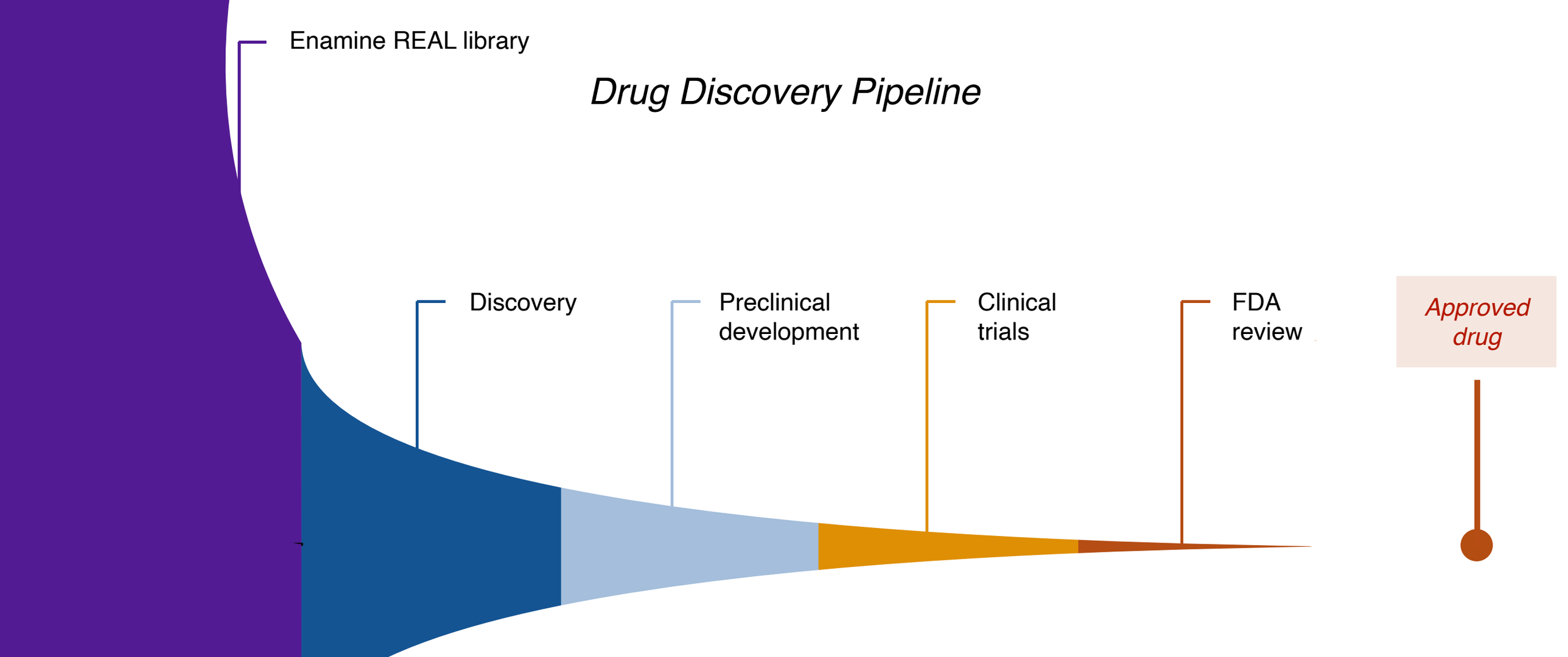
Enamine

May 16th, 2023

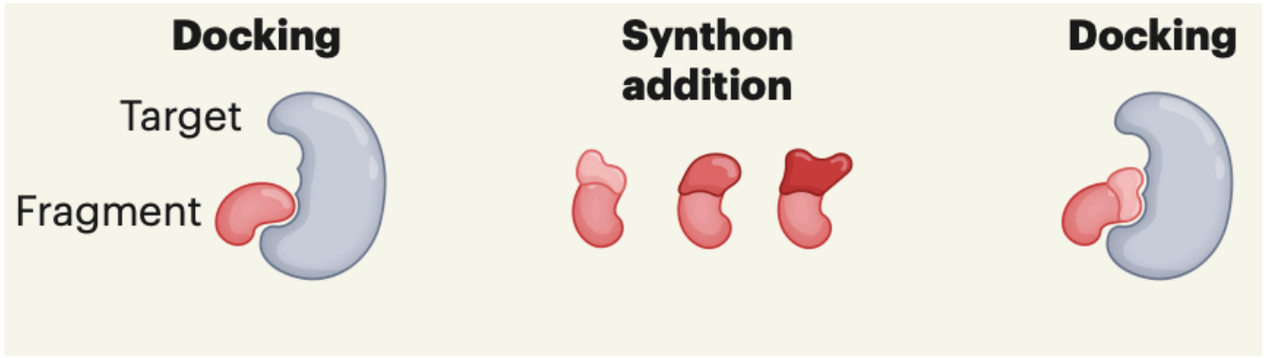
Will Lyon

MacMillan Group

Princeton University


Enamine REAL library
Drug Discovery Pipeline
Discovery
Preclinical development
Clinical trials
FDA review
Approved drug
> 5000 compounds
250 compounds
5 compounds
1 compound
36 billion compounds
How can massive libraries be utilized in drug discovery?

Virtual ligand screening for drug discovery



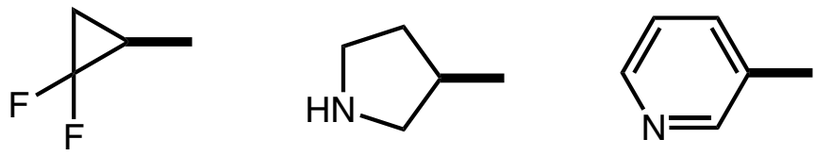
Virtual screening of ligand-receptor docking enables early stage drug discovery

How can this be applied to libraries with billions of compounds?

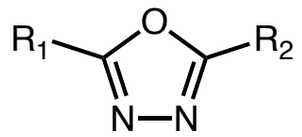
Deane, C.; Mokaya, M. *Nature* **2021**, *601*, 322.; Sadybekov, A. A. et al. *Nature* **2021**, *601*, 452.

Generation of a minimal enumeration library

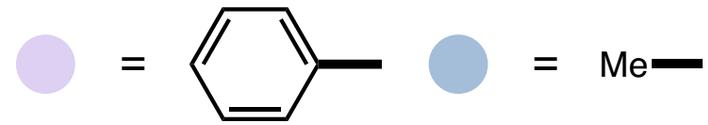
Synthons



Scaffold



Minimal caps



Minimal enumeration library

REAL database: 11 billion compounds

minimal enumeration

600,000 compound library

Sadybekov, A. A. et al. *Nature* **2021**, *601*, 452.

Ligand-receptor docking

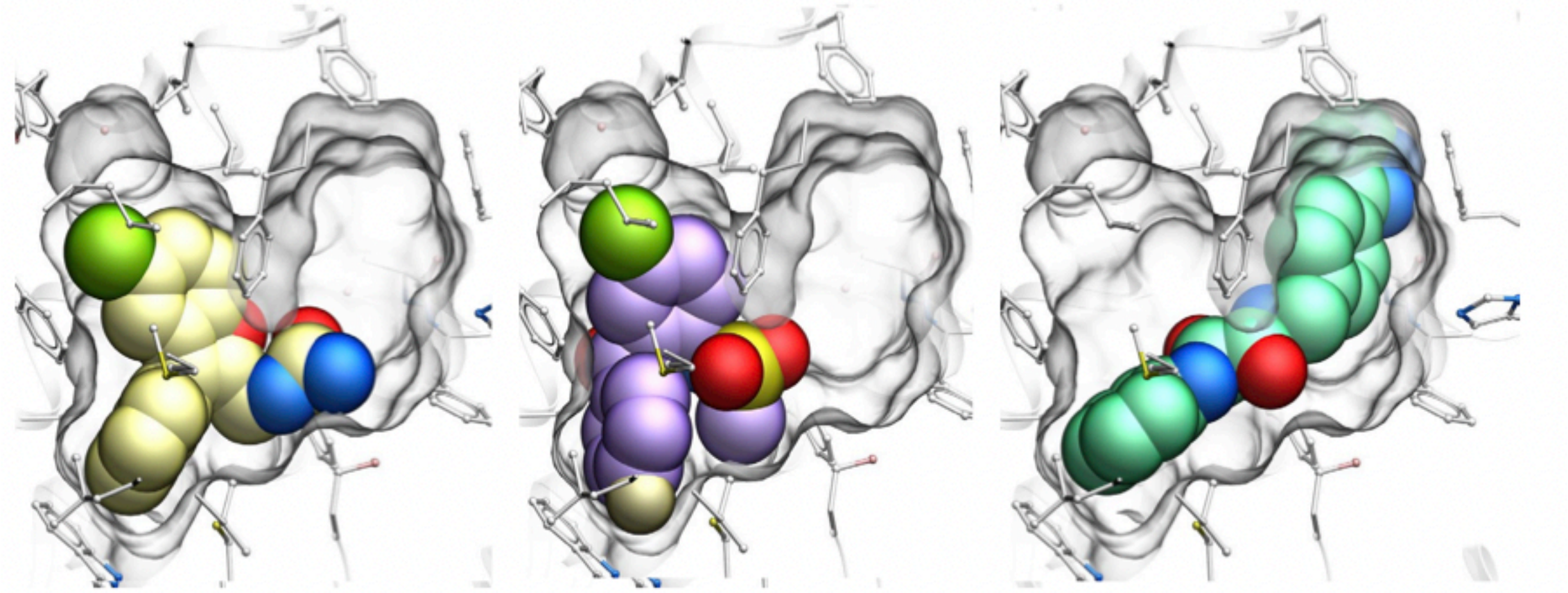
REAL database: 11 billion compounds

minimal enumeration

600,000 compound library

~$800,000 to evaluate

~$50 to evaluate



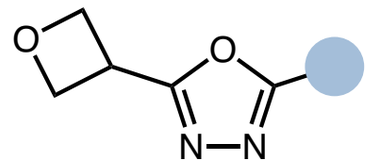
1,000 – 10,000 minimal fragments selected

Sadybekov, A. A. et al. *Nature* **2021**, *601*, 452.

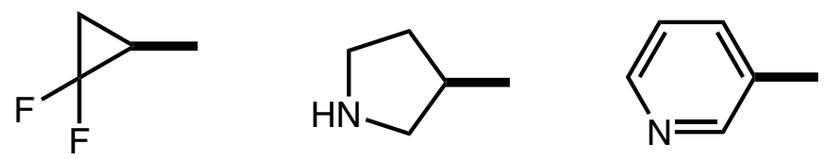
Full enumeration of fragments

1,000 – 10,000 minimal fragments selected

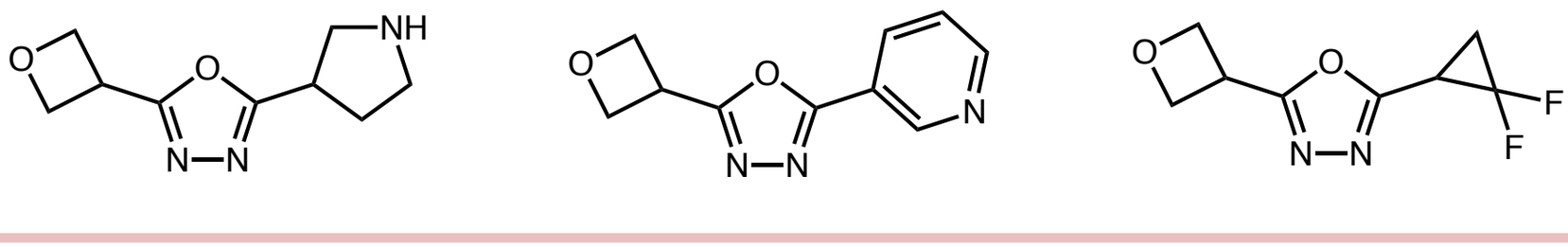
Lead minimal fragment



Synthons



Fully enumerated library

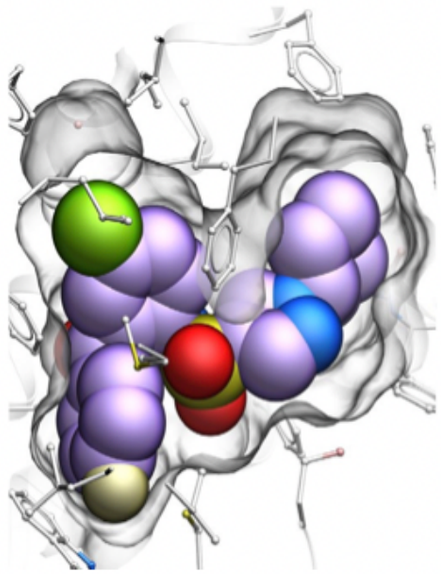


1,000 – 10,000 minimal fragments —***enumeration***→ **1,000,000 enumerated compounds**

Sadybekov, A. A. et al. *Nature* **2021**, *601*, 452.

Candidate generation

1,000,000 enumerated compounds



Docking screen and rigorous filtering (druglikeness, novelty, etc.)

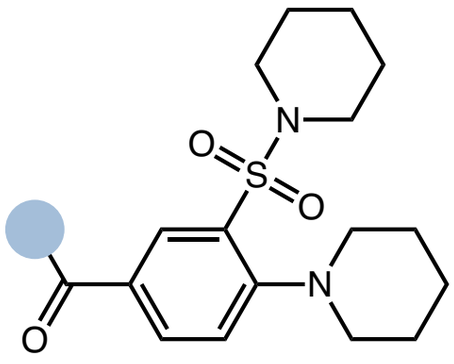
~100 compounds for biological testing

Enamine

88% success rate for REAL compound synthesis

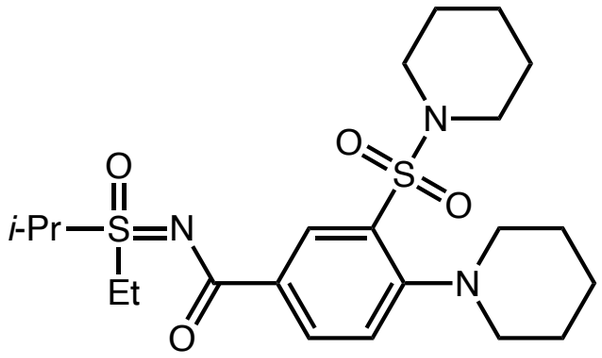
Sadybekov, A. A. et al. *Nature* **2021**, *601*, 452.

Optimization and lead compound development

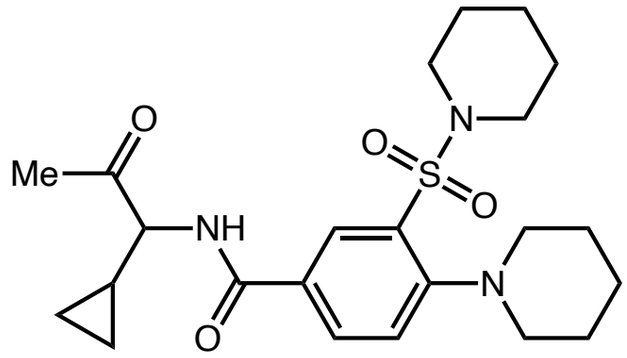


Rapid SAR enabled by REAL space

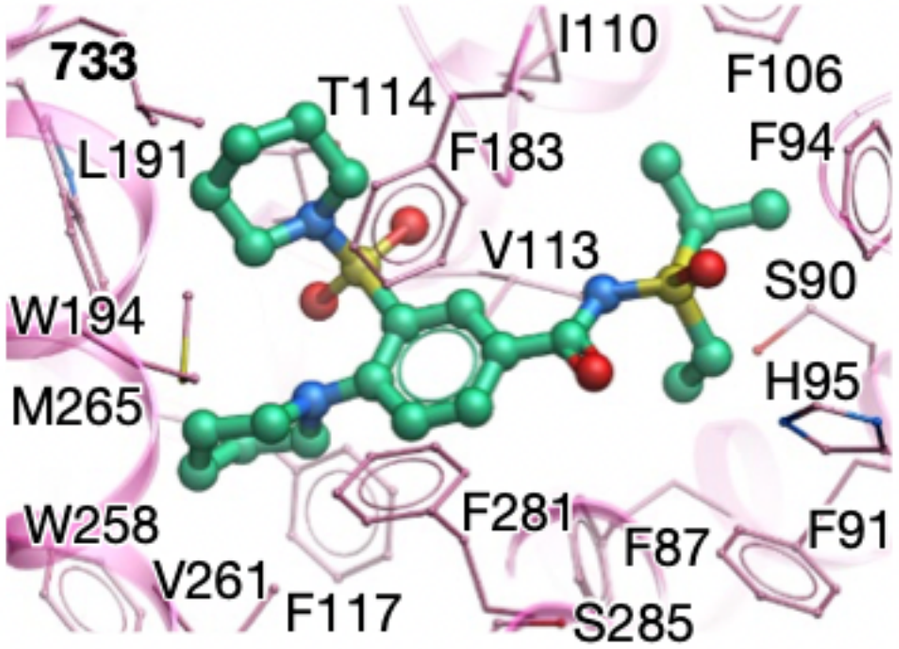
Lead compounds

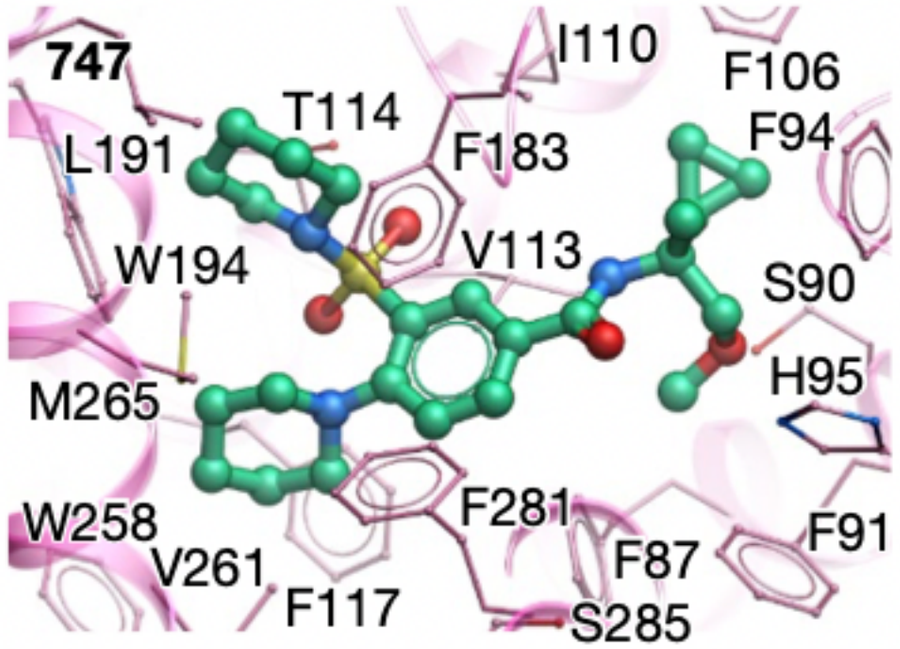


733, K_i = *10.9 nm*



747, K_i = *9.6 nm*





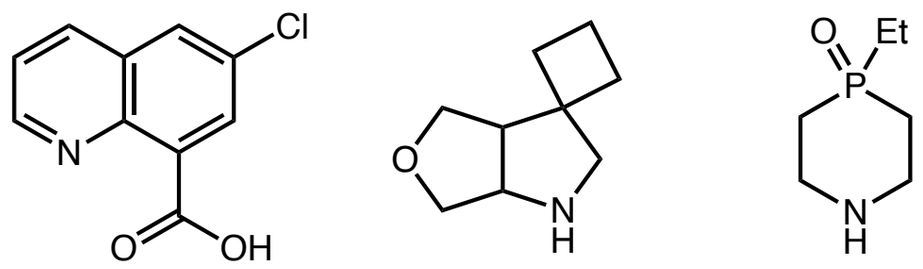
Binding of lead compounds to CB_2 pocket

Sadybekov, A. A. et al. *Nature* **2021**, *601*, 452.

