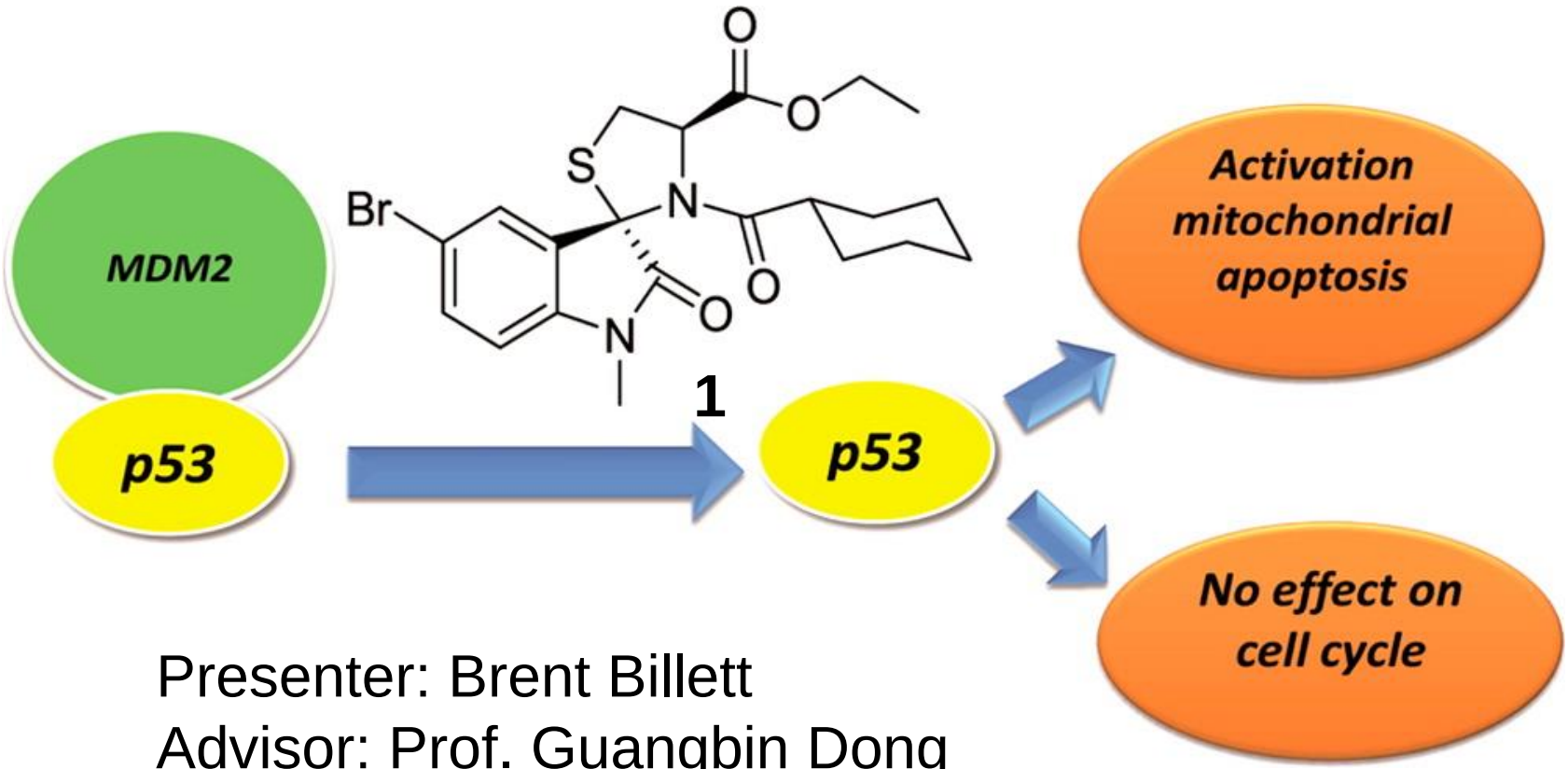
Spirocycles and the Development of a MDM2 antagonist

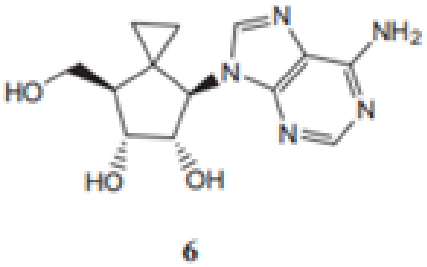


Presenter: Brent Billett
Advisor: Prof. Guangbin Dong
University of Texas at Austin
March 2, 2016

Outline

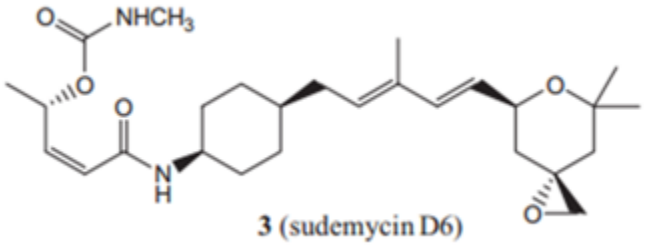
- Introduction
- Examples of spirocycle formation/synthesis
- Examples of thiazolidine formation/synthesis; a reoccurring and potent moiety
- Biological Aside
  - western blot, cell cycle, and P53-MDM2 complex
- Specific thiazolidine drug example

Spirocycle Examples



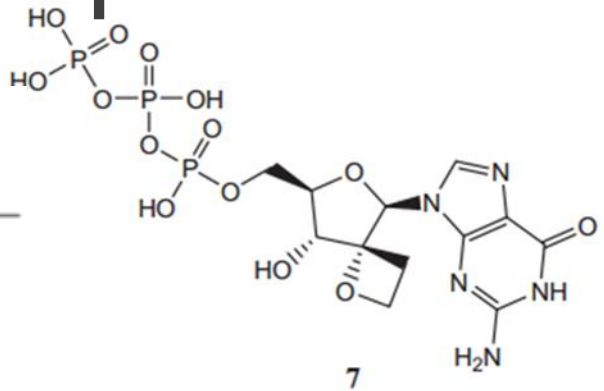
6

cyclopropanes



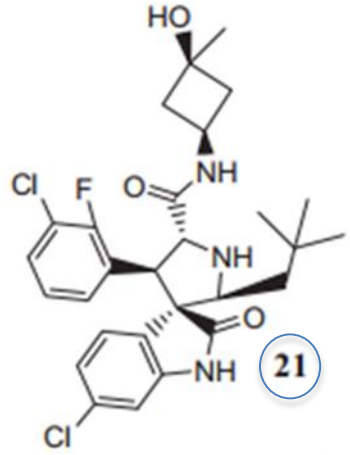
3 (sudemycin D6)

