Spirocyclic Pyrrolidines in Drug Discovery

Key Points

- Combine the characteristics of both pyrrolidines and spirocycles
- Offer enhanced aqueous solubility and other physiochemical properties
- Offer tighter interactions with the target protein thus more potent and selective with fewer off-target effects

Overview

Spirocyclic pyrrolidines combine the characteristics of both pyrrolidines and spirocycles. The pyrrolidine motif on a drug may also offer enhanced aqueous solubility and other physiochemical properties. Meanwhile, spirocyclic pyrrolidines, like most spirocycles, are bestowed with the three advantages over their flat counterparts: (a) inherent three-dimensional geometry offers tighter interactions with the target protein thus more potent and selective with fewer off-target effects; (b) potential superior physiochemical properties; and (c) possible novel intellectual properties.

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Spirocyclic pyrrolidines combine characteristics of both pyrrolidines and spirocycles. For pyrrolidine itself, its N atom may serve as a hydrogen bond acceptor to interact with the drug target protein. Meanwhile, it may offer enhanced aqueous solubility and other physiochemical properties.

Spirocyclization has been taken advantage of to provide rigidification of floppy molecules. Spirocyclic pyrrolidines may be considered a bioisostere of pyrrolidines, but with a three-dimensional (3-D) geometry. Like most 3-D structures, spirocyclic pyrrolidines have three common benefits in comparison to their flat counterparts. (a) Spirocyclic scaffolds project functionalities in all three dimensions to interact more extensively with the drug target proteins of interest, leading to higher potency and fewer off-target effects; (b) They may be associated with more favorable physiochemical properties such as higher aqueous solubility; and (c) Novel intellectual properties may be obtained.

Spirocyclic Pyrrolidine-containing Drugs

Three spirocyclic pyrrolidine-containing drugs are currently on the market. One of the early ones is Sandoz's angiotensin-converting enzyme (ACE) inhibitor spirapril (Renormax, **1**), approved by the FDA in 1995 for the treatment of hypertension.[1]

Daiichi Sankyo's sitafloxacin (Gracevit, **2**) contains a cyclopropane-fused spiropyrrolidine fragment. It is a fluoroquinolone antibiotic only sold in Japan.[2]

Gilead has been a leader in the hepatitis C virus (HCV) therapeutics field. Its HCV non-structural protein 5A (NS 5A) inhibitor ledipasvir (**3**) is combined with sofosbuvir) and was approved for marketing in 2014 with brand name Harvoni.[3]
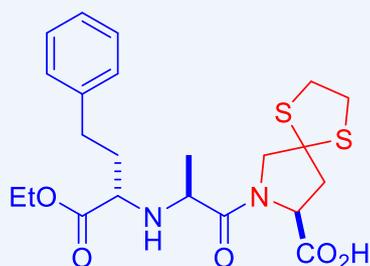
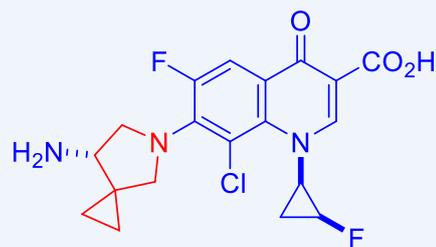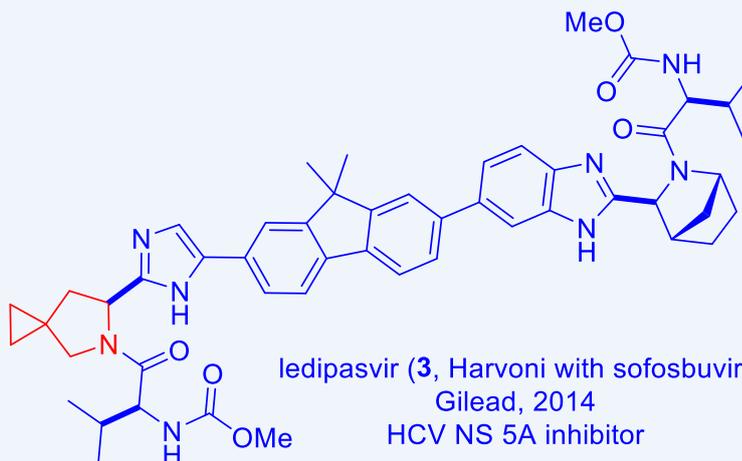## PharmaBlock Products

PBN2011625

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spirapril (Renormax, **1**)
Sandoz, 1995
ACE inhibitor

sitafloxacin (Gracevit, **2**)
Daiichi Sankyo, 2008 in Japan
fluoroquinolone antibiotic

ledipasvir (**3**, Harvoni with sofosbuvir)
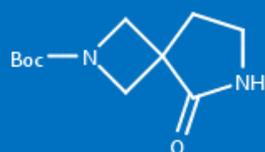Gilead, 2014
HCV NS 5A inhibitor