Enamine REAL database

>250,000 fragments in stock ⊗ ***167 reliable reactions*** — ***1-2 steps*** → **36 billion compounds**

Fragments



⊗

Reliable reactions

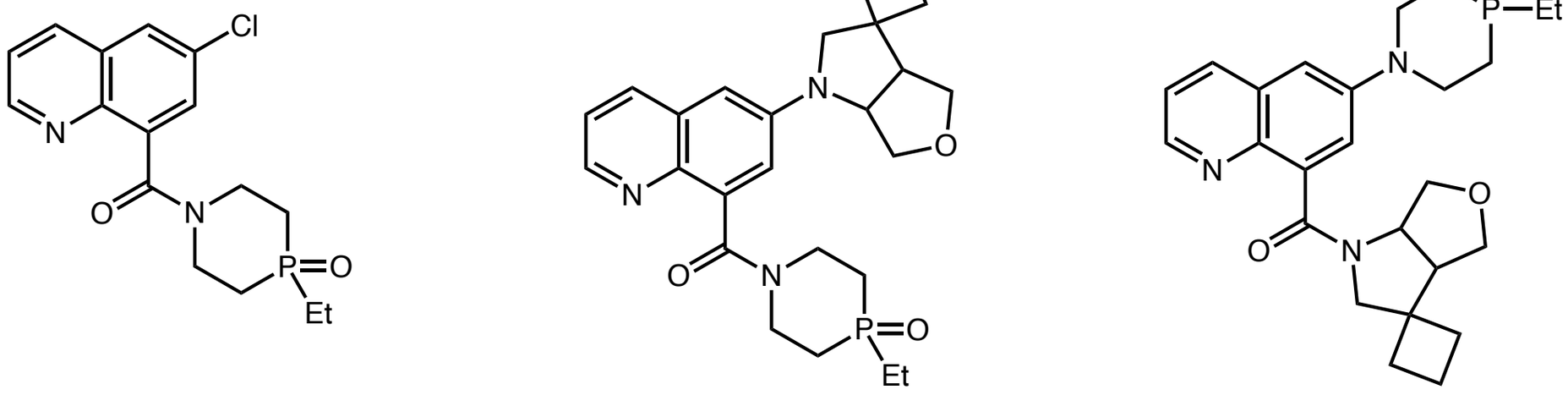
Buchwald-Hartwig

Amide coupling

S_NAr

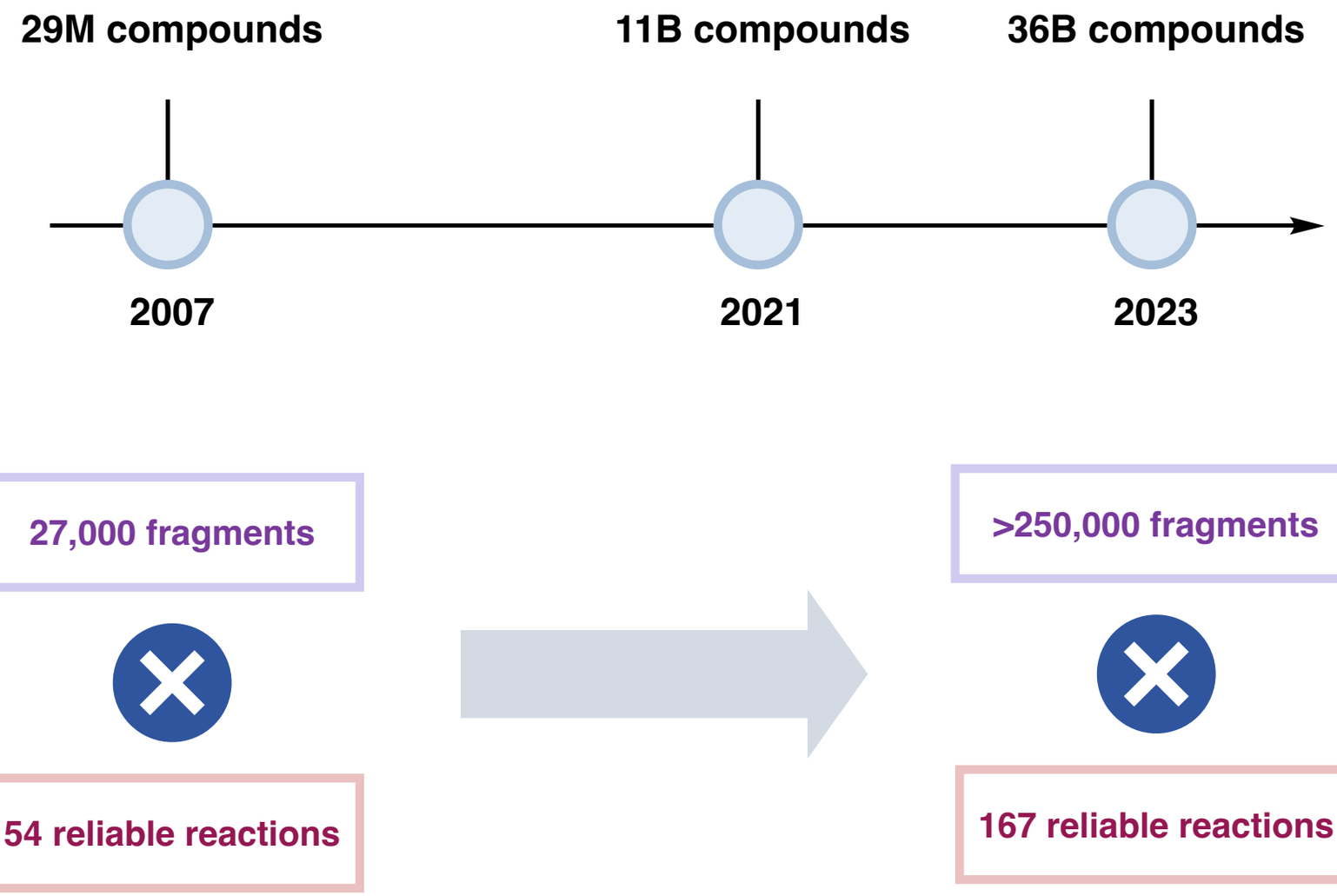
Suzuki-Miyaura

Accessible chemical space



Virtual screening of immense library enabled by REAL database

Development of REAL database

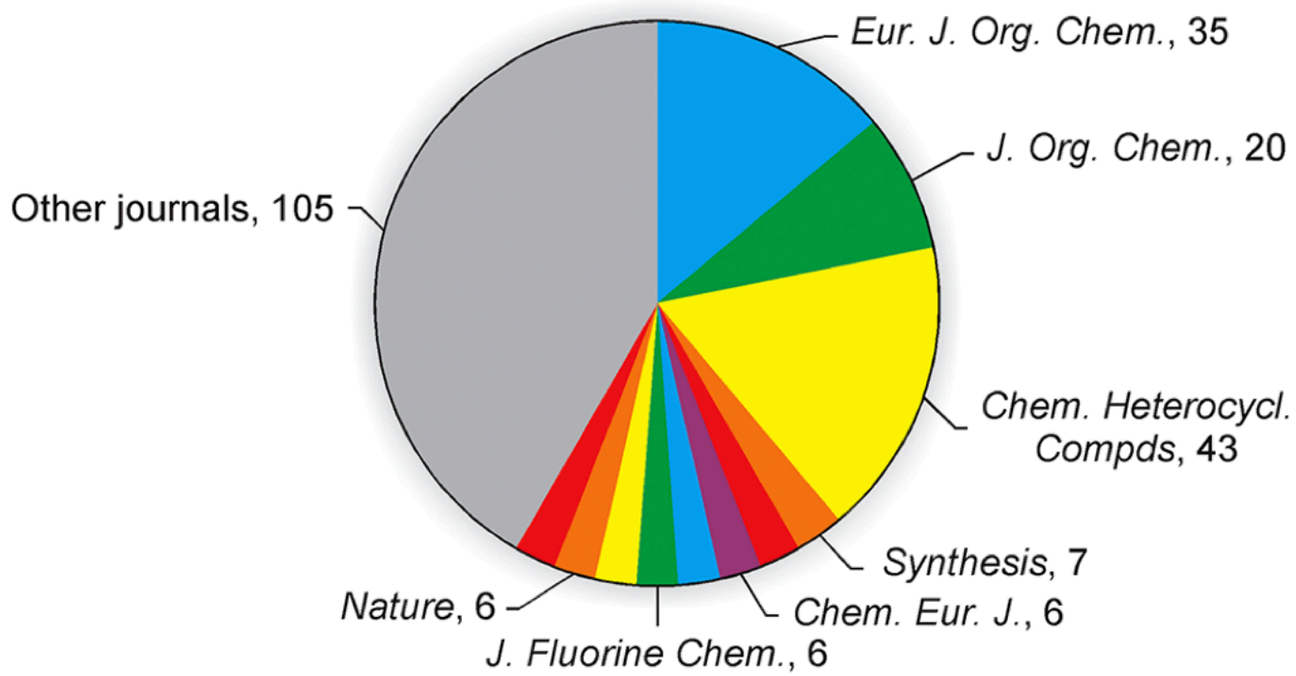


What has driven this great increase in fragments and reactions?

Shivanyuk, A. N. et al. *Chem. Today* **2005**, *25*, 58.; *Real Compounds*. https://enamine.net/compound-collections/real-compounds

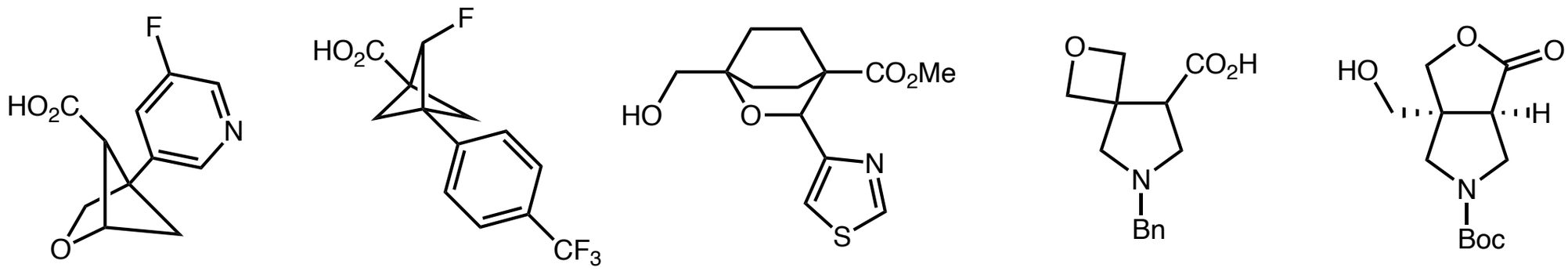
Enamine as a research institution

Pavel Mykhailiuk



Enamine's publications from 2019–2022

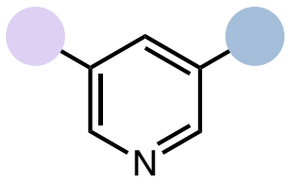
Medicinally driven research

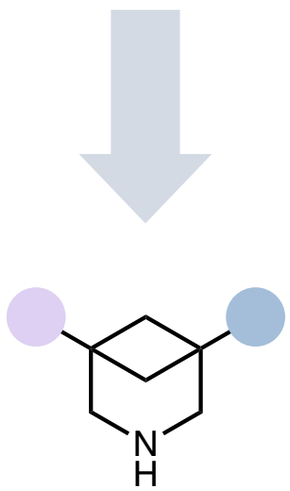


Kondratov, I. S. et al. *ACS Med. Chem. Lett.* **2022**, *13*, 992.