oxiranes

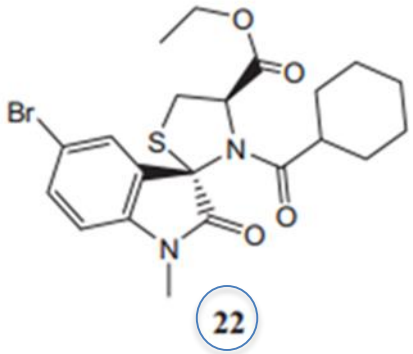


7

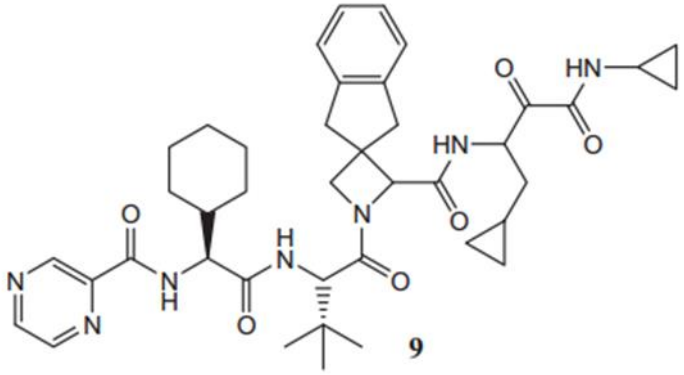
oxetanes



spirooxindol



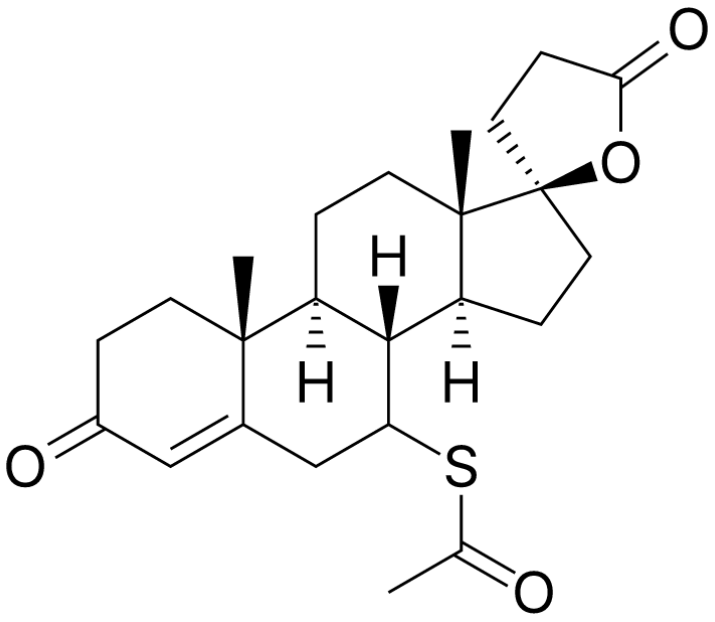
spirooxindol-3,3'-thiazolidine



spiroazetidines

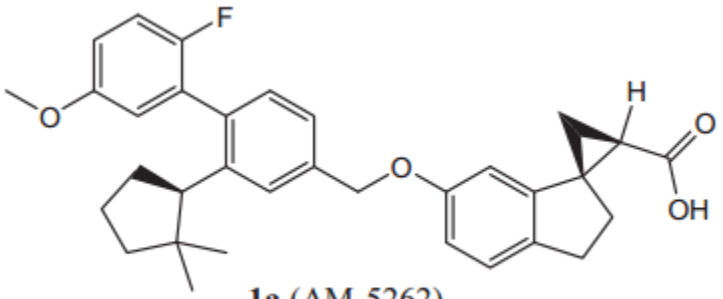
Spirocycle features

- Rigidify ligand conformation upon ligation to target
- Conformation restriction within molecule
- Ligand binding entropy
- Greater 3-dimensionality
  - more physical properties when compared to planar/aromatic derivatives
- Novelty for patentability

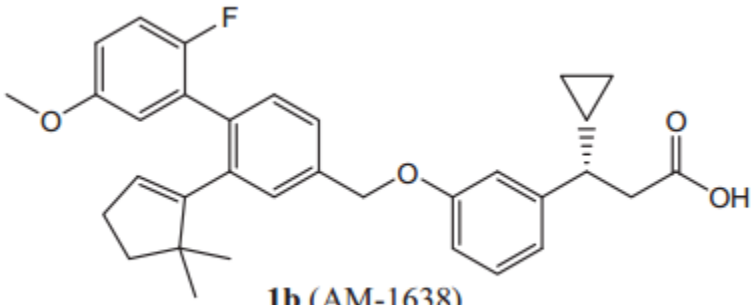


Zheng, Y.; Tice, C. M.; Singh, S. B. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 3673.

