ONE-POT ORGANOCATALYTIC ROUTES TO NITROGEN- AND SULFUR-CONTAINING HETEROCYCLES

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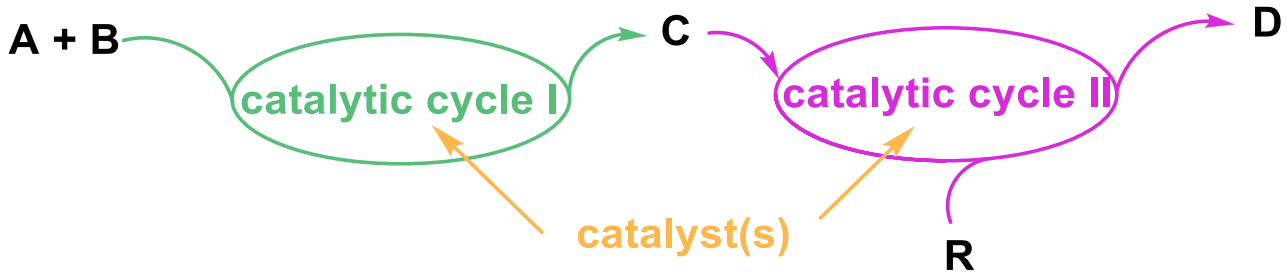
Our central aim: one-pot and tandem (or domino) reactions

Processes that allow us to perform two or more transformations in a single reactor carrying out a single work-up step.

Tandem (or domino) reactions: processes of two (or more) chemical transformations occurring under identical conditions, in which the subsequent transformation takes place at the functionalities obtained in the former transformation.

Tandem catalysis



Advantages towards a more sustainable and Green Chemistry

Recently domino organocatalysed reactions have provided an effective tool to prepare optically enriched heterocyclic molecules

Advantages:

-Avoid separation and purification of intermediates

-Reduced materials costs and time saving, reduced use of solvents

-Significant reduction of the amount of waste

-Mild reaction conditions suitable for practical synthetic applications

Shindoh, N.; Takemoto, Y.; Takasu, K. *Chem. Eur. J.* **2009**, *15*, 12168; Fogg, D. E.; dos Santos, E. N. *Coord. Chem. Rev.* **2004**, *248*, 2365.

Aims

Società Chimica Italiana

SCI2021

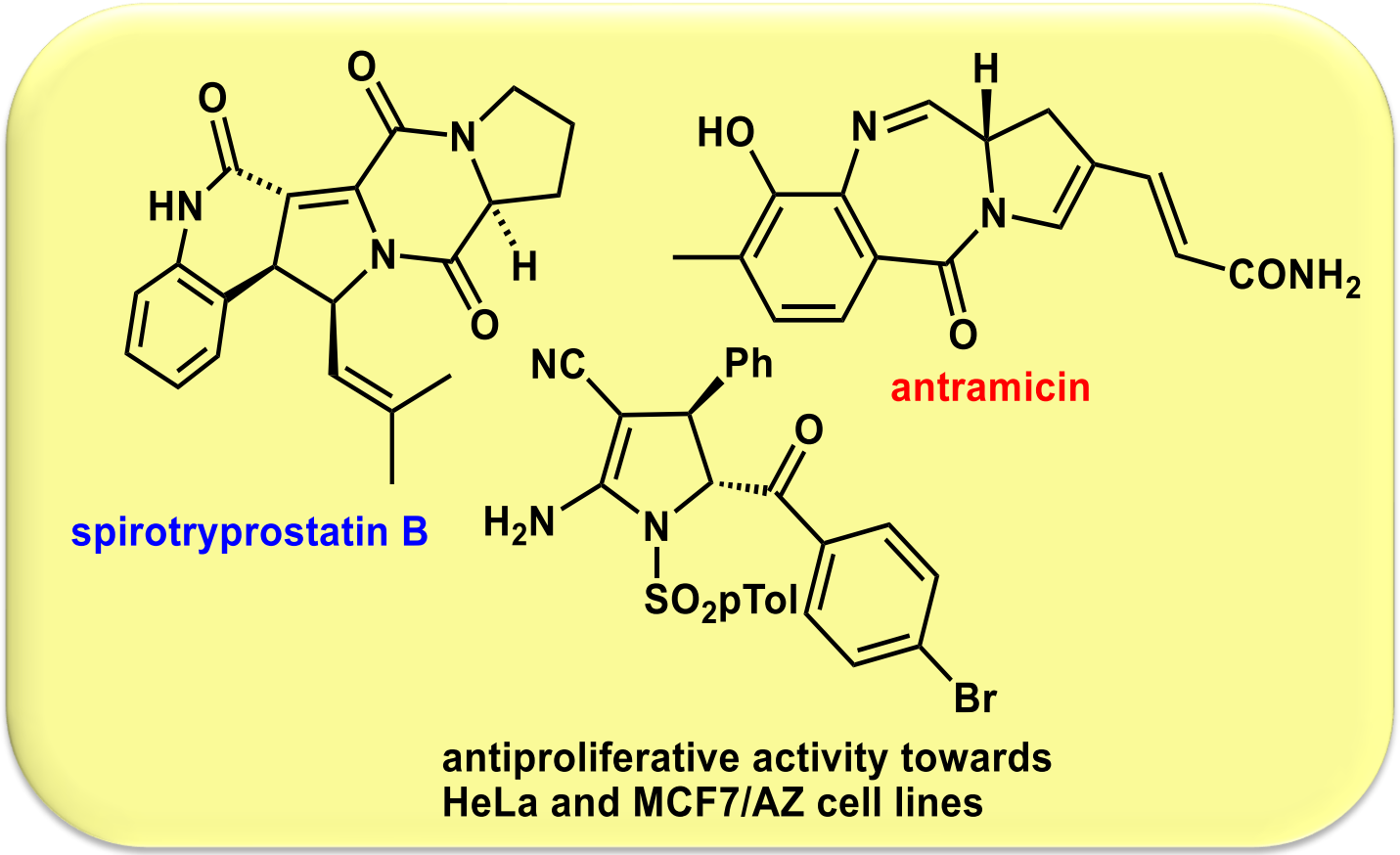
Research Award "Organic Chemistry for the Environment, Energy and Nanosciences" Junior

«For her important contributions to the detailed understanding of the mechanistic aspects of new asymmetric syntheses, with low environmental impact, of heterocyclic compounds of biological interest through the use of organocatalysts from natural sources.»

New approaches aim at:

- ✓ Using cheap metal-free organic promoters
- ✓ of low toxicity, high stability to air and moisture
- ✓ and able to work under mild and less hazardous reaction conditions
- ✓ Green solvents
- ✓ Design of one-pot and tandem methodologies
- ✓ Minimizing waste production, avoiding purification or separation of intermediates

One-pot diastereoselective synthesis of tetrasubstituted 2-pyrrolines



J. Antibiot. **1996**, *49*, 832; *Cancer Res*. **1969**, *29*, 2195; *Nature*, **1979**, *282*, 529; *Bioorg. Med. Chem. Lett*. **2008**, *18*, 1392.

One-pot diastereoselective synthesis of tetrasubstituted 2-pyrrolines

Green Chemistry

COMMUNICATION

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Received 10th November 2014,
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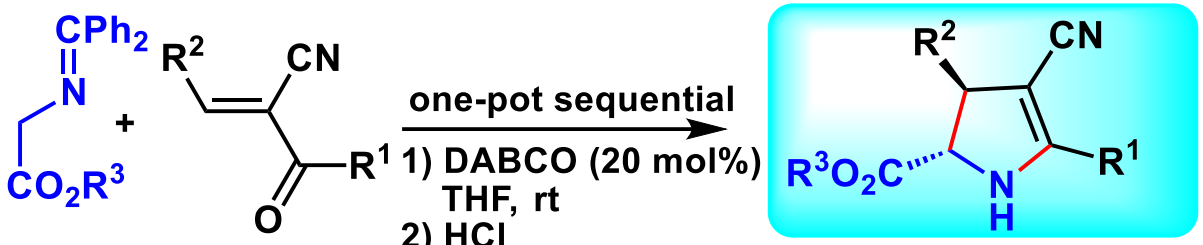
DOI: 10.1039/c4gc02191f

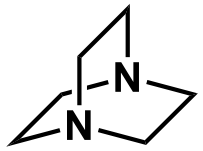
www.rsc.org/greenchem

One-pot highly diastereoselective annulation to N-unprotected tetrasubstituted 2-pyrrolines†

Sara Meninno, Amedeo Capobianco, Andrea Peluso and Alessandra Lattanzi*

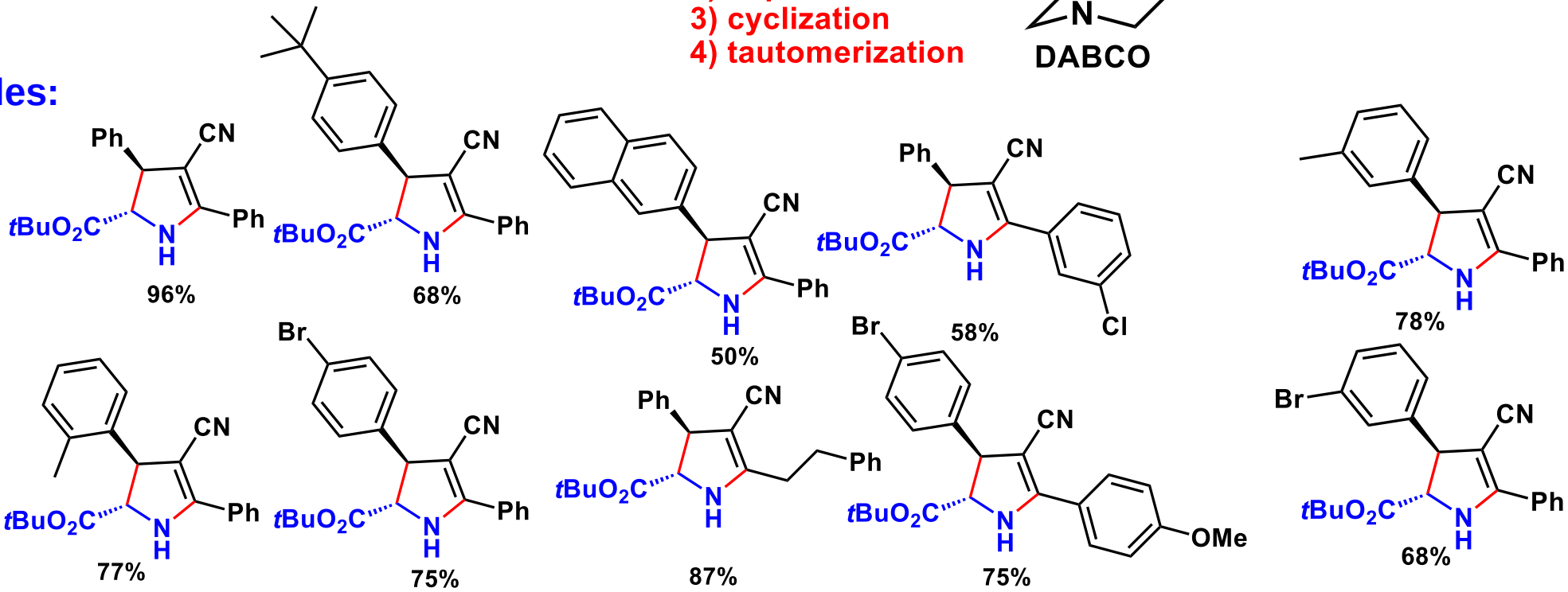


1) Michael addition
2) deprotection
3) cyclization
4) tautomerization



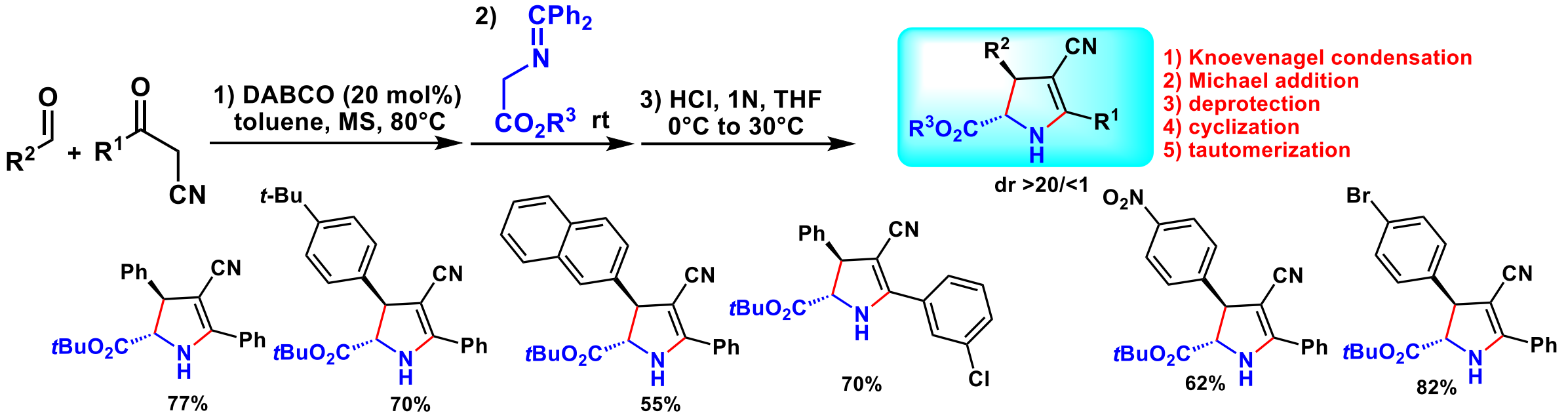
DABCO

Selected examples:

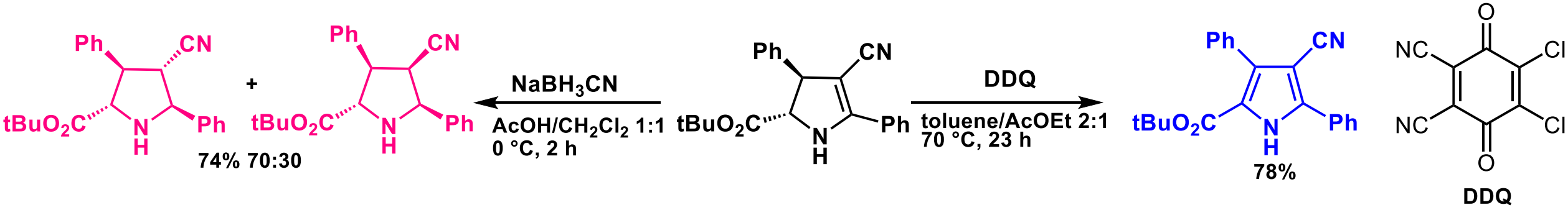


Meninno, S.; Capobianco, A.; Peluso, A.; Lattanzi, A. *Green. Chem.* **2015**, *17*, 2137.

One-pot sequential synthesis of trans-2-pyrrolines from commercially available aldehydes and aroylacetonitriles



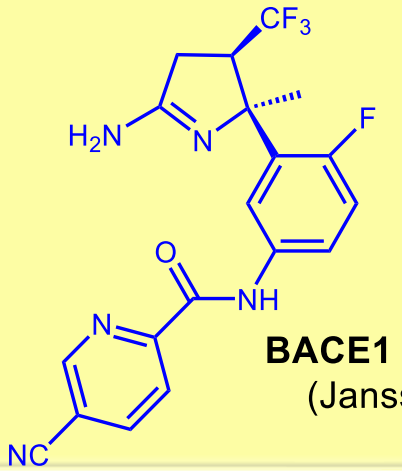
Derivatization of trans-2-pyrrolines to pyrroles and pyrrolidines



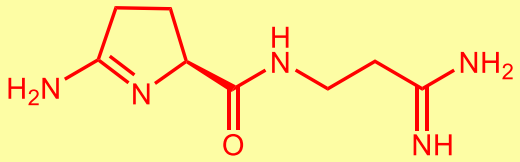
Meninno, S.; Capobianco, A.; Peluso, A.; Lattanzi, A. *Green. Chem.* **2015**, *17*, 2137.

Polysubstituted amidines

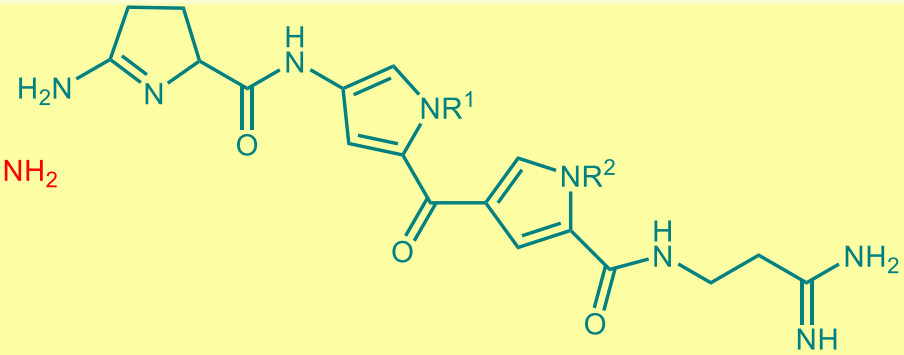
Bioactive and natural products bearing the 2-amino-1-pyrroline core



BACE1 inhibitor
(Janssen)



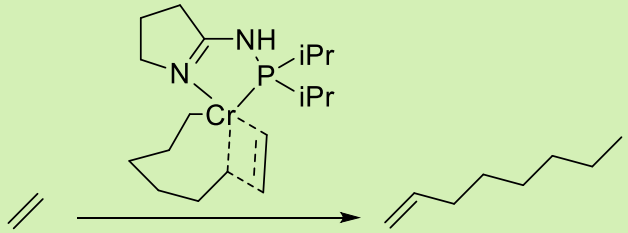
Norformicin



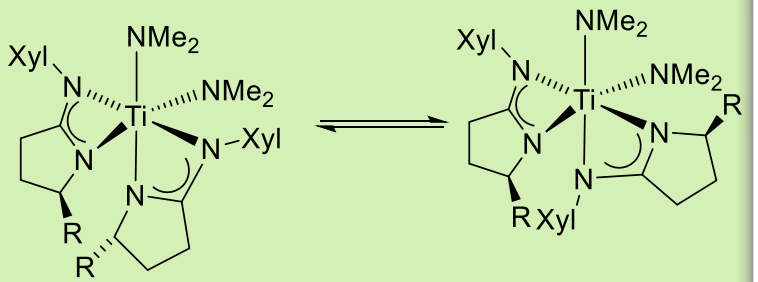
Anthelvencin A, R^1= Me, R^2= H
Anthelvencin B, R^1= R^2= H

Bioorg. Med. Chem. Lett. **2019**, *29*, 761; *Expert Opin. Drug Discov*. **2019**, *14*, 879.

Applications of 2-amino-1-pyrrolines as ligand in metal catalysis



Cr(P,N) catalysts for control of ethylene trimerization and tetramerization

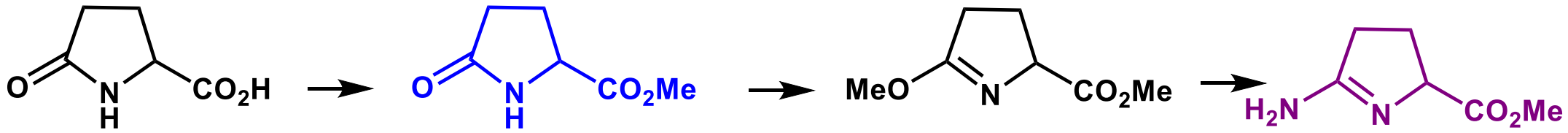


Chiral amidinate ligands for coordination to Ti^{IV}

ACS Catal. **2018**, *8*, 1138; *Inorg. Chem.* **2006**, *45*, 7777.

Synthesis of 5-amino-3,4-dihydro-2H-pyrrole-2-carboxylic acid ester

Lown's work:

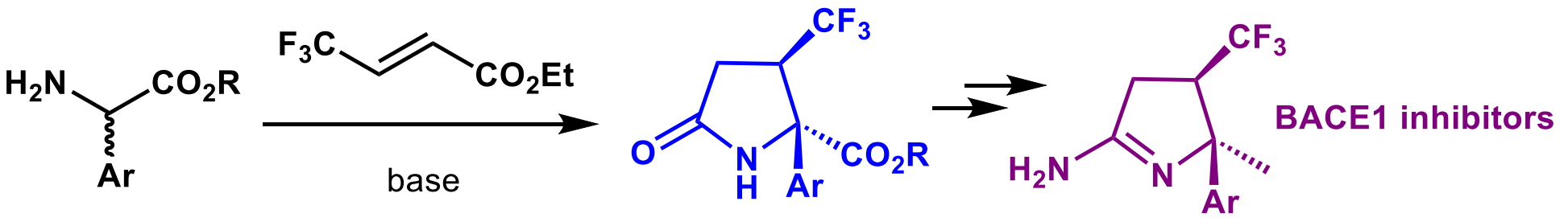


pyroglutamic acid

stepwise approach

J. Org Chem. **1987**, *52*, 5717; *J. Org Chem.* **1988**, *53*, 1855.

Work by Mateu, Delgado and Fustero:



Chem. Eur. J. **2015**, *21*, 11719.