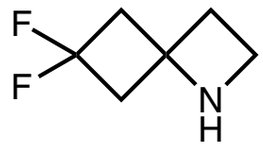
Outline

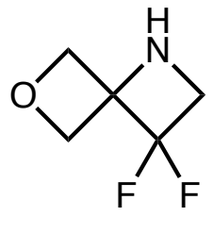
Aromatic bioisosteres

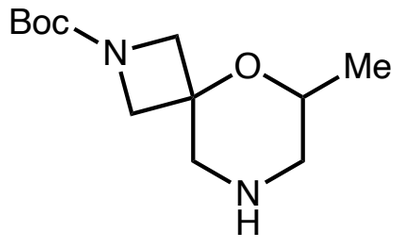




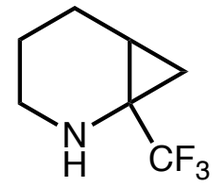
Spirocycles

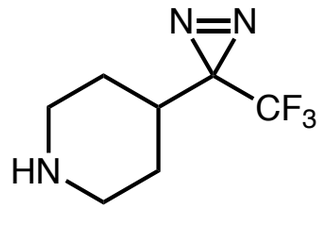


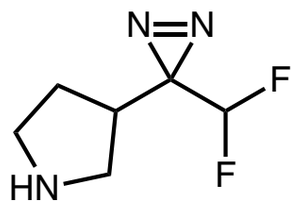




Small rings



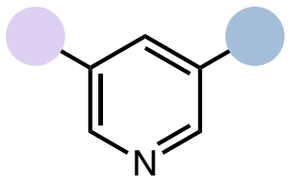


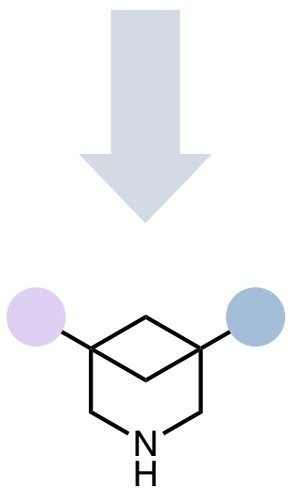


Enamine

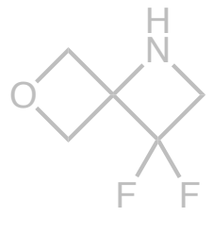
Outline

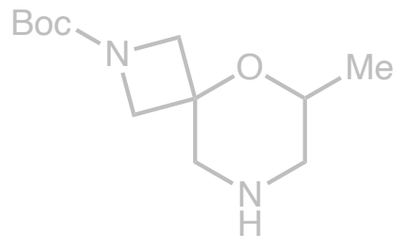
Aromatic bioisosteres



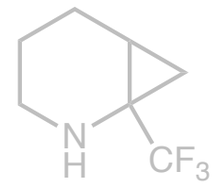


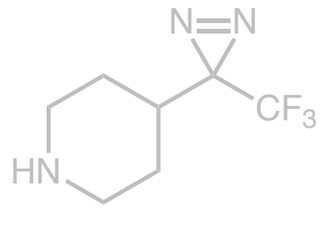
Spirocycles

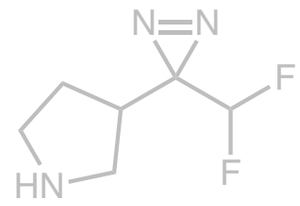




Small rings

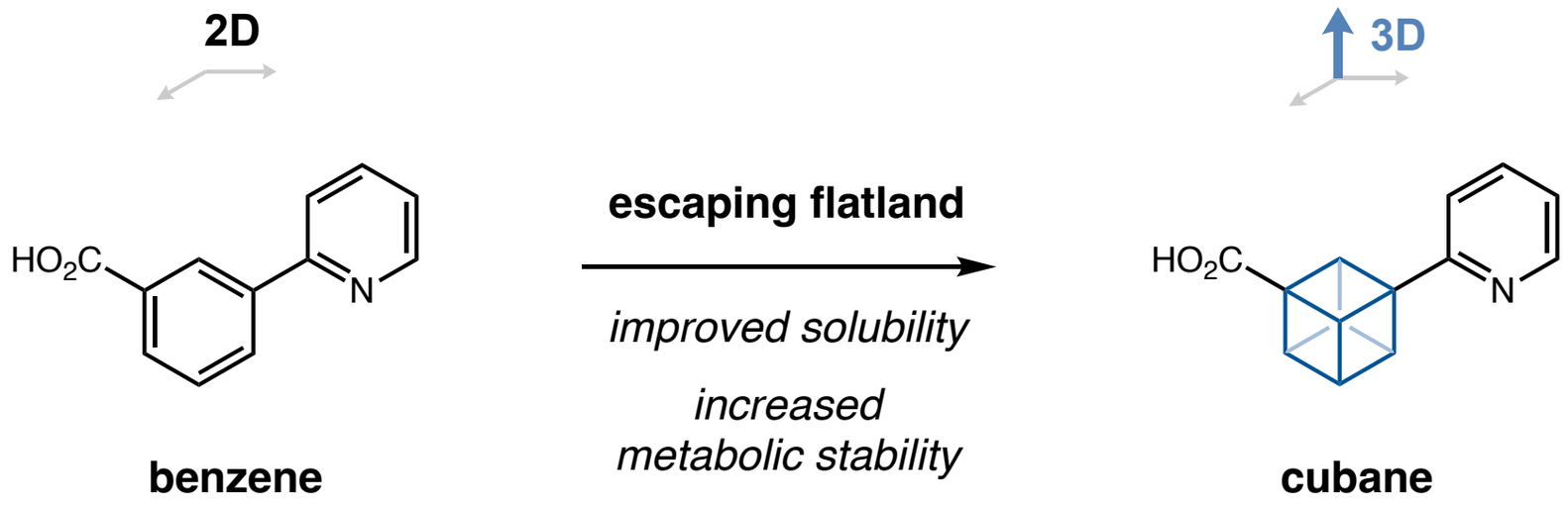


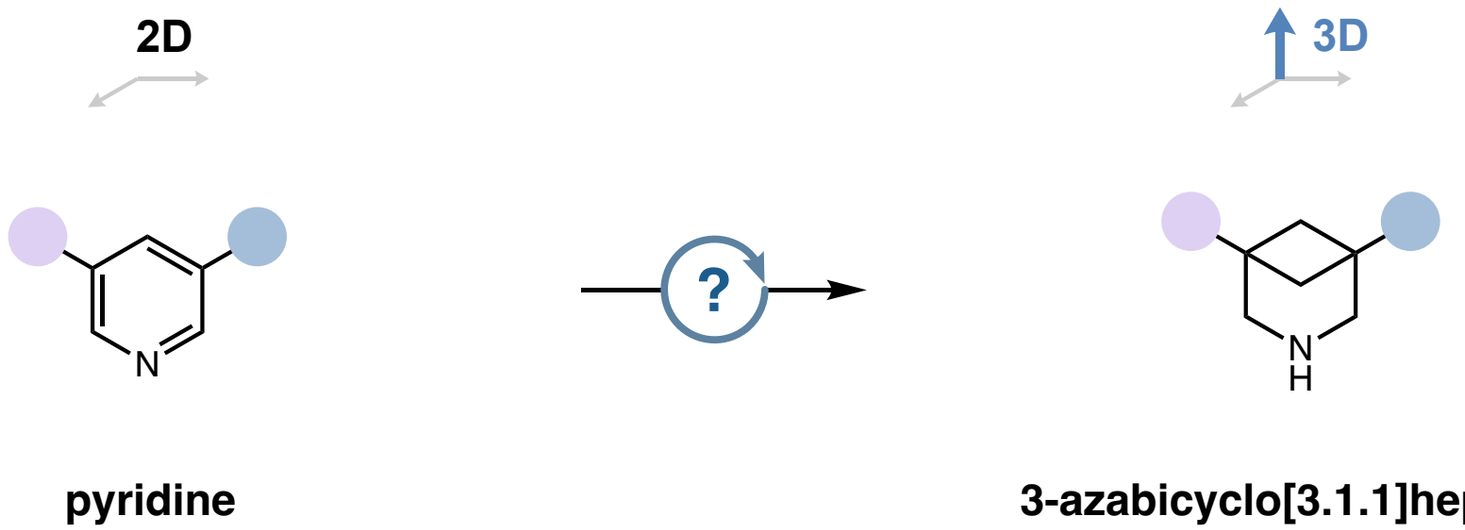




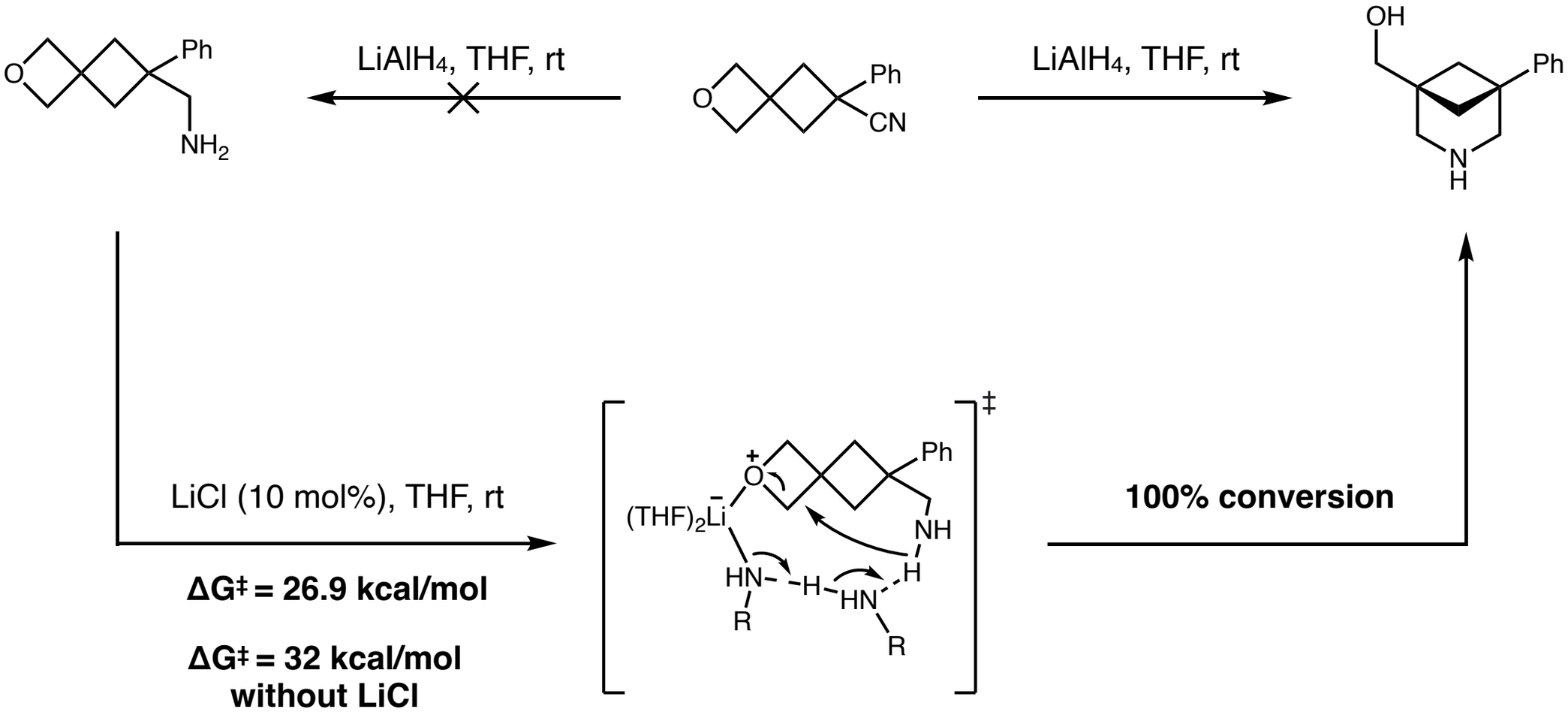
Enamine

Aromatic bioisosteres in medicinal chemistry





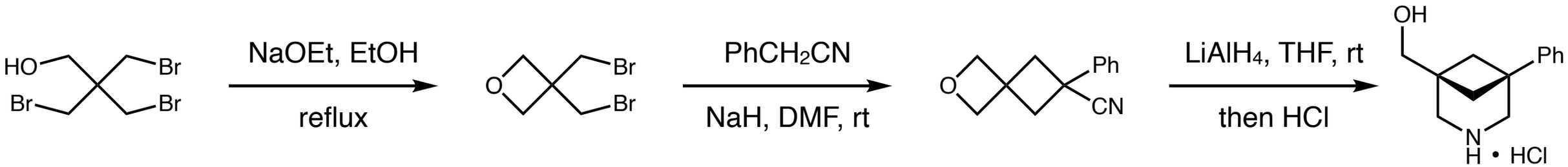
Unexpected discovery of 3-azabicyclo[3.1.1]heptane



Lewis acid coordination accelerates ring-opening

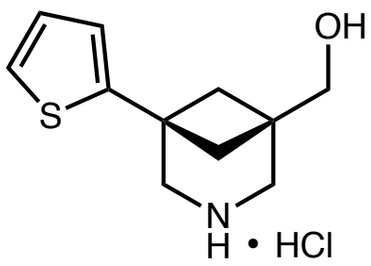
Dibchak, D. et al. *ChemRxiv* **2023**, doi: 10.26434/chemrxiv-2023-jbb0r

Synthesis and scope

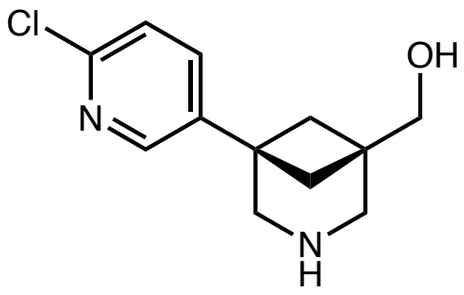


<$1/g | **77% yield** | **$2/g** | **97% yield** | **69% yield (40 g scale)**

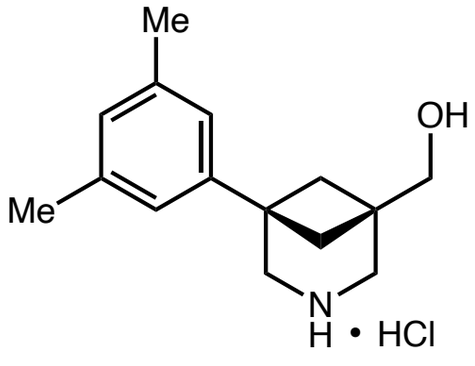
Aryl substitution



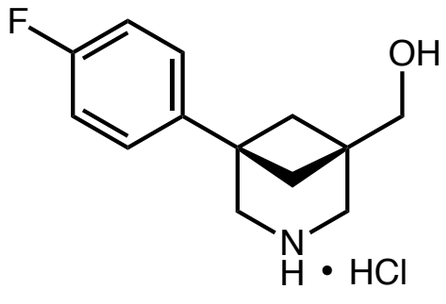
39% yield



37% yield

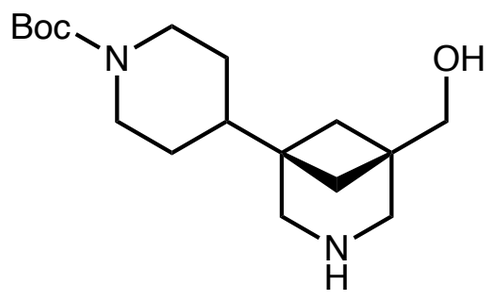


89% yield

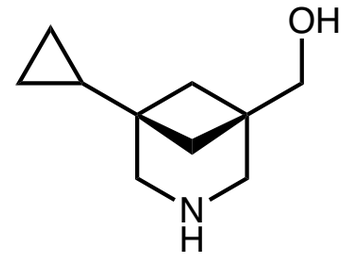


42% yield

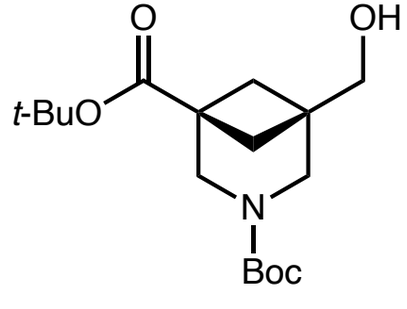
Alkyl substitution



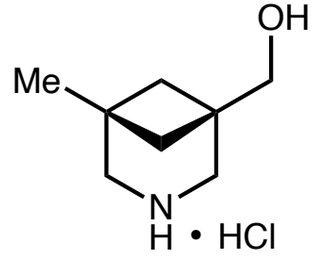
44% yield



82% yield



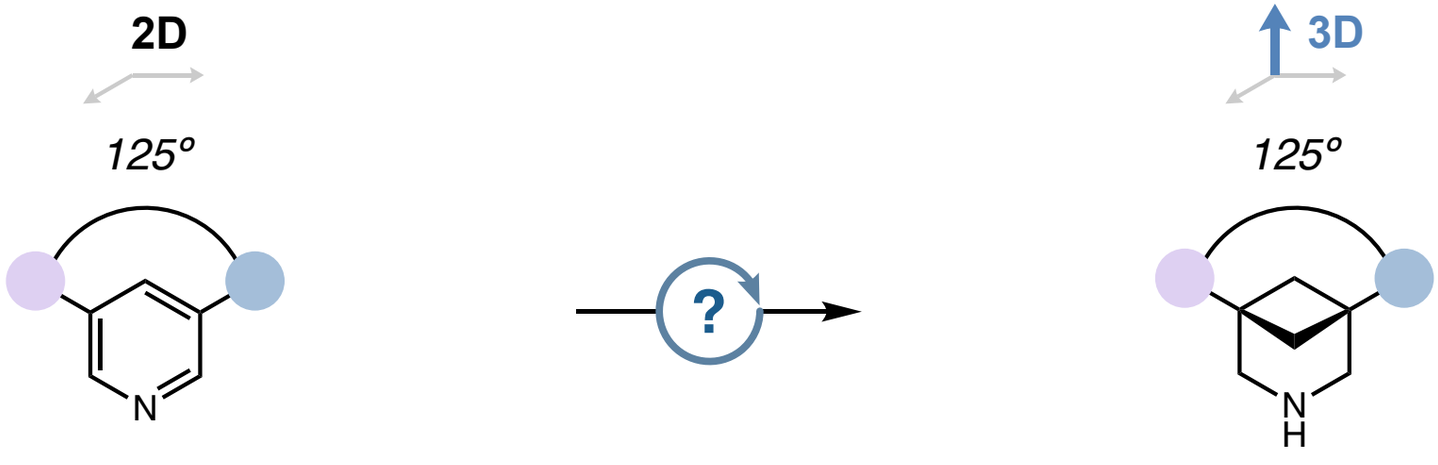
57% yield



77% yield (52 g scale)

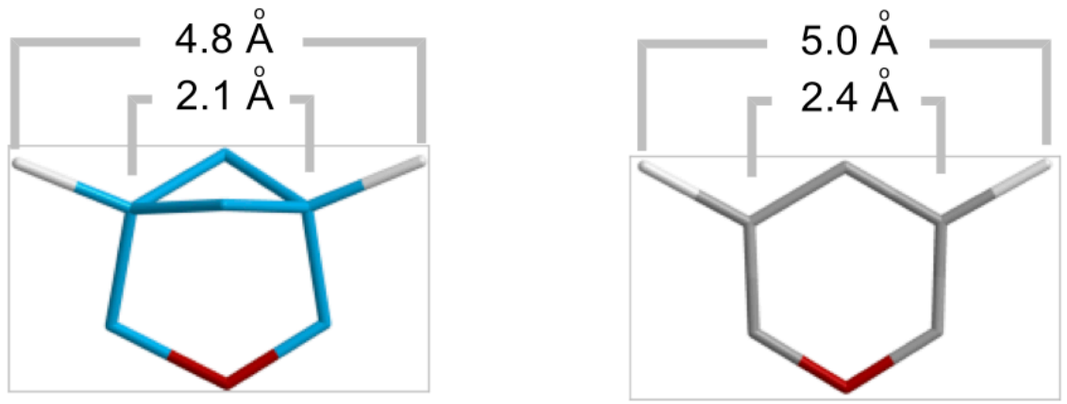
Dibchak, D. et al. *ChemRxiv* **2023**, doi: 10.26434/chemrxiv-2023-jbb0r

Bioisosteric replacement of pyridine ring

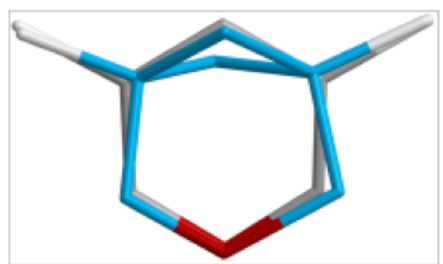


pyridine **3-azabicyclo[3.1.1]heptane**

Geometric parameters



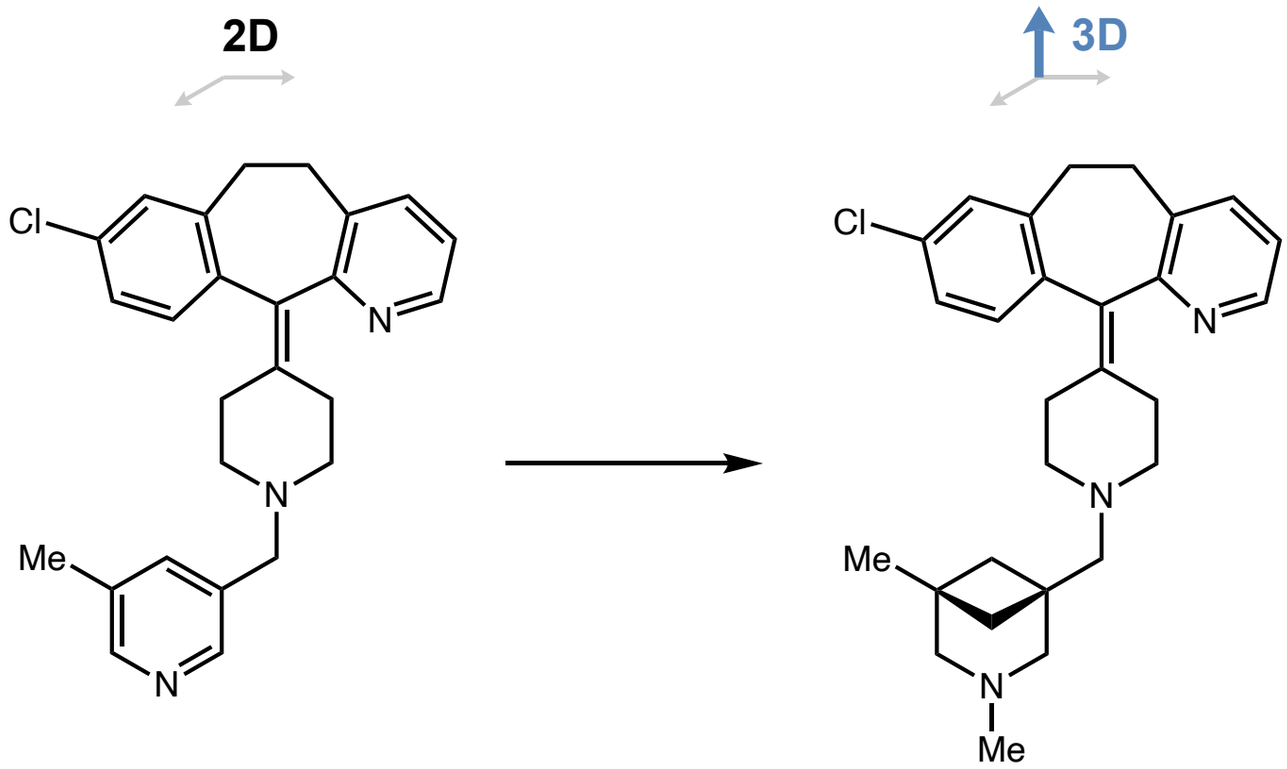
Superposition



How does this bioisostere perform in a biological setting?

Dibchak, D. et al. *ChemRxiv* **2023**, doi: 10.26434/chemrxiv-2023-jbb0r

Direct bioisosteric replacement of rupatadine



rupatadine (antihistamine) **3D-rupatadine**

Physicochemical properties

solubility: 12-fold increase

lipophilicity: 8-fold decrease

metabolic stability: 11-fold increase

3-azabicyclo[3.1.1]heptane is a bioisostere of pyridine

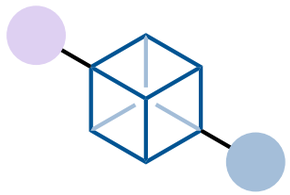
Dibchak, D. et al. *ChemRxiv* **2023**, doi: 10.26434/chemrxiv-2023-jbb0r

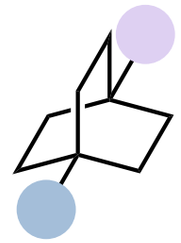
Para-substituted phenyl bioisosteres

para-substituted
benzene

≈

Common bioisosteres





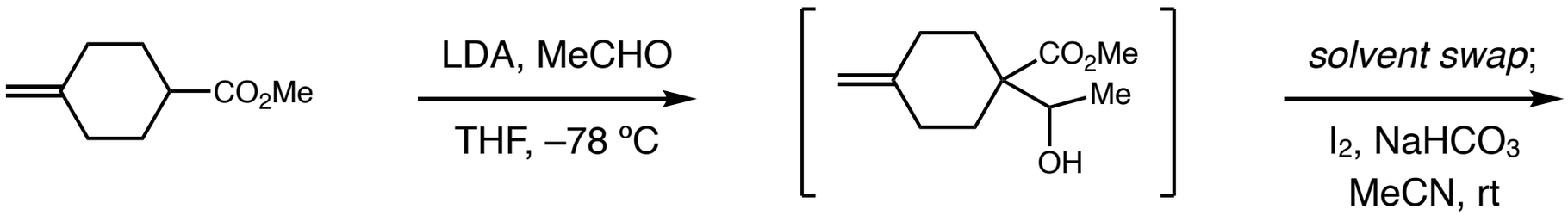
2-oxabicyclo[2.2.2]octane

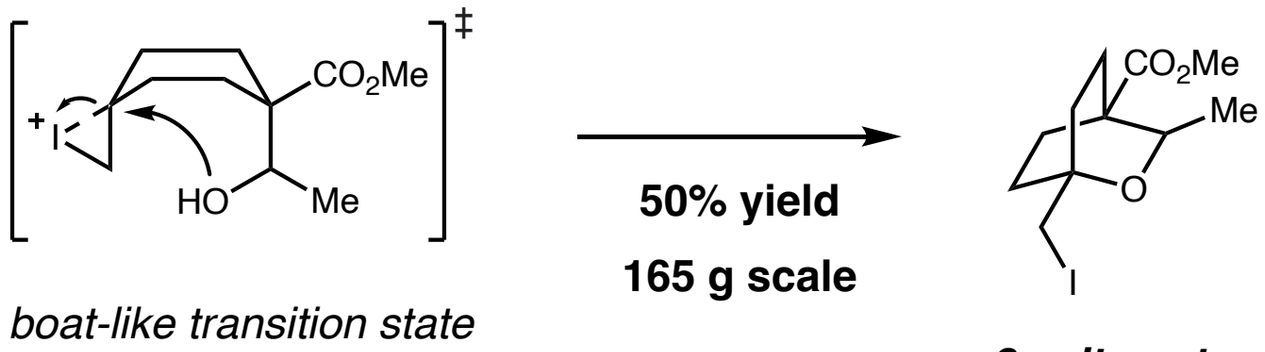
- *increased solubility*
- *decreased lipophilicity*
- *similar geometry*

How can this strained core be readily synthesized?