Spirocyclic Pyrrolidines in Drug Discovery

Some spirocyclic pyrrolidines have been employed as three dimensionally extended pyrrolidines. Merck's ACE inhibitor lisinopril (Zestril, **4**, FDA approval, 1987) was significantly more potent than Squibb's first-in-class ACE inhibitor captopril (Capten, FDA approval, 1980). Lisinopril (**4**) occupies the S_1 hydrophobic pocket on the ACE enzyme not taken advantage of by the captopril. Sandoz scientists took one step further to explore additional hydrophobic binding pockets. They achieved success with spirapril (**1**) by installing a spirocyclic proline where the dithioketal extended deeper to the S_2' pocket. Apparently it offered more extensive binding to the enzyme because spirapril (**1**)'s *in vitro* inhibition was 80-fold more potent than lisinopril (**4**). This has also translated to *in vivo* efficacy where spirapril (**1**) was tested 3.5-fold more efficacious than lisinopril (**4**) in rat and anesthetized dog models.[2]
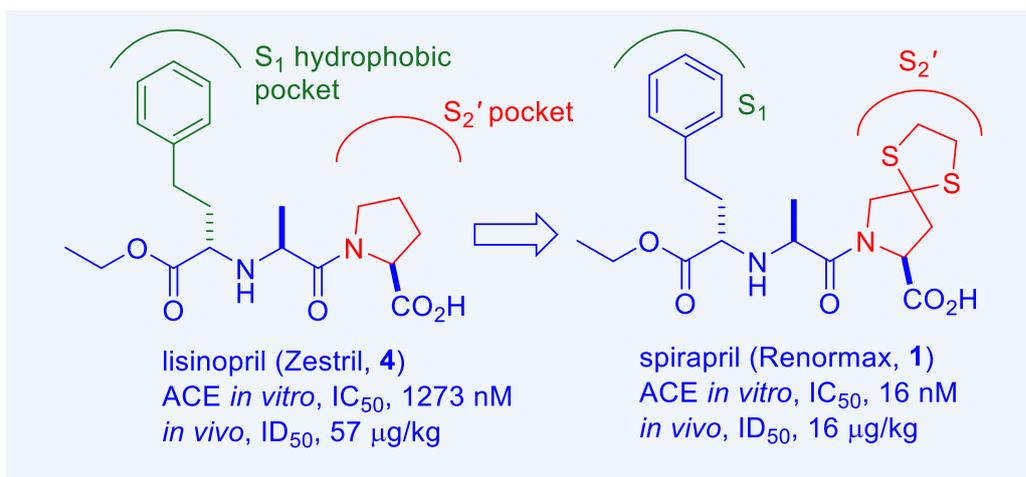
lisinopril (Zestril, **4**)
ACE *in vitro*, IC_{50}, 1273 nM
in vivo, ID_{50}, 57 µg/kg

spirapril (Renormax, **1**)
ACE *in vitro*, IC_{50}, 16 nM
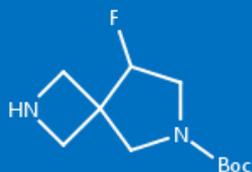*in vivo*, ID_{50}, 16 µg/kg

Drug discovery is extremely challenging for the first-in-class drugs. It is relatively easier when it comes to "me-too" drugs, especially if you know what you are doing.

The genesis of quinolone antibiotics traces back to Sterling–Winthrop's nalidixic acid (Nevigramon). Bayer's ciprofloxacin (Cipro, **5**), a second-generation quinolone antibiotic, is likely the "best-in-class" drug, possibly because its cyclopropyl motif helps boosting its bioavailability and subsequently efficacy. Third-generation quinolone antibiotic tosufloxacin (Ozex, **6**) contains a 3-amino-pyrrolidine moiety. Sandoz's sitafloxacin (**2**) incorporates features of both ciprofloxacin (**5**)'s cyclopropane and tosufloxacin (**6**)'s 3-amino-pyrrolidine. If one cyclopropyl group is good, two is probably even better. Sandoz decided to add a spirocyclic cyclopropyl fragment to the 3-amino-pyrrolidine group, and arrived at tosufloxacin (**6**). Like all fluoroquinolones, sitafloxacin (**2**)'s mechanism of action (MoA) is through inhibiting bacterial DNA gyrase and topoisomerase IV.[2]

Two HCV NS3/4A serine protease inhibitors are on the market. One is Schering–Plough/Merck's boceprevir (Victrelis, 2011), and the other is Vertex's telaprevir (Incivek, 2011). Building on the success of boceprevir, Schering–Plough/Merck scientists pursued superior backup HCV NS3/4A inhibitors. Linear molecule **7** is a reasonably good HCV NS3/4A inhibitor. However, it can adopt many conformations as an open chain molecule. Restraining the molecule would significantly rigidify the molecule and limit the number of conformations binding to the enzyme.[4]
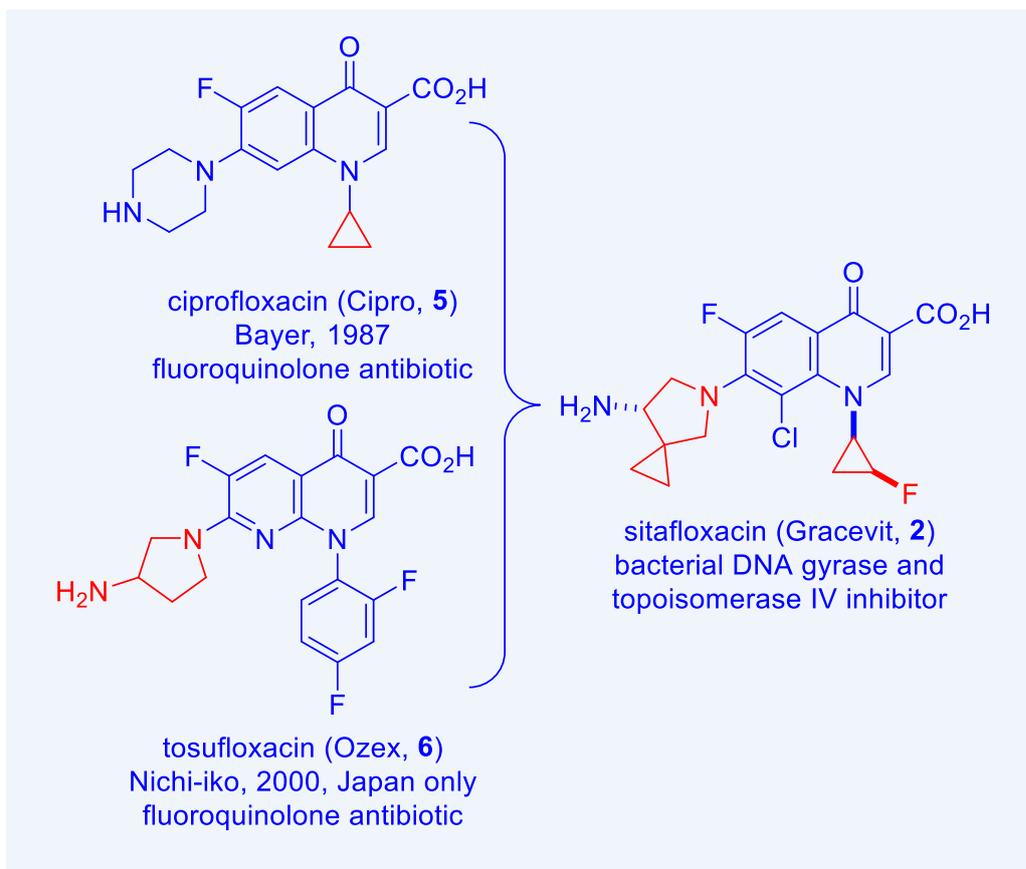
ciprofloxacin (Cipro, **5**)
Bayer, 1987
fluoroquinolone antibiotic