Spirocycle feature: constraint/rigidity



1a (AM-5262)
Amgen

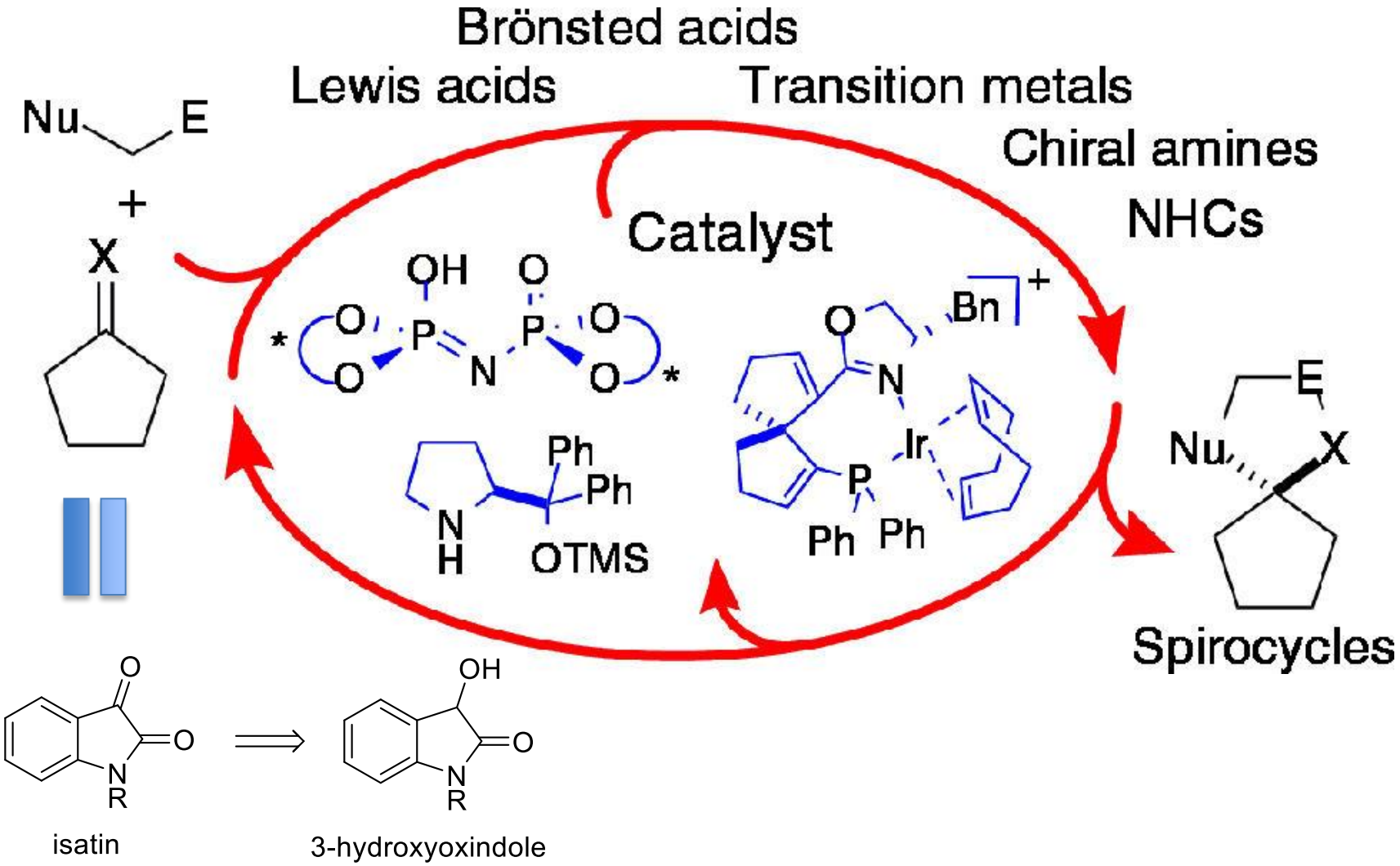


1b (AM-1638)
Amgen

1a is twice as potent and more selective than early lead **1b** due to 3 membered spiro constraint.

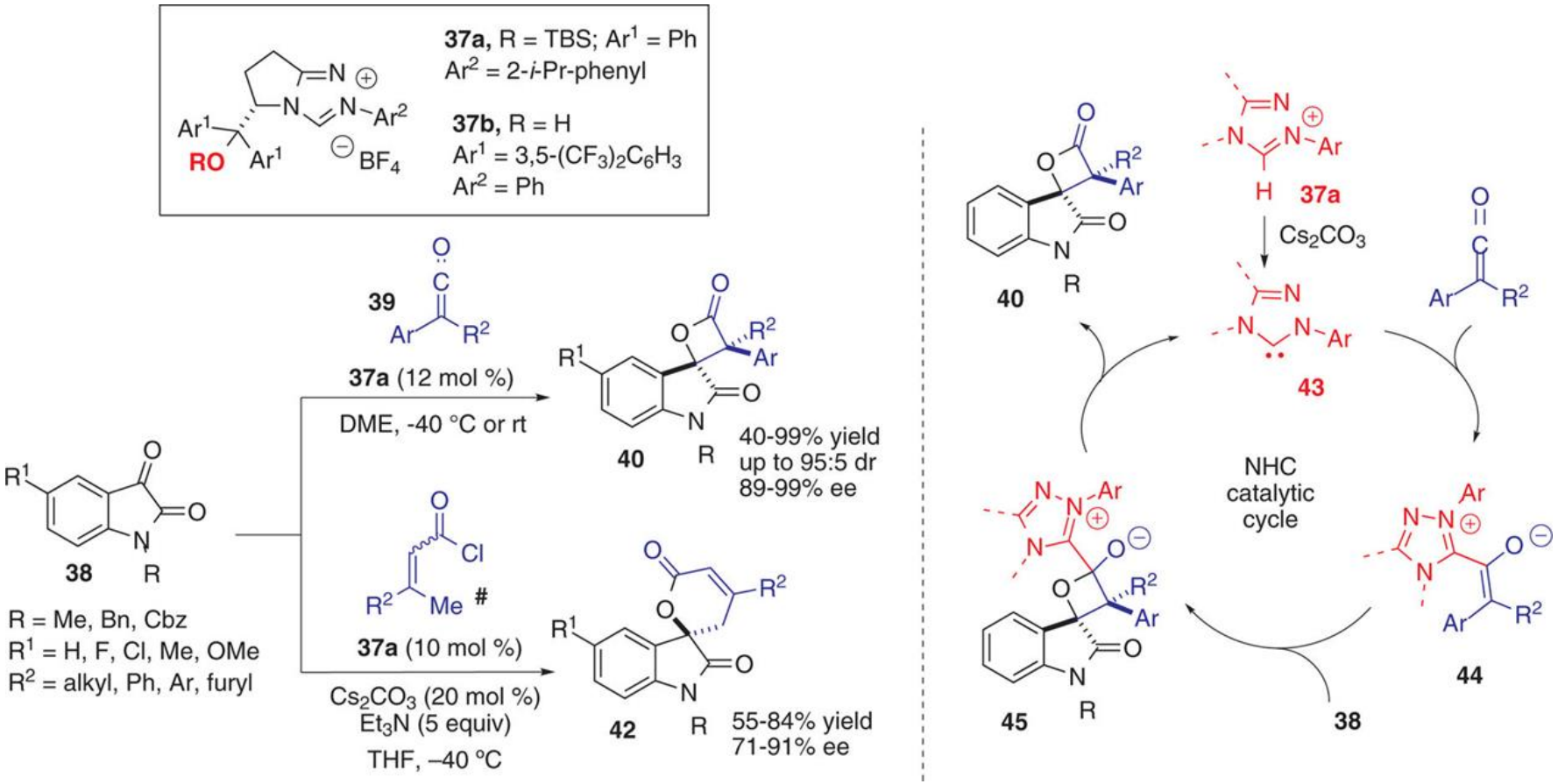
Zheng, Y.; Tice, C. M.; Singh, S. B. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 3673.