Levterov, V. V. et al. *ChemRxiv* **2023**, doi: 10.26434/chemrxiv-2023-rbgz3

Synthesis of 2-oxabicyclo[2.2.2]octane cores





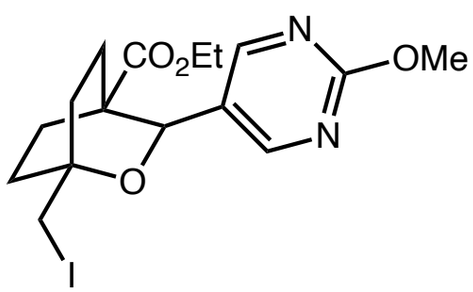
boat-like transition state

50% yield

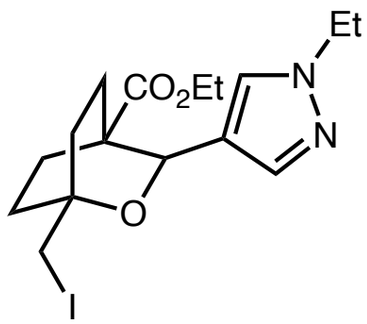
165 g scale

3 exit-vectors

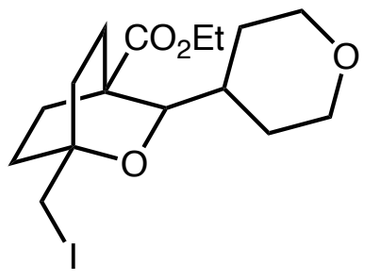
Selected scope



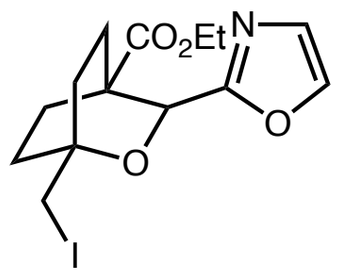
63% yield



39% yield



51% yield

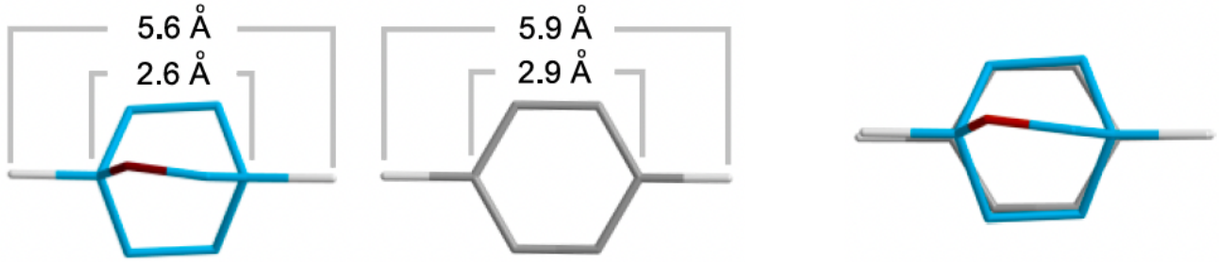


55% yield

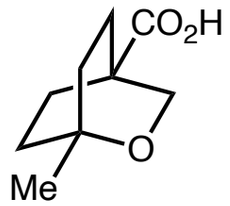
Levterov, V. V. et al. *ChemRxiv* **2023**, doi: 10.26434/chemrxiv-2023-rbgz3

Bioisosteric replacement of phenyl ring

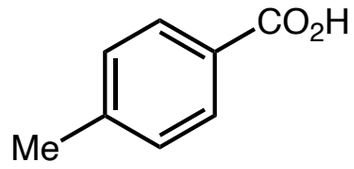
Comparison of phenyl ring with bioisostere



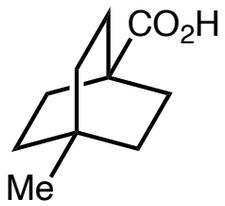
pK_a considerations



pK_a = 4.4

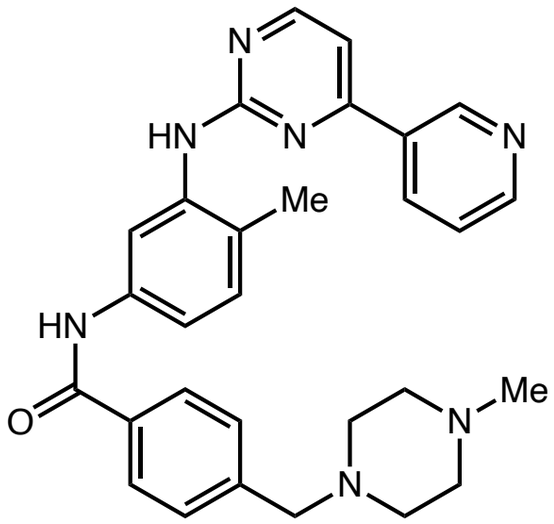


pK_a = 4.5

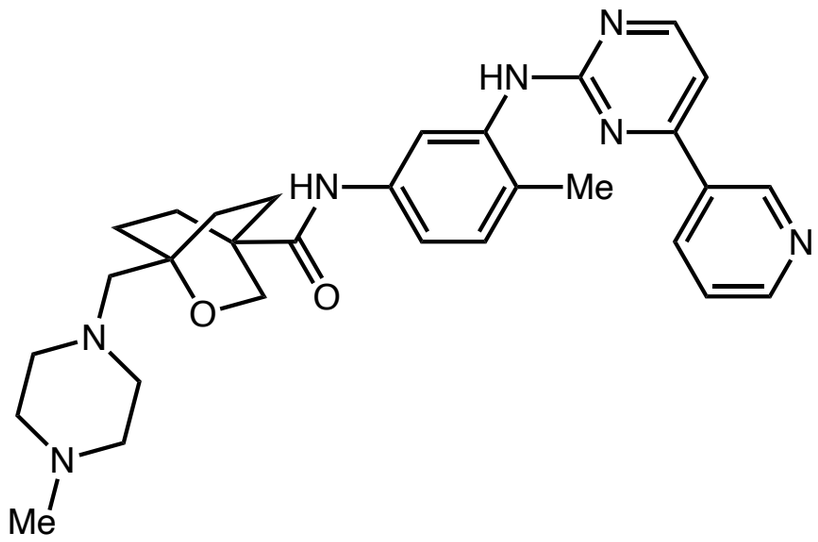


pK_a = 5.6

Geometric and electronic parameters match closely



imatinib (anticancer)



3D-imatinib (anticancer)

Physicochemical properties

solubility: 1.1-fold increase

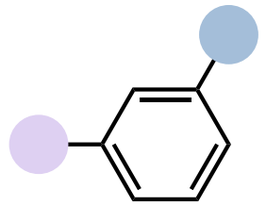
lipophilicity: 8-fold decrease

metabolic stability: 1.5-fold increase

Levterov, V. V. et al. *ChemRxiv* **2023**, doi: 10.26434/chemrxiv-2023-rbgz3

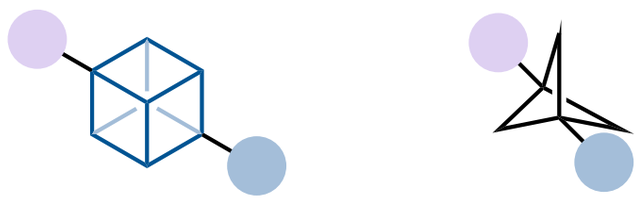
Meta-substituted phenyl bioisosteres

para-substituted
benzene

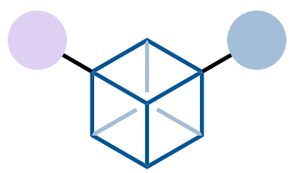


meta-substituted
benzene

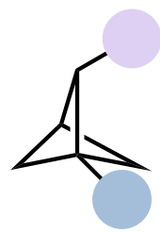
Commonly invoked



Fewer methods



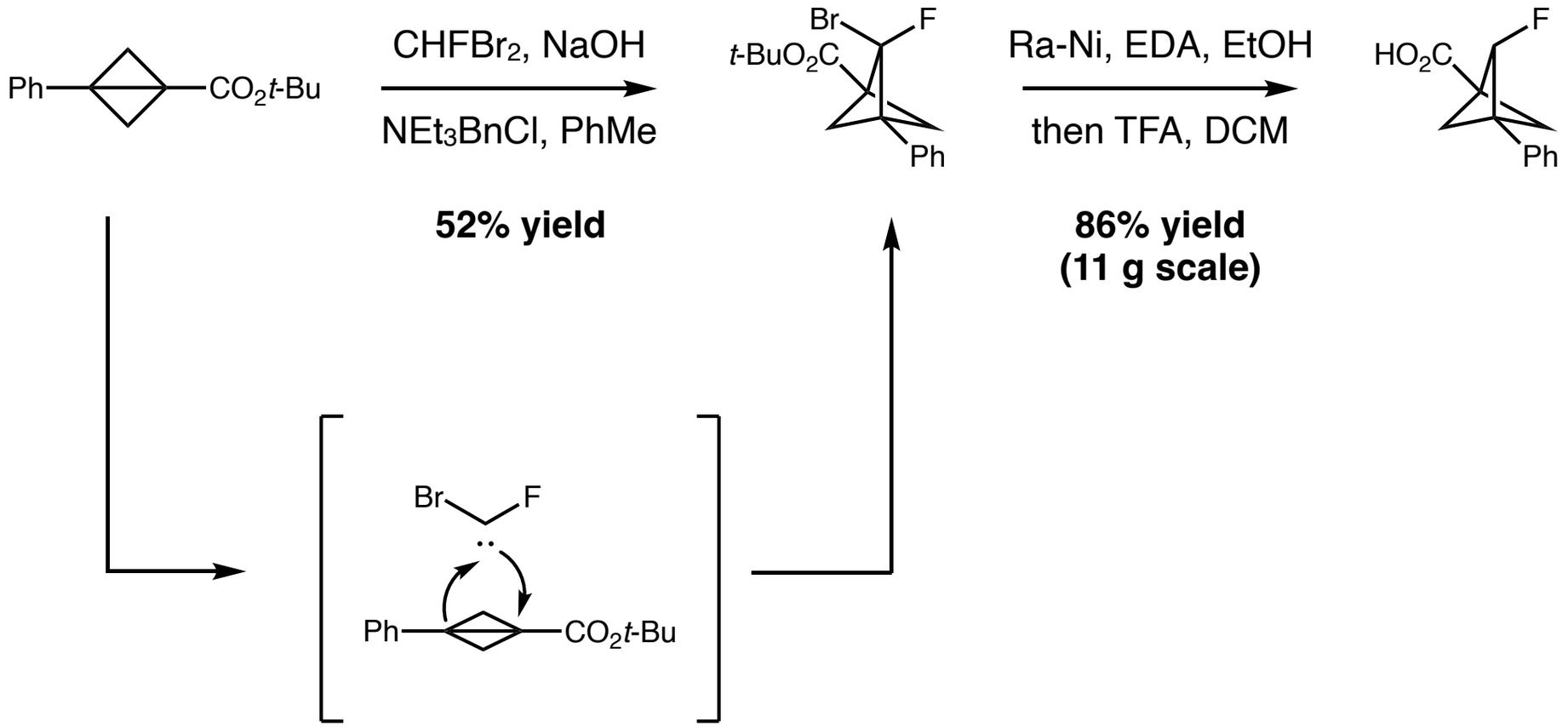
Nature, **2023**



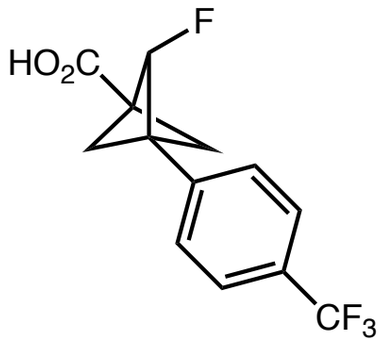
JACS, **2023**

What other methods exist for forming 2-substituted BCPs?

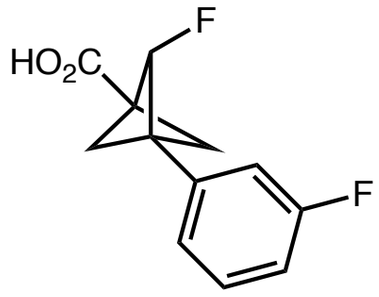
2-Fluoro-substituted BCP synthesis and scope



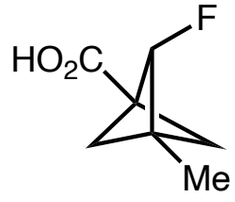
Selected scope



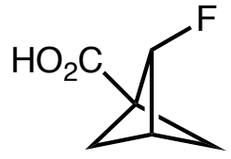
40% yield



46% yield



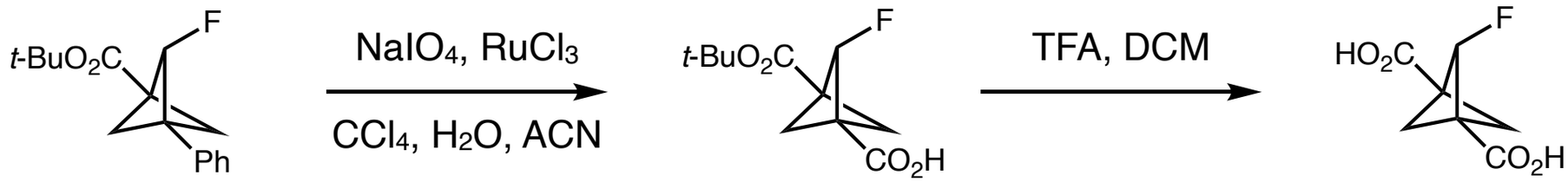
0% yield



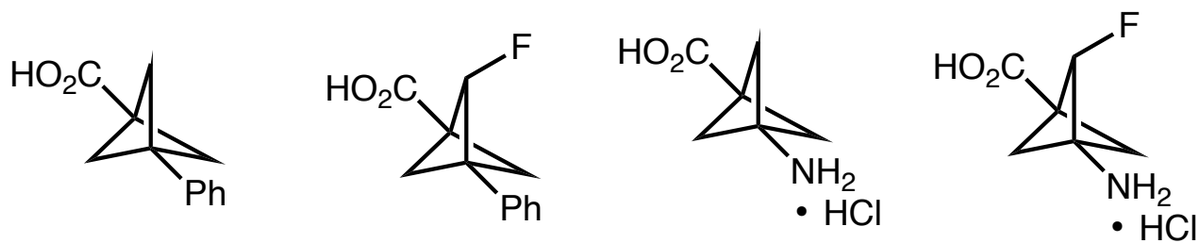
0% yield

Bychek, R.; Mykhailiuk, P. K. *Angew. Chem. Int. Ed.* **2022**, *61*, e202205103

2-Fluoro-substituted BCP applications

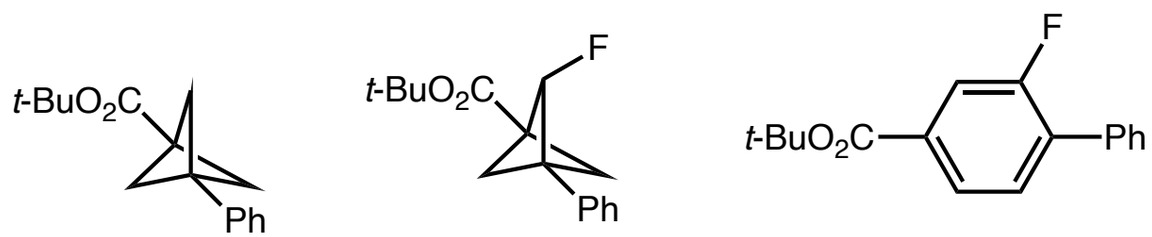


pK_a considerations



pK_a = 4.2 *pK_a = 3.5* *pK_a = 8.2* *pK_a = 6.5*

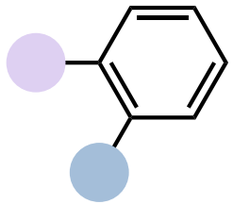
Lipophilicity



cLogP = 3.5 *cLogP = 3.3* *cLogP = 5.4*

Bychek, R.; Mykhailiuk, P. K. *Angew. Chem. Int. Ed.* **2022**, *61*, e202205103

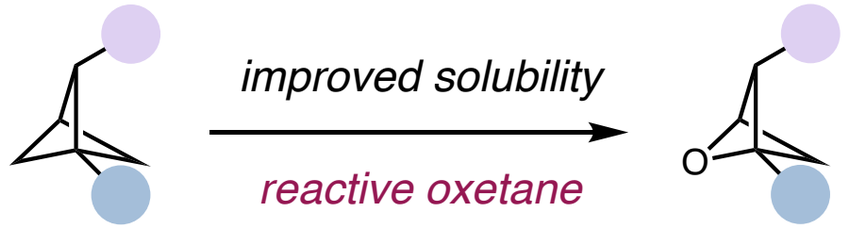
Ortho-substituted phenyl bioisosteres

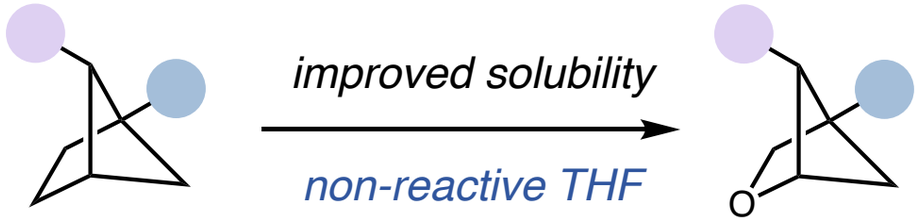


≈

ortho-substituted benzene

Common bioisosteres

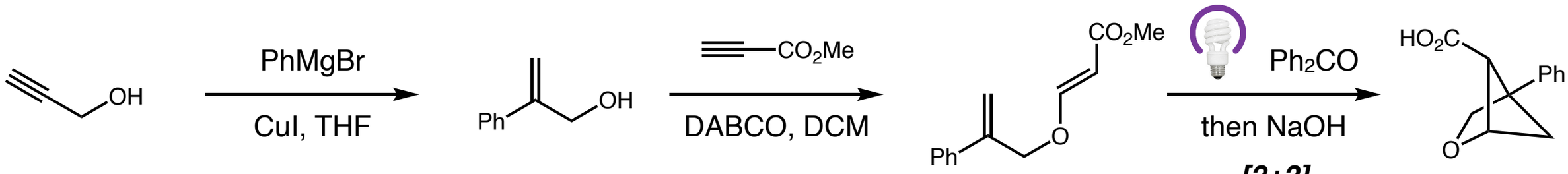




How can this oxabicyclohexane be synthesized?