tosufloxacin (Ozex, **6**)
Nichi-iko, 2000, Japan only
fluoroquinolone antibiotic

sitafloxacin (Gracevit, **2**)
bacterial DNA gyrase and
topoisomerase IV inhibitor

Through molecular modeling around the P2 region, it was observed that a spirocyclization of quinolone moiety of **7** onto the proline could make favorable van der Waals contact with histidine (H57), an invariant catalytic residue, which may result in an improved mutant profile. *Spirocyclization would also impart greater conformational rigidity to the molecule and may provide an advantage with reduced entropic cost of binding by biasing it toward the bioactive conformation*. Indeed, the resulting spirocyclic proline **8** was tested significantly more potent in both enzymatic assay and replicon efficacy assay. More important, minor optimization led to the discovery of MK-8831 (**44**, *vide infra*), a novel spiro-proline macrocycle as a pan-HCV NS3/4A protease inhibitor with good pharmacokinetic profile and excellent safety. MK-8831 (**44**) was carried to phase I clinical trials in 2015.[4] This tactic has been employed to discover additional P2–P4 macrocycles containing this unique spirocyclic proline core structure.[5]
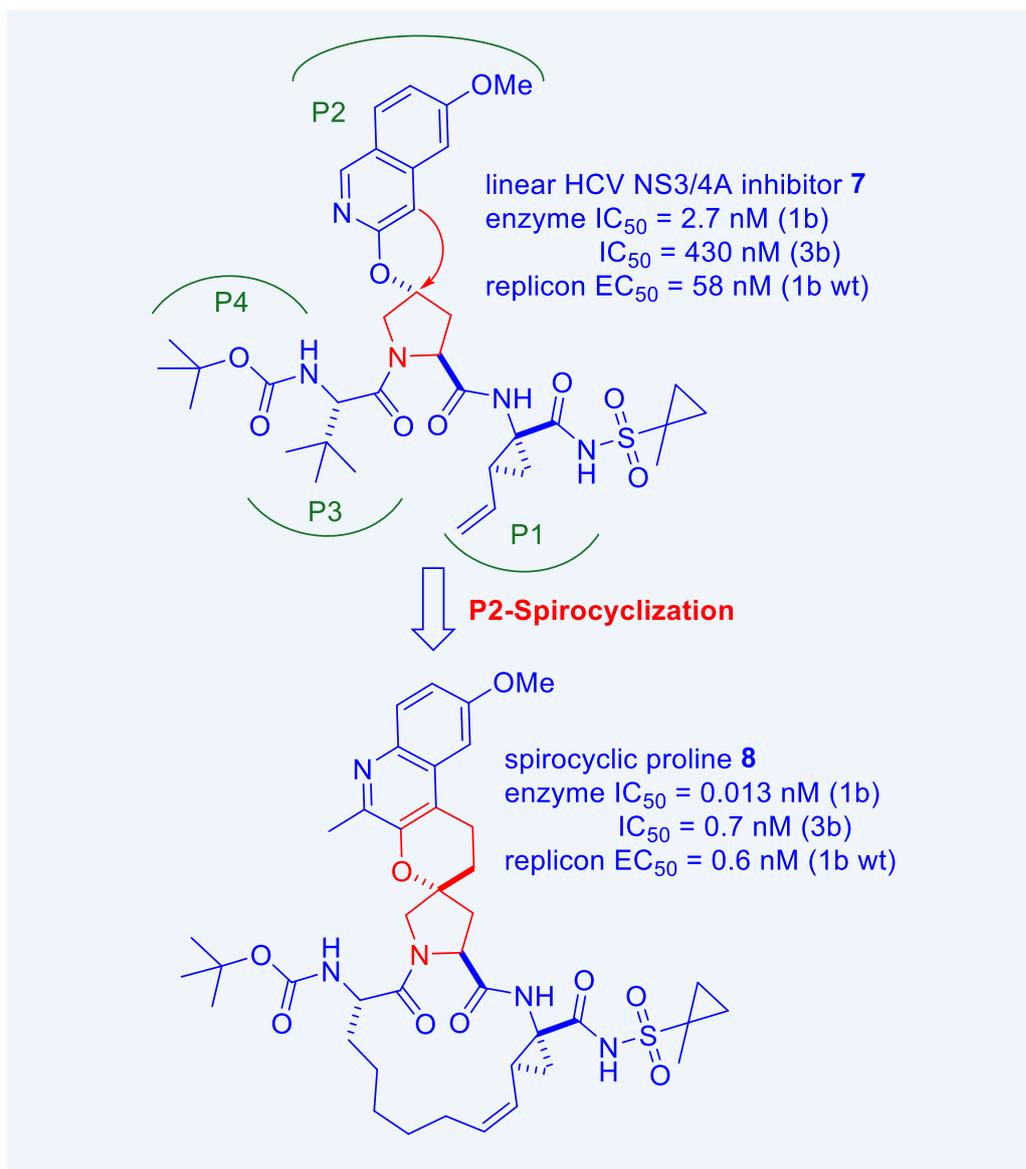
PharmaBlock Products