Synthesis of spirocycles



Franz, A. K.; Hanhan, N. V.; Ball-Jones, N.R. *ACS Catal.* **2013**, *3*, 540

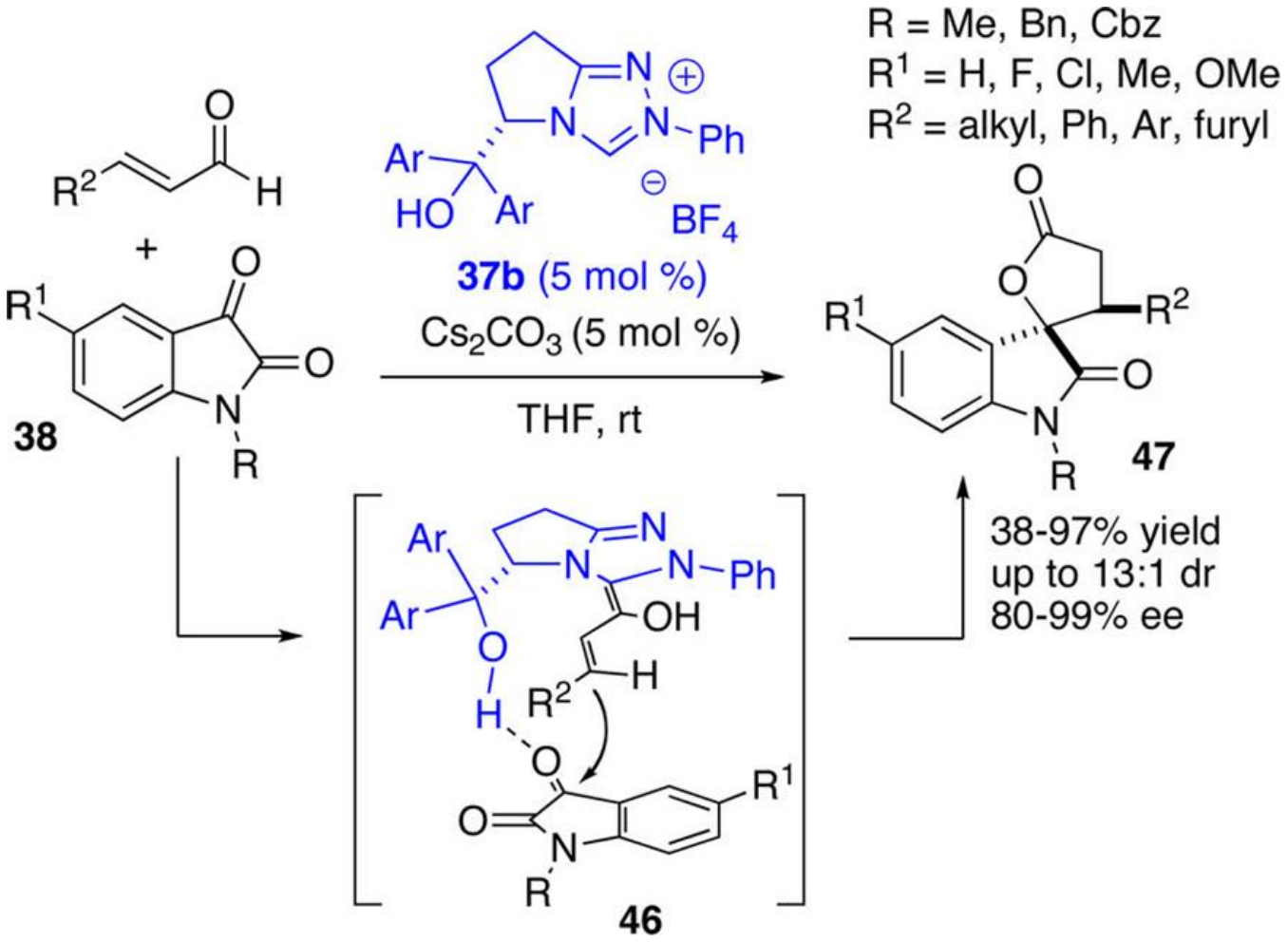
NHC-catalyzed cycloaddition/Annulation



Franz, A. K.; Hanhan, N. V.; Ball-Jones, N.R. *ACS Catal.* **2013**, *3*, 540
Wang, X.; Zhang, Y.; Ye, S. *Adv. Synth. Catal.* **2010**, 352, 1892

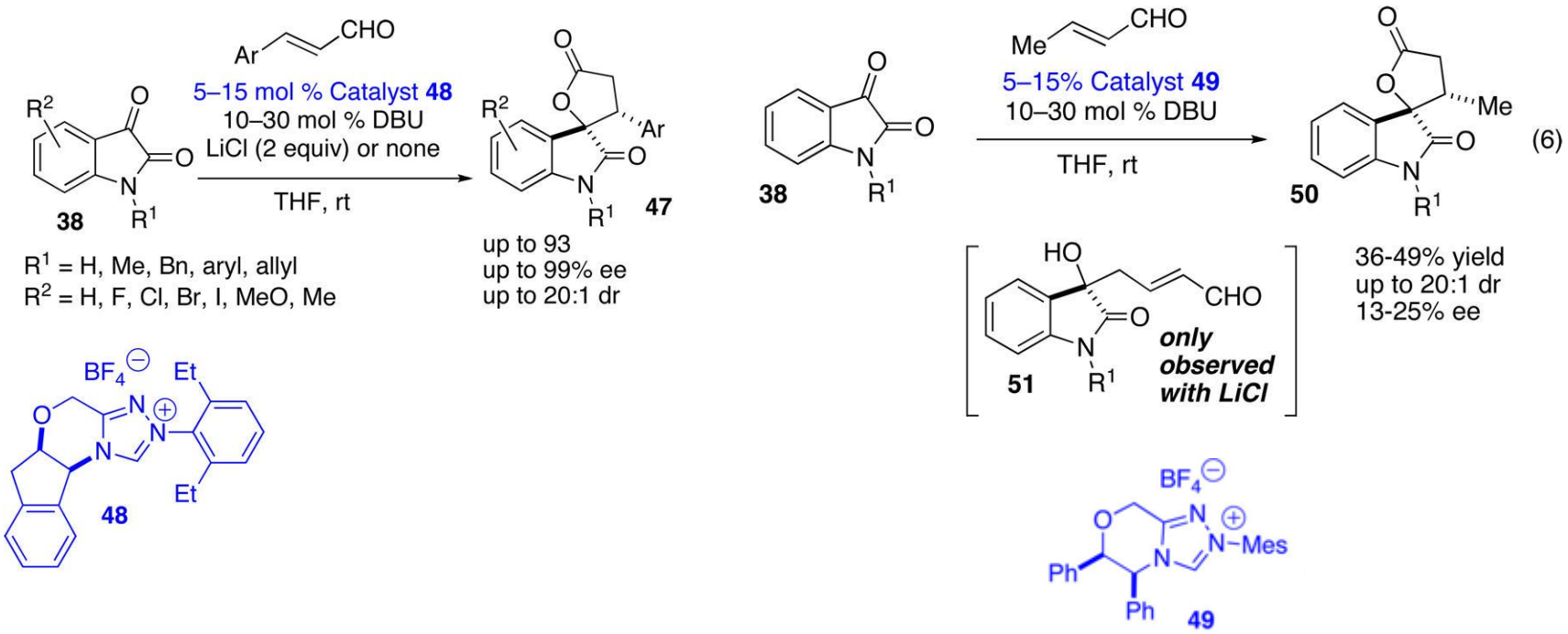
NHC-Catalyzed [3+2]

alcohol directed



Franz, A. K.; Hanhan, N. V.; Ball-Jones, N.R. *ACS Catal.* **2013**, *3*, 540
Sun, L. H.; Shen, L.T.; Ye, S. *Chem. Commun.* **2011**, 47, 10136