Adv. Synth. Catal. **2018**, *360*, 4362.

One-pot aza-Michael-lactamization approach

One-pot approach to functionalized 2-amino-1-pyrrolines

Chemistry–A European Journal | Communication | doi.org/10.1002/chem.202005262

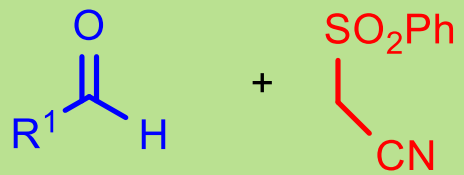
Catalysis

Diastereoselective Synthesis of Functionalized 5-Amino-3,4-Dihydro-2*H*-Pyrrole-2-Carboxylic Acid Esters: One-Pot Approach Using Commercially Available Compounds and Benign Solvents

Sara Meninno,*[a] Mario Carratù,[a] Jacob Overgaard,[b] and Alessandra Lattanzi*[a]

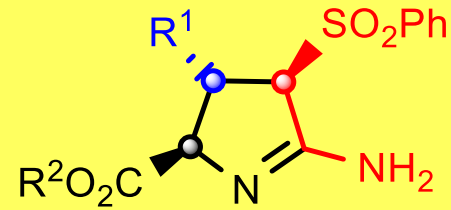
Abstract: A novel three-step four-transformation approach to highly functionalized 5-amino-3,4-dihydro-2*H*-pyrrole-2-carboxylic acid esters, starting from commercially available phenylsulfonylacetonitrile, aldehydes, and *N*-(diphenylmethylene)glycine *tert*-butyl ester, was developed. The one-pot strategy delivered this class of amidines bearing, for the first time, three contiguous stereocenters, in good to high yield and diastereoselectivity. The entire sequence was carried out using diethyl carbonate and 2-methyl tetrahydrofuran as benign solvents, operating under metal-free conditions. The process could be conveniently scaled-up, and the synthetic utility of the products was demonstrated.

Our targets:



✓ Simple commercially available reagents

- ✓ one-pot process
- ✓ metal-free
- ✓ scalability to gram scale
- ✓ green solvents

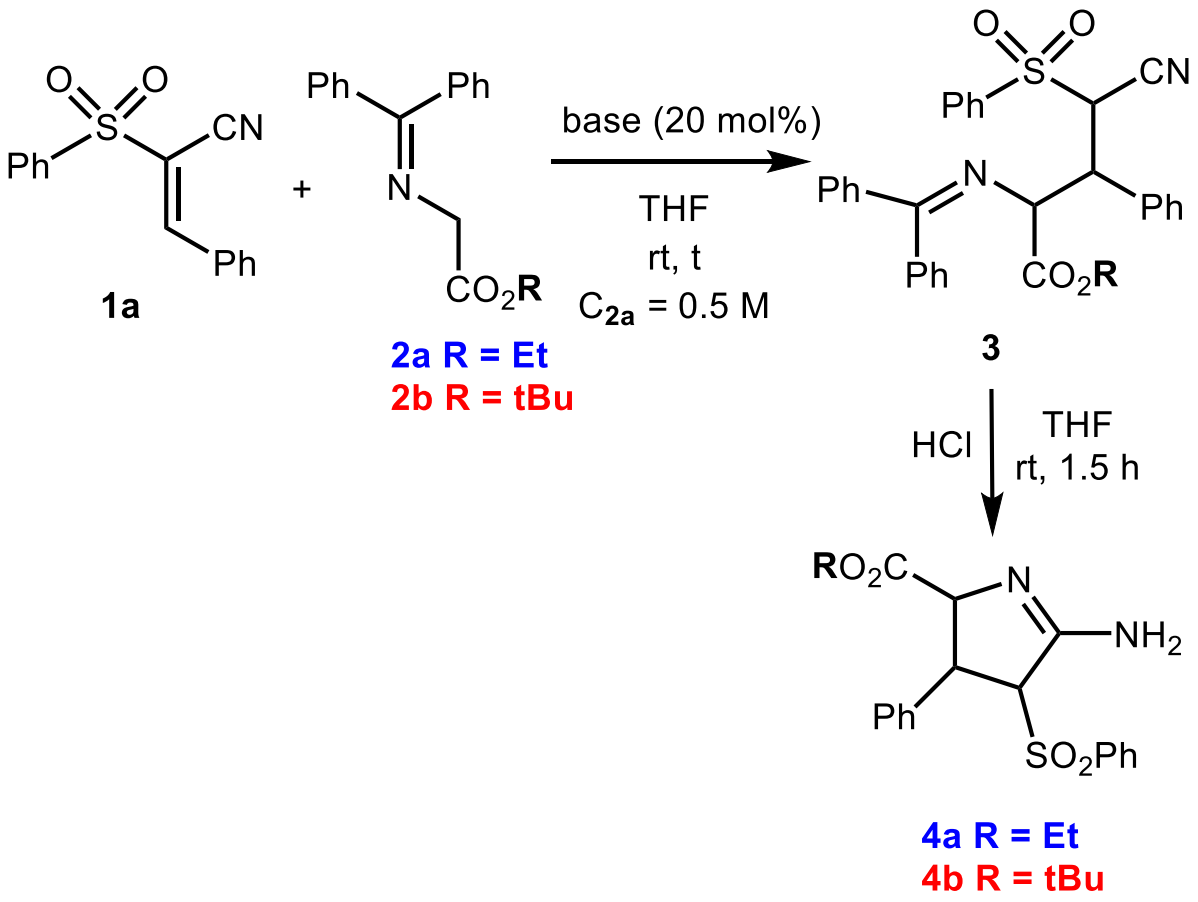


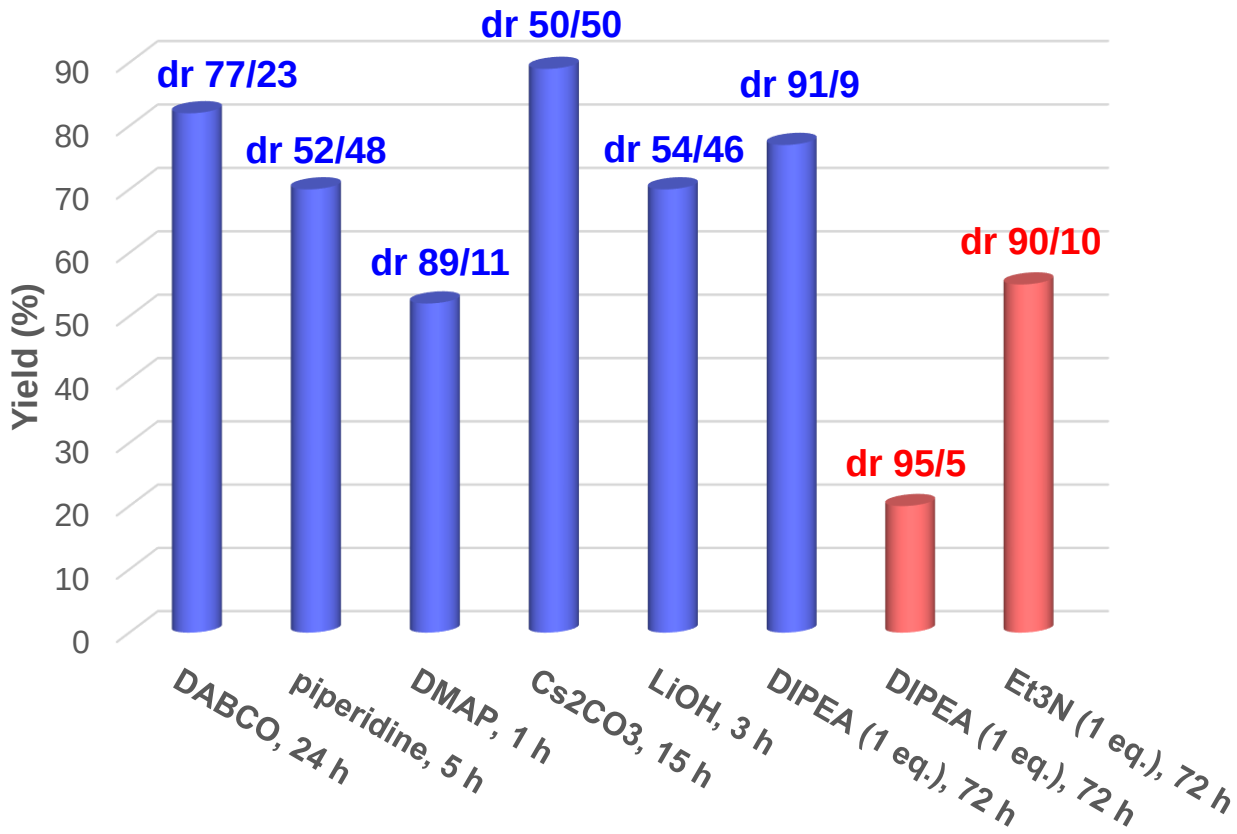
✓ highly functionalized five-membered amidines

Including biomass derived aldehydes

One-pot approach to functionalized 2-amino-1-pyrrolines

Optimization study for the Michael addition

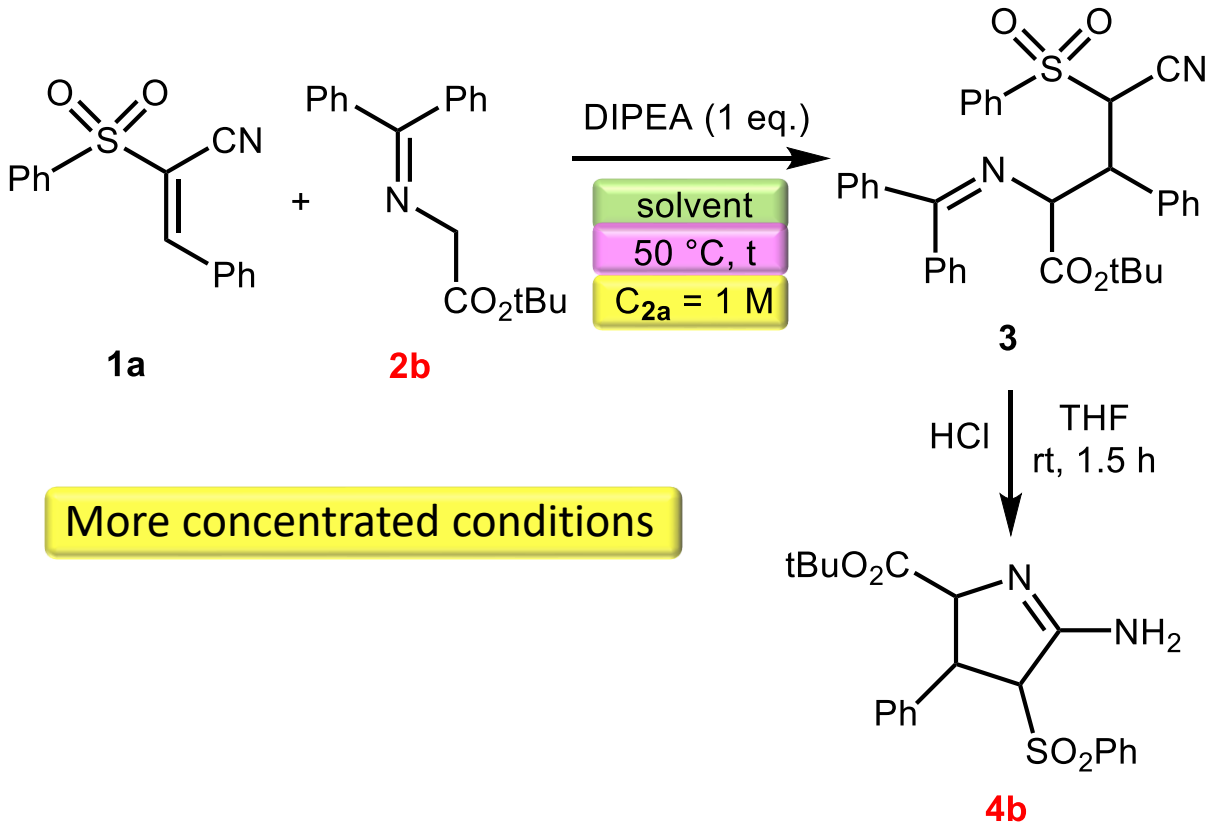




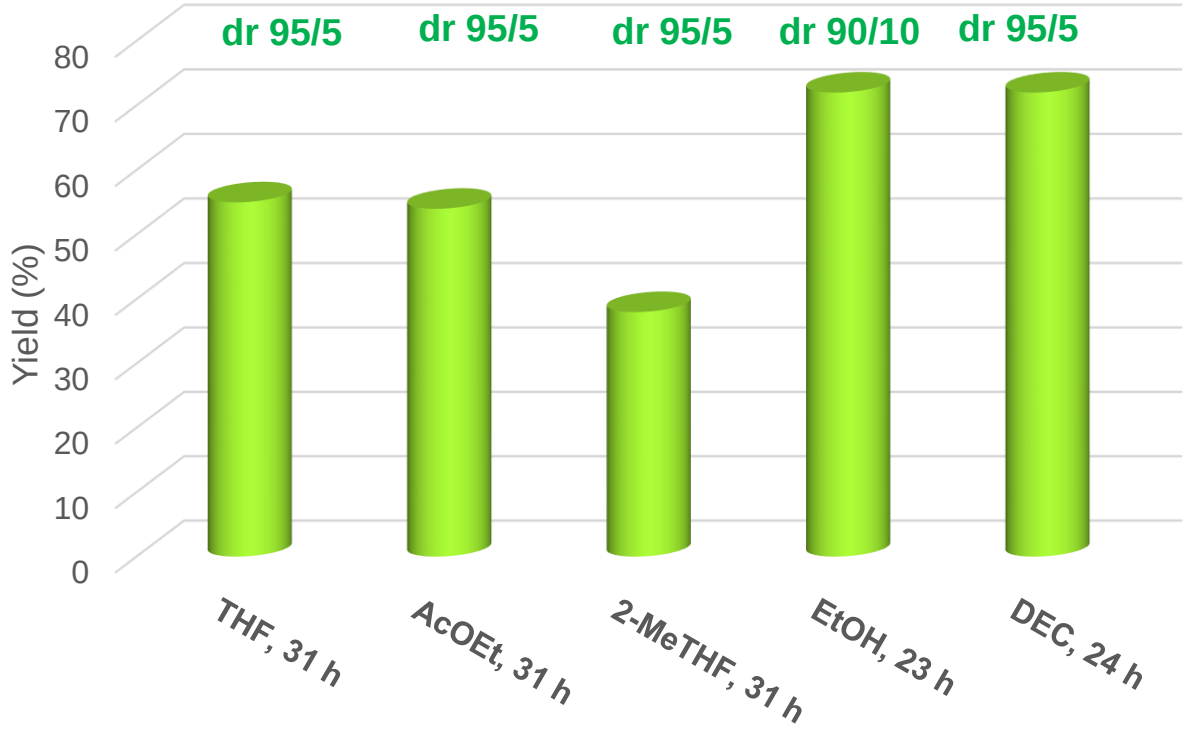
Meninno, S.; Carratù, M.; Overgaard, J.; Lattanzi A. *Chem. Eur. J.* **2021**, *27*, 4573.

One-pot approach to functionalized 2-amino-1-pyrrolines

Optimization study for the Michael addition



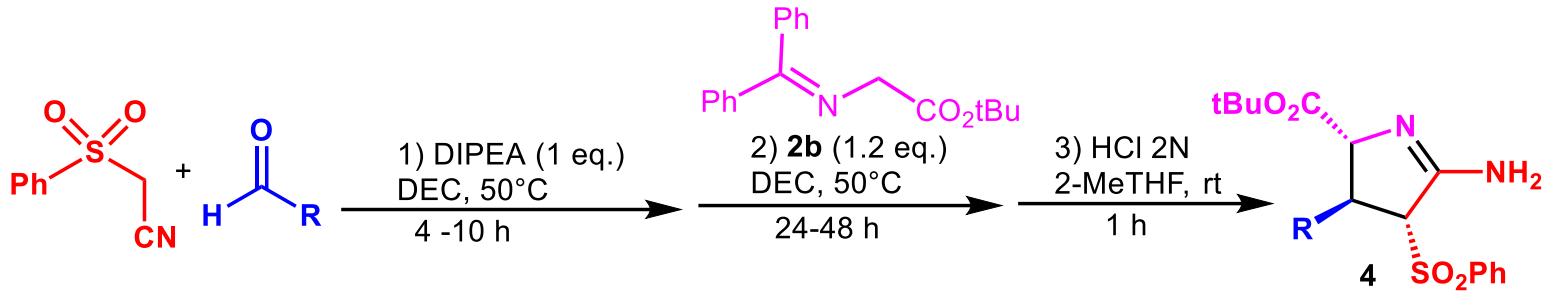
More concentrated conditions

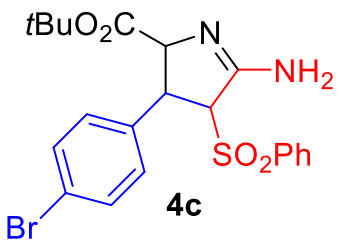


Meninno, S.; Carratù, M.; Overgaard, J.; Lattanzi A. *Chem. Eur. J.* **2021**, *27*, 4573.

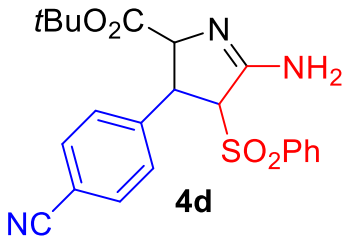
One-pot approach starting from commercial reagents

Substrate scope

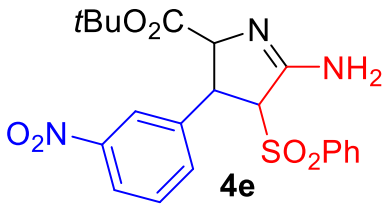




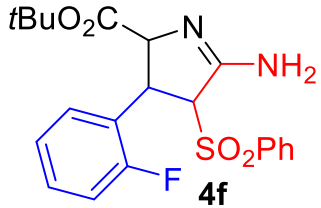
64%, dr 90/10



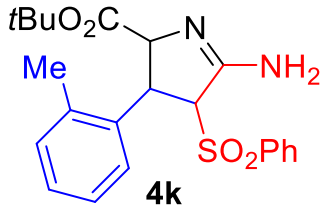
55%, dr 92/8



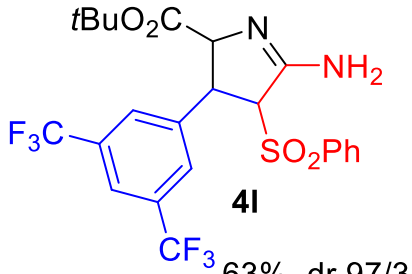
54%, dr 92/8



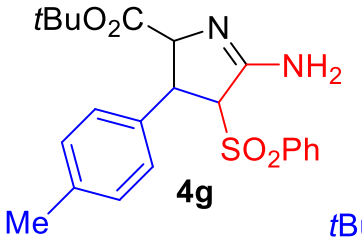
51%, dr 96/4



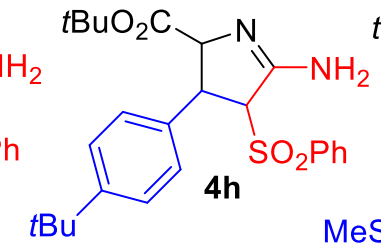
87%, dr 92/8



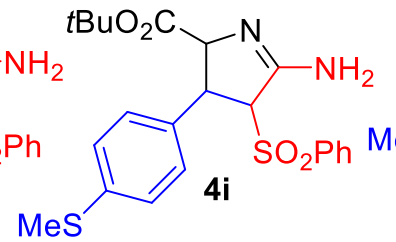
63%, dr 97/3



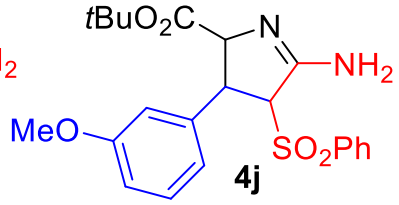
73%, dr 91/9



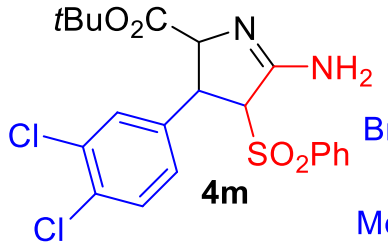
73%, dr 92/8



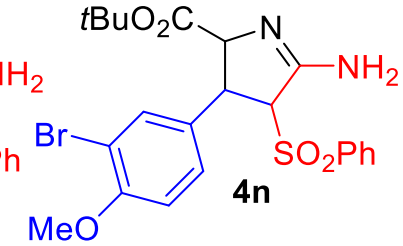
72%, dr 94/6



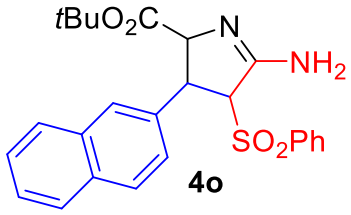
89%, dr 93/7



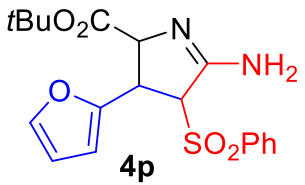
55%, dr 91/9



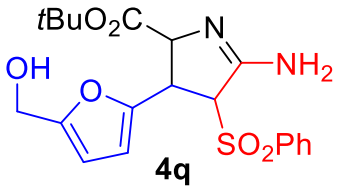
65%, dr 91/9



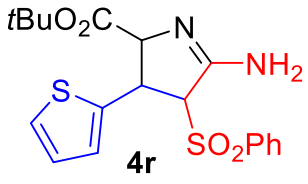
76%, dr 93/7



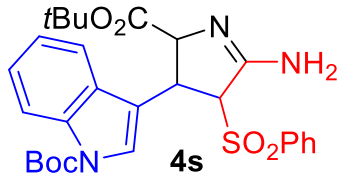
67%, dr 77/23



44%, dr 70/30



55%, dr 87/13



55%, dr 83/17

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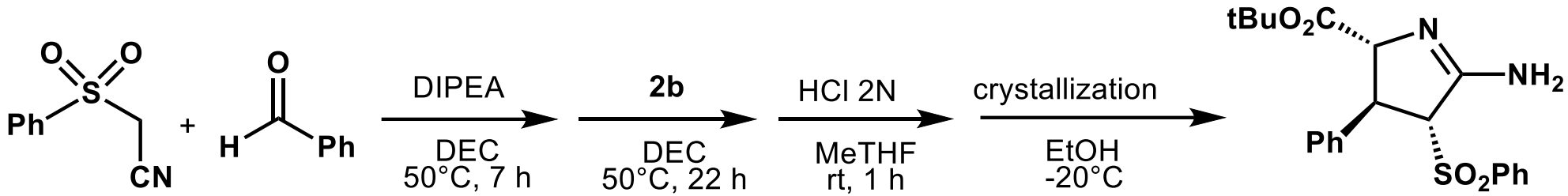
Scale-up procedure and post-functionalizations of amidines