PBZ2863

PBU4564

linear HCV NS3/4A inhibitor **7**
enzyme IC$_{50}$ = 2.7 nM (1b)
IC$_{50}$ = 430 nM (3b)
replicon EC$_{50}$ = 58 nM (1b wt)

P2-Spirocyclization

spirocyclic proline **8**
enzyme IC$_{50}$ = 0.013 nM (1b)
IC$_{50}$ = 0.7 nM (3b)
replicon EC$_{50}$ = 0.6 nM (1b wt)

A group of my former colleagues at Pfizer in Ann Arbor discovered a series of novel and selective spiroindole-based inhibitors of Sky, a tyrosine kinase receptor. For example spiroindole **9** binds in the ATP-binding site and exhibits high level of kinome selectivity through filling the Ala571-subpocket. In addition, the nitrogen on the pyrrolidine sandwiched between indoline and the top pyrrolidine also forms two hydrogen bonds with Asp663 on the target Sky protein. In all, the spirocyclic pyrrolidine motif provides at least two advantages here: (a) The 3-D geometry offers selectivity for the target Sky protein; (b) The nitrogen atom on pyrrolidine serves as a hydrogen bond acceptor. Spiroindole **9** exhibits moderate bioavailability in the rat due to low absorption across gut wall.[6]
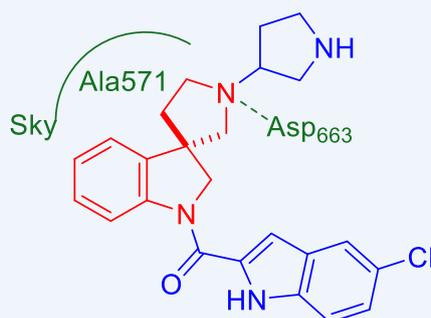
spiroindole **9**
Sky IC_{50} = 495 nM
solubility = 5.3 μM
$t_{1/2}$ = 3.1 h
Cl = 60 mL/min/kg
V_{dss} = 13 L/kg
F% = 14%
HLM $t_{1/2}$ = 1 h

Another Pfizer project also involved spirocyclic pyrrolidines: it was the β-secretase (BACE1) inhibitors project employing the fragment-based drug design (FBDD) technique. An X-ray-based fragment screen of Pfizer's proprietary fragment library of 340 compounds identified spiropyrrolidine **10** as the only BACE binder. The nitrogen atom on pyrrolidine forms two hydrogen bonds with Asp32 and Asp228, respectively, on the BACE enzyme. Despite exhibiting only weak inhibitory activity against the BACE enzyme, spiropyrrolidine hit **10** was verified by biophysical and NMR-based methods as a genuine BACE inhibitor.[7]

Identification of a viable vector led to extension at the 5' position of the pyrrolidine fragment. Guided by structure-based drug design (SBDD), computational prediction of physiochemical properties, installation of a series of esters gave rise to the optimized spiropyrrolidine inhibitor **11**, which was an approximately 1000-fold improvement in potency over fragment **10**. It also has a high ligand efficiency (LE) and properties predictive of good permeability and low P-gp liability.[7]


spiropyrrolidine hit **10**
BACE1, K_D = 1.4 mM

spiropyrrolidine inhibitor **11**
BACE1, IC_{50} = 4 μM
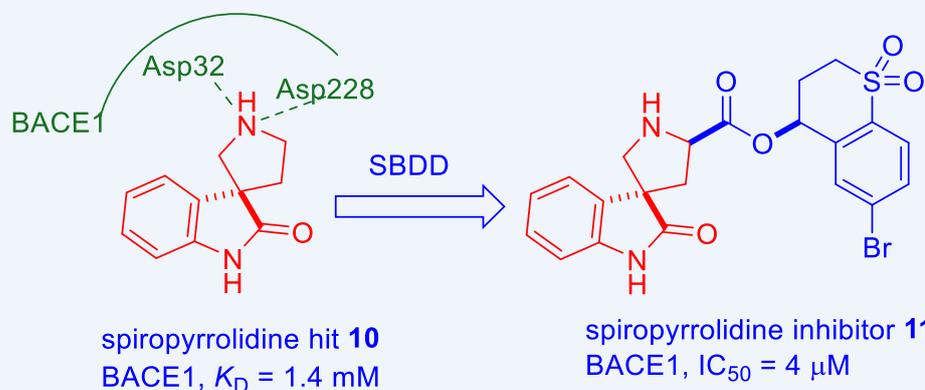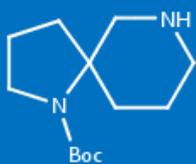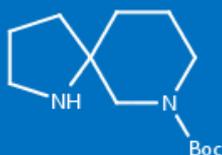
Blockbuster antiplatelet drug clopidogrel (Plavix) works as a $P2Y_{12}$ antagonist. Similarly, $P2Y_1$ antagonists have potential as blood thinners as well. BMS scientists used a high throughput screen (HTS) hit, a urea-containing compound, as their starting point and carried out an extensive drug discovery campaign. The fruit of their labor was spirocyclic pyrrolidine **12** as a potent $P2Y_1$ antagonist with good *in vitro* binding and functional activities. Moreover, spirocyclic pyrrolidine **12** demonstrated a favorable PK profile and was the first $P2Y_1$ antagonist that inhibited arterial and venous thrombosis at doses that produced a limited prolongation of bleeding time via oral as well as IV dosing.[8]
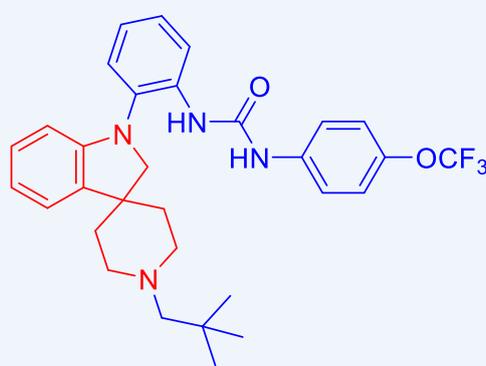