Cooperative NHC/Lewis Acid Strategy

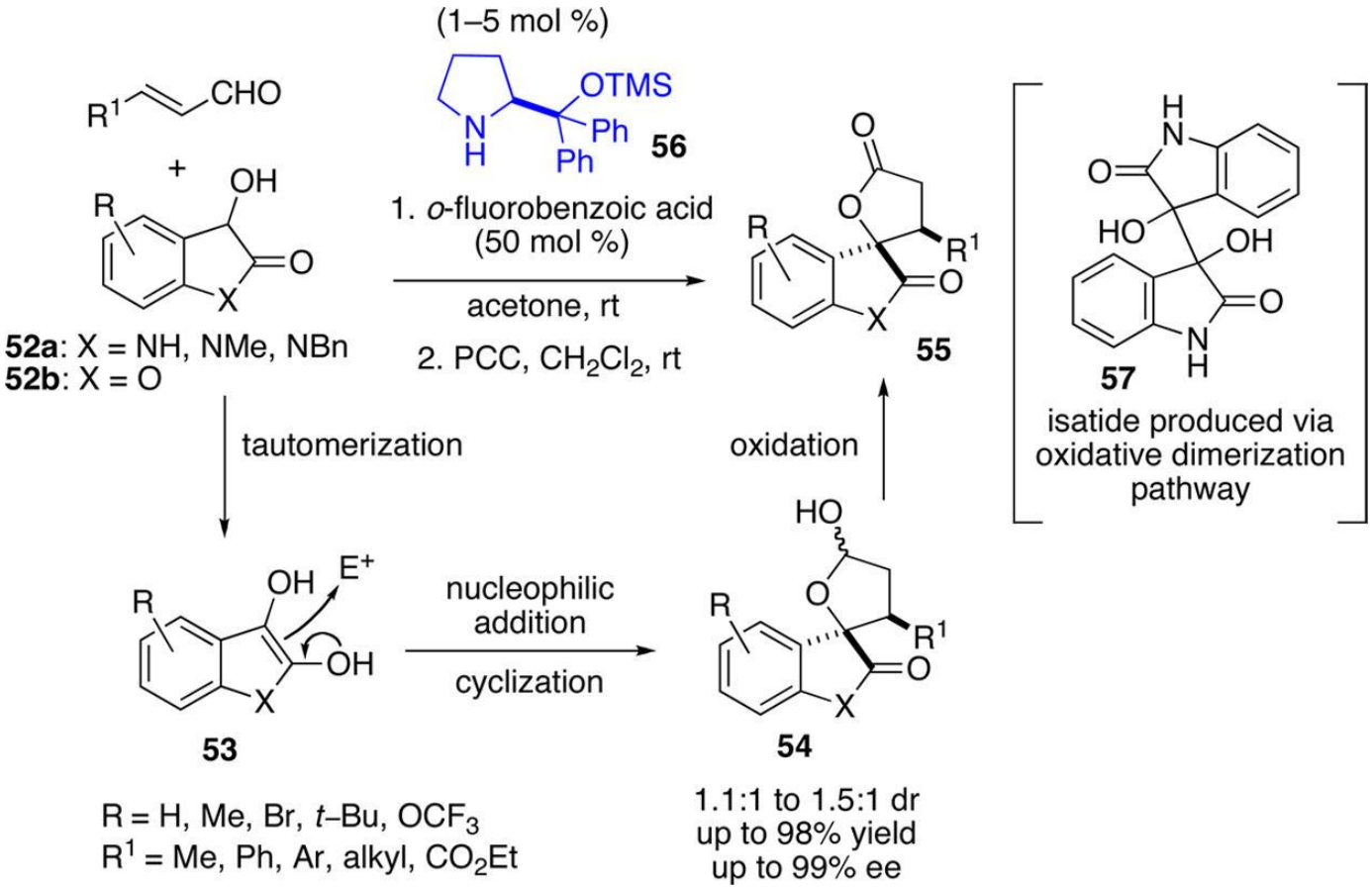


Question: Please provide a stereochemical model for the observed stereochemistry in the NHC/Lewis Acid strategy case. (the conversion of benzoquinone **38** to spirolactone **47**)

Franz, A. K.; Hanhan, N. V.; Ball-Jones, N.R. *ACS Catal.* **2013**, *3*, 540
Scheidt, K.A. *Chem. Sci.* **2012**, *3*, 53 and *Angew. Chem. Int. Ed.* **2012**, *51*, 4963