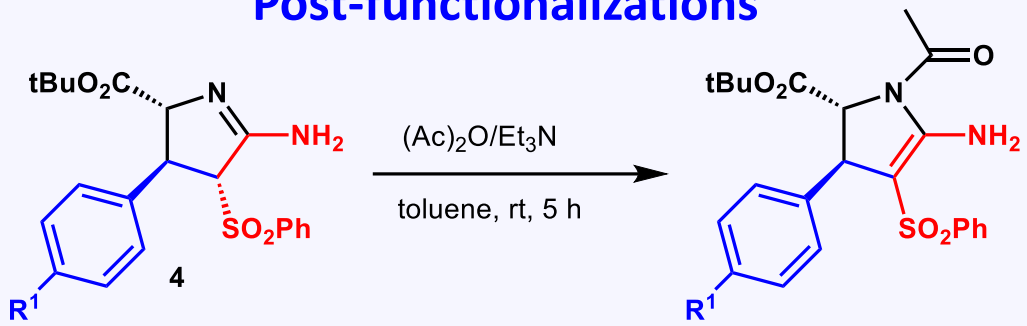
Scale-up



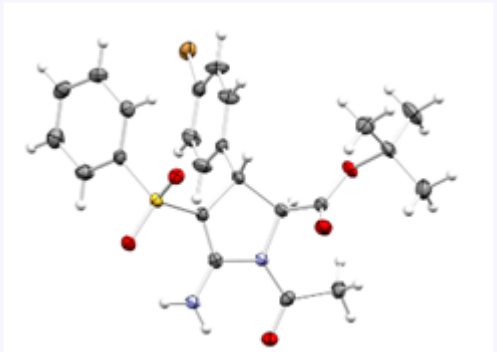
1g, 5.6 mmol

1.33 g, 60%
only (*trans*,*trans*)-**4b**

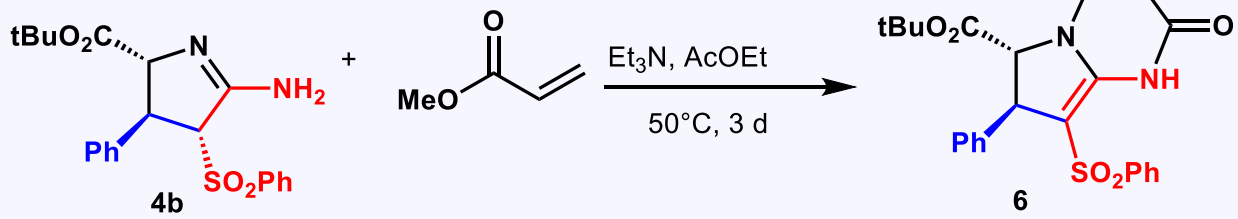
Post-functionalizations



R^1 = H 69%, dr >20/<1 **5a**
R^1 = Br 72%, dr >20/<1 **5b**
R^1 = SCH_3 53%, dr >20/<1 **5c**



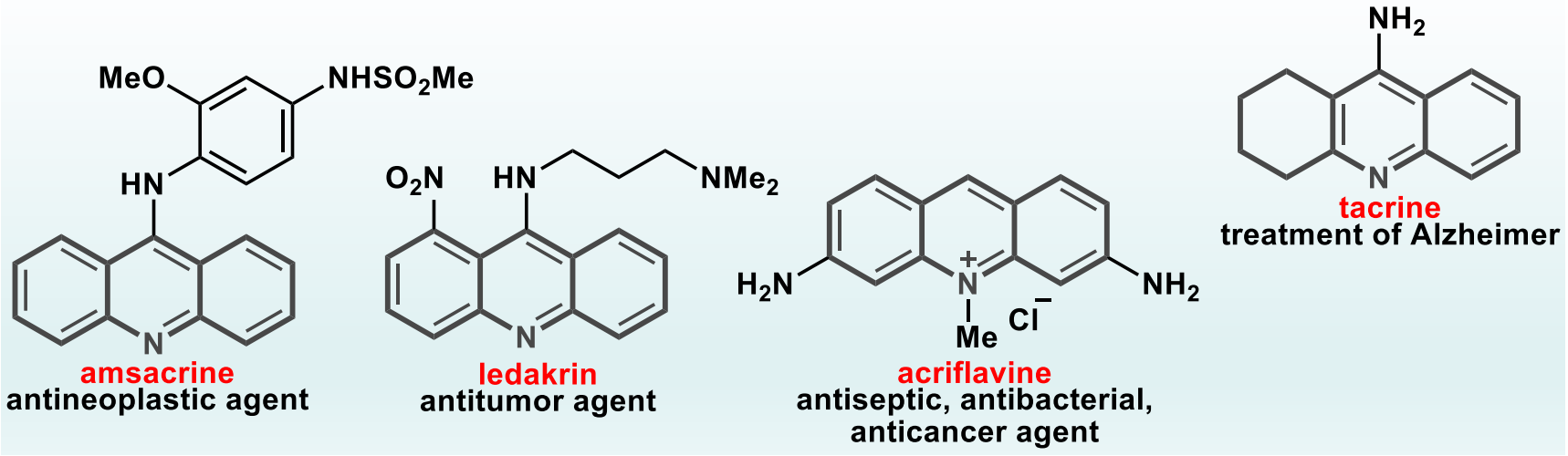
5b



69%, dr >20/<1

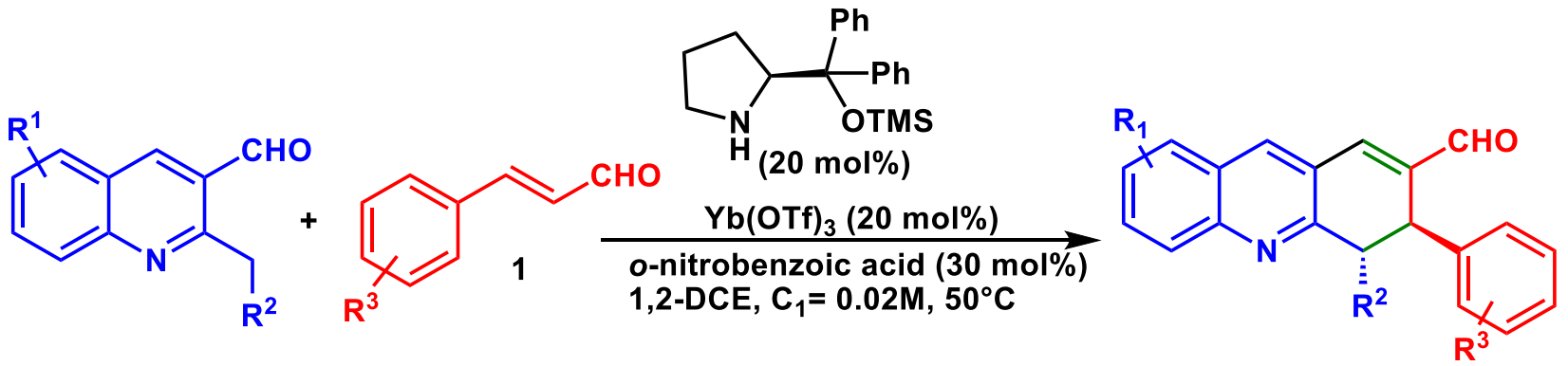
Meninno, S.; Carratù, M.; Overgaard, J.; Lattanzi A. *Chem. Eur. J.* **2021**, *27*, 4573.

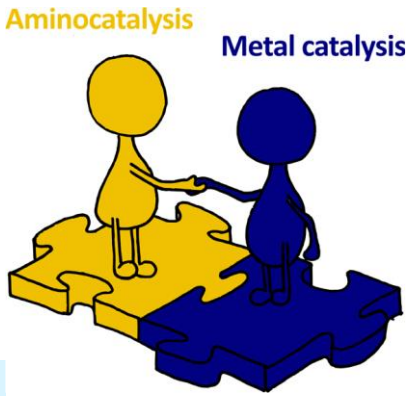
Synergistic catalysis: highly enantioselective cascade reaction for the synthesis of dihydroacridines



Our approach

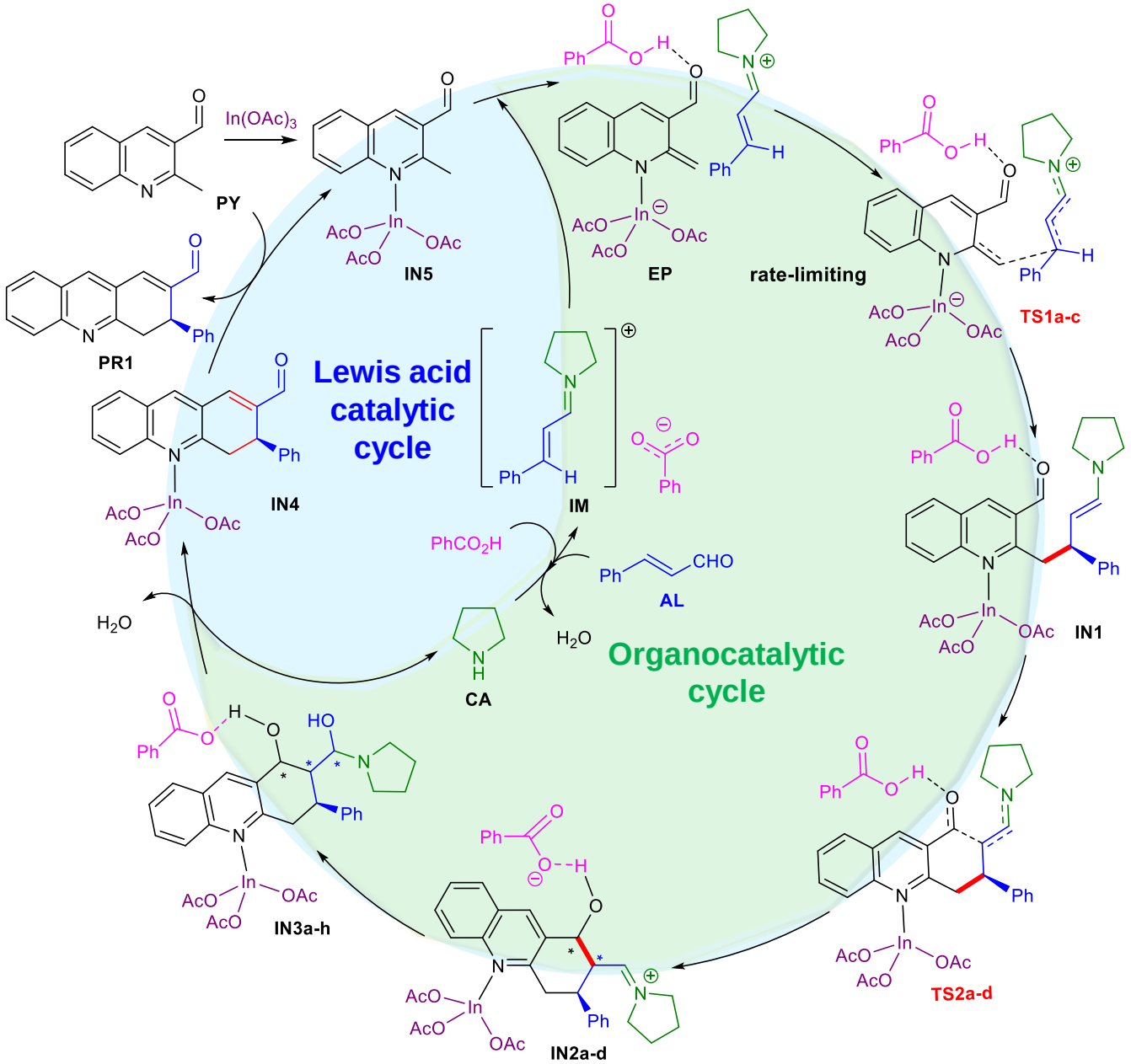
Synergistic Catalysis: merging covalent organocatalysis (aminocatalysis) and transition metal catalysis





Meninno, S.; Meazza, M.; Yang, J., M.; Tejero, T.; Merino-Gomez, P.; Rios, R. *Chem. Eur. J.* **2019**, *25*, 7623.

Cascade reaction for the synthesis of dihydroacridines



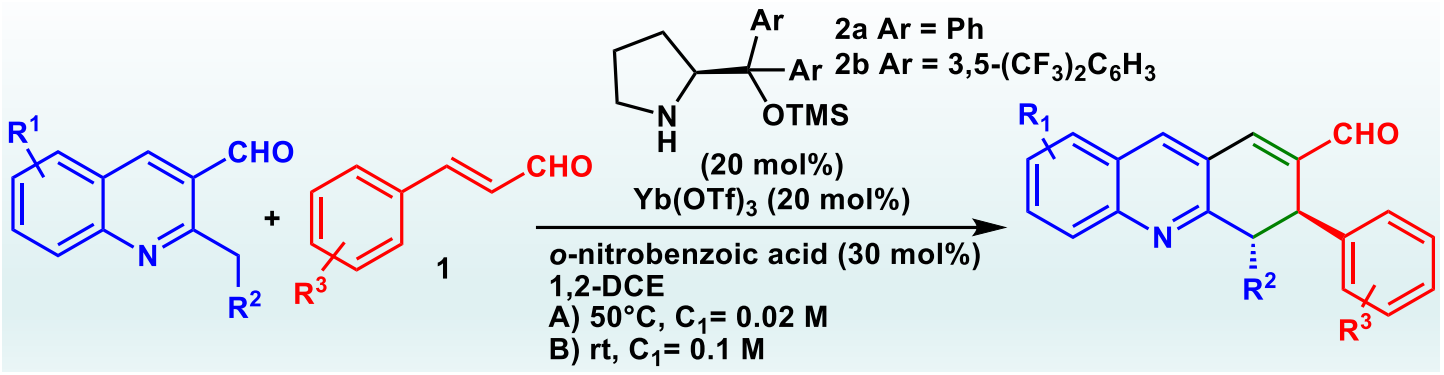
Cascade process

Michel reaction | Aldol reaction | Dehydra-tation

- ✓ High enantioselectivity
- ✓ Very simple catalysts
- ✓ No need of expensive chiral ligands or complex organocatalyst to induce the stereocontrol
- ✓ Activation of otherwise unreactive substrates under benign reaction conditions

Meninno, S.; Meazza, M.; Yang, J., M.; Tejero, T.; Merino-Gomez, P.; Rios, R. *Chem. Eur. J.* **2019**, *25*, 7623.

Cascade reaction for the synthesis of dihydroacridines



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European Chemical Societies Publishing

Communication

Synergistic Catalysis: Highly Enantioselective Cascade Reaction for the Synthesis of Dihydroacridines

Dr. Sara Meninno, Dr. Marta Meazza, Prof. Jung Woon Yang, Prof. Tomas Tejero, Prof. Pedro Merino, Dr. Ramon Rios

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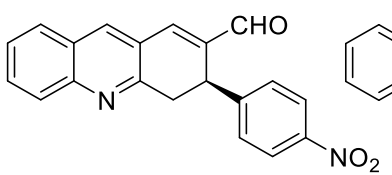
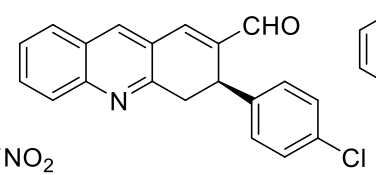
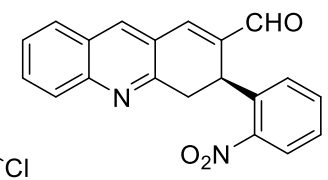


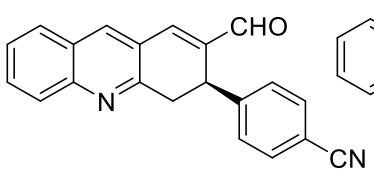
A 75% yield, 94% ee



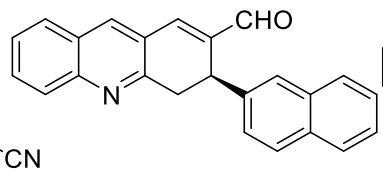
A 60% yield, 91% ee



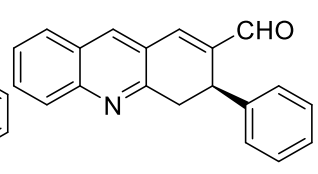
A 73% yield, 95% ee



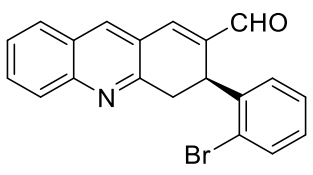
A 59% yield, 83% ee



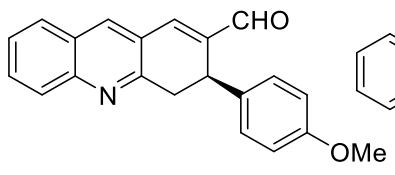
A 65% yield, 92% ee



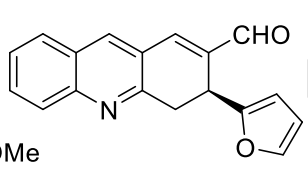
A 74% yield, 92% ee
B 55% yield, 96% ee



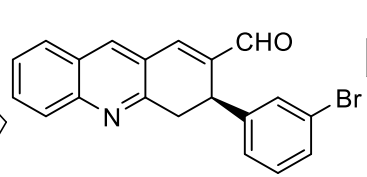
A 51% yield, 99% ee
B 70% yield, 95% ee



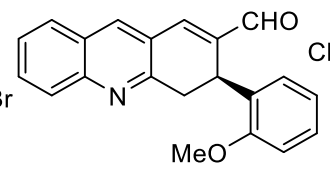
A 59% yield, 87% ee



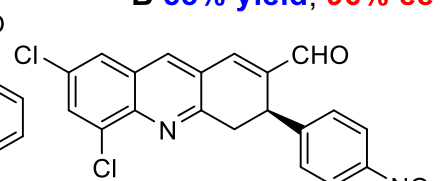
A 46% yield, 86% ee



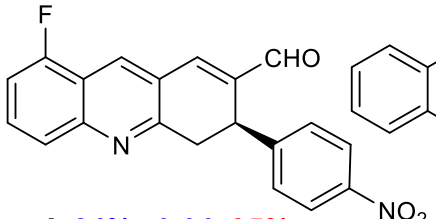
A 37% yield, 90% ee
B 23% yield, 96% ee



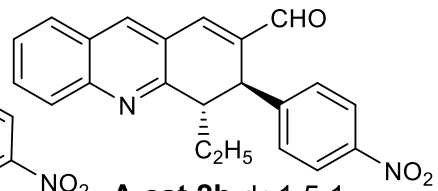
B 57% yield, 97% ee



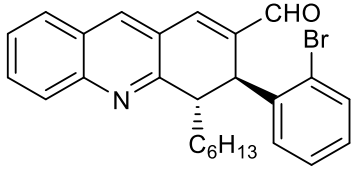
A 38% yield, 88% ee



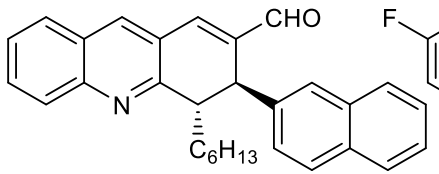
A 64% yield, 95% ee



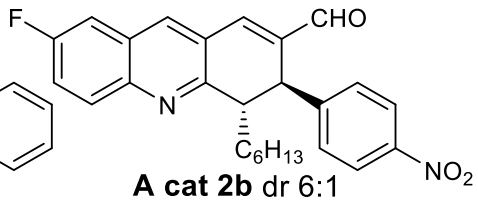
A cat 2b dr 1.5:1
78% yield (major), 94% ee
15% yield (minor)