Denisenko, A. et al. *ChemRxiv* **2022**, doi: 10.26434/chemrxiv-2022-tln0p-v2

Synthesis and scope of 2-oxabicyclo[2.1.1]hexane cores

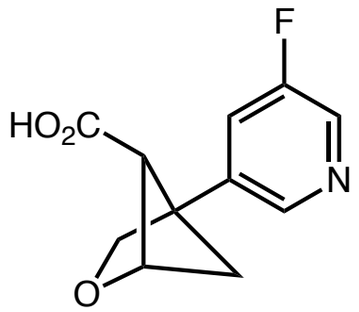


71% yield

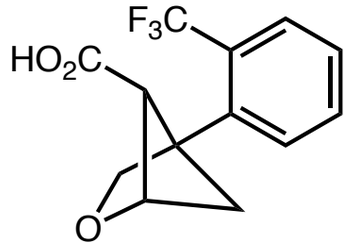
crude

71% yield (3 steps, 10 g scale)

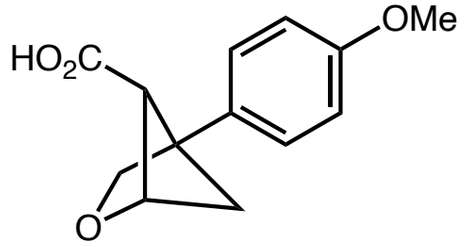
Selected scope



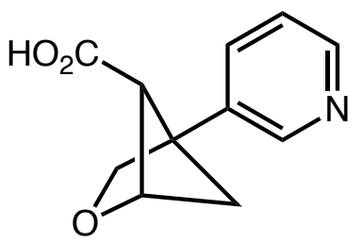
71% yield



59% yield



74% yield



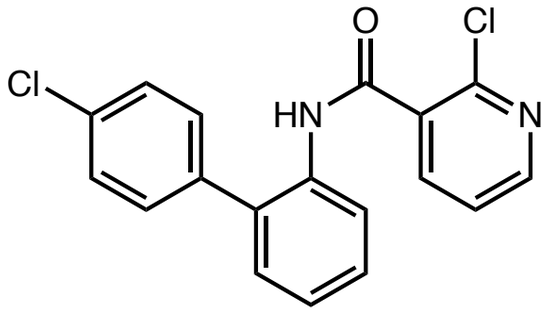
74% yield

Limited to aryl substituents (triplet sensitization of styrene)

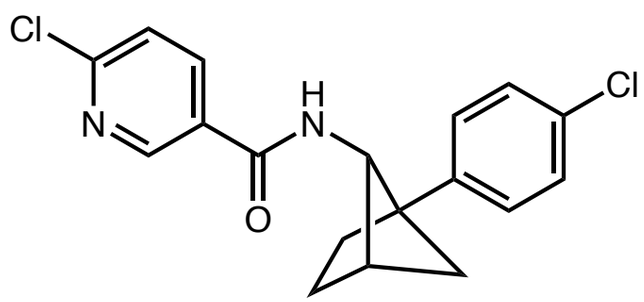
Denisenko, A. et al. *ChemRxiv* **2022**, doi: 10.26434/chemrxiv-2022-tln0p-v2

Replacement of ortho-substituted phenyl ring

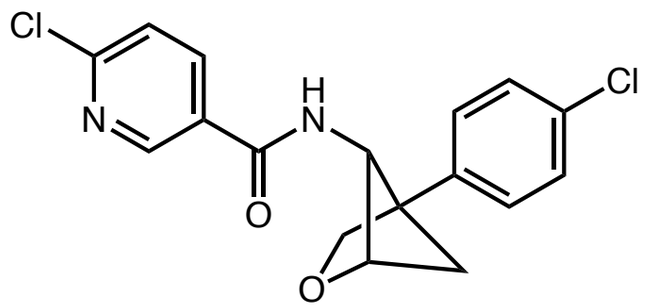
Physicochemical properties



boscalid (fungicide)



BCH-boscalid



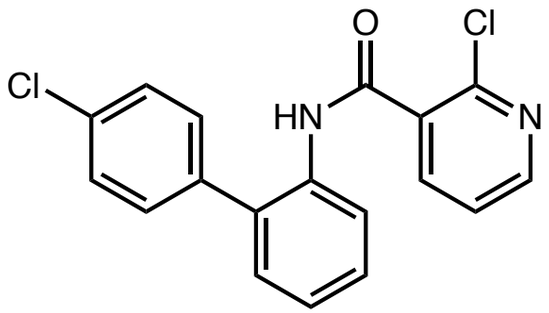
oxa-BCH-boscalid

solubility (μM)	11	17	**152**
logD (lipophilicity)	3.6	3.5	**2.7**
Cl_{int} *(μL/min/mg) (metabolic stability)*	26	12	**3**

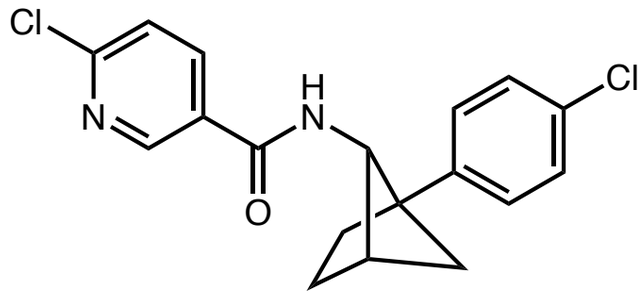
Are these BCH bioisosteres still potent?

Denisenko, A. et al. *ChemRxiv* **2022**, doi: 10.26434/chemrxiv-2022-tln0p-v2

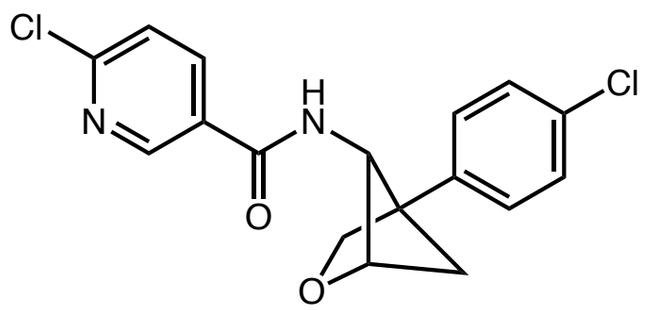
Replacement of ortho-substituted phenyl ring



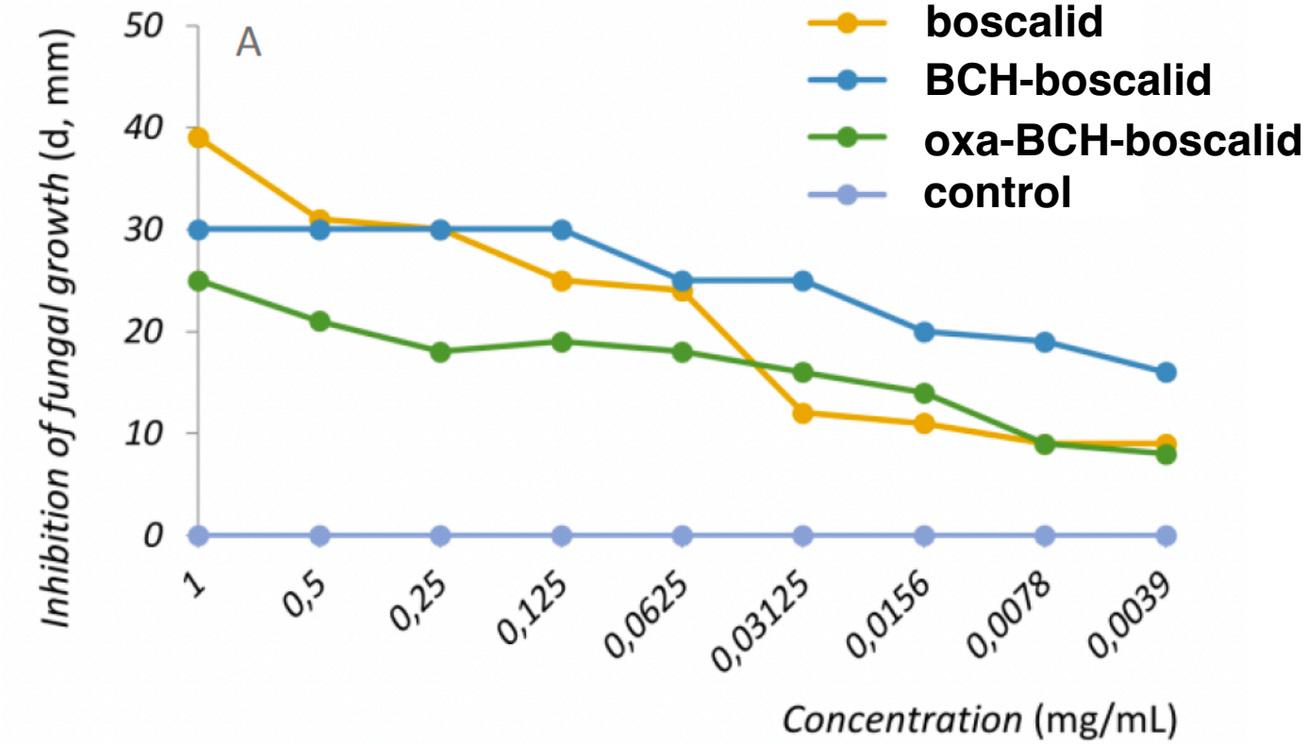
boscalid (fungicide)



BCH-boscalid



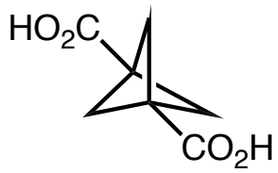
oxa-BCH-boscalid



Both BCH and oxa-BCH derivatives are competent fungicides

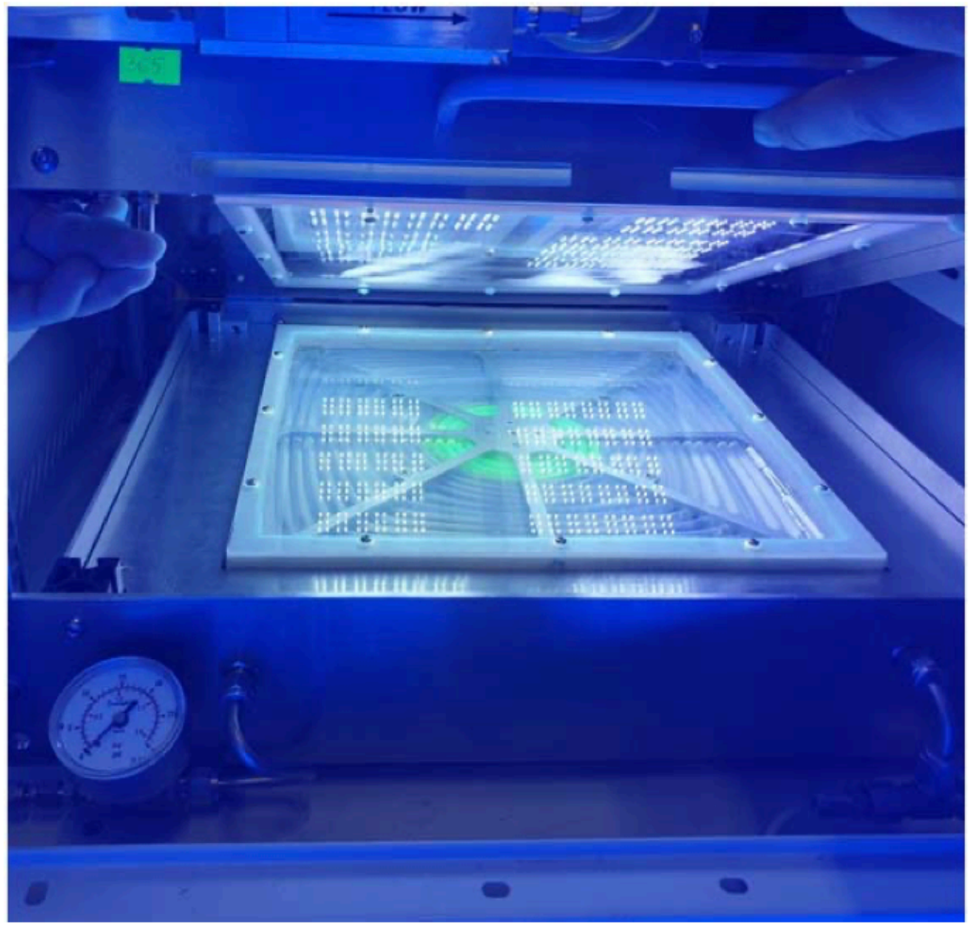
Denisenko, A. et al. *ChemRxiv* **2022**, doi: 10.26434/chemrxiv-2022-tln0p-v2

Bicyclopentane 1,3-diacid



$18/g - in stock

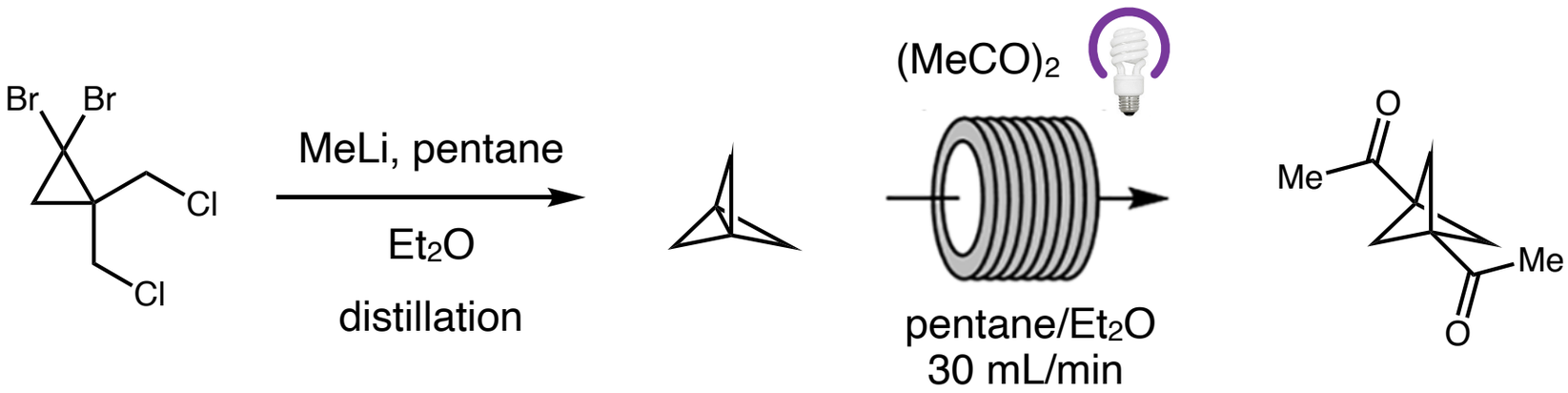
How is this made industrially? What drives the price down?



photochemical flow chemistry

Ripenko, V. et al. *J. Org. Chem.* **2021**, *86*, 14061.

Large-scale synthesis of bicyclopentane 1,3-diacid



70 – 78% yield **94% yield, 1 kg (6 hours)**

propellane + diacetyl

flow photoreactor

product

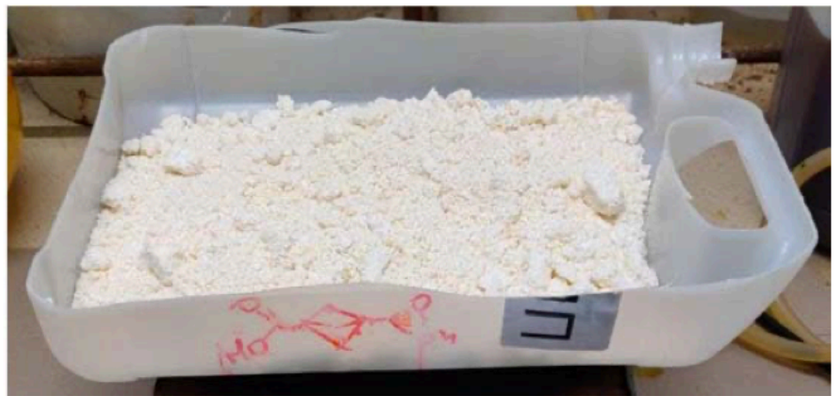
Ripenko, V. et al. *J. Org. Chem.* **2021**, *86*, 14061.

Large-scale synthesis of bicyclopentane 1,3-diacid

70 – 78% yield

94% yield, 1 kg (6 hours)

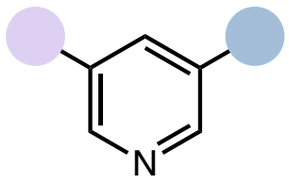
45 – 51% yield, >100 g scale

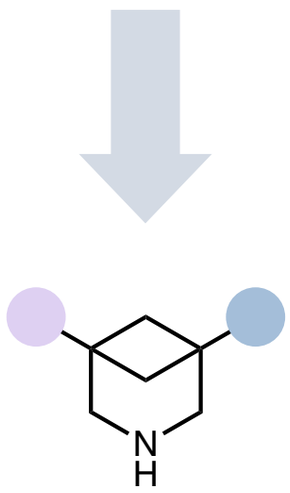


Ripenko, V. et al. *J. Org. Chem.* **2021**, *86*, 14061.

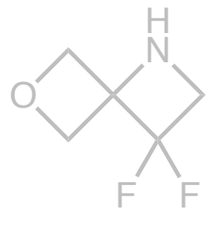
Outline

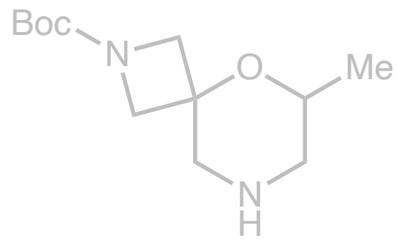
Aromatic bioisosteres



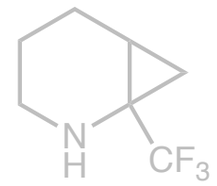


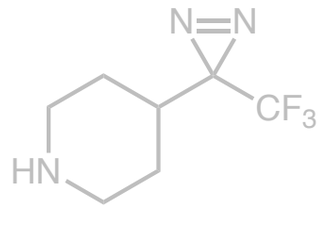
Spirocycles

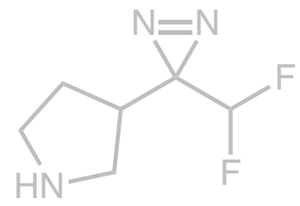




Small rings



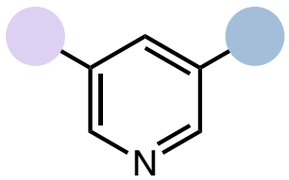


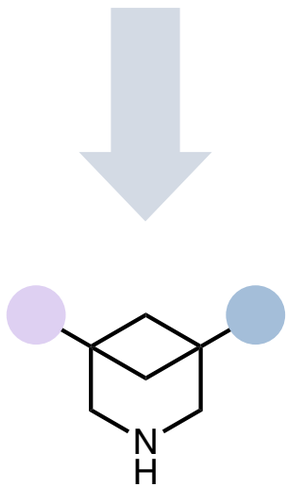


Enamine

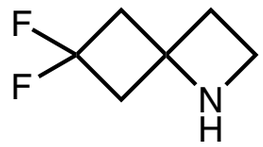
Outline

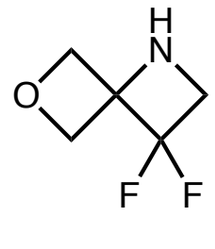
Aromatic bioisosteres

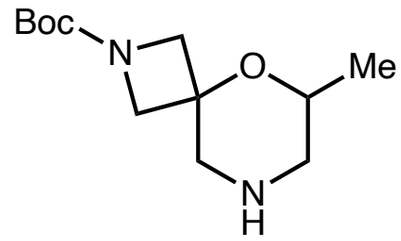




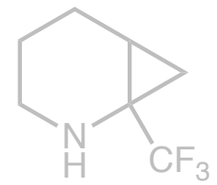
Spirocycles

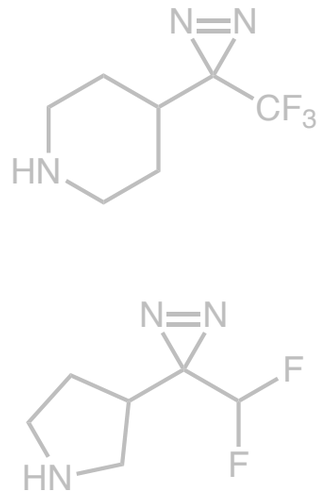






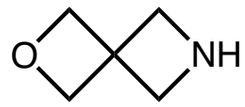
Small rings





Enamine

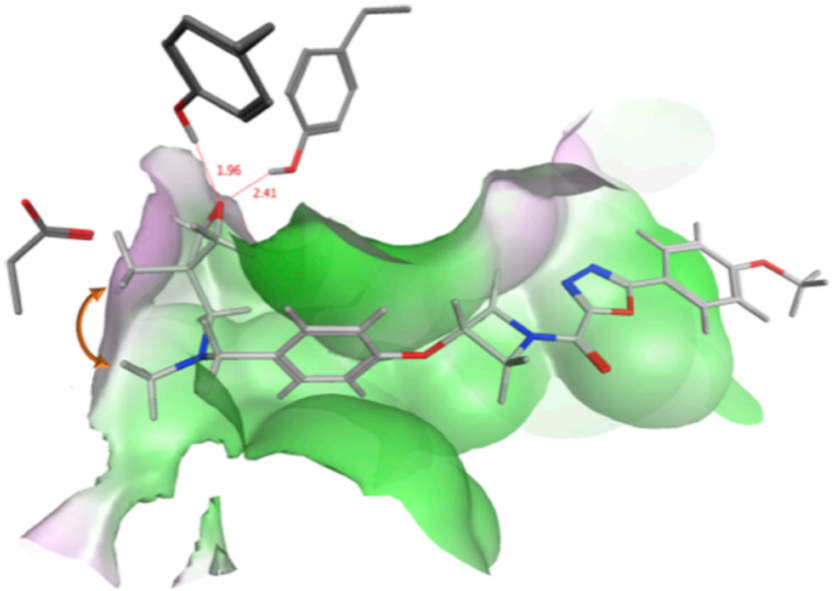
Spirocycles are privileged bioactive molecules