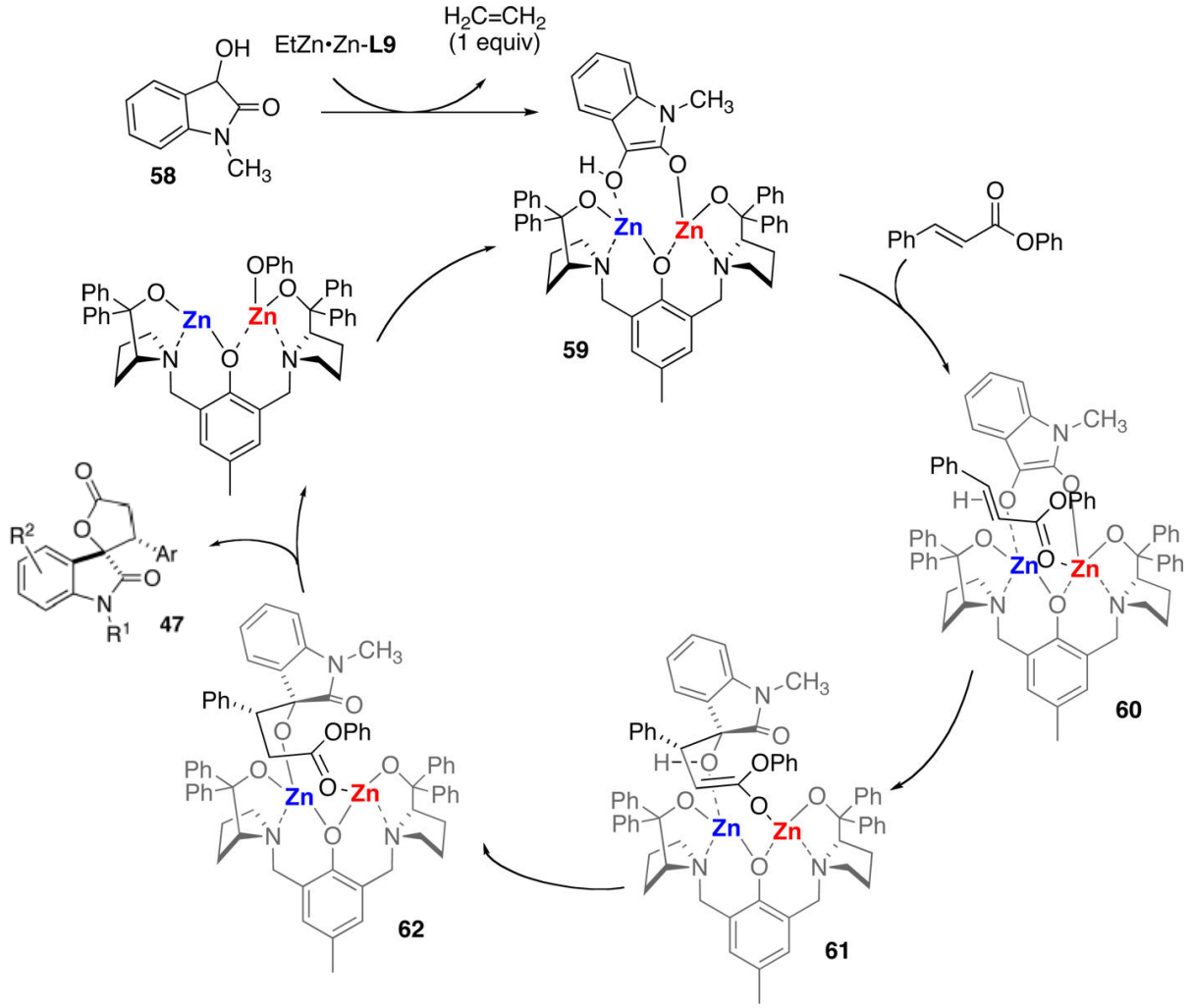
Michael Reaction Strategy

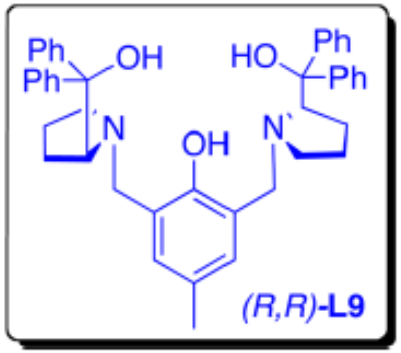
complementary spirolactone formation



Franz, A. K.; Hanhan, N. V.; Ball-Jones, N.R. *ACS Catal.* **2013**, *3*, 540
Bergonzini, G.; Melchiorre, P. *Angew. Chem.* **2012**, *51*, 995

Zn-ProPhenol Spirolactone Synthesis

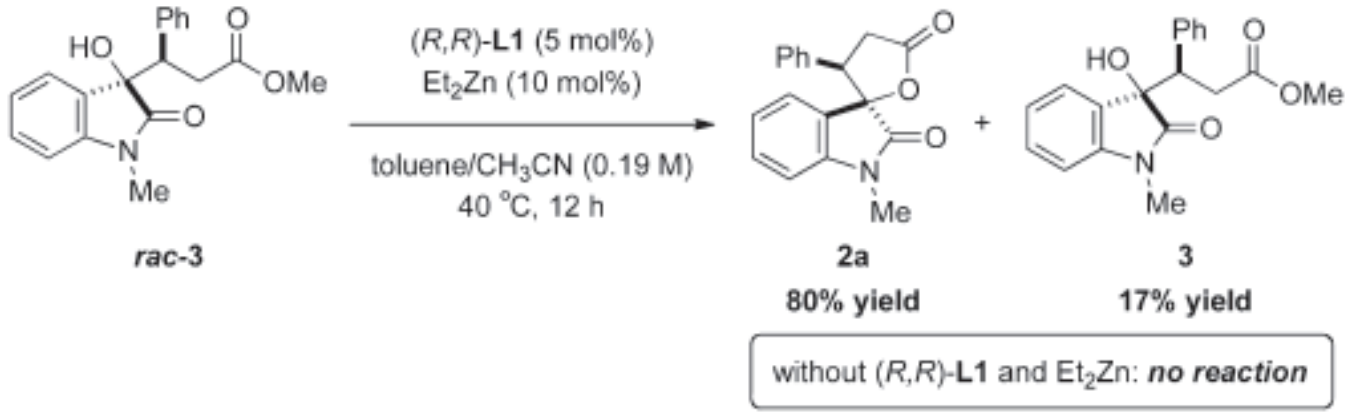




Franz, A. K.; Hanhan, N. V.; Ball-Jones, N.R. *ACS Catal.* **2013**, *3*, 540
Trost, B. M.: Hirano, K. *Org. Lett.* **2012**, *14*, 2446

Zn-ProPhenol Spirolactone Synthesis

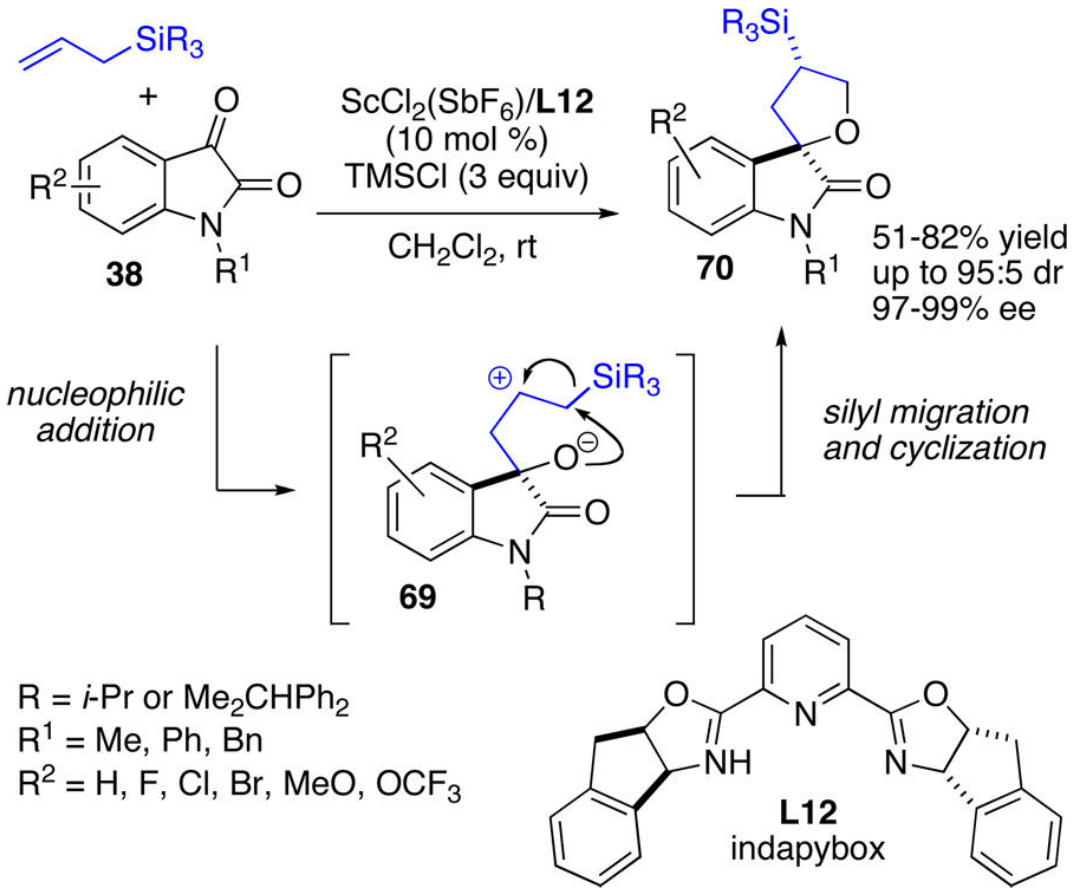
Scheme 4. Zinc-ProPhenol Complex Is Necessary for the Transesterification