A cat 2b dr 4:1
46% yield (major), 80% ee



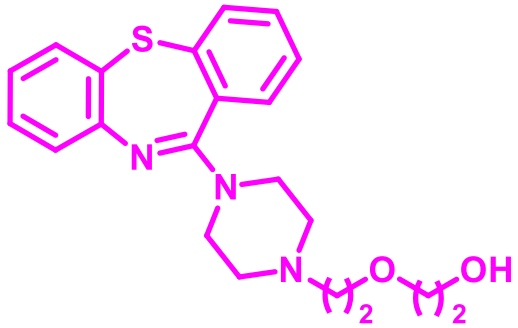
A cat 2b dr 6:1
48% yield (major), 84% ee



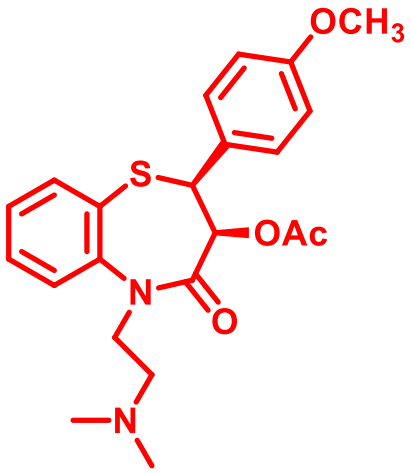
A cat 2b dr 6:1
80% yield (major/minor)
96% ee (major)

In the other entries, 2a was used

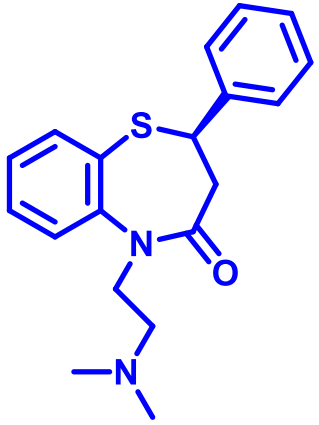
Benzothiazepines: pharmaceuticals applications



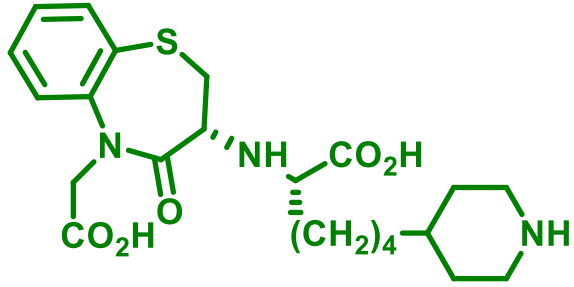
quetiapina



diltiazem



tiazesim

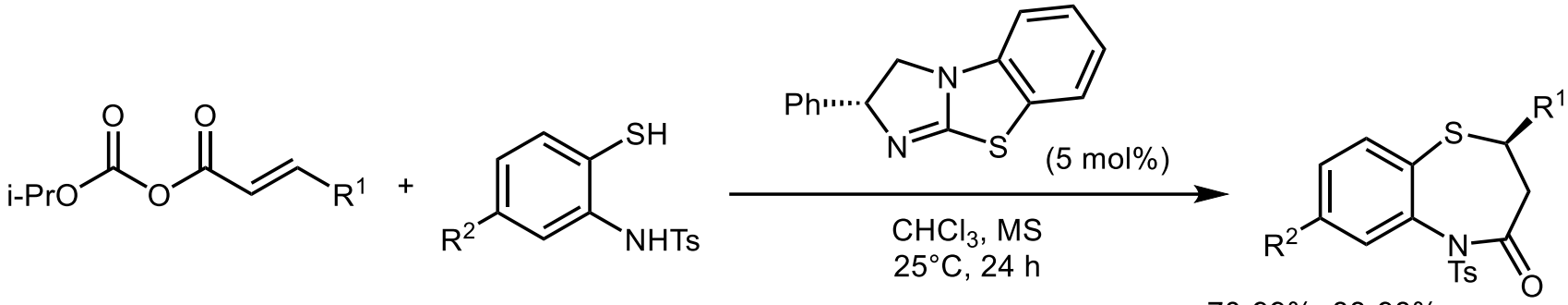


CV-5975

(a) Lévai, A.; Kiss-Szikszai, *ARKIVOC* **2008**, *8*, 65; (b) Saha, D.; Jain, J.; Sharma, A. *RSC Adv.* **2015**, *5*, 70619.

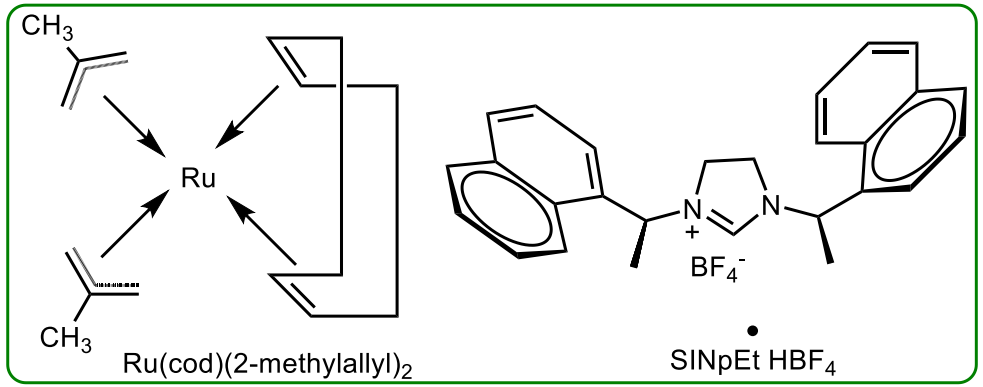
Synthesis of benzothiazepines: literature precedents

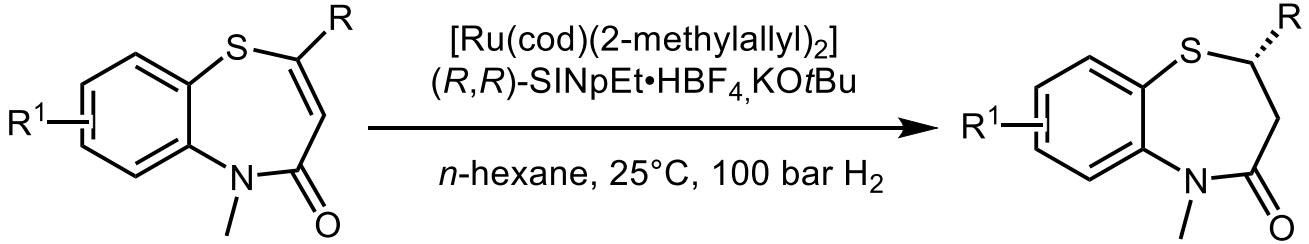
[4 + +3] Cycloaddition of anhydrides and aminothiophenols using a chiral isothiourea catalyst:



70-99%, 88-98% ee

Enantioselective ruthenium-*N*-heterocyclic-carbene-catalyzed hydrogenation of heterocyclic vinyl thioethers:



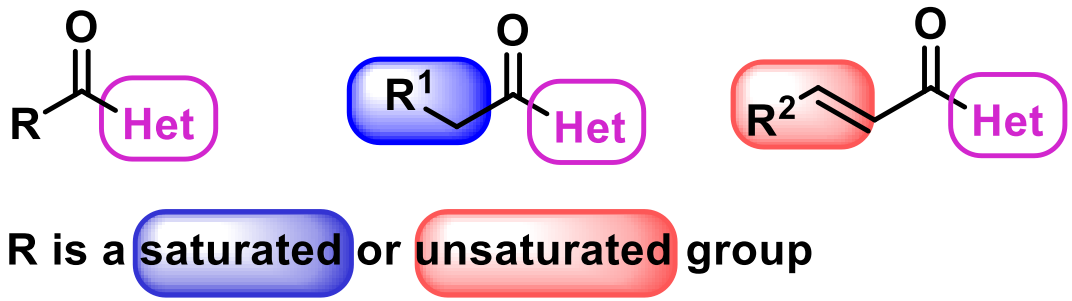


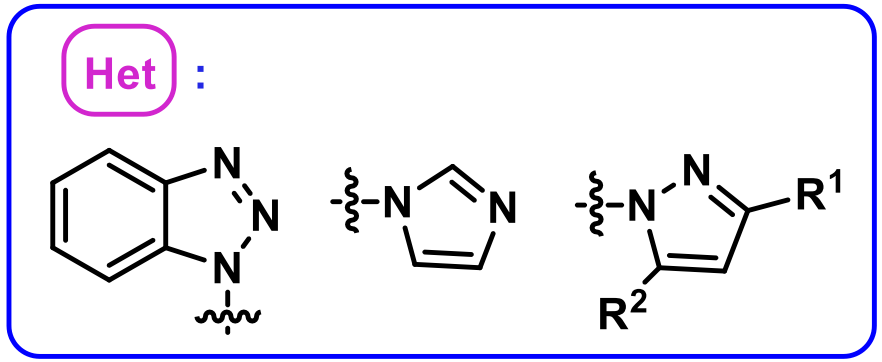
69-99%, 90-95% ee

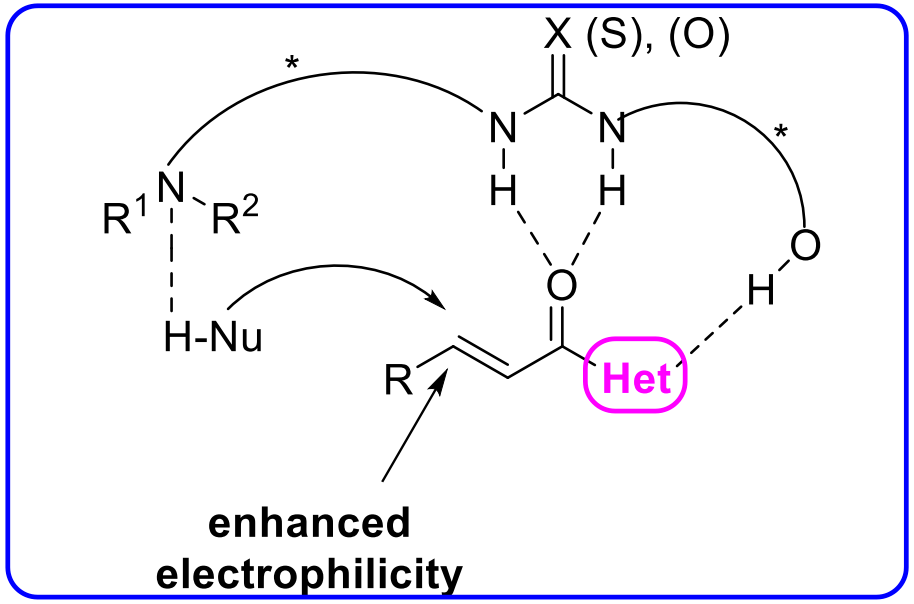
(a) Fukata, Y.; Asano, K.; Matsubara, S. *J. Am. Chem. Soc.* **2015**, *137,* 5320. (b) Li, W.; Schlepphorst, C.; Daniliuc, C.; Glorius, F. *Angew. Chem. Int. Ed.* **2016**, *55*, 3300.

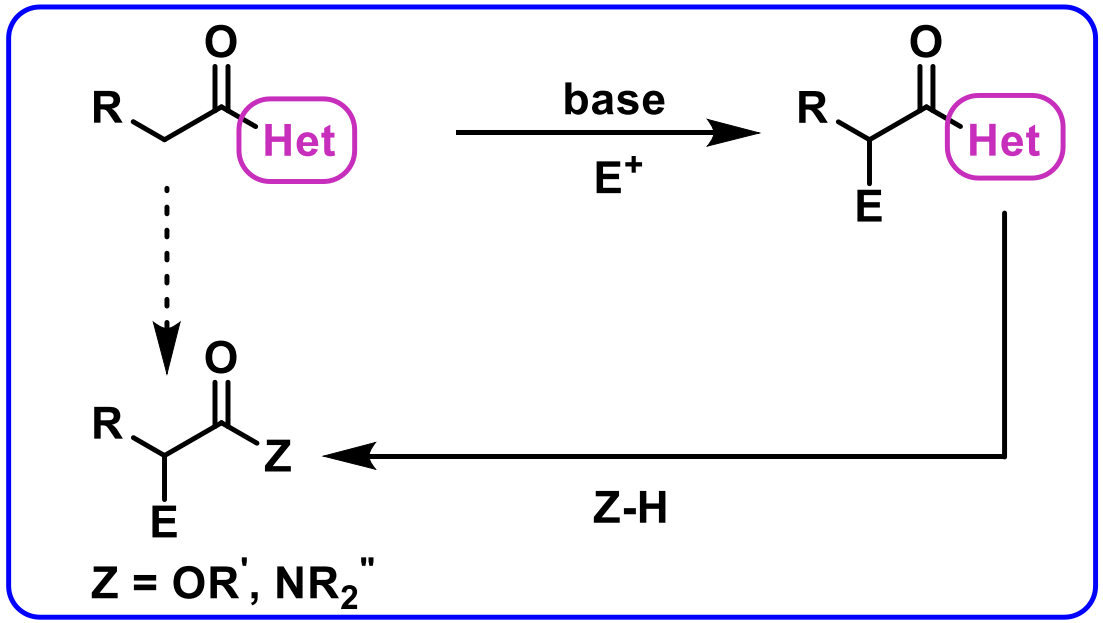
N-H Free 1,5-Benzothiazepines: our asymmetric approach via ester surrogates

***N*-Acyl aza-heterocycles as "masked bricks" to build new scaffolds**



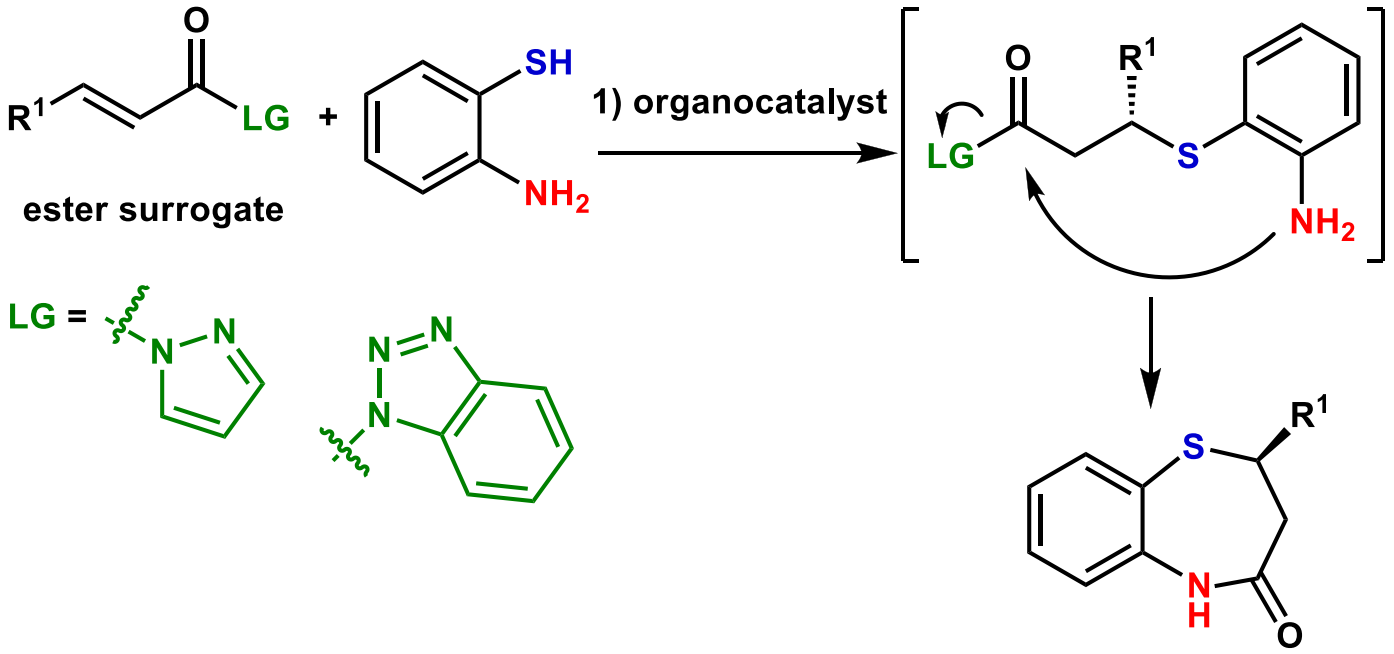


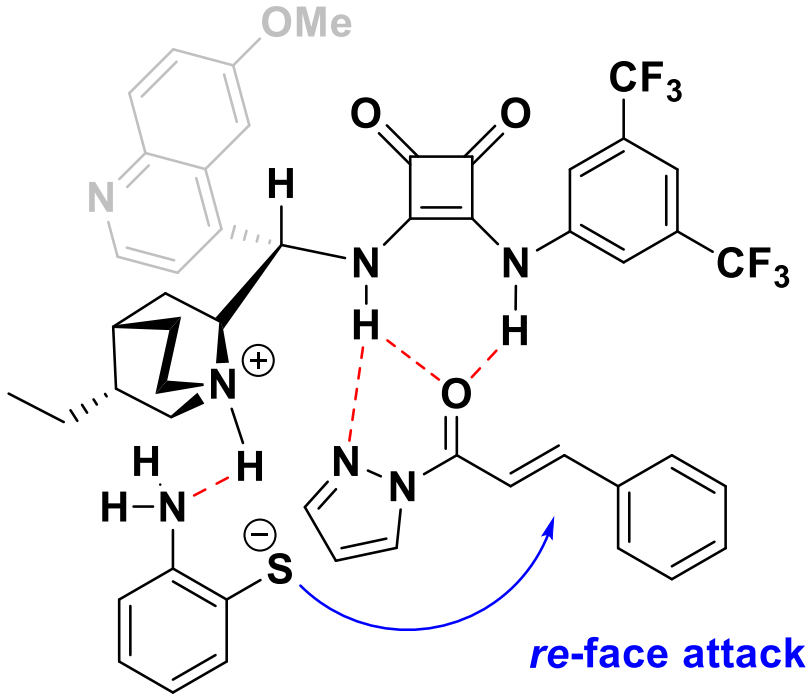




Reviews on ester surrogates in asymmetric synthesis: Monge, D.; Jiang, H.; Alvarez-Casao, Y. *Chem. Eur. J.* **2015**, *21,* 4494; Desimoni, G.; Faita, G.; Quadrelli, P. *Chem. Rev.* **2015**, *115*, 9922.