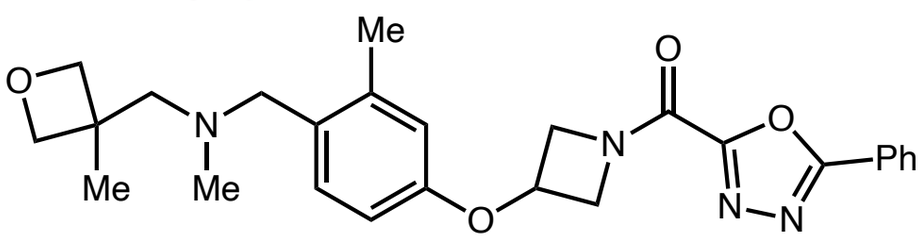


Morpholine bioisostere

Increased conformational rigidity

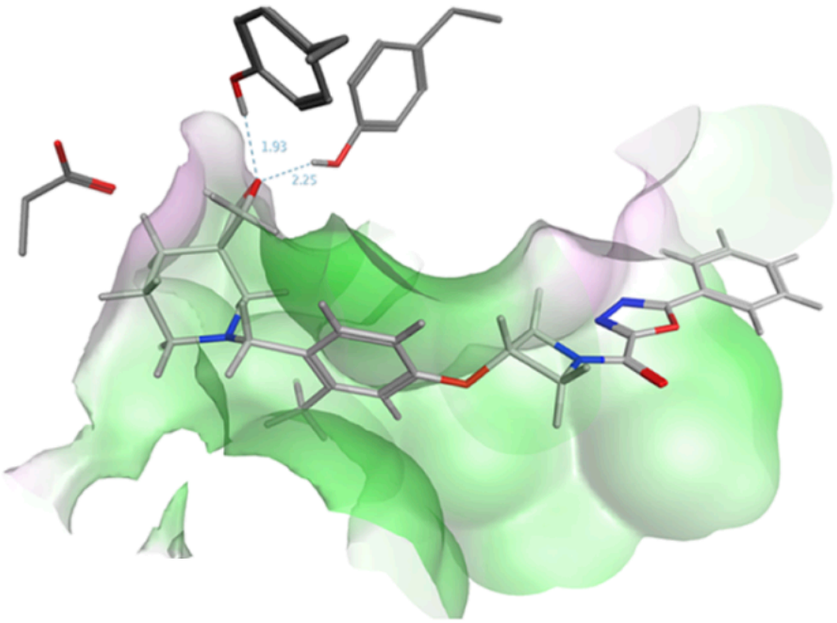
Improved solubility

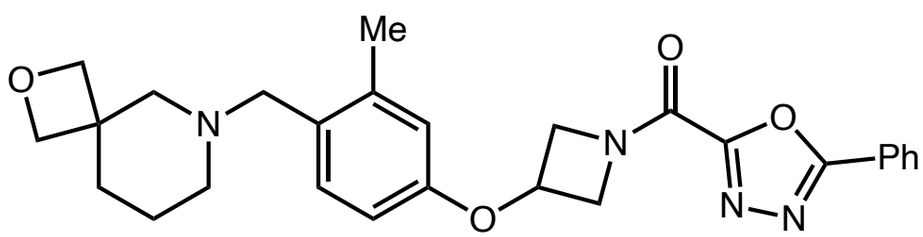




89% hydrolysis

Ring closure





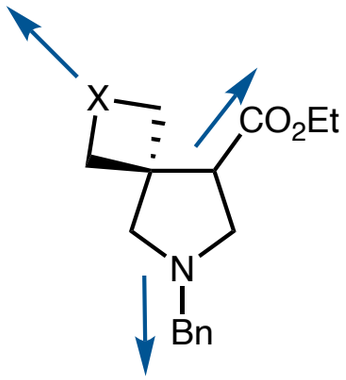
98% hydrolysis

Increased conformational rigidity promotes binding to key Tyr residues

Toselli, F. et al. *J. Med. Chem.* **2019**, *62*, 7383.

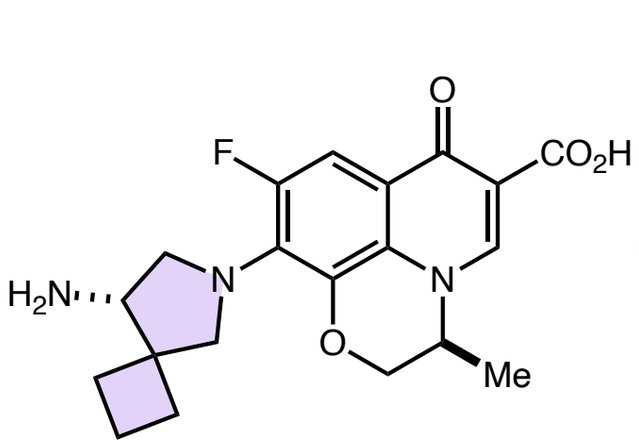
Spirocyclic pyrrolidines in medicinal chemistry

Spirocyclic pyrrolidine

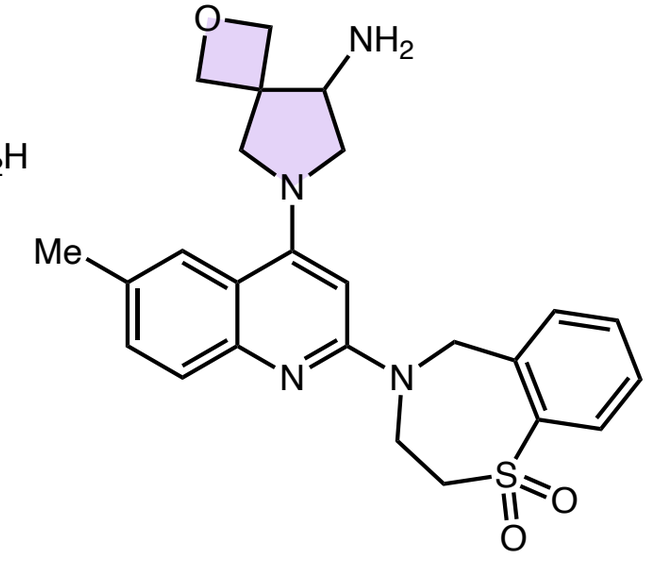


- *3 exit vectors*
- *modular substituents*
- *conformationally restricted*

Examples from medicinal chemistry



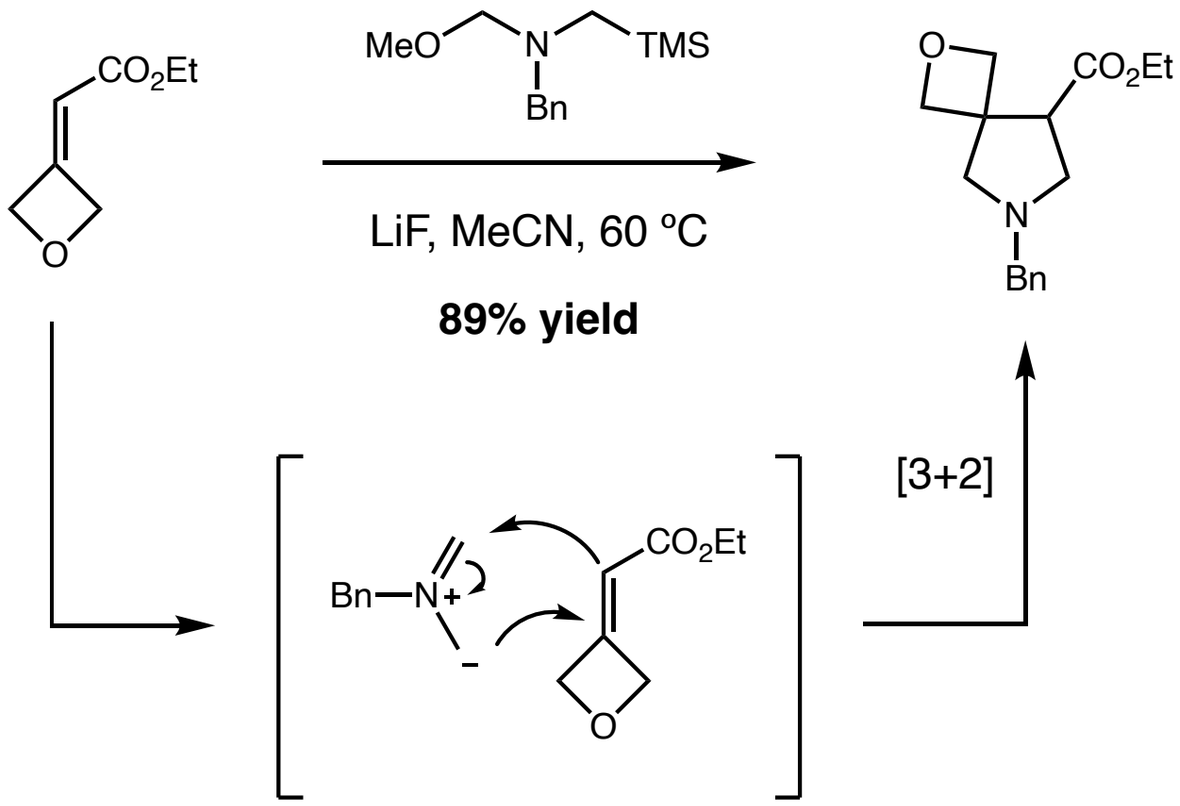
antibacterial agent



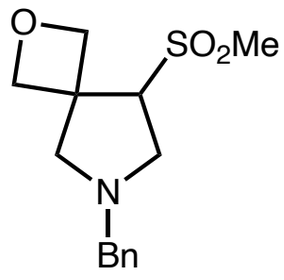
antiviral agent

Chalyk, B. A. et al. *Eur. J. Org. Chem.* **2017**, 4530.

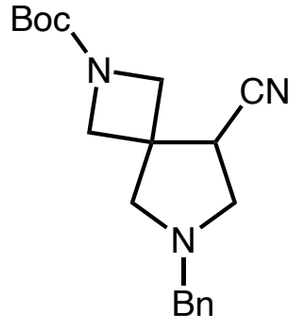
Synthesis of spirocyclic pyrrolidines



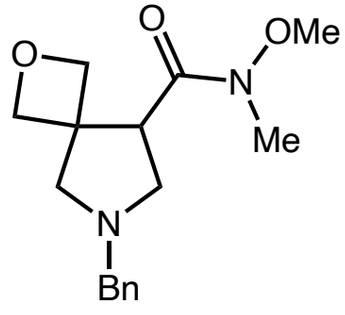
Selected scope



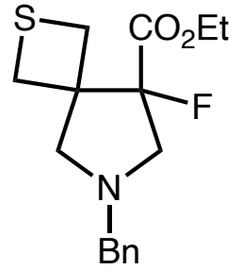
87% yield



80% yield



76% yield

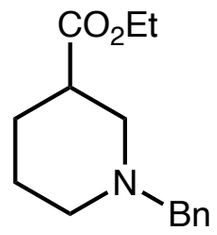
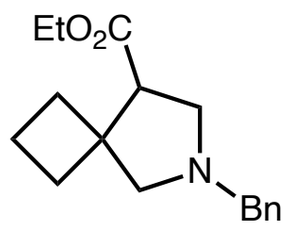
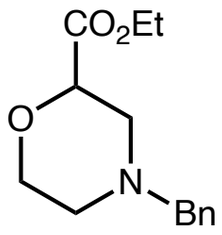
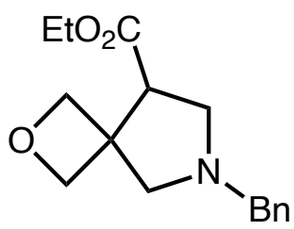


88% yield

Chalyk, B. A. et al. *Eur. J. Org. Chem.* **2017**, 4530.

Physicochemical properties

Physicochemical properties

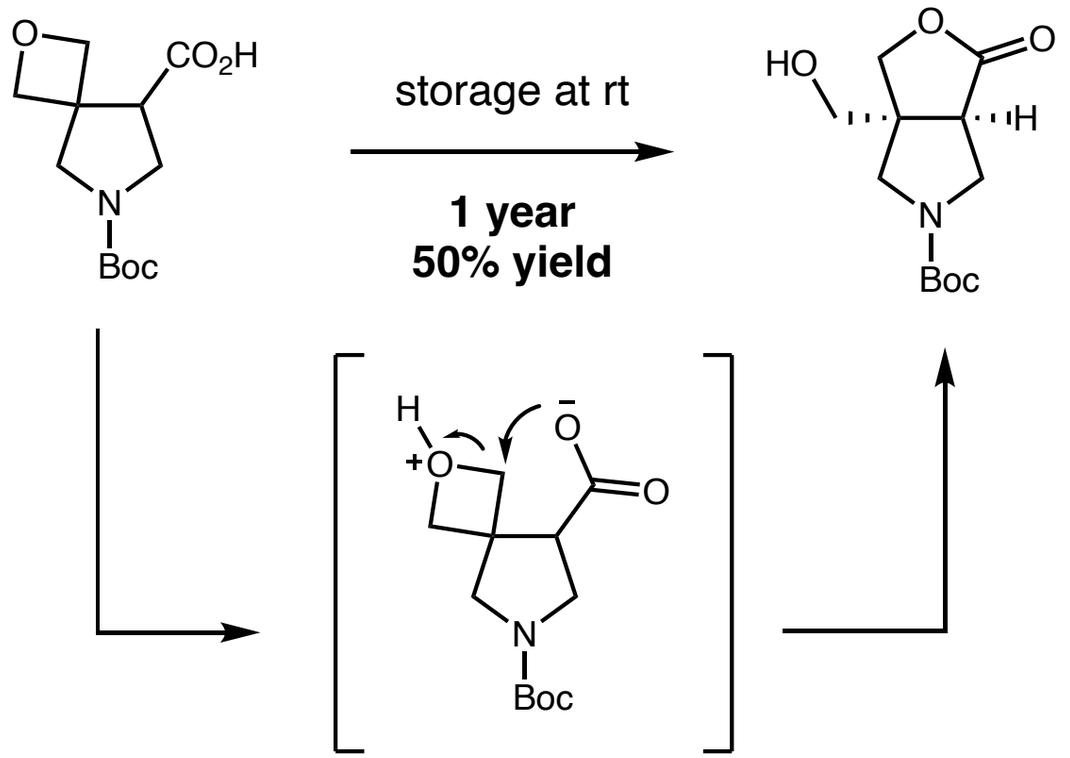
				
solubility (μM)	7	2	**>10**	**>10**
logD (lipophilicity)	2.6	2.9	**2.1**	**2.2**
Cl_{int} (μL/min/mg) (metabolic stability)	decomp.	**120**	decomp.	**50**

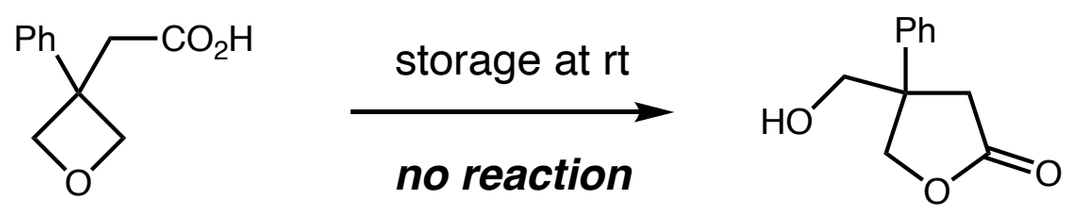
Spirocyclic core imparts desirable physiochemical properties

Chalyk, B. A. et al. *Eur. J. Org. Chem.* **2017**, 4530.

An unexpected reaction

Ring opening/expansion



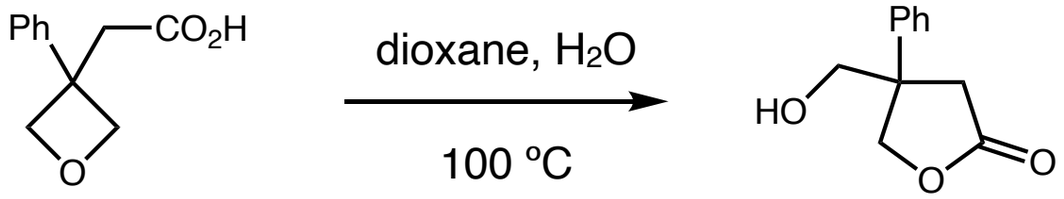


Bulky substituents stabilize oxetane-carboxylic acid

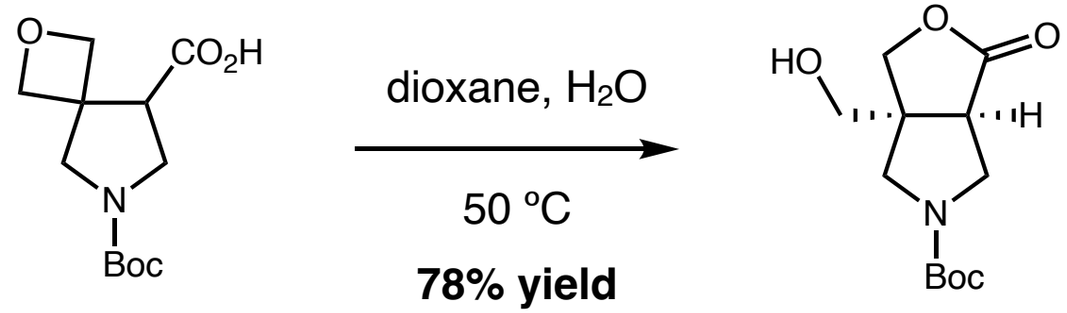
Chalyk, B. et al. *Org. Lett.* **2022**, *24*, 4722.

Reaction development

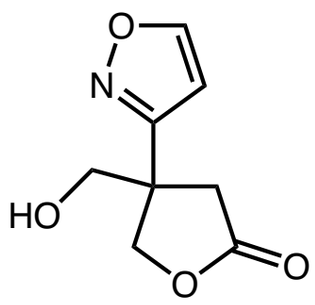
Stable acids



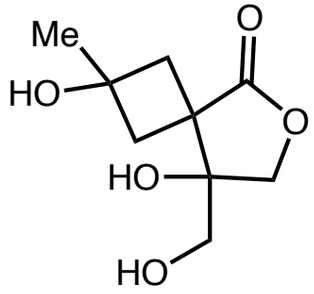
Unstable acids



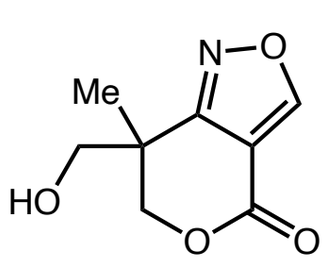
Selected scope



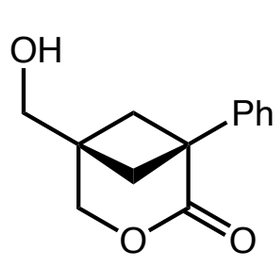
95% yield



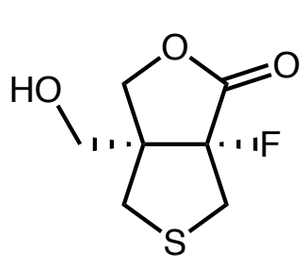
74% yield



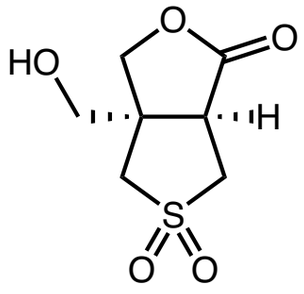
93% yield



91% yield



94% yield



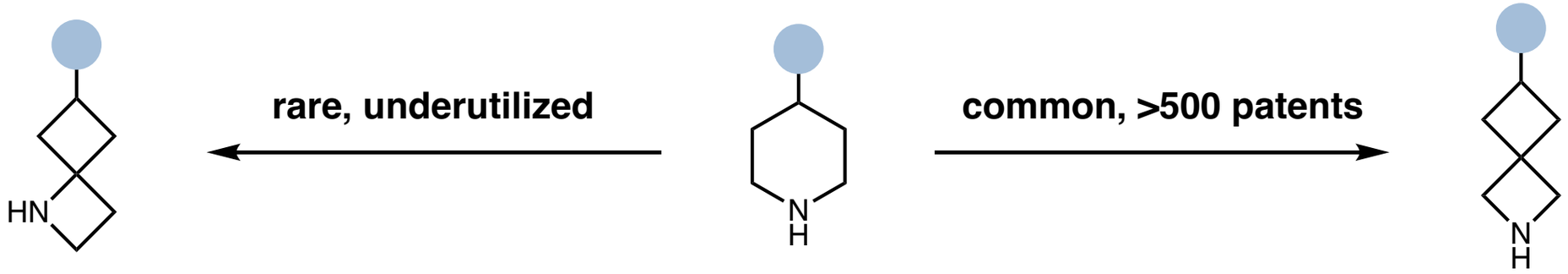
82% yield

Structurally diverse lactones produced efficiently

Chalyk, B. et al. *Org. Lett.* **2022**, *24*, 4722.