racemic racemic 11% ee

Trost, B. M.: Hirano, K. *Org. Lett.* **2012**, *14*, 2446

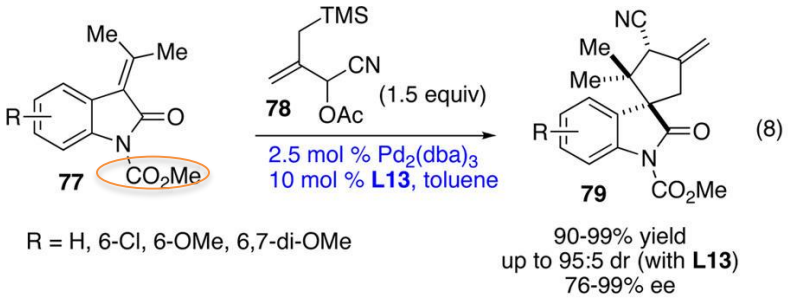
Asymmetric [3+2] allylsilane annulation

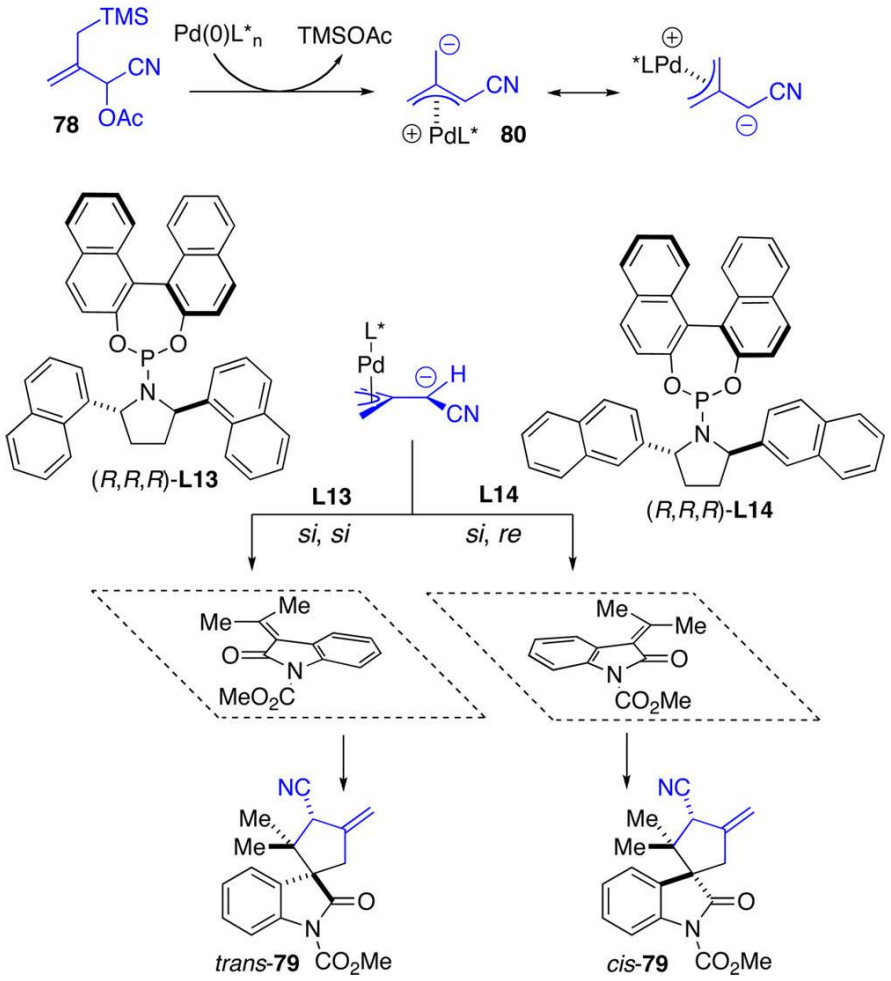


Takes advantage of the inherent enantioselectivity of allylsilanes

Franz, A. K.; Hanhan, N. V.; Ball-Jones, N.R. *ACS Catal.* **2013**, *3*, 540
Franz, A.K. *Angew. Chem. Int. Ed.* **2012**, *51*, 989

