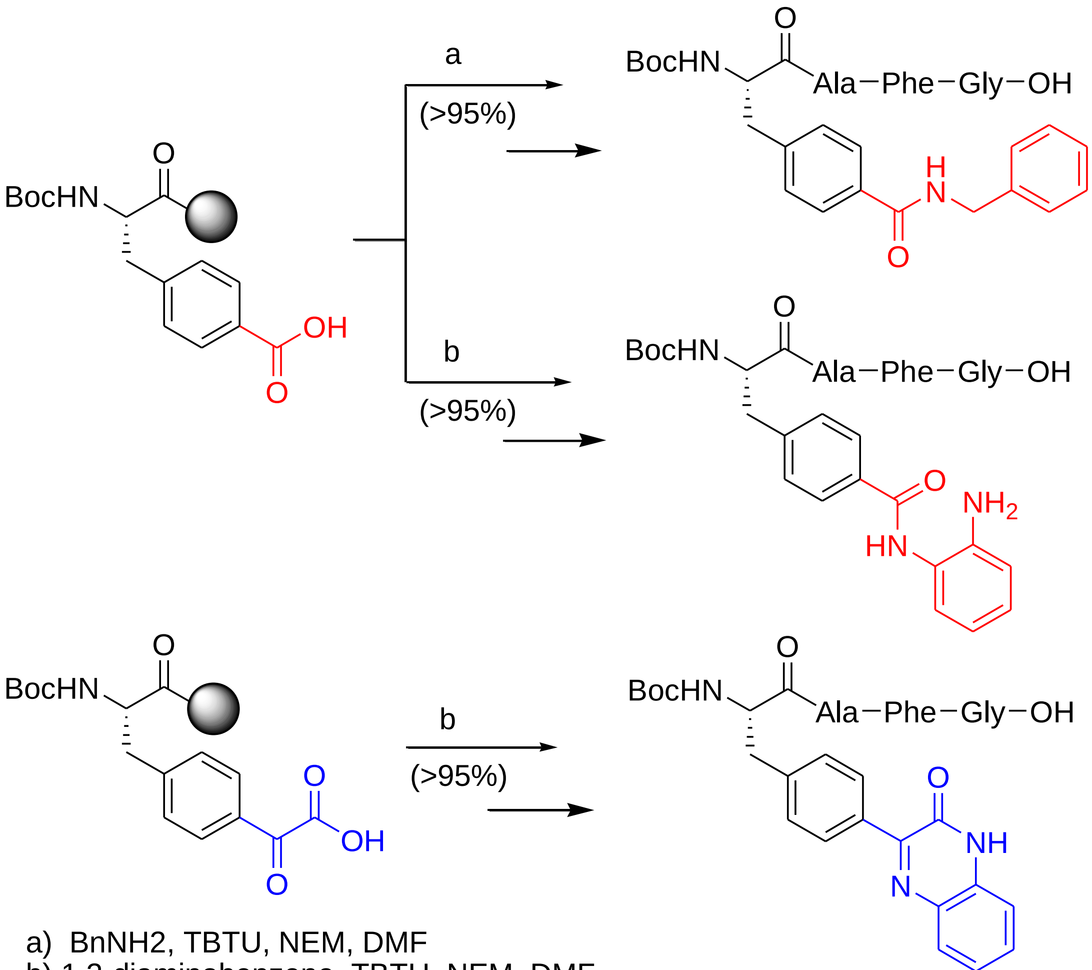
a) 95% TFA (aq)

R	product, purity (%)
	>95
	>95
	>95
	>95
	>95
	>95
	>95

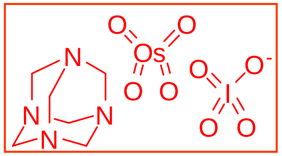
Diversity from aromatic acetylenes

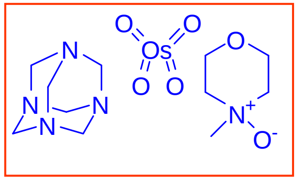


Diversity from aromatic acetylenes



a) BnNH2, TBTU, NEM, DMF
b) 1,2-diaminobenzene, TBTU, NEM, DMF





Thanks

Conclusion:
Screening molecular properties for cellular control
Monoclonal GPCR – reporter gene assays
N-acyliminium ion mediated reactions
New small molecule scaffolds from peptides

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Lamine Bouakaz
Grith Hagel