Azaspiro[3.3]heptanes as piperidine bioisosteres

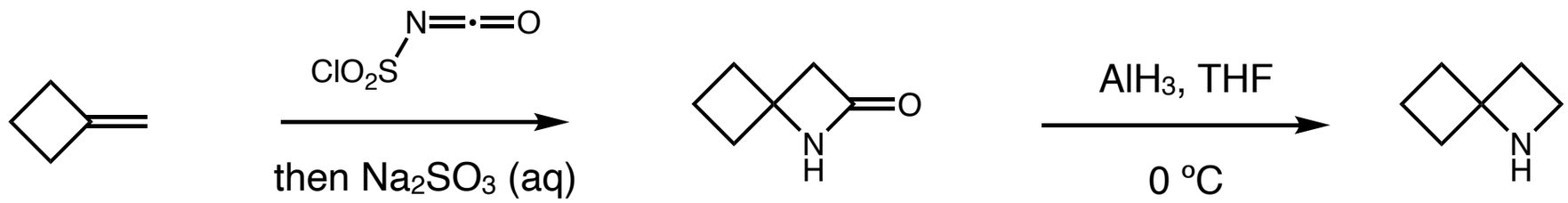
Piperidine: 3rd most abundant ring in drug molecules



How can these potential bioisosteres be readily synthesized?

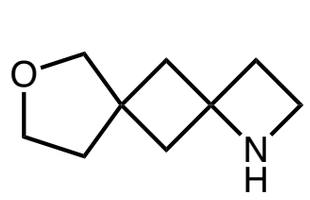
Shearer, J. et al. *J. Med. Chem.* **2022**, *65*, 8699.; Kirichok, A. A. et al. *ChemRxiv* **2023**, doi: 10.26434/chemrxiv-2023-rpjld

Synthesis of 1-Azaspiro[3.3]heptanes

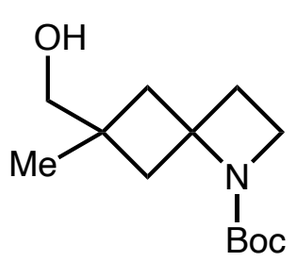


used crude

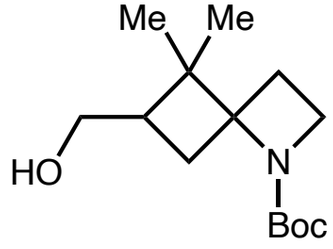
Selected scope



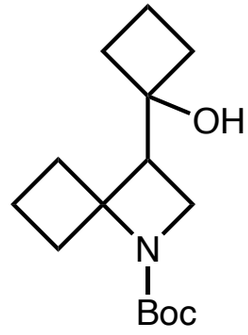
29% yield



57% yield



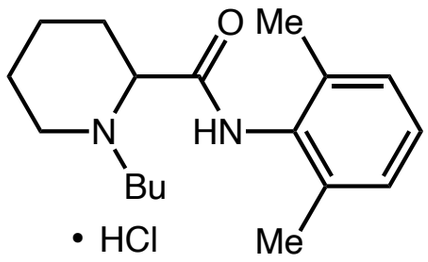
62% yield



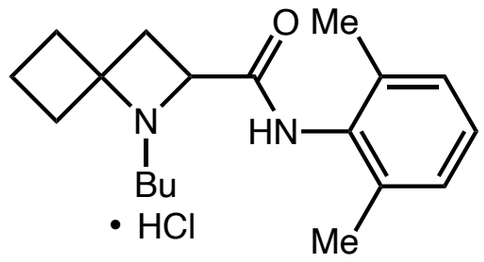
54% yield
via LDA alkylation

Kirichok, A. A. et al. *ChemRxiv* **2023**, doi: 10.26434/chemrxiv-2023-rpjld

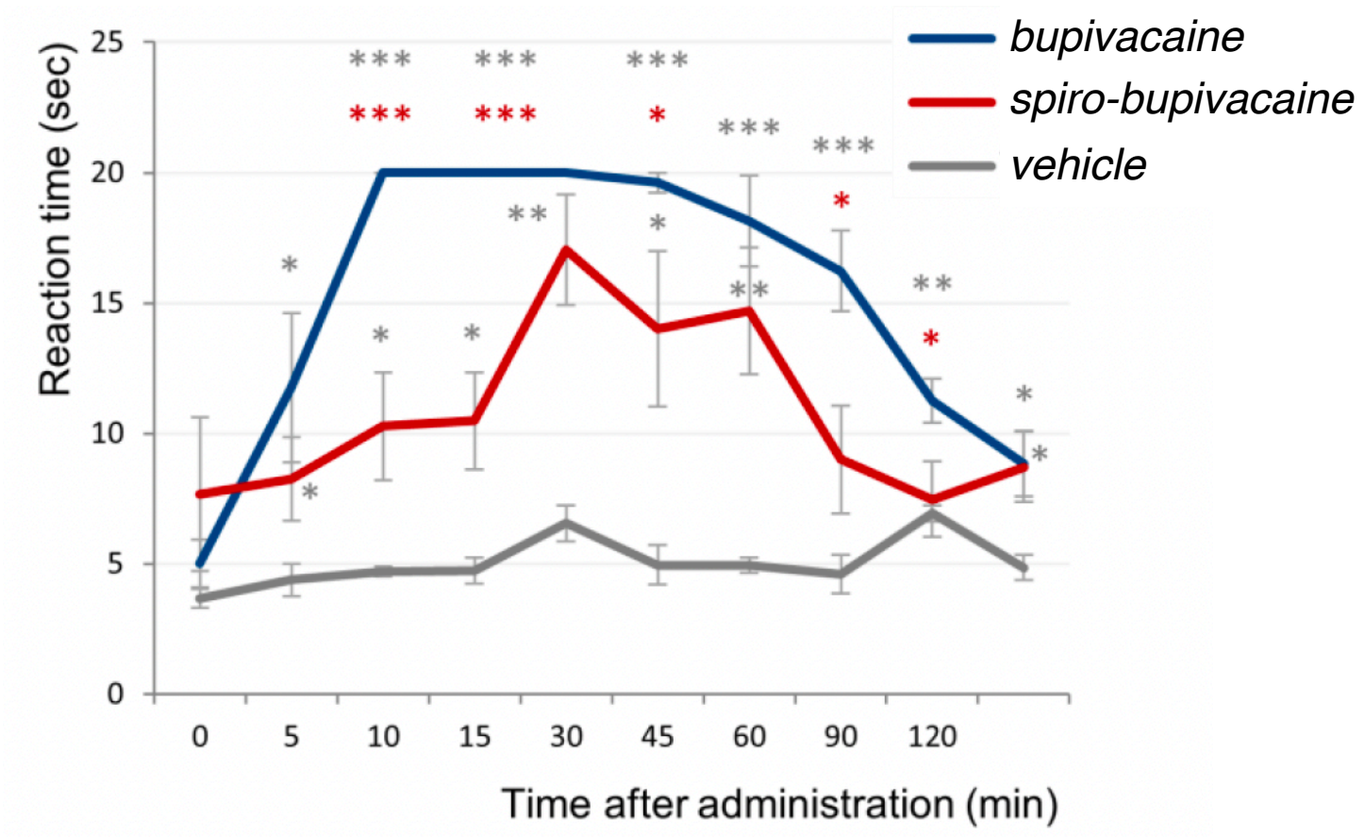
Isosteric replacement of piperidine with 1-Azaspiro[3.3]heptane



bupivacaine (local anesthetic)



spiro-bupivacaine

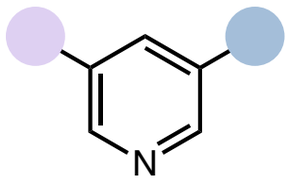


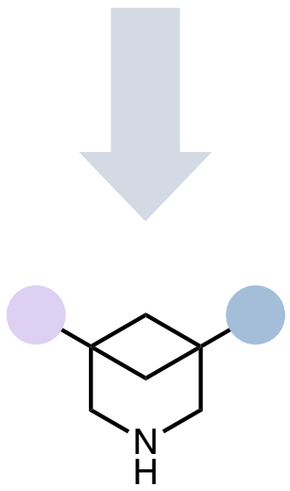
Spiro-bupivacaine shows significant analgesic activity

Kirichok, A. A. et al. *ChemRxiv* **2023**, doi: 10.26434/chemrxiv-2023-rpjld

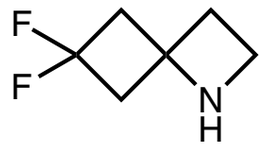
Outline

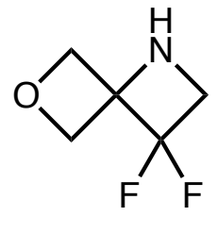
Aromatic bioisosteres

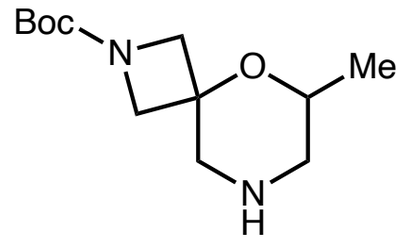




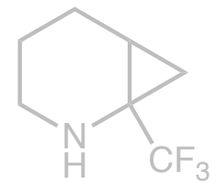
Spirocycles

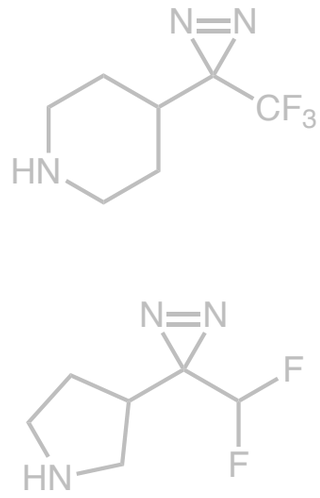






Small rings

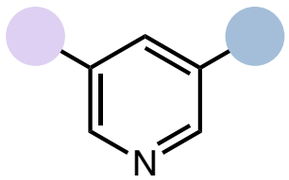


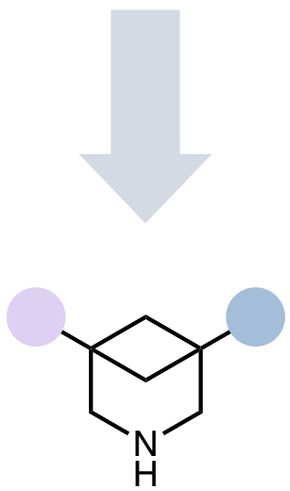


Enamine

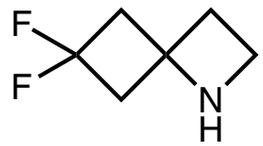
Outline

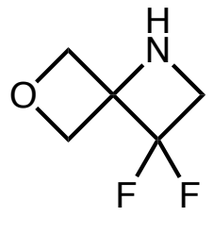
Aromatic bioisosteres

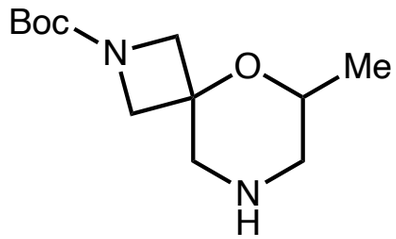




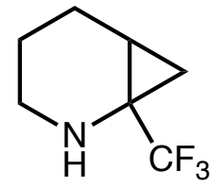
Spirocycles

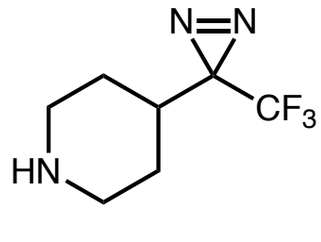


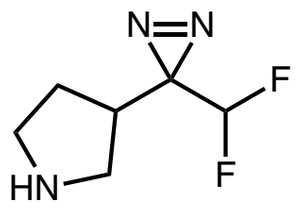




Small rings



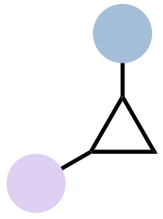




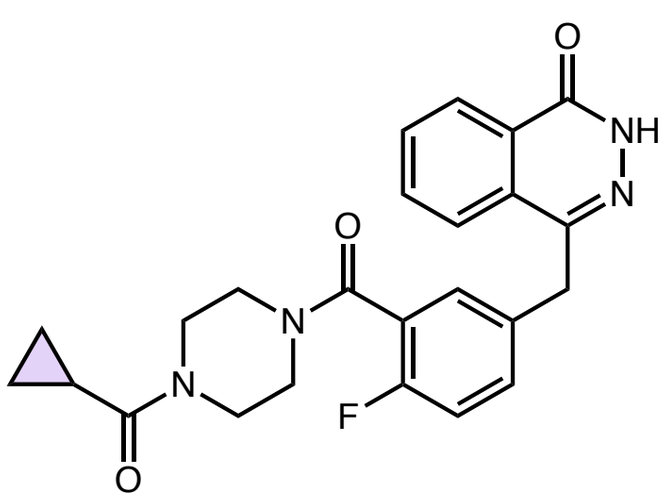
Enamine

Cyclopropanes in medicinal chemistry

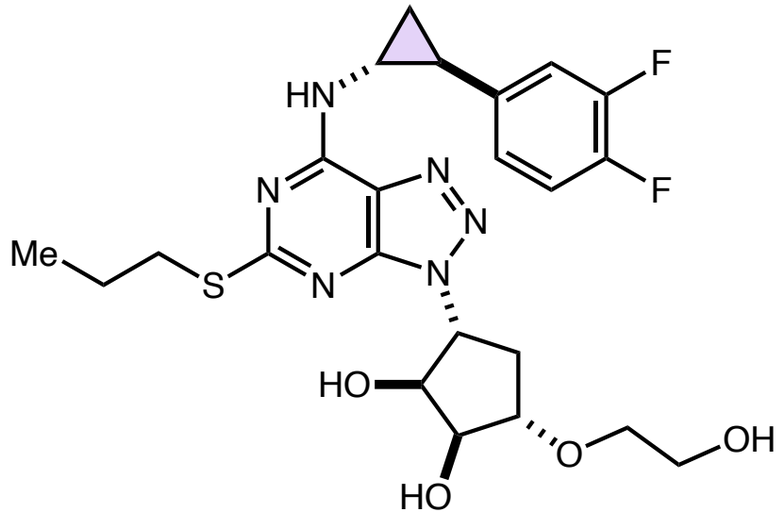
Cyclopropanes



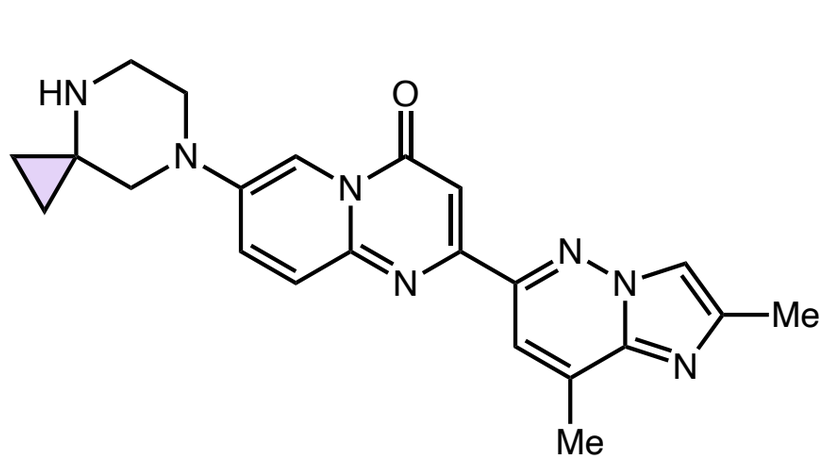
- *6th-most abundant ring in drugs*
- *Metabolic stability*
- *Entropically favorable binding*



olaparib (oncology)



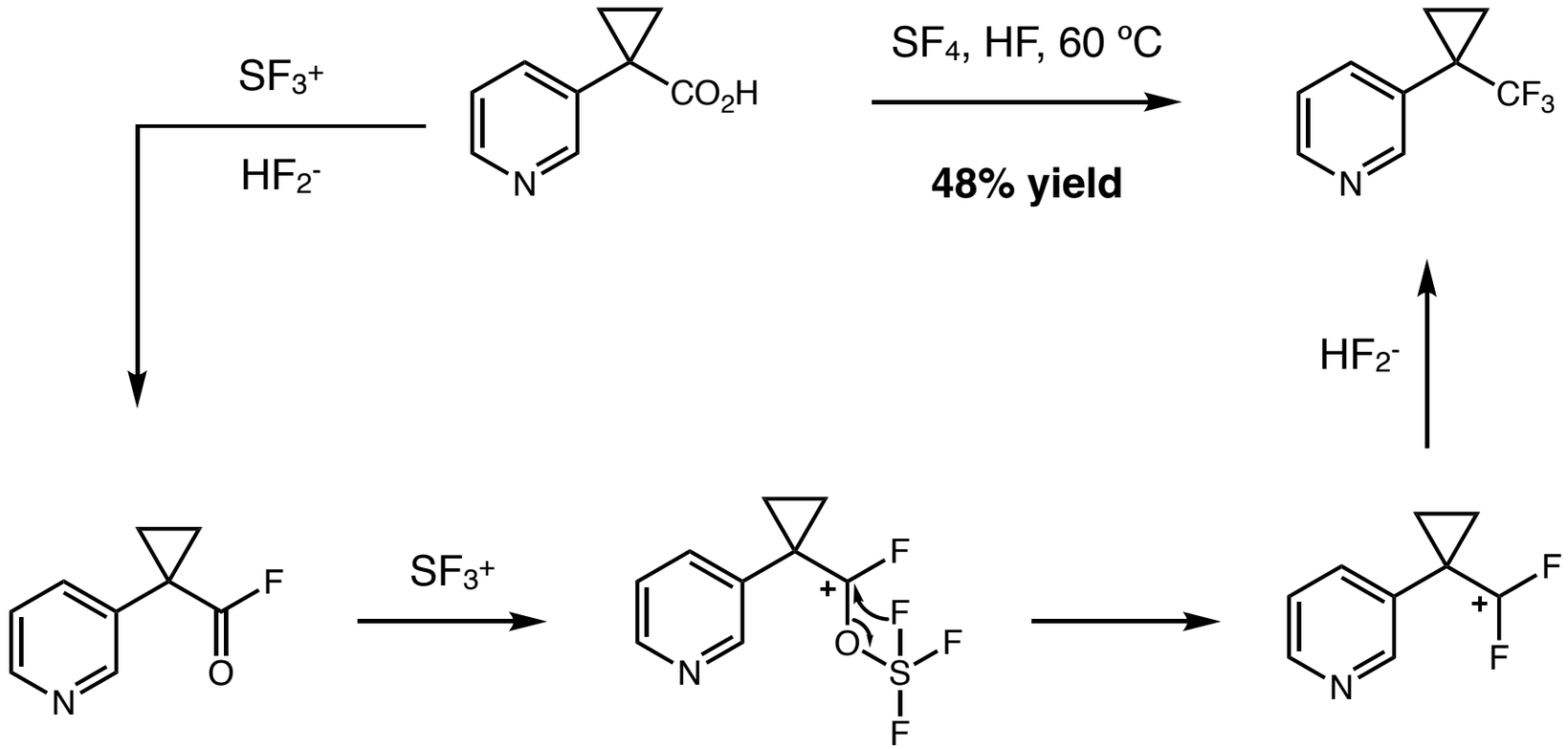
ticagrelor (cardiovascular)



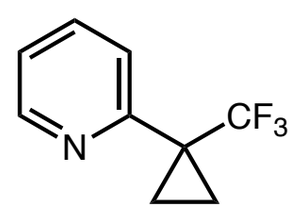
risdiplam (neurology)

Shearer, J. et al. *J. Med. Chem.* **2022**, *65*, 8699.; Talele, T. T. *J. Med. Chem.* **2016**, *59*, 8712.