$P2Y_1$ antagonist **12**
K_i = 4.3 nM
IC_{50} = 4.9 μM
HLM, 89%
CI = 5.3 mL/min/kg
V_{dss} = 3.1 L/kg
$t_{1/2}$ = 9.7 h
F% = 55%

Proteolysis targeting chimera (PROTAC) functions as a protein degrader. It uses hetero-bifunctional small molecules to remove specific proteins from cells to achieve targeted protein degradation. Wang and coworkers recently published a spirocyclic pyrrolidine PROTAC MD-224 (**13**). It links a murine double minute 2 (MDM2)–p53 inhibitor on the left and immunomodulatory drug (IMiD) lenalidomide as E3 ligase binders on the right, as a result of careful linker optimization. The CRL4–CRBN E3 ubiquitin ligase on **13** degraded MDM2, but co-treatment of **13** with lenalidomide, a cereblon (CRBN) binder, effectively blocked MDM2 degradation via competitive displacement of cereblon from the ternary complex, confirming the drug was on target. PROTAC **13** was tested as a nanomolar drug in cell and efficacious in RS4; 11 xenograft animal models when given multiple IV-dosing at 25 mg/kg every second day (Q2D).[9]
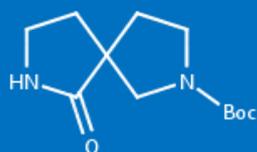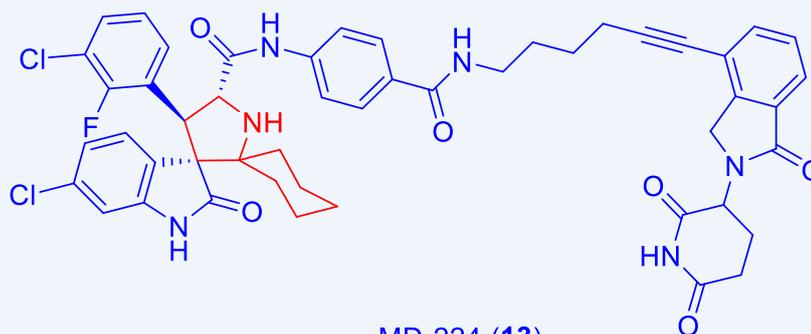
MD-224 (**13**)
RS4;11 cell growth inhibit. IC_{50} = 1.5 nM
RS4;11 xenograft = 50% regression
at 25 mpk, iv Q2D dosing

Synthesis of Some Spirocyclic Pyrrolidine-containing Drugs

For synthesis of Daiichi's sitafloxacin (**2**), the key intermediate is a cyclopropane-fused spirocyclic pyrrolidine **22**. Initially, fragment **22** was prepared via a chiral auxiliary [(R)-1-phenylethan-1-amine]-aided separation. Later on, a more efficient and greener route was developed via asymmetric microbial reduction. As shown beneath, N-benzyl glycine (**14**) was protected as its Boc analog **15**. After treating **15** with carbonyldiimidazole, the intermediate was exposed to the magnesium enolate of hydrogen ethyl malonate to afford the corresponding β-keto-γ-amino ester **16** in 84% yield. Cyclopropanation of **16** with 1,2-dibromoethane and potassium carbonate in acetone gave **17** in 72% yield. Treatment of **17** with TFA was followed by refluxing in toluene produced the key intermediate **18** in 72% yield.[10]