N-H Free 1,5-Benzothiazepines: our asymmetric approach via ester surrogates



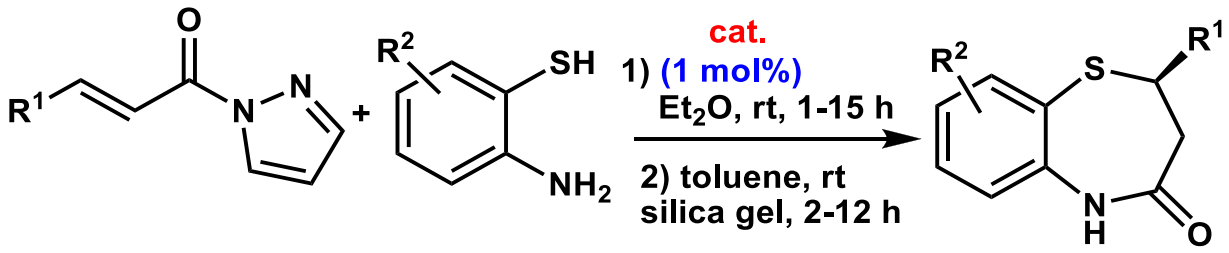


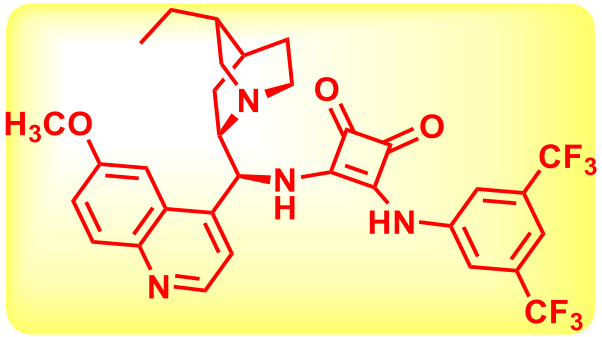
Challenges to address:

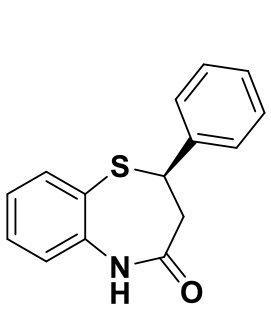
-Selective sulfa- over aza-1,4-addition and sulfa-1,2-addition

- Racemization of the product via potential reversible thio-Michael reaction during the lactamization step

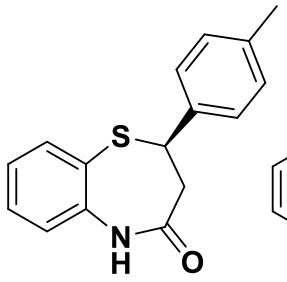
One-Pot catalytic asymmetric route to N-H Free 1,5-Benzothiazepines



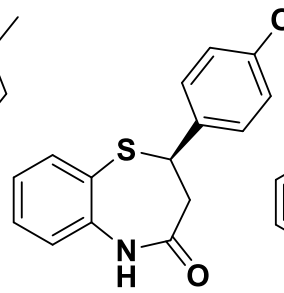




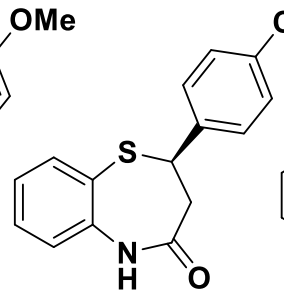
97%, 93% ee
3 mmol scale
94%, 90% ee



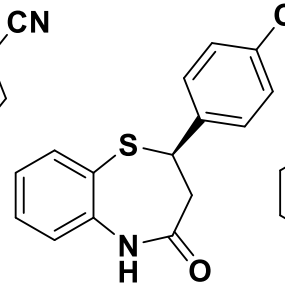
98%, 91% ee



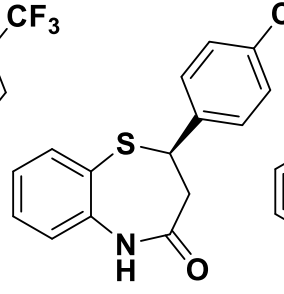
92%, 87% ee



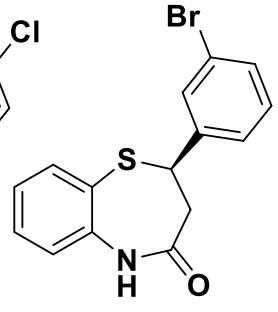
98%, 96% ee



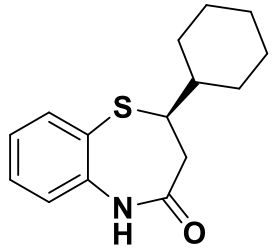
92%, 94% ee



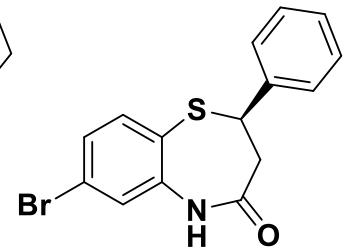
98%, 92% ee



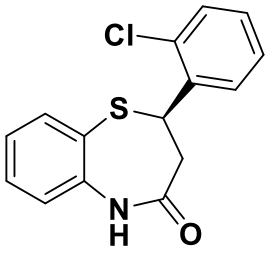
80%, 92% ee



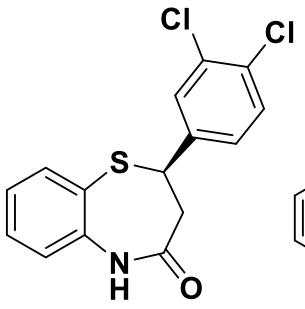
77%, 94% ee



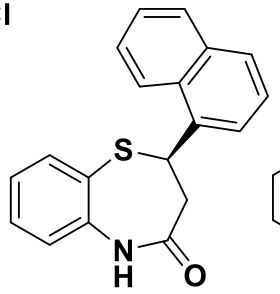
70%, 91% ee



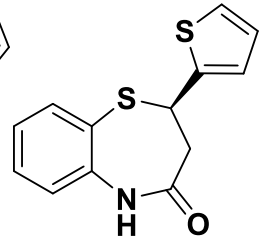
96%, 77% ee



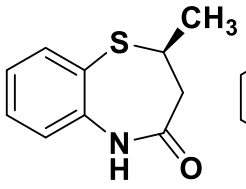
99%, 92% ee



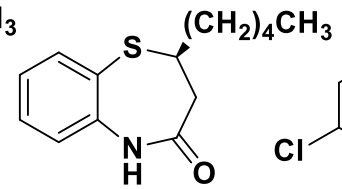
91%, 92% ee



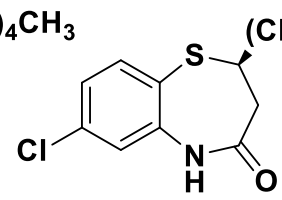
80%, 83% ee



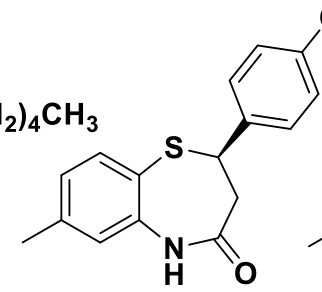
98%, 92% ee



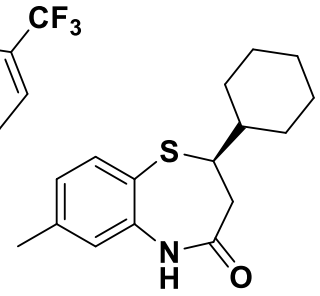
77%, 92% ee



65%, 89% ee



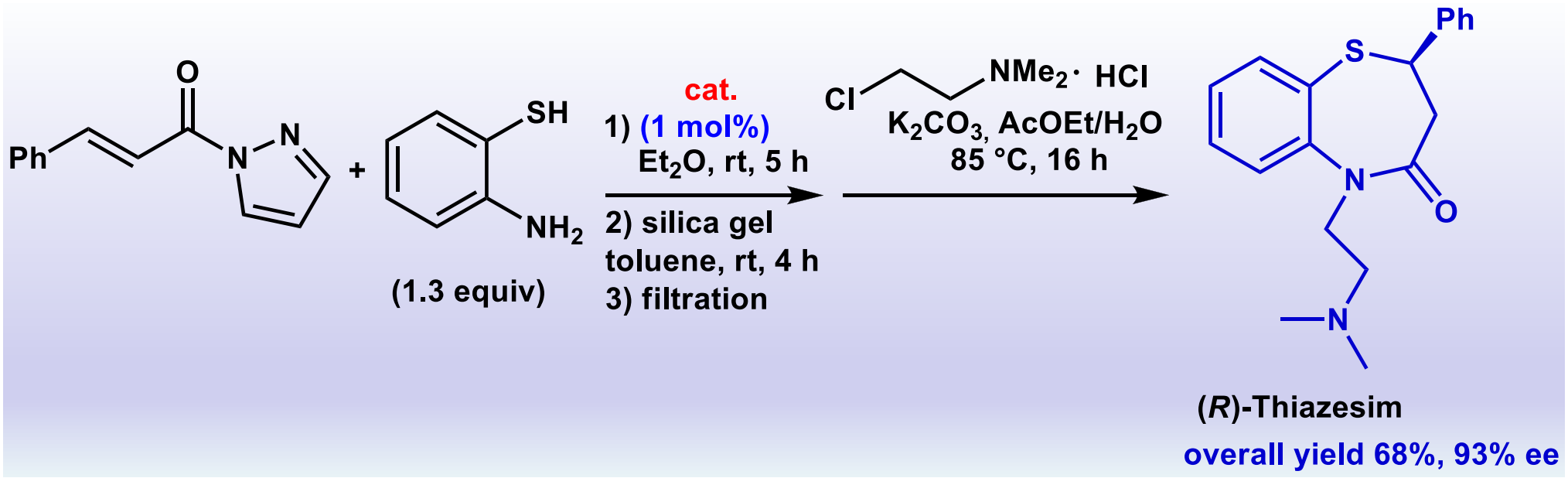
80%, 91% ee



89%, 95% ee

Meninno, S.; Volpe, C.; Lattanzi, A. *Chem. Eur. J.* **2017**, *23*, 4547.

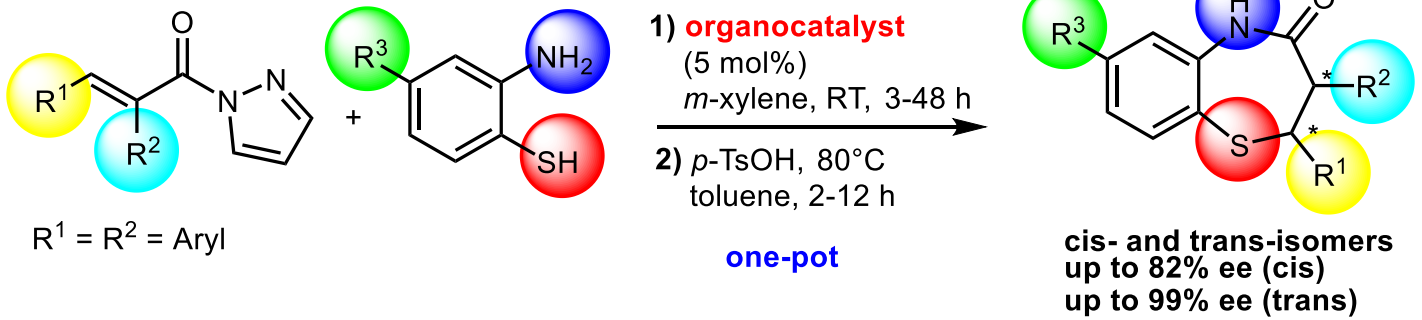
Medicine made easy: concise synthesis of (*R*)-Thiazesim

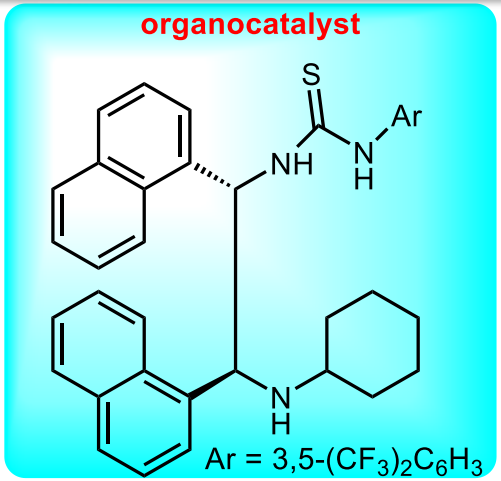


Meninno, S.; Volpe, C.; Lattanzi, A. *Chem. Eur. J.* **2017**, *23*, 4547.

Catalytic enantioselective one-pot synthesis of cis and trans 2,3-disubstituted 1,5-Benzothiazepines

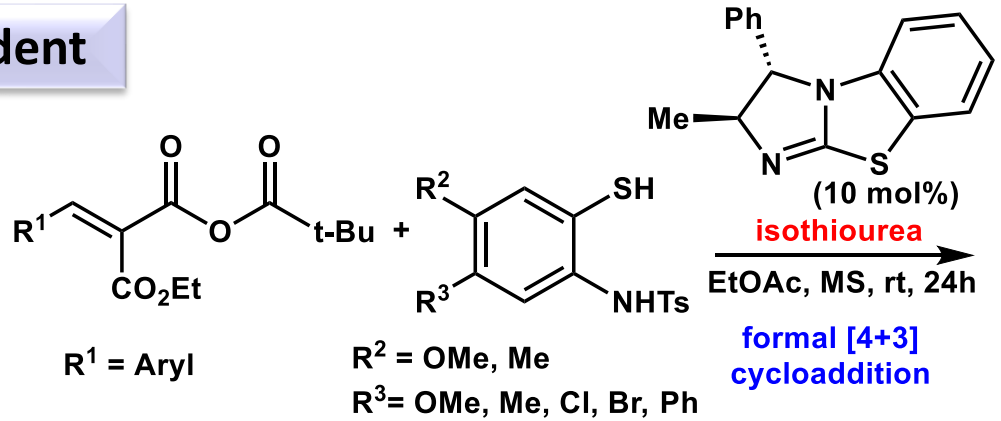
Our proposal of an one-pot approach to *cis*- and *trans*-2,3-diaryl substituted 1,5-benzothiazepines

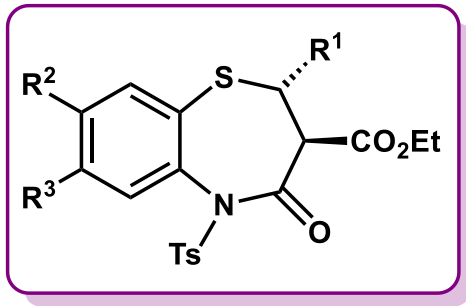




Meninno, S.; Quaratesi, I.; Volpe, C.; Mazzanti, A., Lattanzi, A. *Org. Biomol. Chem.* **2018**, *16*, 6923.

Literature precedent

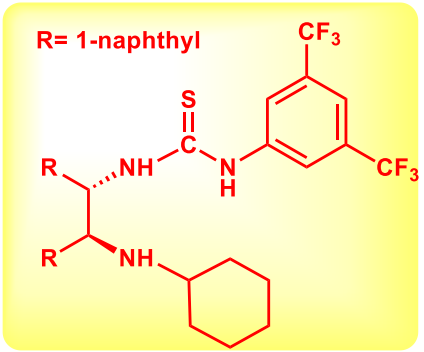


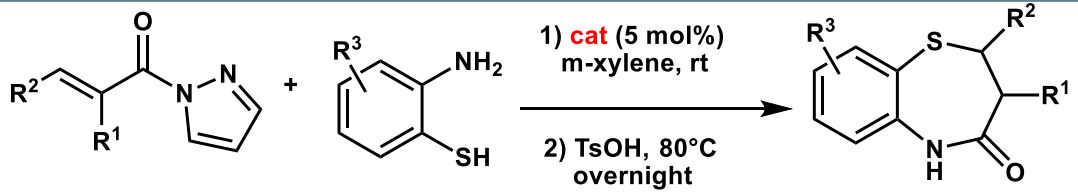


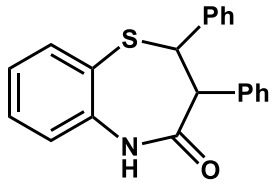
single *trans*-isomer
45-87%, 57-88% ee

Fukata, Y.; Yao, K.; Miyaji, R.; Asano, K.; Matsubara, S. *J. Org. Chem.* **2017**, *82*, 12655.

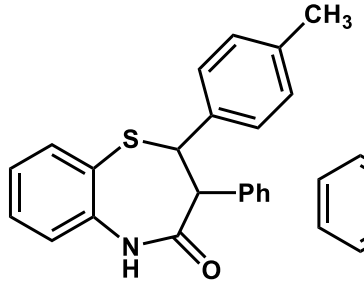
Catalytic enantioselective one-pot synthesis of cis and trans 2,3-disubstituted 1,5-Benzothiazepines



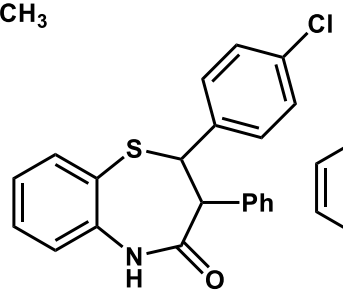




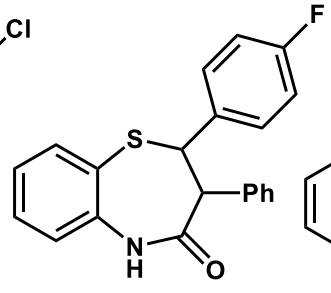
7 h, 70%,
cis/trans 53/47
79% ee (cis)
94% ee (trans)



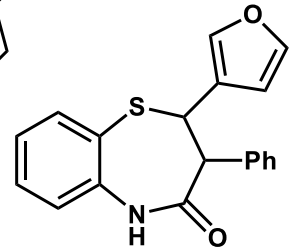
8 h, 71%,
cis/trans 54/46
74% ee (cis)
93% ee (trans)



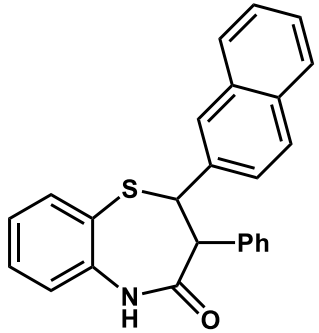
6 h, 73%,
cis/trans 48/52
59% ee (cis)
92% ee (trans)



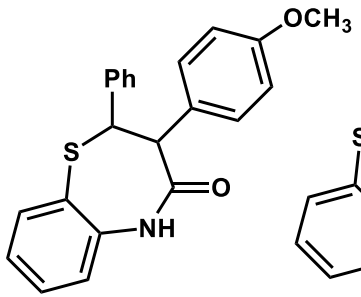
3 h, 78%,
cis/trans 54/46
80% ee (cis)
94% ee (trans)



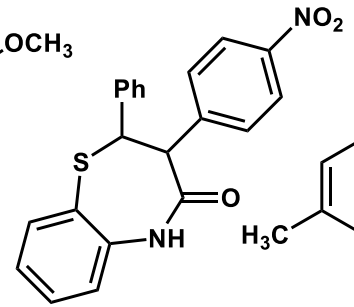
24 h, 47%,
cis/trans 62/38
67% ee (cis)
99% ee (trans)



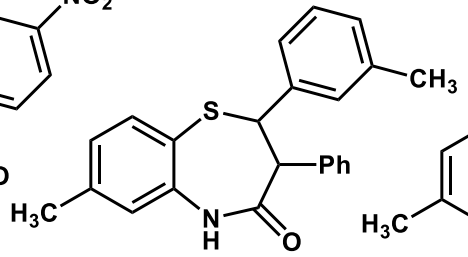
6 h, 72%,
cis/trans 46/54
45% ee (cis)
55% ee (trans)



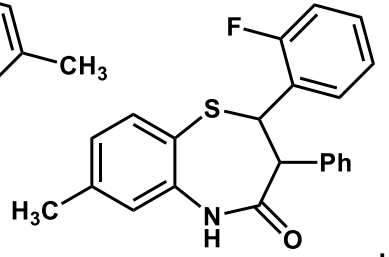
30 h, 80%,
cis/trans 45/55
55% ee (cis)
99% ee (trans)



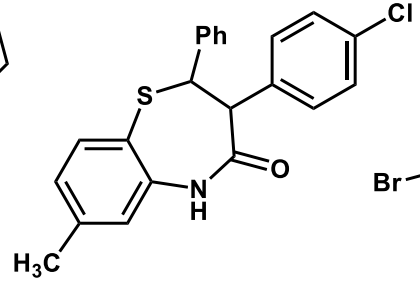
4 h, 51%,
cis/trans 66/34
73% ee (cis)
77% ee (trans)



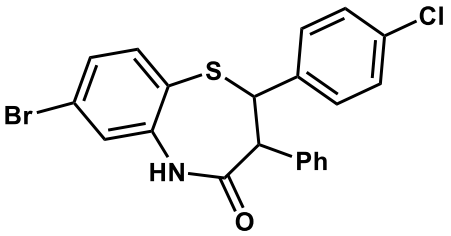
26 h, 43%,
cis/trans 65/35
70% ee (cis)
95% ee (trans)



24 h, 48%,
cis/trans 50/50
66% ee (cis)
94% ee (trans)



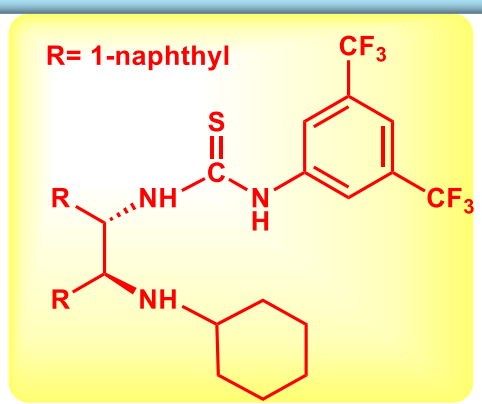
23 h, 59%,
cis/trans 63/37
73% ee (cis)
95% ee (trans)

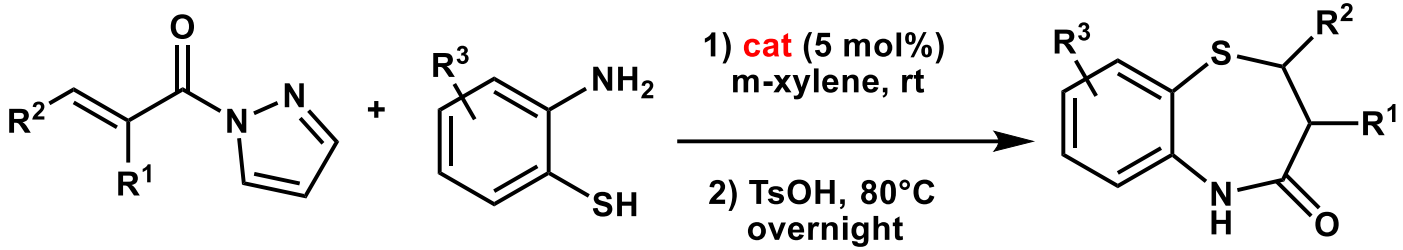


30 h, 67%,
cis/trans 47/53
72% ee (cis)
99% ee (trans)

Meninno, S.; Quaratesi, I.; Volpe, C.; Mazzanti, A., Lattanzi, A. *Org. Biomol. Chem.* **2018**, *16*, 6923.