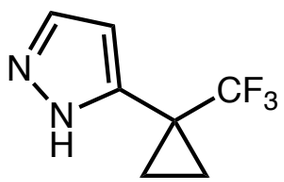
Synthesis of trifluoromethylated cyclopropane building blocks



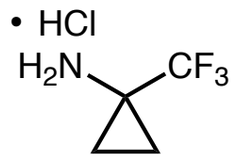
Selected scope



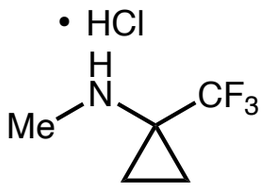
95% yield



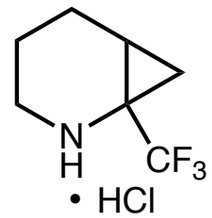
74% yield



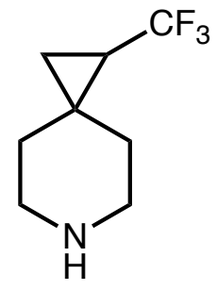
93% yield



91% yield



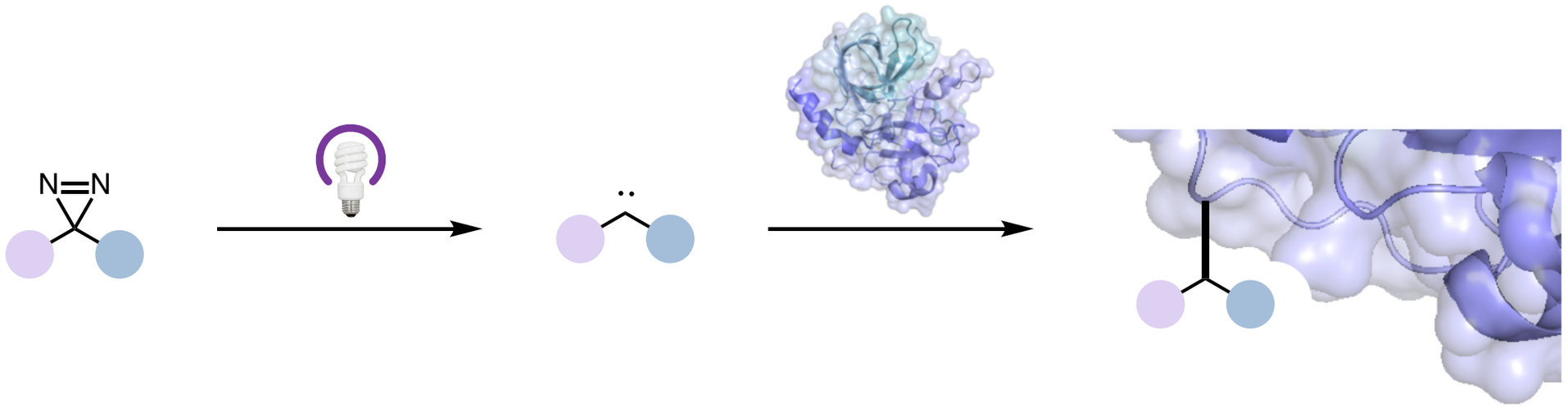
94% yield



0% yield

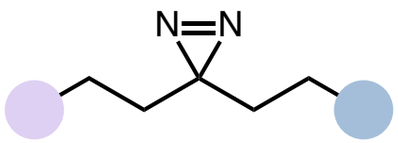
Ahunovych, V. et al. *J. Org. Chem.* **2023**, *88*, 3859.

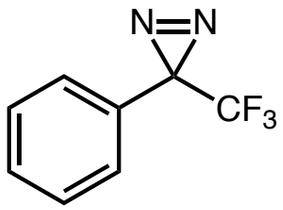
Diazirines in chemical biology



X–H insertion products

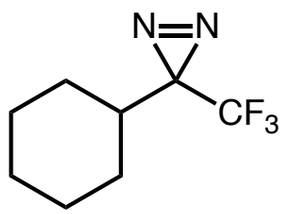
Well-established diazirines

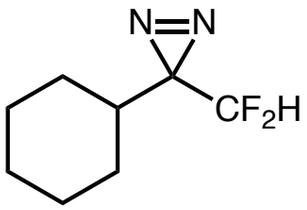




>3,000 of each known in literature

Unprecedented diazirines

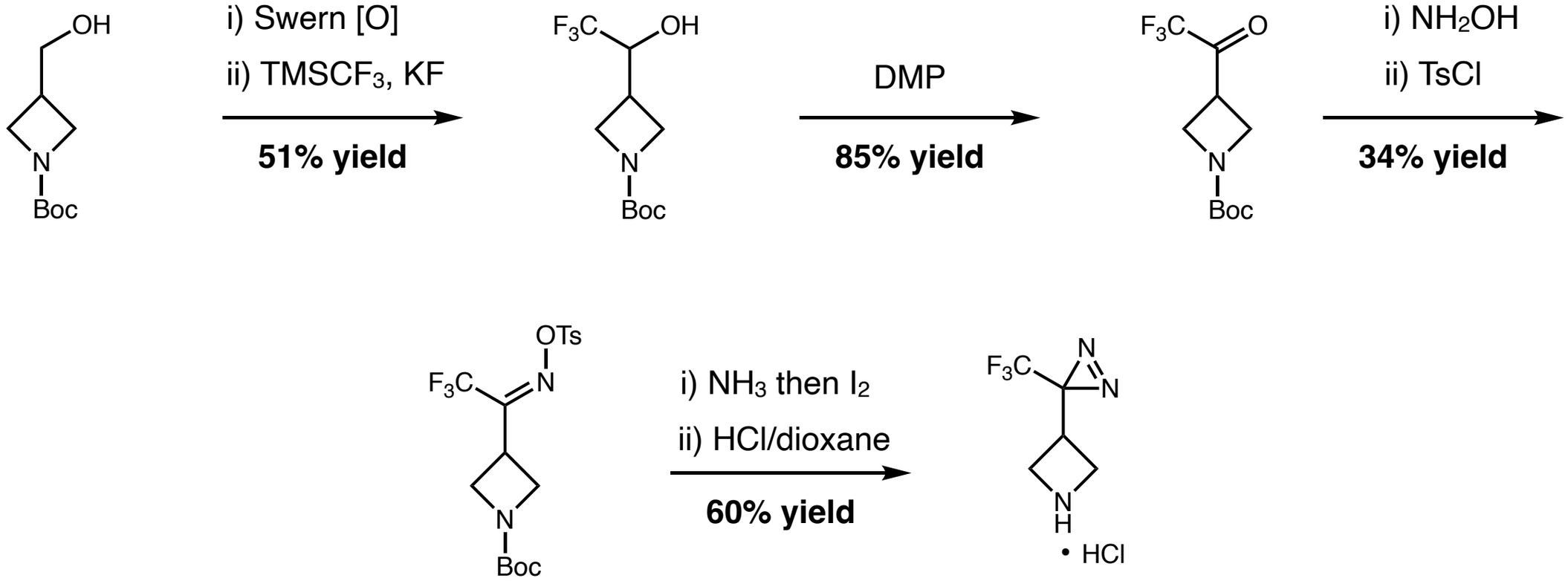




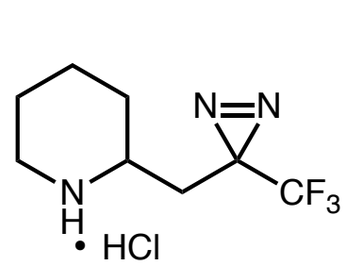
Are these aliphatic fluorinated diazirines useful in chemical biology?

Kornii, Y. et al. *J. Org. Chem.* **2023**, *88*, 1.

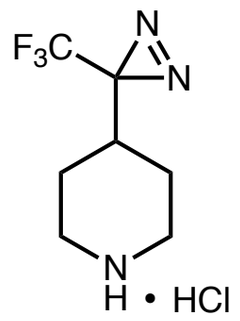
Synthetic route to aliphatic CF_3-substituted diazirines



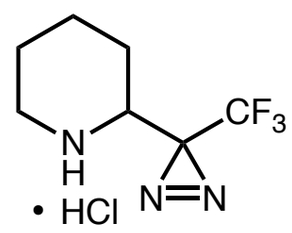
Selected scope



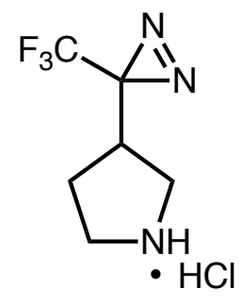
26% yield (13 g)



28% yield (12 g)



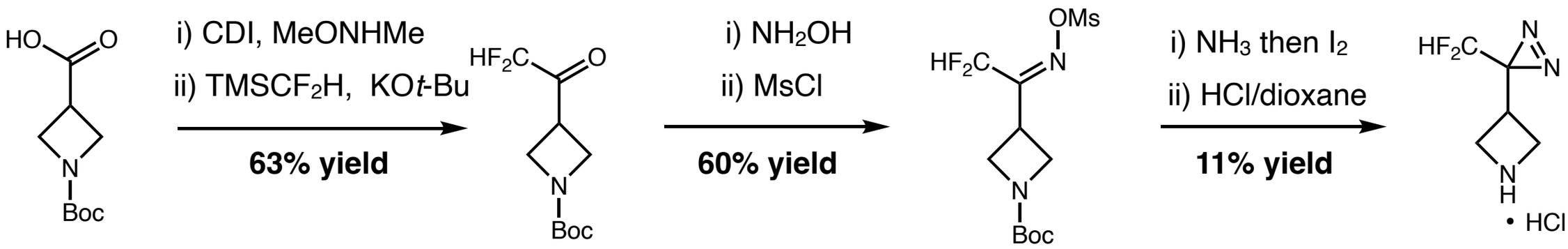
20% yield (10 g)



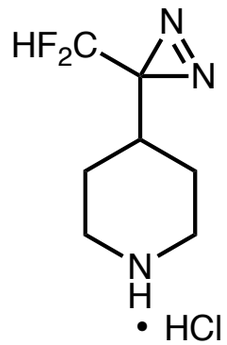
15% yield (9 g)

Kornii, Y. et al. *J. Org. Chem.* **2023**, *88*, 1.

Synthetic route to aliphatic CF_2H-substituted diazirines



Scope

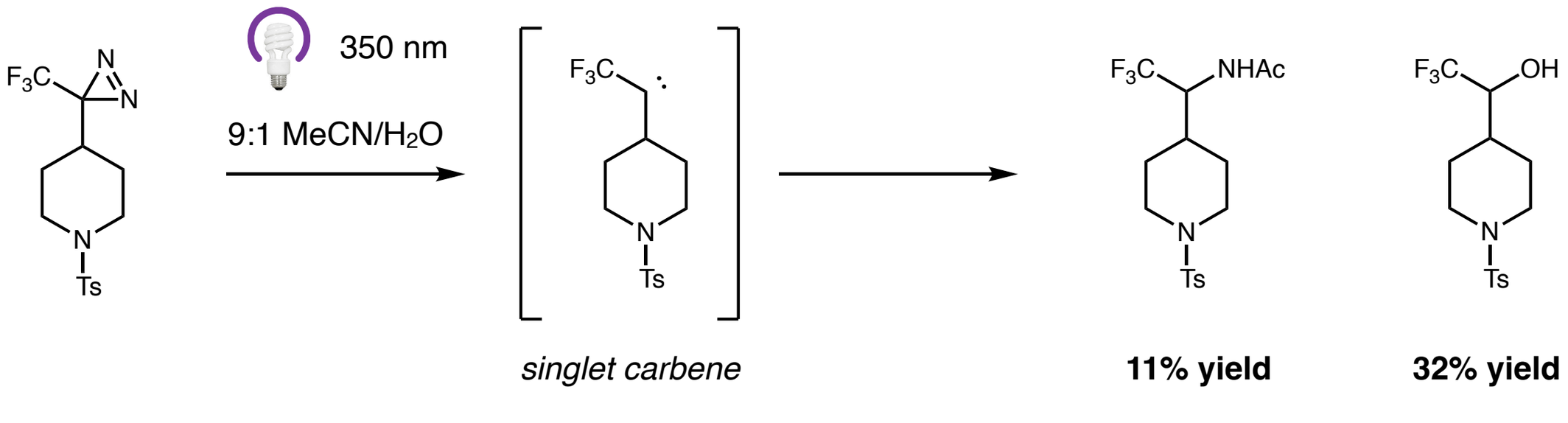


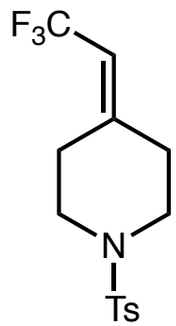
24% yield (20 g)

How do these novel diazirines perform in biological settings?

Kornii, Y. et al. *J. Org. Chem.* **2023**, *88*, 1.

Evaluation of aliphatic CF_3-substituted diazirines





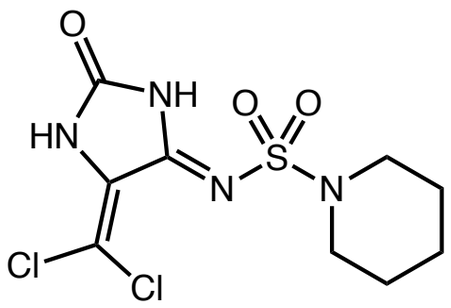
undesired β-hydride elimination

57% yield

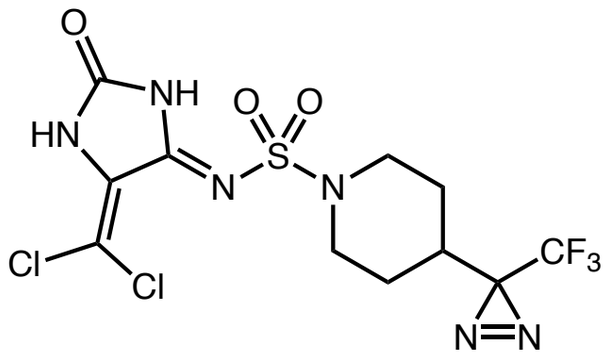
Aliphatic CF_3-substituted diazirines are suitable for labelling experiments

Kornii, Y. et al. *J. Org. Chem.* **2023**, *88*, 1.

Evaluation of aliphatic CF_3-substituted diazirines on drug molecules



anticancer agent



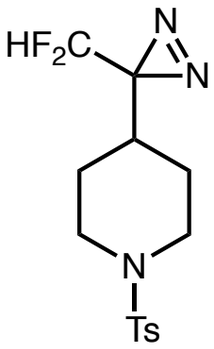
N_2-anticancer agent

Cell line		
K-562 (leukemia)	**–52% growth**	**+8% growth**
NCI-H522 (lung cancer)	**–82% growth**	**–58% growth**
HCT-15 (colon cancer)	**–79% growth**	**–61% growth**
SW-620 (colon cancer)	**–62% growth**	**–36% growth**
OVCAR-3 (ovarian cancer)	**–57% growth**	**+40% growth**

Aliphatic CF_3-substituted diazirines can be compatible with drug molecules

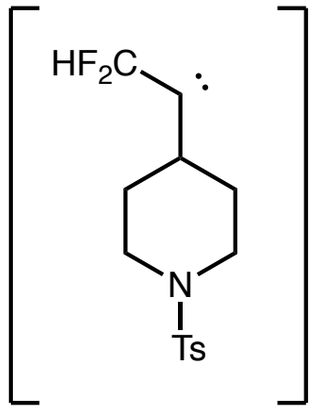
Kornii, Y. et al. *J. Org. Chem.* **2023**, *88*, 1.

Evaluation of aliphatic CF_2H-substituted diazirines

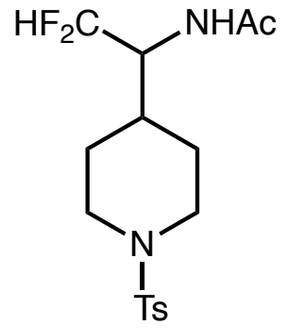


350 nm

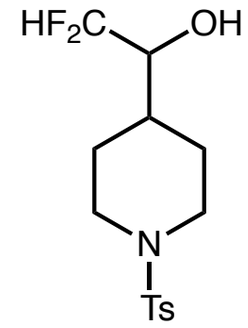
9:1 MeCN/H_2O



singlet carbene

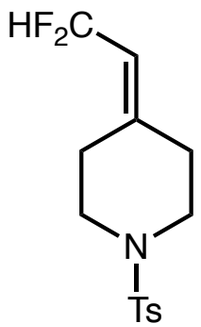


0% yield

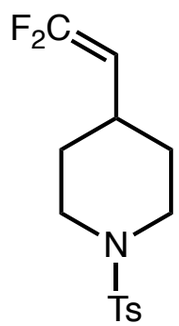


0% yield

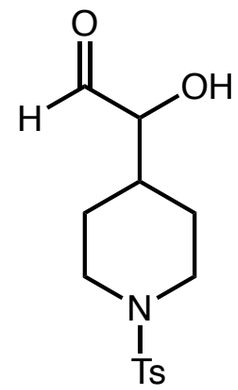
desired X-H insertion products



29% yield



10% yield



17% yield

undesired elimination products

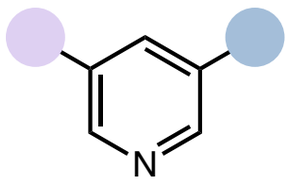
C–F bond hydrolysis

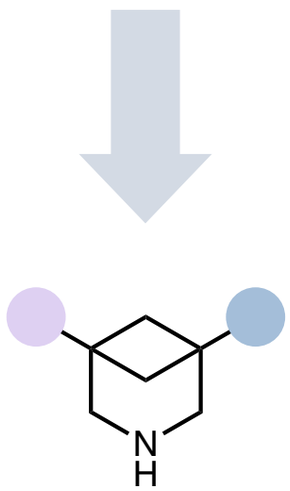
Aliphatic CF_2H-substituted diazirines are not suitable for labelling experiments

Kornii, Y. et al. *J. Org. Chem.* **2023**, *88*, 1.

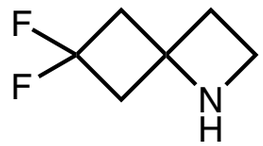
Questions?

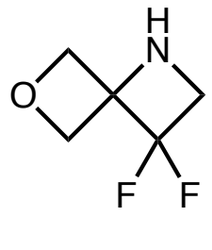
Aromatic bioisosteres

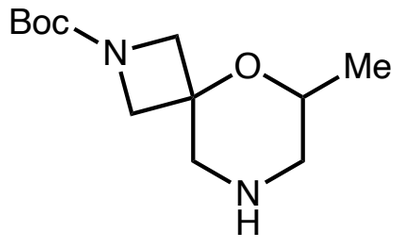




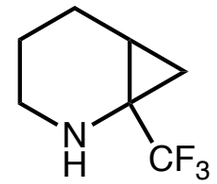
Spirocycles

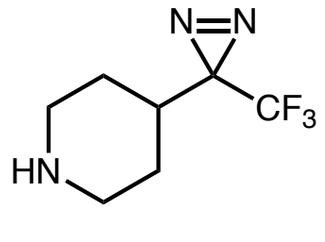


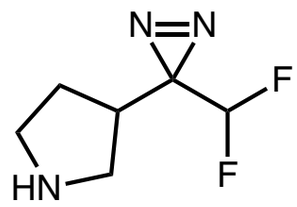




Small rings







Enamine