With substrate keto-lactam **18** in hand, optimization of the microbial reduction conditions led to the use of *Phaeocreopsis* sp. JSM 1880, giving rise to chiral alcohol **19**. Subsequently, a Mitsunobu reaction using DPPA afforded the corresponding azide, which was reduced to amine **20**. After protection, the resulting Boc analog **21** was subjected to hydrogenolysis to deliver cyclopropyl-pyrrolidine **22**, which was used to prepare sitafloxacin (**2**).[10]
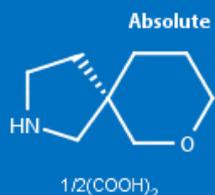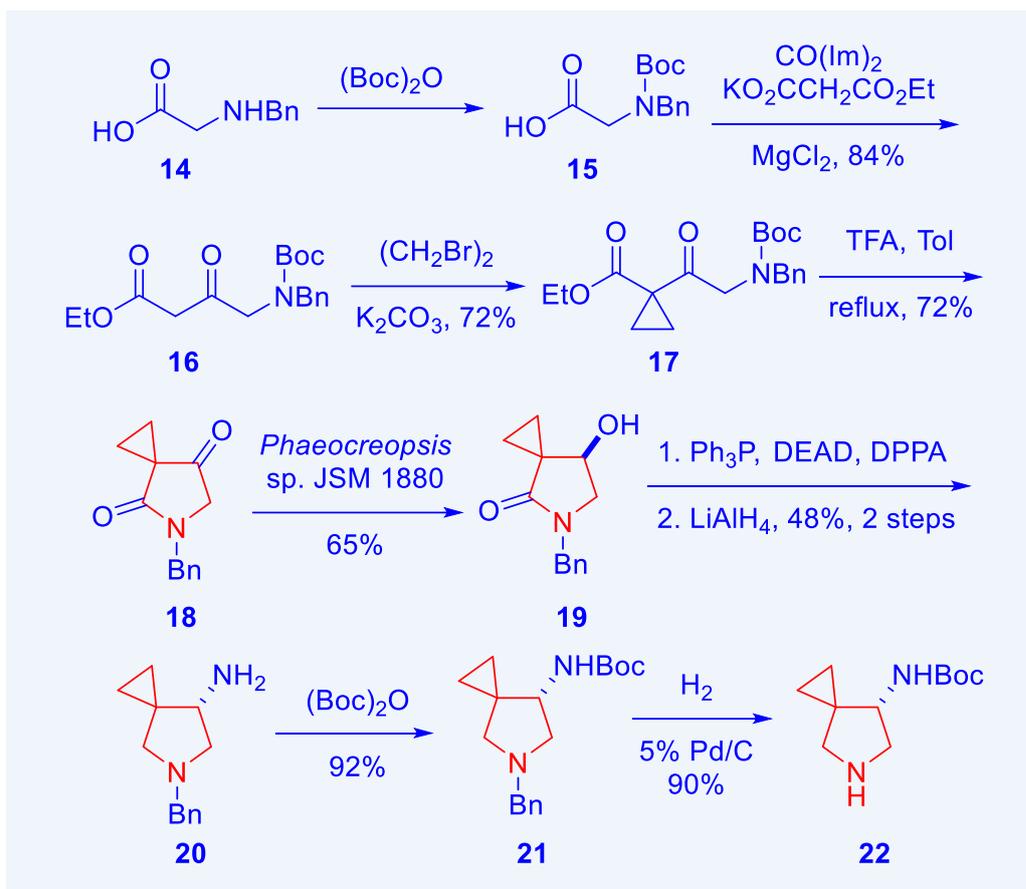
For the synthesis of Gilead's HCV NS 5A inhibitor ledipasvir (**3**), here we only focus on the synthesis of the closely related cyclopropyl-proline portion **28**. Thus, diol **23** was iodinated using the routine combination of iodine, triphenylphosphine, and imidazole to prepare di-iodide **24**. Spirocyclopropyl-proline **26** was assembled as a racemate treating N-Boc-glycine ethyl ester (**25**) with sodium hydride followed by di-iodide **24**. Saponification of ester **26** followed by a classical resolution with (1S,2R)-aminoindanol provided enantiomerically pure salt **27** after recrystallization in 2-methyltetrahydrofuran. After liberation of the free carboxylic acid, treatment with potassium *tert*-butoxide produced enantiopure potassium **28**, ready to be used as a building block to synthesize ledipasvir (**3**).[11]