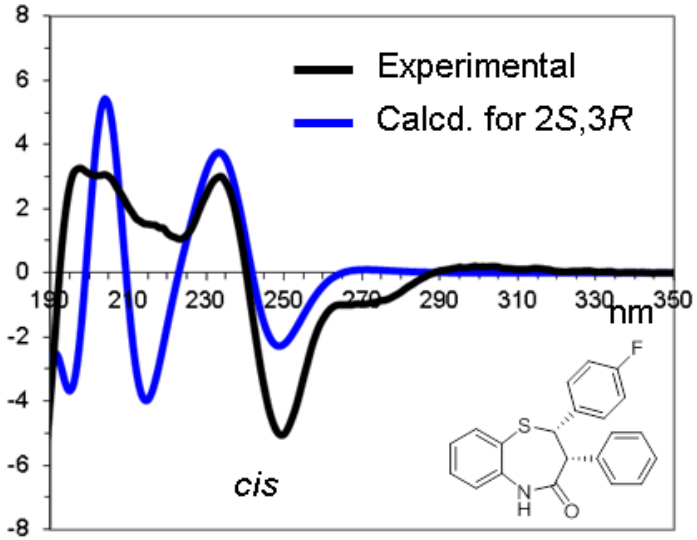
Catalytic enantioselective one-pot synthesis of cis and trans 2,3-disubstituted 1,5-Benzothiazepines

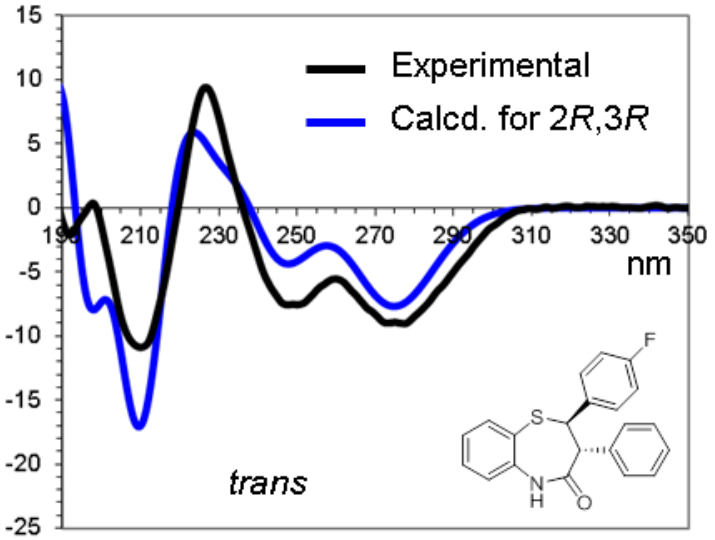




Assignment of the relative and absolute configuration

An hybrid approach based on NMR spectroscopy and Electronic Circular Dichroism (**ECD**), supported by DFT conformational analysis and TD-DFT calculation of ECD spectra was employed to assign the relative and absolute configuration of *trans* and *cis* compounds.





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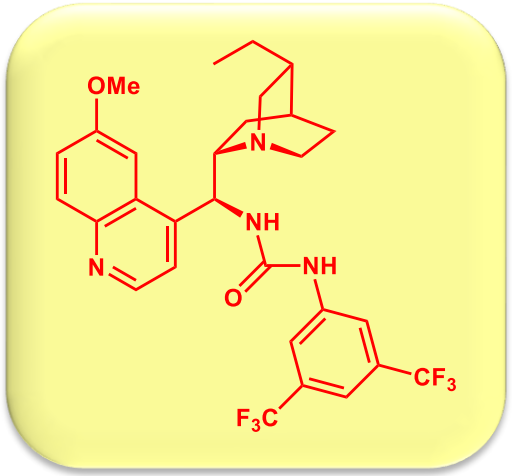
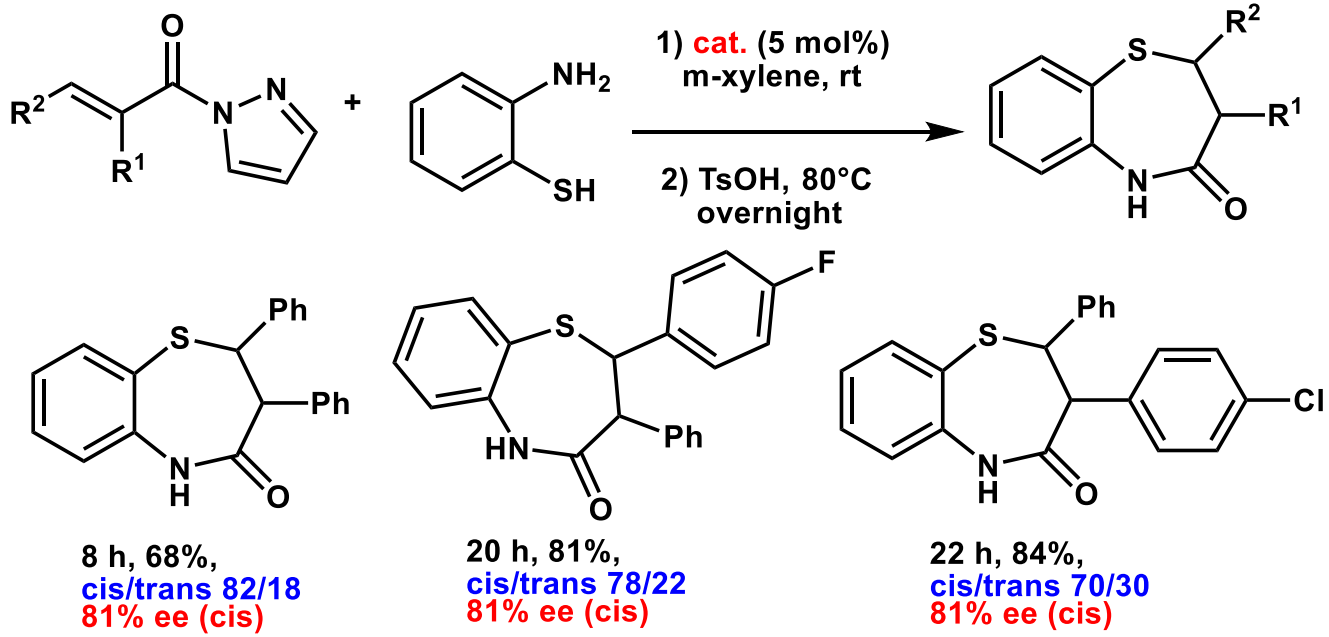
Catalytic enantioselective one-pot synthesis of cis and trans 2,3-disubstituted 1,5-Benzothiazepines





8 h, 68%,
cis/trans 82/18
81% ee (cis)

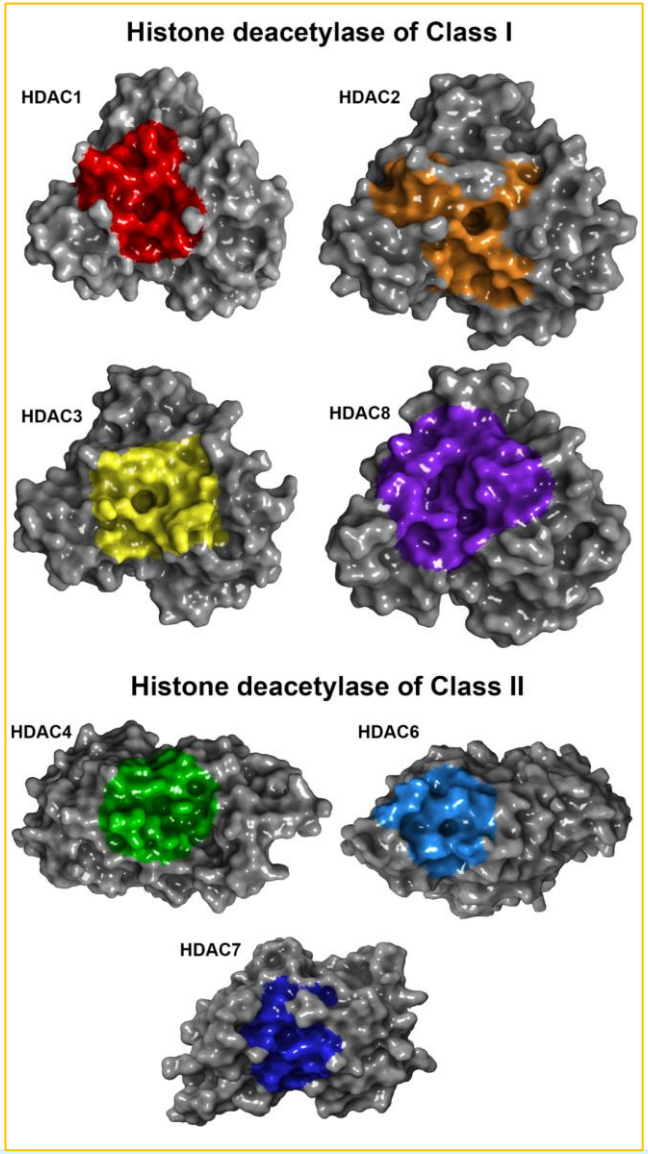
20 h, 81%,
cis/trans 78/22
81% ee (cis)

22 h, 84%,
cis/trans 70/30
81% ee (cis)

Meninno, S.; Quaratesi, I.; Volpe, C.; Mazzanti, A., Lattanzi, A. *Org. Biomol. Chem.* **2018**, *16*, 6923.

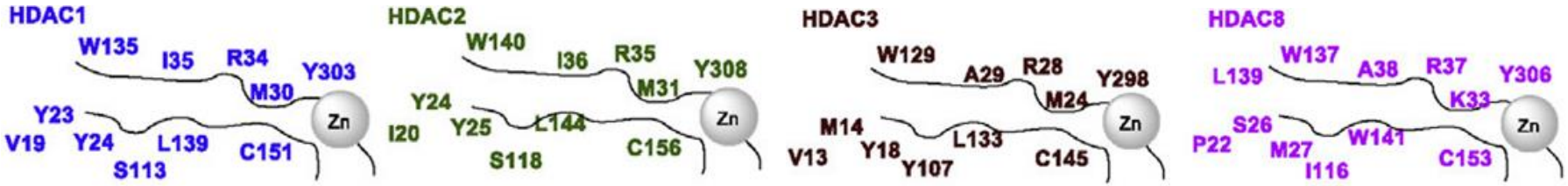
2-Substituted 1,5-Benzothiazepine-based Histone Deacetylase (HDAC) Inhibitors: Anticancer activities on human solid and acute myeloid leukemia cell lines

Involvement of Histone Deacetylases (HDACs) in cancer



Molecular surface of the Histone Deacetylase proteins (Class I and II) represented by the molecular surface and colored according to the key binding region residues at the top of the catalytic sites.

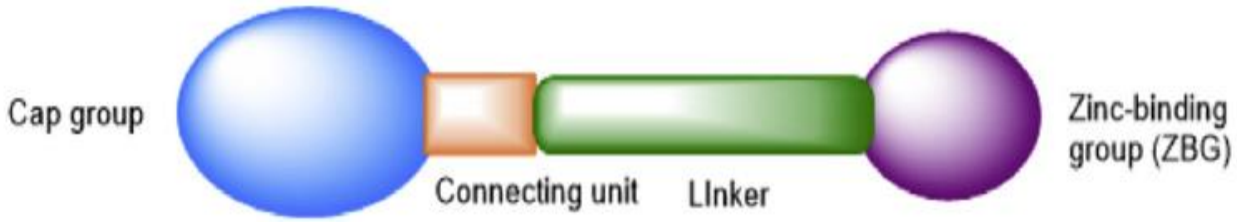
key residues delineating the cavities in class I HDACs:

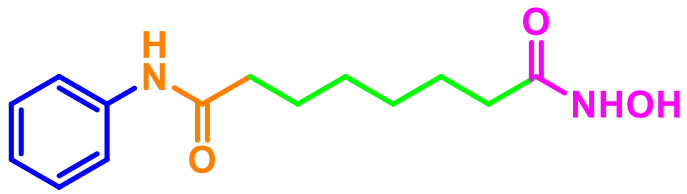


	Isoform	Expression in tumor tissues
Class I	HDAC1	Gastric, pancreatic, colorectal, prostate cancer, hepatocellular carcinoma
	HDAC2	Colorectal cancer, cervical cancer; gastric and prostate cancer
	HDAC3	Gastric, prostatic, colorectal cancer
	HDAC8	Infant neuroblastoma
Class IIA	HDAC4	Breast cancer
	HDAC7	Colorectal cancer
Class IIB	HDAC6	Oral squamous cell tumor, breast cancer

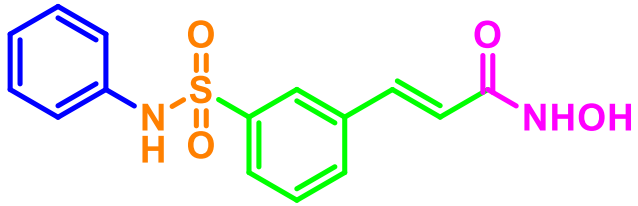
(a) Di Micco, S.; Chini, M.G.; Terracciano, S.; Bruno, I.; Riccio, R.; Bifulco, G. *Bioorg. Med. Chem.* **2013**, *21*, 3795; (b) Witt, O.; Deubzer, H.E.; Milde, T.; Oehme, I. *Cancer Lett.* **2009**, *277*, 8; (c) Roche, J.; Bertrand, P. *Eur. J. Med. Chem.* **2016**, *121*, 451.

Histone Deacetylase (HDAC) Inhibitors

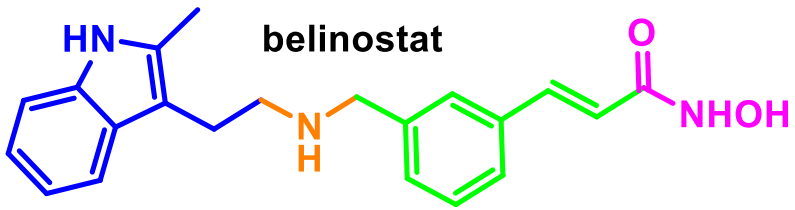




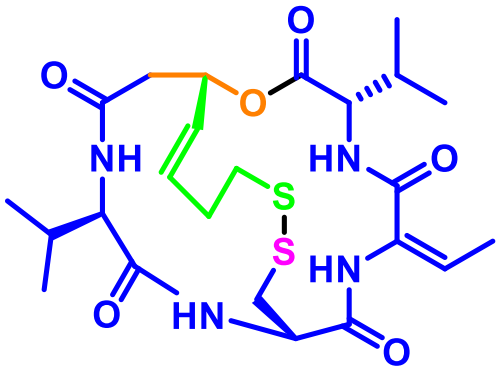
SAHA, vorinostat



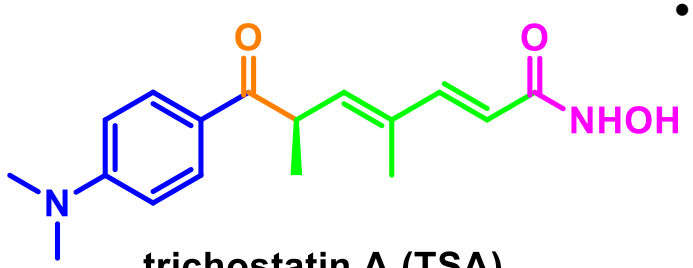
belinostat



panabinostat



romidespin



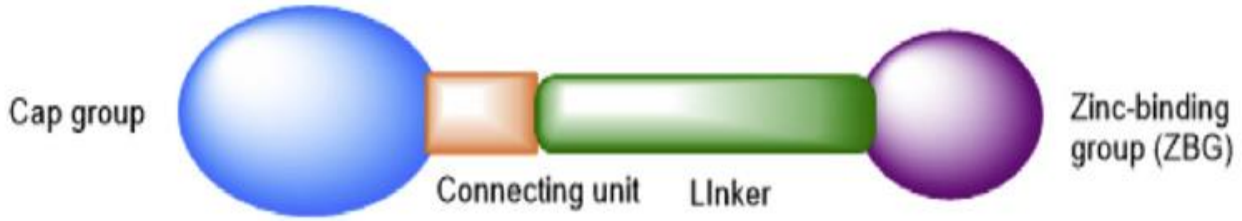
trichostatin A (TSA)

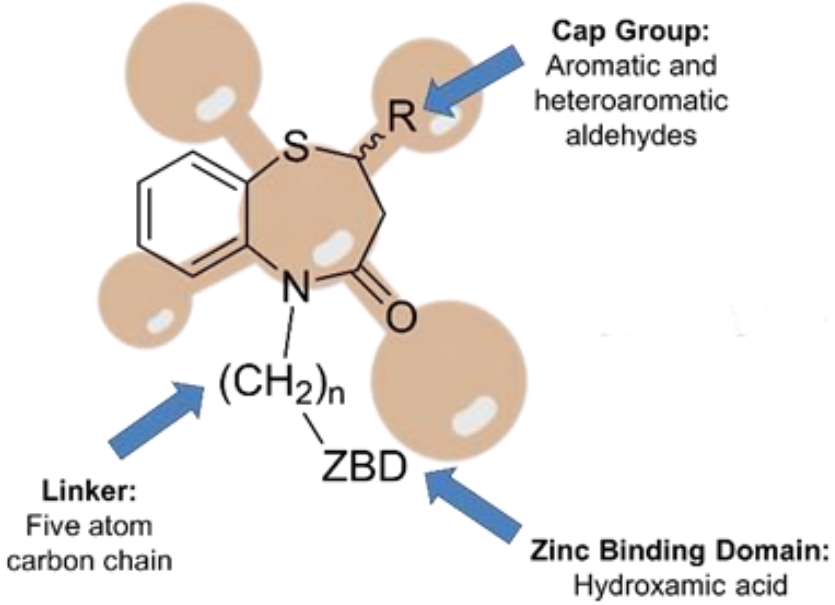
- FDA approved drugs are confined mainly to haematological malignancies

- the employment of HDAC inhibitors in solid tumours is still a very important target in anticancer research.

(a) Di Micco, S.; Chini, M. G.; Terracciano, S.; Bruno, I.; Riccio, R.; Bifulco, G. *Bioorg. Med. Chem.* **2013**, *21*, 3795; (b) Witt, O.; Deubzer, H.E.; Milde, T.; Oehme, I. *Cancer Lett.* **2009**, *277*, 8; (c) Roche, J.; Bertrand, P. *Eur. J. Med. Chem.* **2016**, *121*, 451.

A new series of 2-substituted 1,5-Benzothiazepine Histone Deacetylase (HDAC) Inhibitors: in silico design, synthesis and biological evaluation



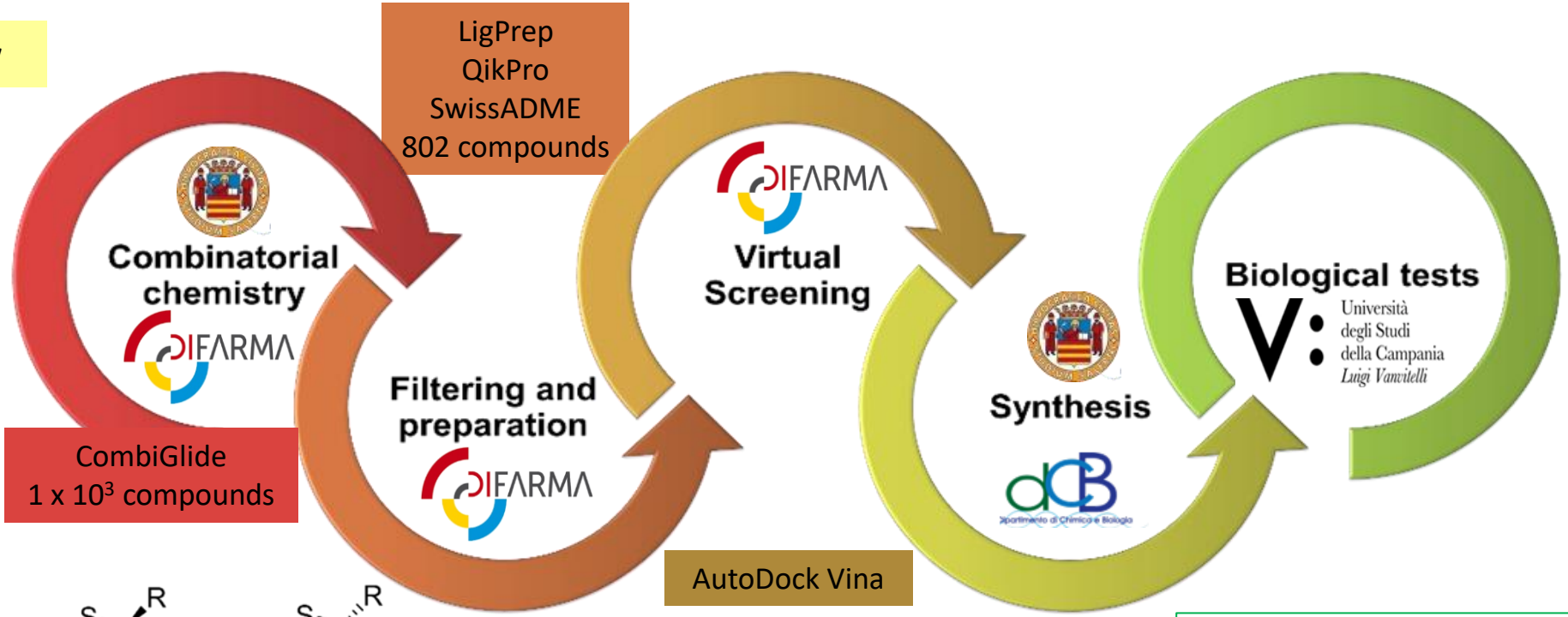


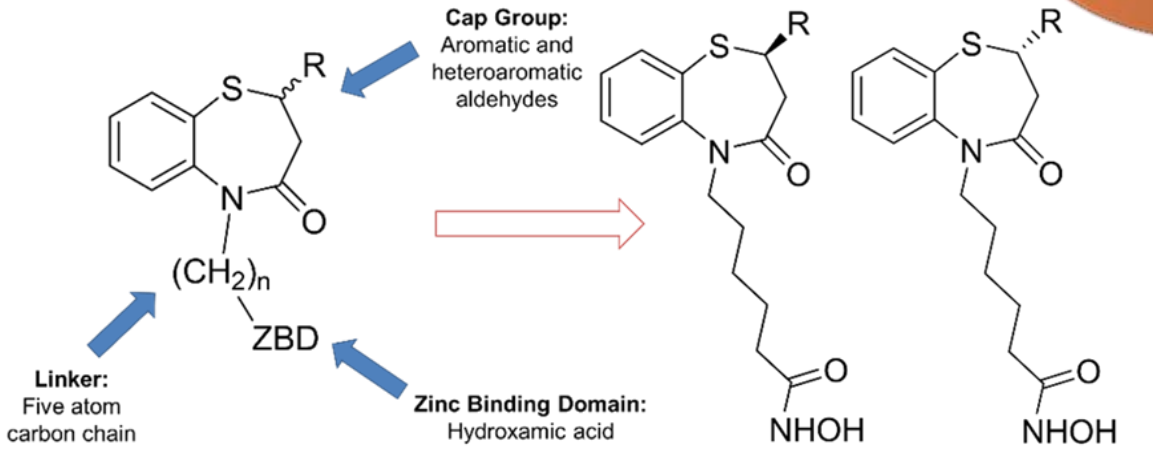
(a) Bertrand P. *Eur. J. Med. Chem.* **2010**, *45*, 2095; (b) Melesina, J.; Simoben, C.V.; Praetorius, L; Bülbül, E.F.; Robaa, D.; Sippl, W. *ChemMedChem*, **2021**, *16,* 1336; (c) Di Micco, S.; Chini, M. G.; Terracciano, S.; Bruno, I.; Riccio, R.; Bifulco, G. *Biorg. Med. Chem*. **2013**, *21*, 3795.

A new series of 2-substituted 1,5-Benzothiazepine Histone Deacetylase (HDAC) Inhibitors: in silico design, synthesis and biological evaluation

Multidisciplinary workflow

In collaboration with
Prof. Bifulco research group
Department of
Pharmaceutical Science
University of Salerno





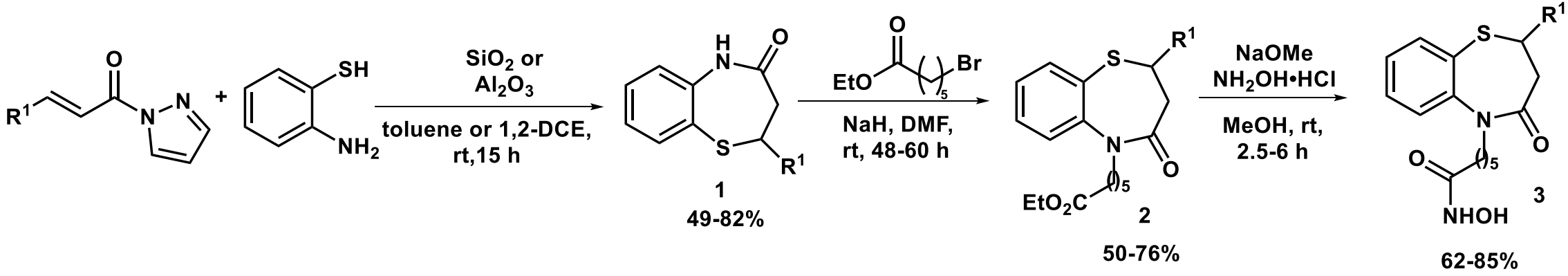
Library 1: 802 molecules **Library 2:** 802 molecules

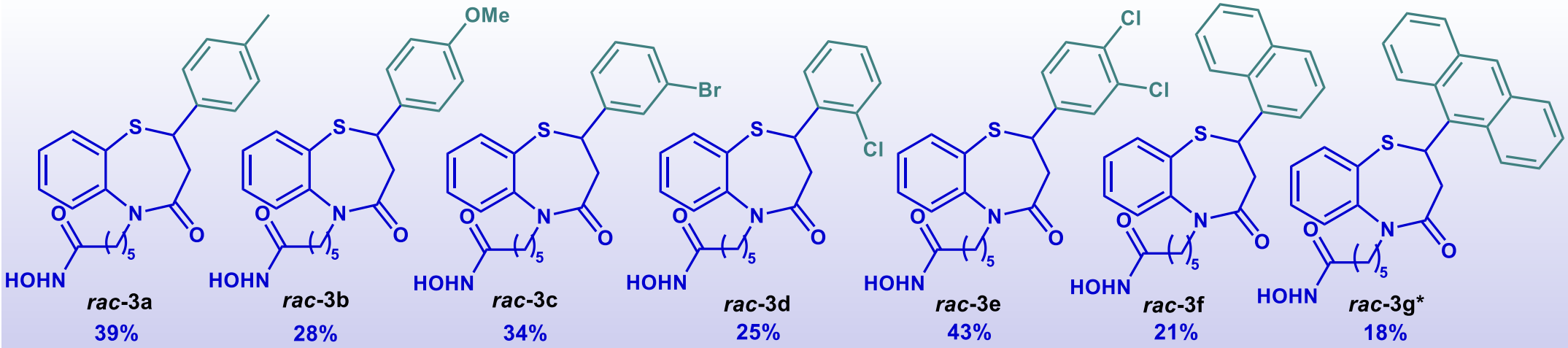
seven HDAC isoforms (1, 2, 3, and 8 for Class I; 4, 6, and 7 for class II) were considered

In collaboration with
Prof. Nebbioso research group
Departement of Precision Medicine University of Campania "Luigi Vanvitelli"

Scuola di Medicina e Chirurgia
Dipartimento di Medicina Sperimentale

A new series of 2-substituted 1,5-Benzothiazepine Histone Deacetylase (HDAC) Inhibitors: in silico design, synthesis and biological evaluation



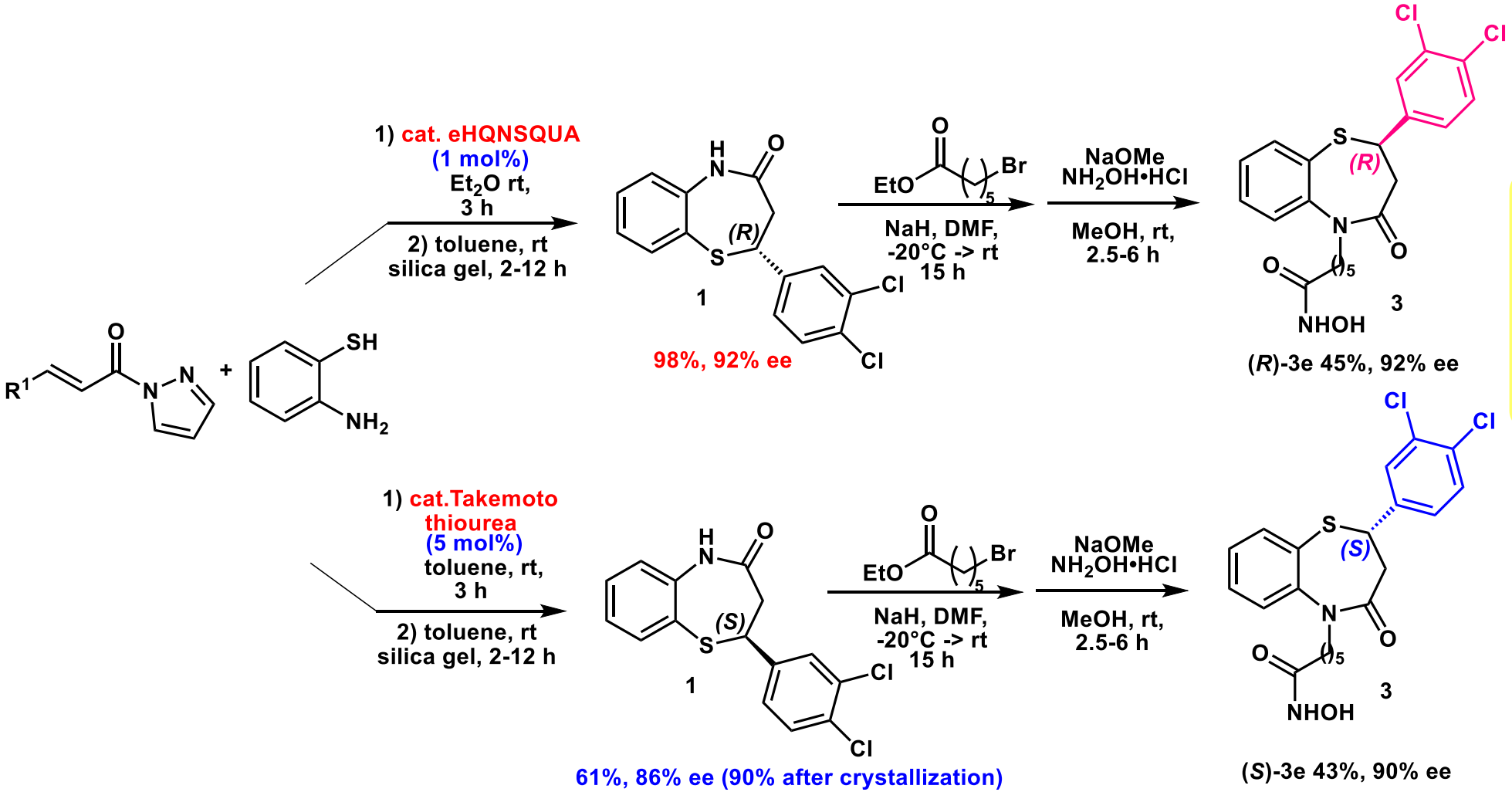


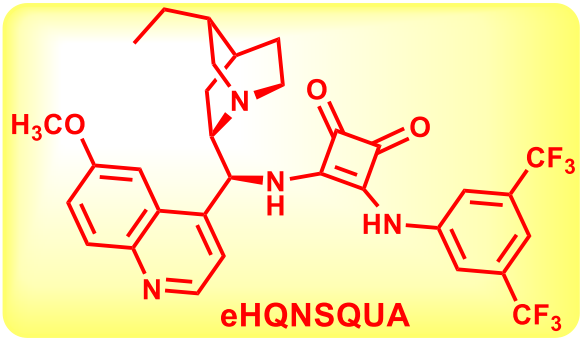
* yields over three steps

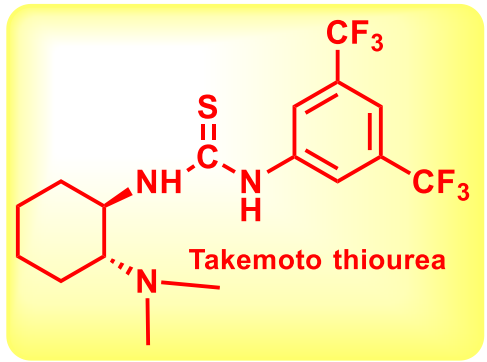
*alkylation reaction condition: K_2CO_3, 6-bromohexanoate, EtOAc/DMF 2/1, 70 °C, 40 h

De Vita, S.; Meninno, S.; Chini, M. G.; Lauro, G.; Rinaldi, R.; Sian, V.; Capasso, L.; Nebbioso, A.; Lattanzi, A.; Bifulco, G. *submitted for publication*

A new series of 2-substituted 1,5-Benzothiazepine Histone Deacetylase (HDAC) Inhibitors: in silico design, synthesis and biological evaluation





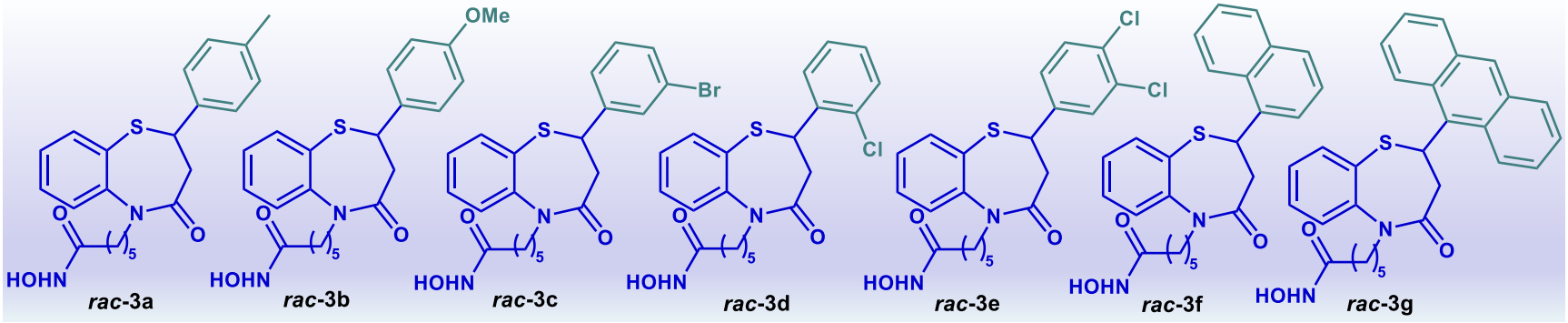


De Vita, S.; Meninno, S.; Chini, M. G.; Lauro, G.; Rinaldi, R.; Sian, V.; Capasso, L.; Nebbioso, A.; Lattanzi, A.; Bifulco, G. *submitted for publication*

In vitro assessment of HDAC binding and selectivity of 2-substituted 1,5-Benzothiazepine

The calculated residual enzyme activity on the nuclear extract of HeLa cells compared to control using fluorogenic peptide as substrate. Compounds were tested in duplicate at 10 µM and DMSO was used as control.

Compound ID	% Residual enzyme activity (Relative to DMSO Controls) (mean ± SD)
3a	1.34 ± 0.12
3b	1.87 ± 0.03
3c	2.94 ± 0.09
3d	2.06 ± 0.12
***rac*-3e**	4.90 ± 0.03
(*R*)-3e	2.86 ± 0.00
(*S*)-3e	12.56 ± 0.32
3f	2.39 ± 0.09
3g	2.16 ± 0.07



Compounds with promising inhibitory capabilities

Compound	IC50 (µM) (mean ± SD)
***rac*-3a**	0.123 ± 0.0140
***rac*-3b**	0.118 ± 0.0113
***rac*-3d**	0.109 ± 0.0190
(*R*)-3e	0.274 ± 0.0572
***rac*-3f**	0.259 ± 0.0681
***rac*-3g**	0.246 ±0.0451
Trichostatin A	0.006 ± 0.0008

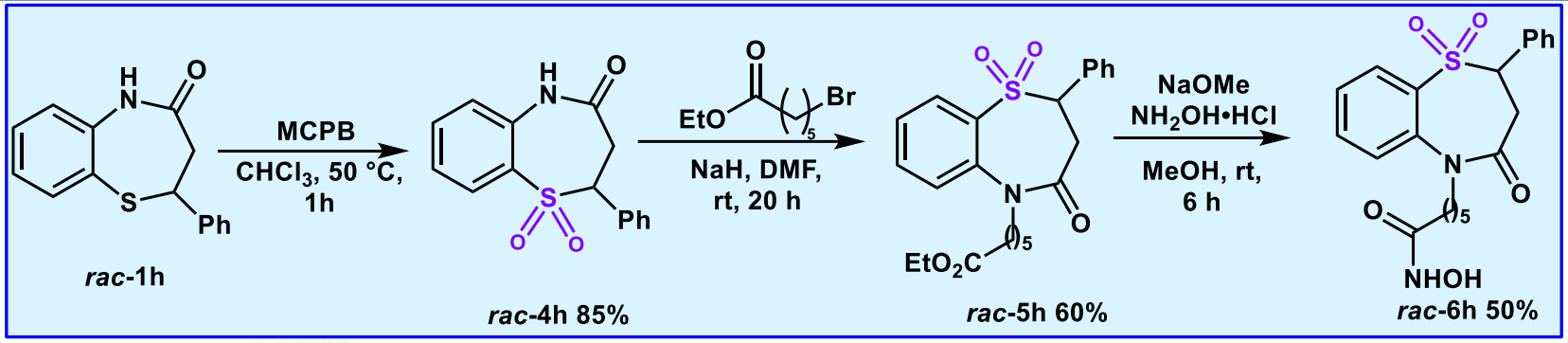
The calculated IC50 values using Trichostatin A as the reference compound and a fluorogenic peptide as substrate.

https://www.reactionbiology.com/

REACTION BIOLOGY

De Vita, S.; Meninno, S.; Chini, M. G.; Lauro, G.; Rinaldi, R.; Sian, V.; Capasso, L.; Nebbioso, A.; Lattanzi, A.; Bifulco, G. *submitted for publication*

In silico evaluation of sulfone compound

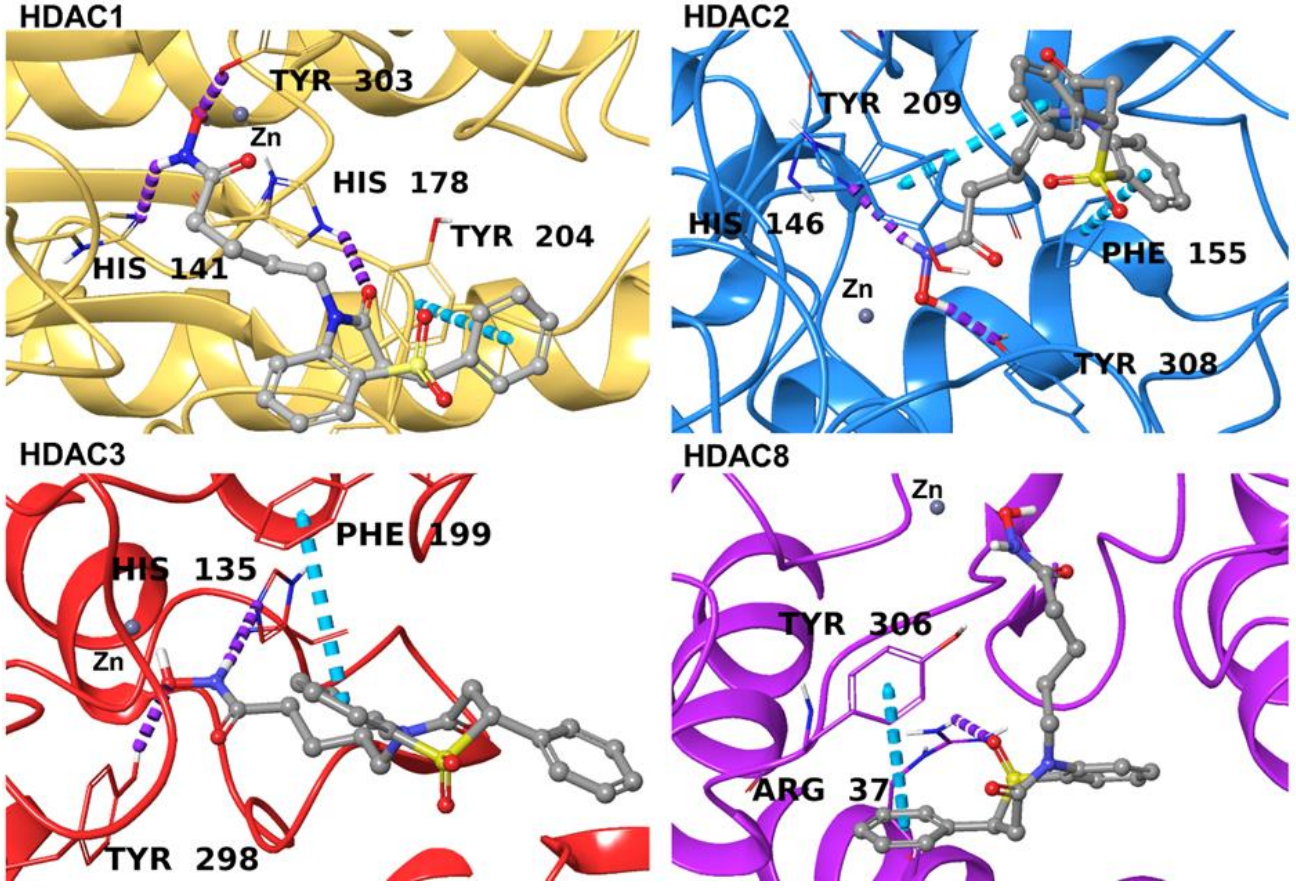


rac-1h

rac-4h 85%

rac-5h 60%

rac-6h 50%



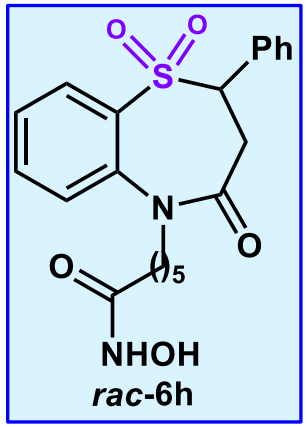
Molecular docking study results:

- **Slight differences between enantiomeric forms**
- **Compound 6h interacts well with Class 1 HDACs (1, 2, 3, and 8) and HDAC 6**

Binding poses of **6h** inside the binding pocket of Class 1 HDACs (1, 2, 3 and 8). Purple and cyan dotted lines represent hydrogen bonds and π-π interactions, respectively. Interacting residues are labeled and the zinc ion is depicted as a grey sphere.