Synthesis of spiroindole **9**, Pfizer's Sky kinase inhibitor, resorted to an interesting rearrangement reaction. When treated with NCS, tetrahydrocarboline **29** was chlorinated at its 3-position to give chloride **30**. Subsequently, unstable chloride **30** underwent a rearrangement to afford spirocyclic pyrrolidine **31** as a racemate, which was separated by chiral SFC to give enantiomerically pure **32**.[6]
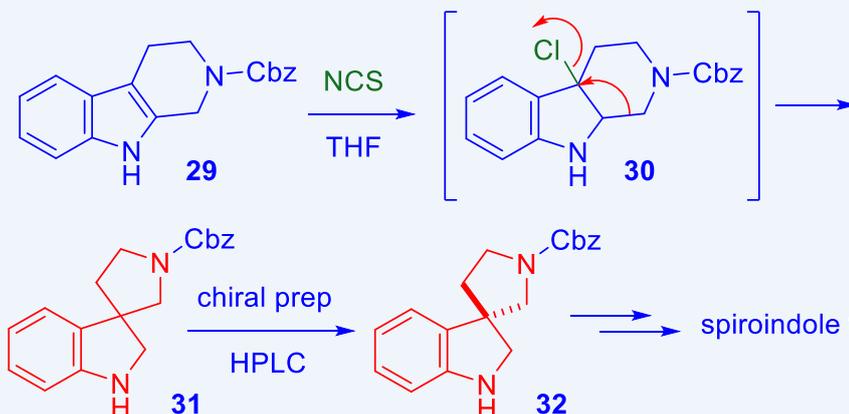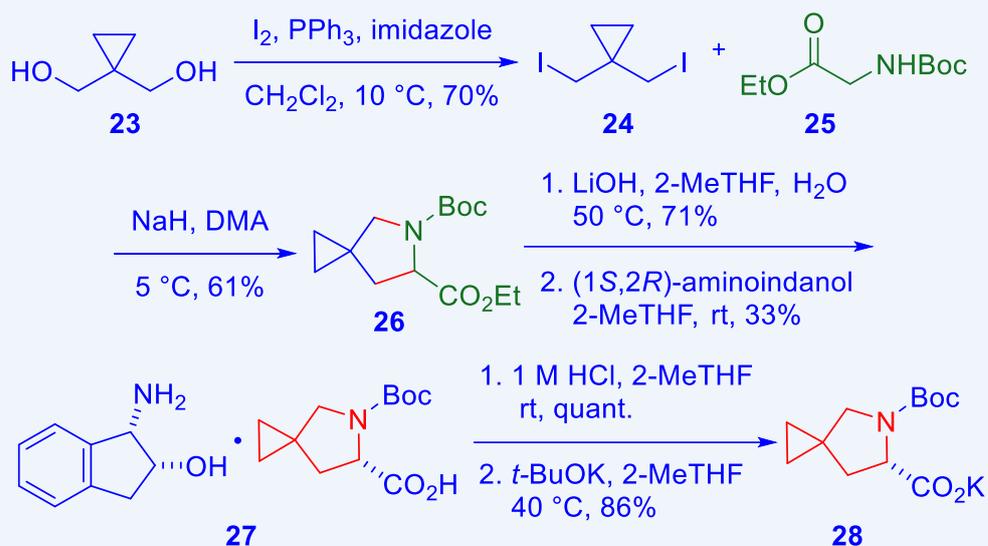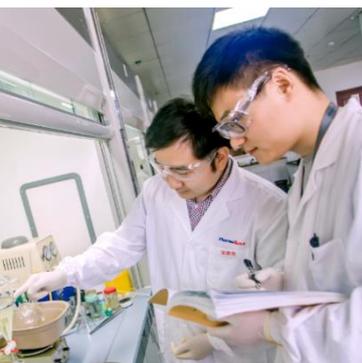
PharmaBlock Products
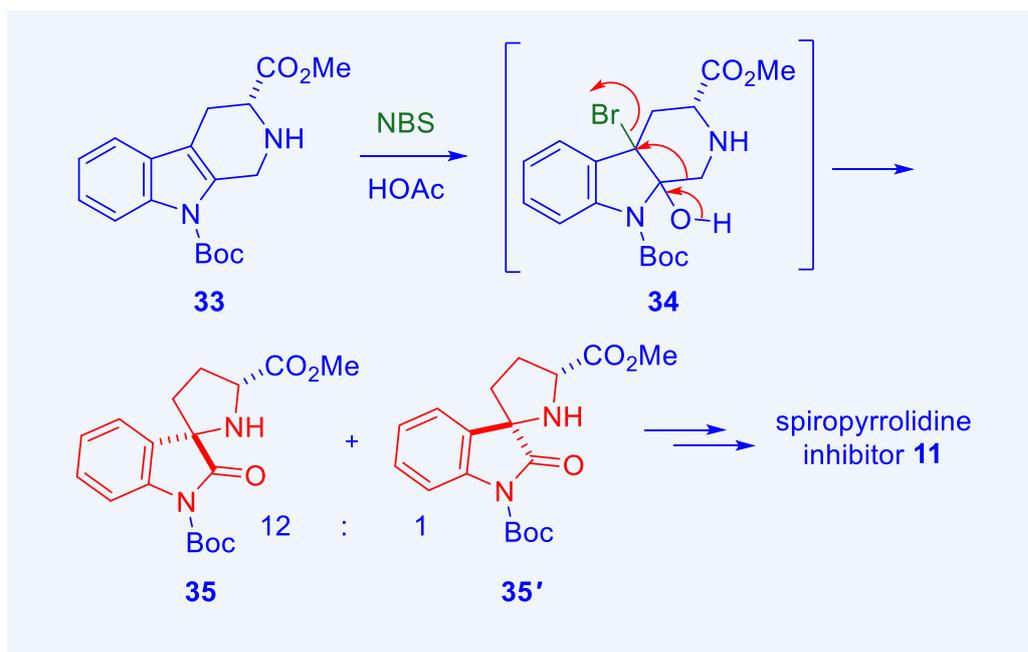

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1/2(COOH)₂
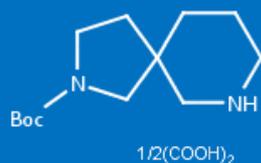
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Oxindoles are privileged structures in drug discovery. Pfizer's BACE1 inhibitor spiropyrrolidine **11** contains a spirocyclic oxindolyl-pyrrolidine moiety. Preparation **11** also resorted the fascinating rearrangement reaction employed to make spirocyclic pyrrolidine **35** for another Pfizer project on Sky kinase inhibitors. Substrate tetrahydrocarboline **33** was treated with NBS in acetic acid. The NBS-induced oxidative rearrangement, via the intermediacy of **34**, furnished the desired spirocyclic pyrrolidine intermediate as a mixture of diastereomers **35** and **35'**. The diastereoselectivity favored the desired diastereomer **35**, which was readily separated via normal flash chromatography and eventually converted to the desired spiropyrrolidine **11** as a potent and bioavailable BACE1 inhibitor.[7]
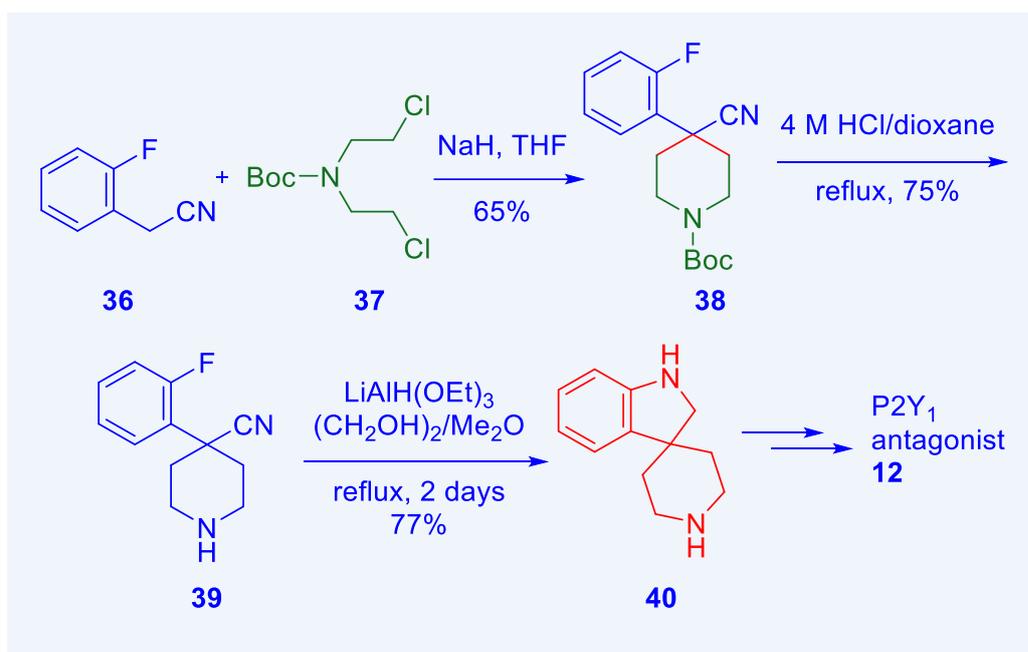
BMS's P2Y$_1$ antagonist **12** contains a spirocyclic piperidinyl indoline core structure **40**. Assembly of the key intermediate **40** began with treating 2-fluorobenzeneacetonitrile (**36**) with bis-chloride **37** to prepare piperidine **38**. After deprotection of the Boc group, the resulting nitrile **39** was reduced by LiAlH(OEt)$_3$, freshly prepared by LiAlH$_4$ and HOEt in ethylene glycol and dimethyl ether, to effect spirocyclization, giving rise to spirocyclic piperidinyl indoline **40**.[8]

MK-8831 (**44**), a novel spiro-proline macrocycle as a pan-HCV NS3/4A protease inhibitor, evolved from spiro-proline **8**. Spirocyclization was carried out by condensing keto-phenol **41** with keto-proline derivative **42** to assemble the spirocyclic pyranone-proline **43**. Several steps of further manipulations then delivered MK-8831 (**44**).[12]
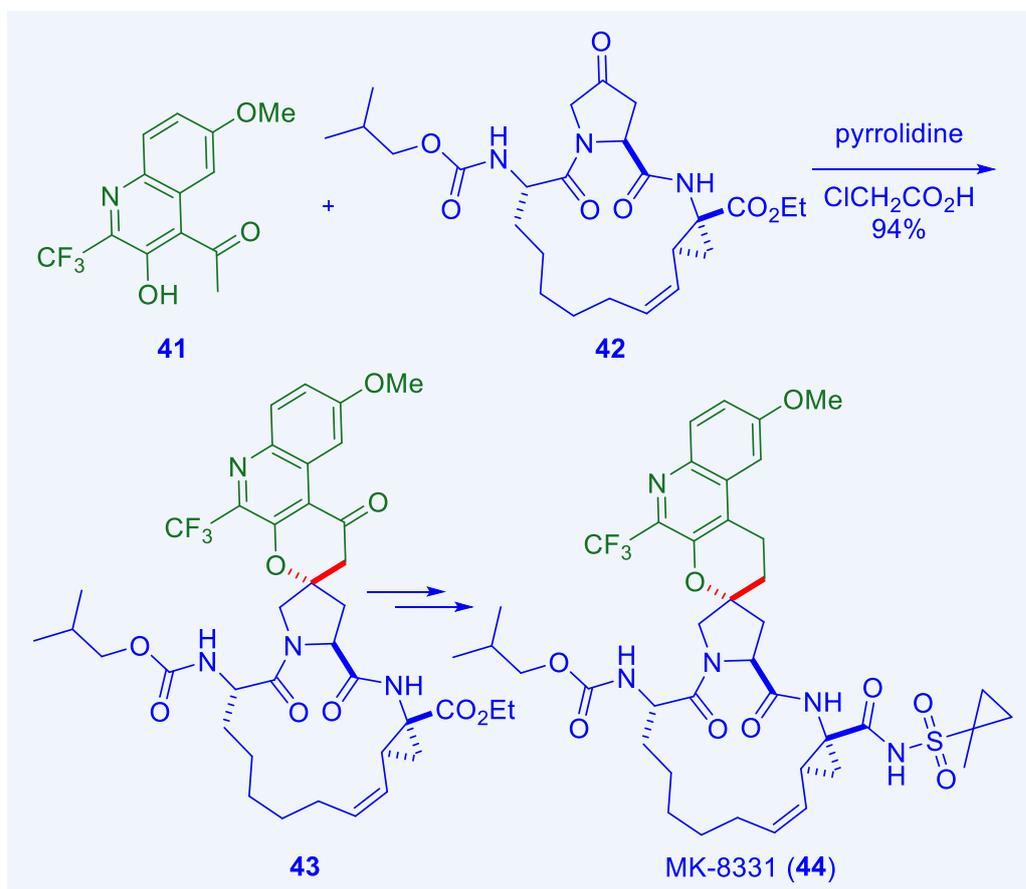
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In summary, spirocyclic pyrrolidines combine the characteristics of both pyrrolidines and spirocycles. The nitrogen atom on the pyrrolidine may serve as a hydrogen bond acceptor, interacting with the target protein. The pyrrolidine motif on a drug may also offer enhanced aqueous solubility and other physiochemical properties. Meanwhile, spirocyclic pyrrolidines, like most spirocycles, are bestowed with the three advantages over their flat counterparts: (a) inherent three-dimensional geometry offers tighter interactions with the target protein thus more potent and selective with fewer off-target effects; (b) potential superior physiochemical properties; and (c) possible novel intellectual properties.

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