Data confirmed by *in vitro* assays on the different HDAC isoforms

In vitro assessment of HDAC binding and selectivity of sulfone compound



rac-6h

Compound ID	% Residual enzyme activity (relative to DMSO controls) (mean ± SD)
6h	1.17 ± 0.17

The calculated residual enzyme activity on the nuclear extract of HeLa cells compared to control using fluorogenic peptide as substrate. Compounds were tested in duplicate at 10 µM and DMSO was used as control.

Compound	IC50 (µM) (mean ± SD)
rac-6h	0.039 ± 0.0102
Trichostatin A	0.006 ± 0.0008

The calculated IC50 values using Trichostatin A as the reference compound and a fluorogenic peptide as substrate.

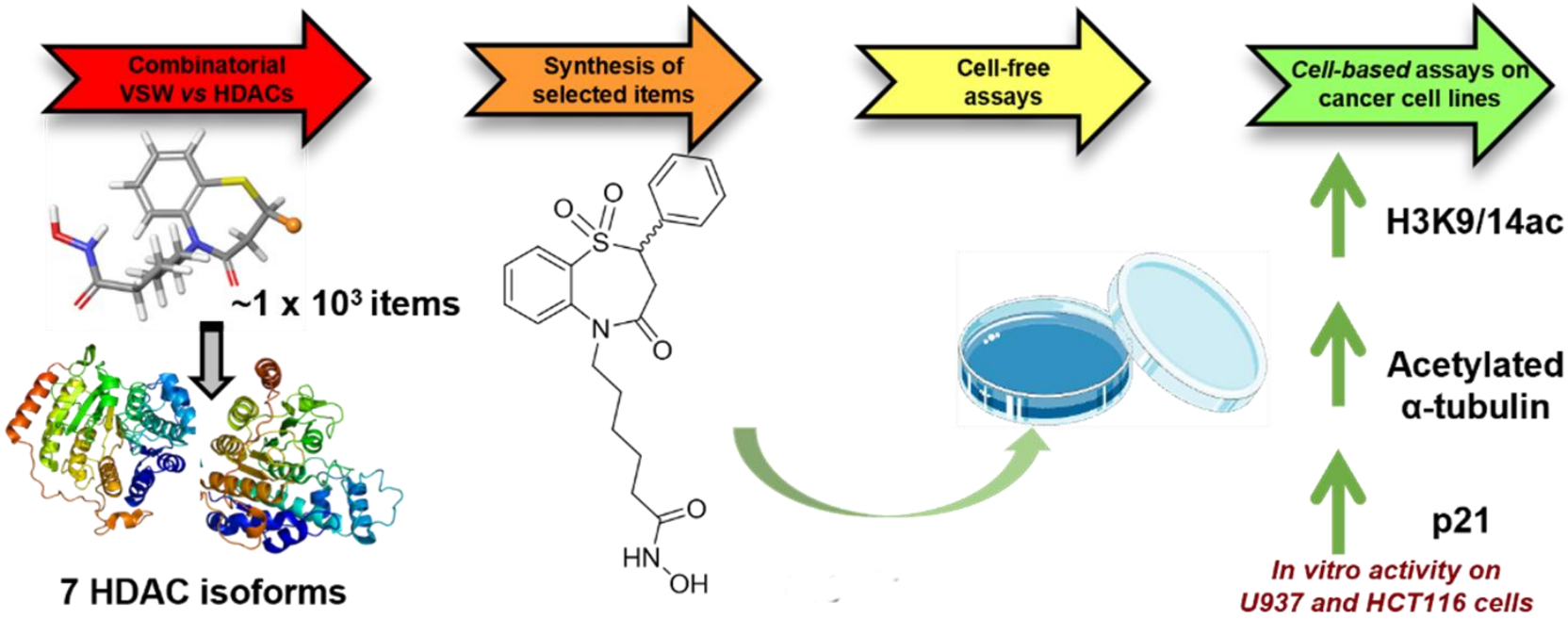
Selectivity profile of racemic 6h

	% Residual enzyme activity (relative to DMSO controls) (mean ± SD)
Target	Compound 6h
HDAC1	0.97 ± 0.55
HDAC2	-3.09 ± 0.29
HDAC3	0.68 ± 0.07
HDAC4	58.61 ± 1.33
HDAC6	1.18 ± 0.03
HDAC7	41.08 ± 2.21
HDAC8	12.75 ± 0.42

Docking results confirmed by the selectivity profile and significant inhibition of the activity of HDAC 1, HDAC 2, HDAC 3 and HDAC 6 disclosed for rac-6h assessed *in vitro* by Reaction Biology

De Vreese, R.; Galle, L.; Depetter, Y.; Franceus, J.; Desmet, T.; Van Hecke, K.; Benoy, V.; Van Den Bosch, L.; D'Hooghe, M. *Chem. Eur. J.* **2017**, *23*, 128.
De Vita, S.; Meninno, S.; Chini, M. G.; Lauro, G.; Rinaldi, R.; Sian, V.; Capasso, L.; Nebbioso, A.; Lattanzi, A.; Bifulco, G. *submitted for publication*

Effects of 2-substituted 1,5-benzothiazepines on cancer cells: identification of novel putative epigenetic drugs



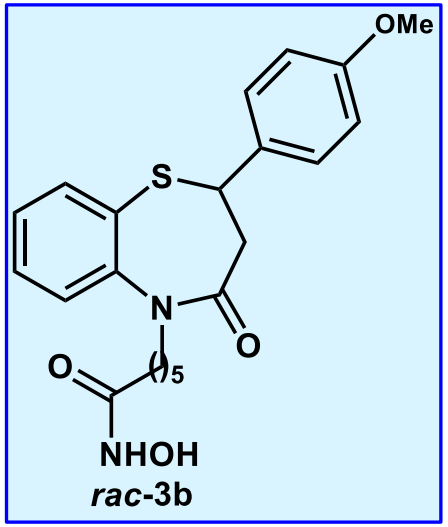
HDAC inhibitors in both racemic (***rac*-3a-g**, ***rac*-6h**) and enantiomeric (**(*R*)-3e** and **(*S*)-3e**) forms were tested (at 1 and 5 µM for 24 h) and compared to the FDA-approved reference compound SAHA on

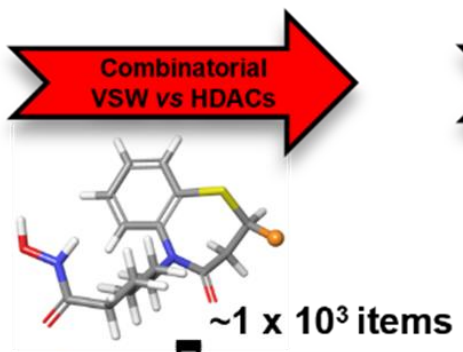
- **Cell cycle progression and cell death**
- **Capability of the compounds to inhibit HDAC enzymatic activity, HDAC6 inhibition**
- **Modulation of non-histone targets of HDAC inhibitors**

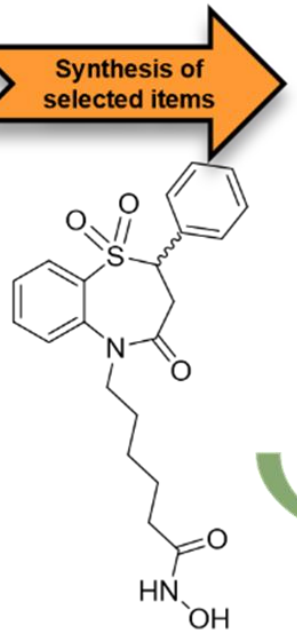
On both

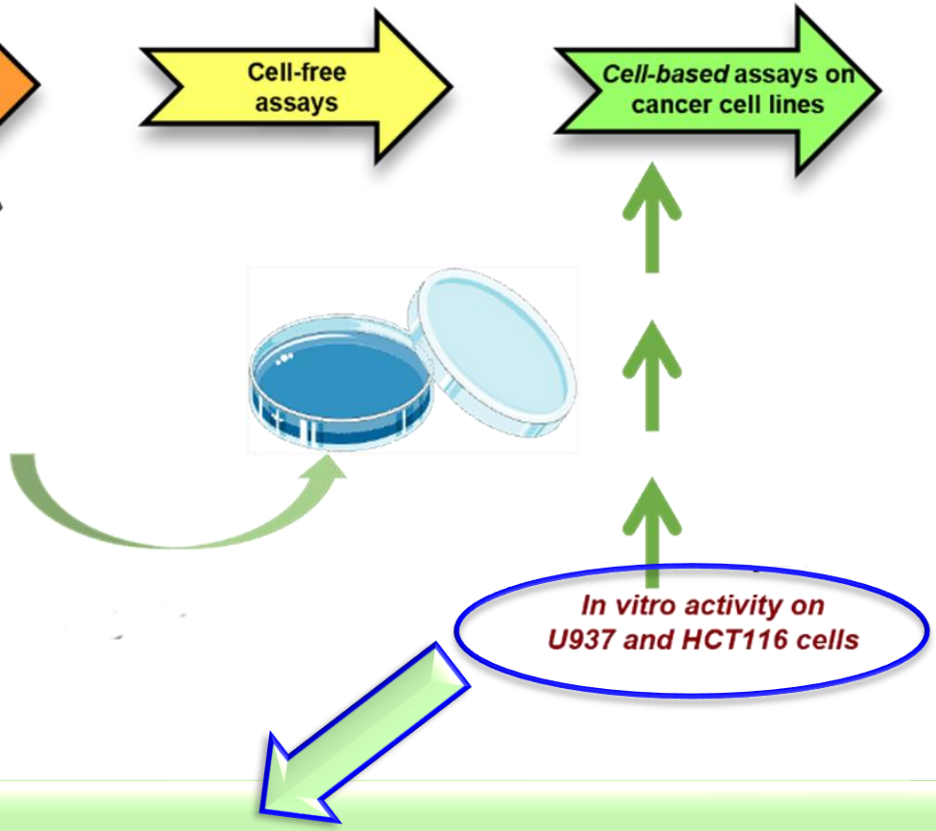
- **human acute myeloid leukemia U937 (hematological malignancy)**
- **colorectal cancer HCT116 cell lines (solid tumour)**

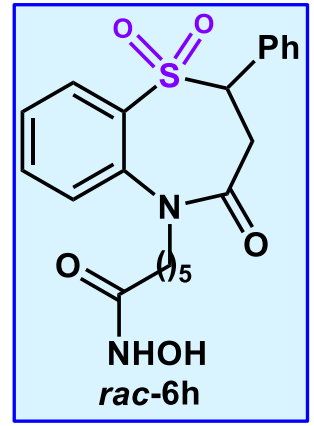
Effects of 2-substituted 1,5-benzothiazepines on cancer cells: identification of novel putative epigenetic drugs







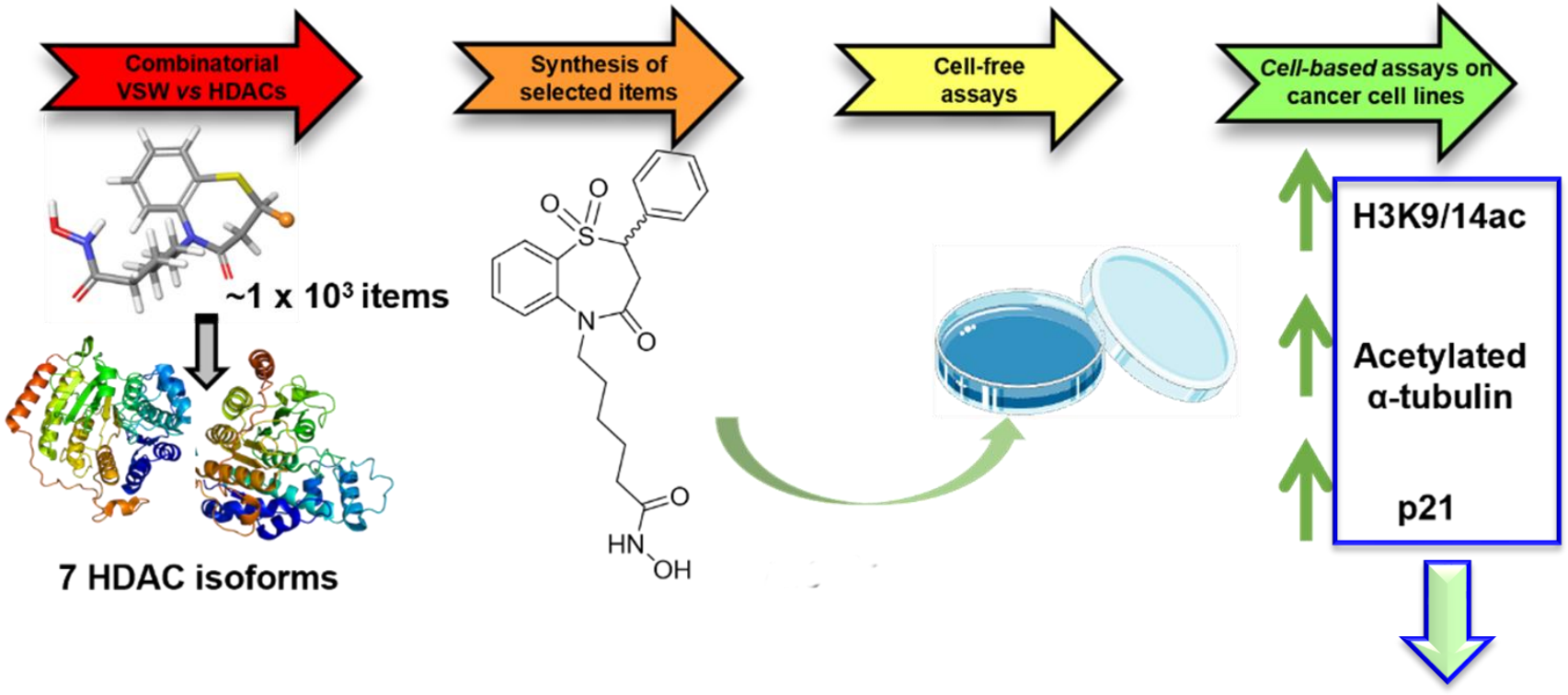




Effects on **cell death** and **cell cycle progression**:

- ✓ All the investigated molecules showed a dose- and cell-dependent cytotoxic effect, with HCT116 being more sensitive than U937 cells.
- ✓ Concerning the impact on the cell cycle progression, all compounds arrested the cell cycle in U937 cells in a way comparable to SAHA.
- ✓ Notably, compounds *rac*-**3b** and *rac*-**6h** showed a stronger effect than the reference compound in HCT116.

Effects of 2-substituted 1,5-benzothiazepines on cancer cells: identification of novel putative epigenetic drugs



Immunoblotting analyses to assess the capability of the compounds to inhibit HDAC enzymatic activity were carried out for histone (histone H3) and non-histone targets (α-tubulin and expression level of p21) on both cell lines:

- ✓ The deacetylation rate of H3K9/14ac decreases for most of the molecules in both the cancer cell lines
- ✓ Sulfone-derivative 6h is the most promising HDAC6 inhibitor as demonstrated by the levels of α-tubulin
- ✓ Expression levels of p21 increased in both cancer cell lines in a dose-dependent manner comparable to SAHA

2-Substituted 1,5-benzothiazepine: an unprecedented chemotype for HDAC inhibitors
Conclusions and future directions

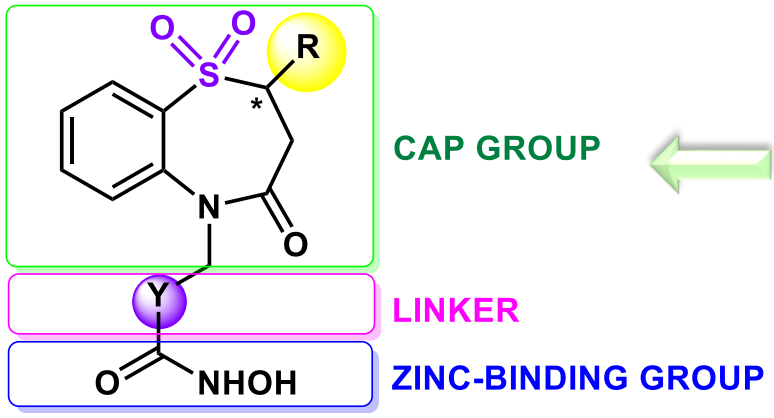
- 2-Substituted 1,5-Benzothiazepine-based HDAC inhibitors exert anticancer activities on human solid and acute myeloid leukemia cell lines

- Compound bearing sulfone moiety showed the most interesting and promising biological anticancer activity profile compared to the FDA-approved reference compound SAHA

Further developments: investigating a novel class of HDAC6-selective inhibitors

Multidisciplinary scientific workflow (combinatorial chemistry, docking studies, synthesis and biological tests) aimed to limit the investment/loss ratio.

Developing much more targeted and specific inhibitors



Expanding the chemical diversity around the 1,5-benzothiazepine sulfone scaffold

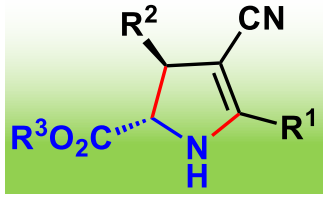
Conclusions

➢ **Design, plan and development of:**

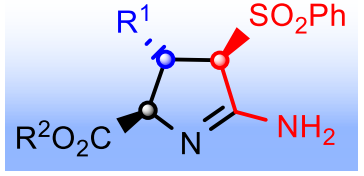
✓ **Mild and convenient protocols that use cheap, available and easily to handle reagents**

✓ **New one-pot and cascade stereoselective protocols for the synthesis of highly functionalized nitrogen- and sulfur-containing heterocycles**

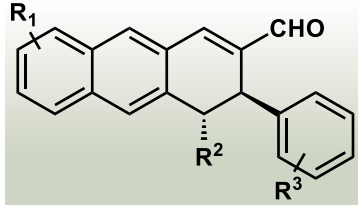
✓ **2-Pyrrolines**



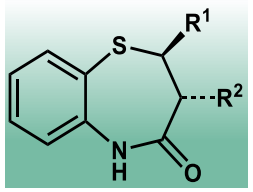
✓ **Five-terms Amidines**



✓ **Dihydroacridines**



✓ **1,5-Benzothiazepines**



Libraries of difficult to access nitrogen and sulfur heterocycles of potential utility in medicinal and synthetic chemistry are obtained

✓ **starting from readily and commercially available starting materials**

✓ **Minimizing the number of chemical operations**

✓ **Using cheap and readily available organic promoters**

✓ **Under mild and benign reaction conditions**