Pd catalyzed spirocyclic-pentanes

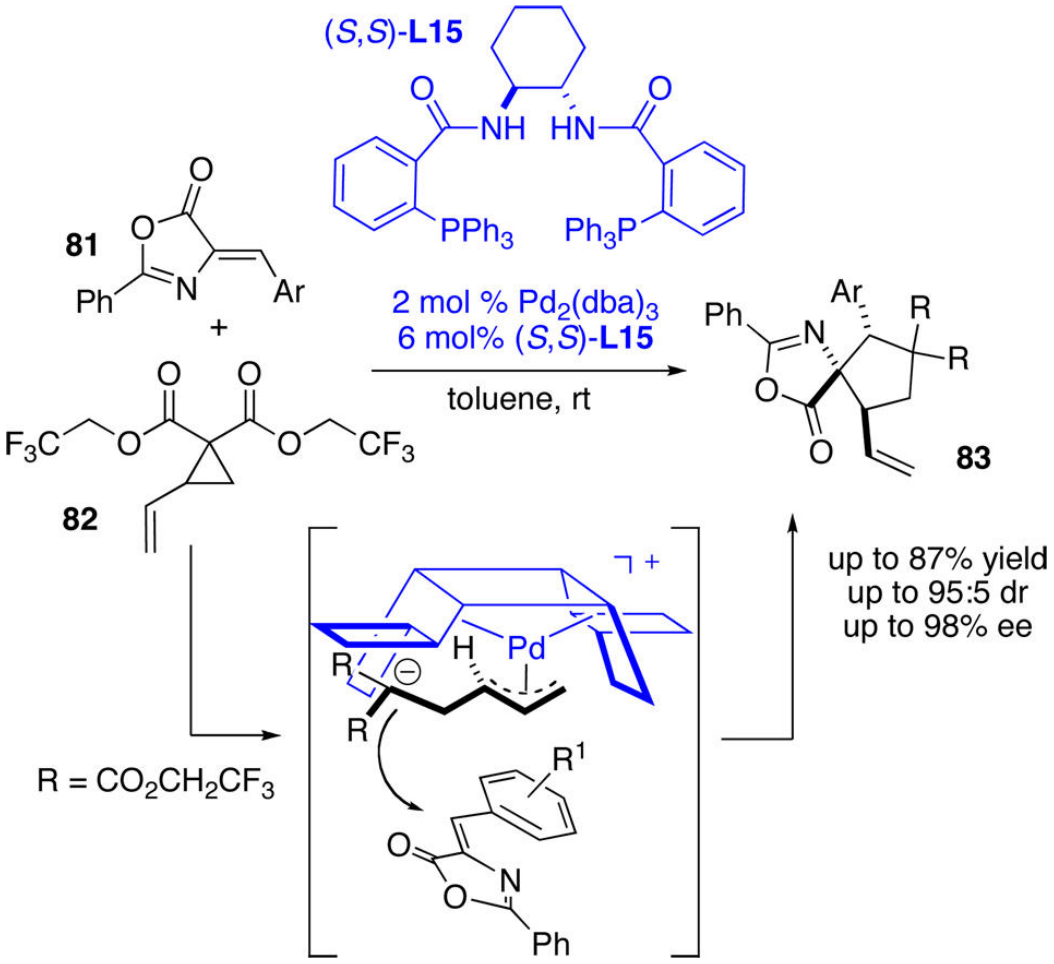




Using achiral ligand affords 2:1 trans/cis diastereomers

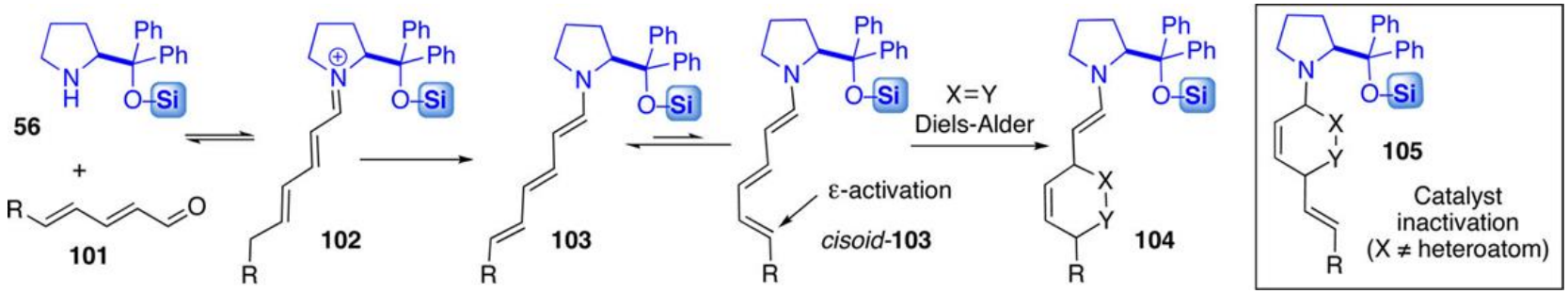
Franz, A. K.; Hanhan, N. V.; Ball-Jones, N.R. *ACS Catal.* **2013**, *3*, 540
Trost, B. M.; *J. Am. Chem Soc.* **2007**, *129*, 12396

Wall and Flap Model



Franz, A. K.; Hanhan, N. V.; Ball-Jones, N.R. *ACS Catal.* **2013**, *3*, 540
Trost, B. M.; *Angew. Chem., Int. Ed.* **2011**, *50*, 6167

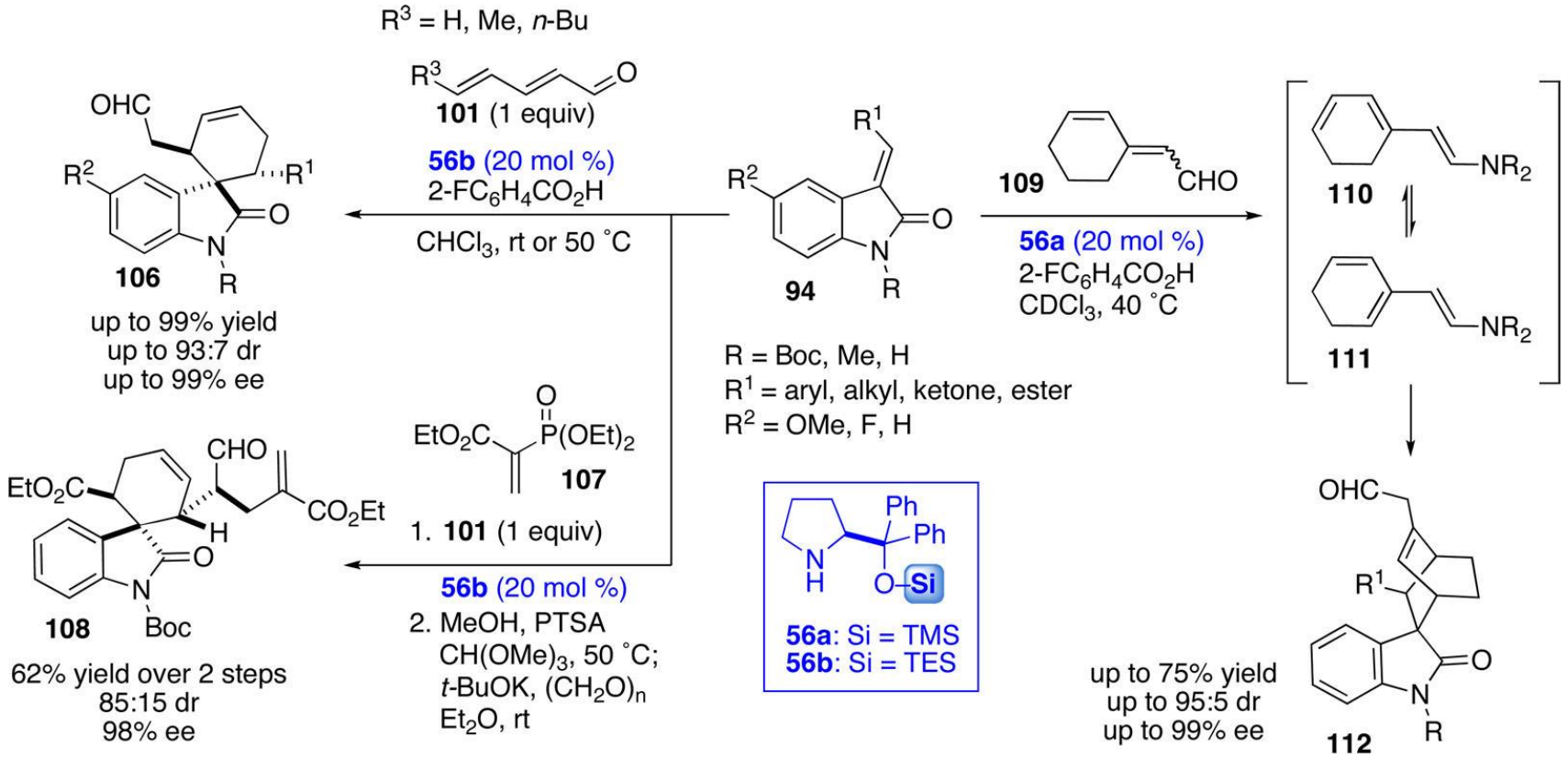
Trienamine/DA approach



"remarkably broad strategy for the enantioselective synthesis of complex spirocycles"

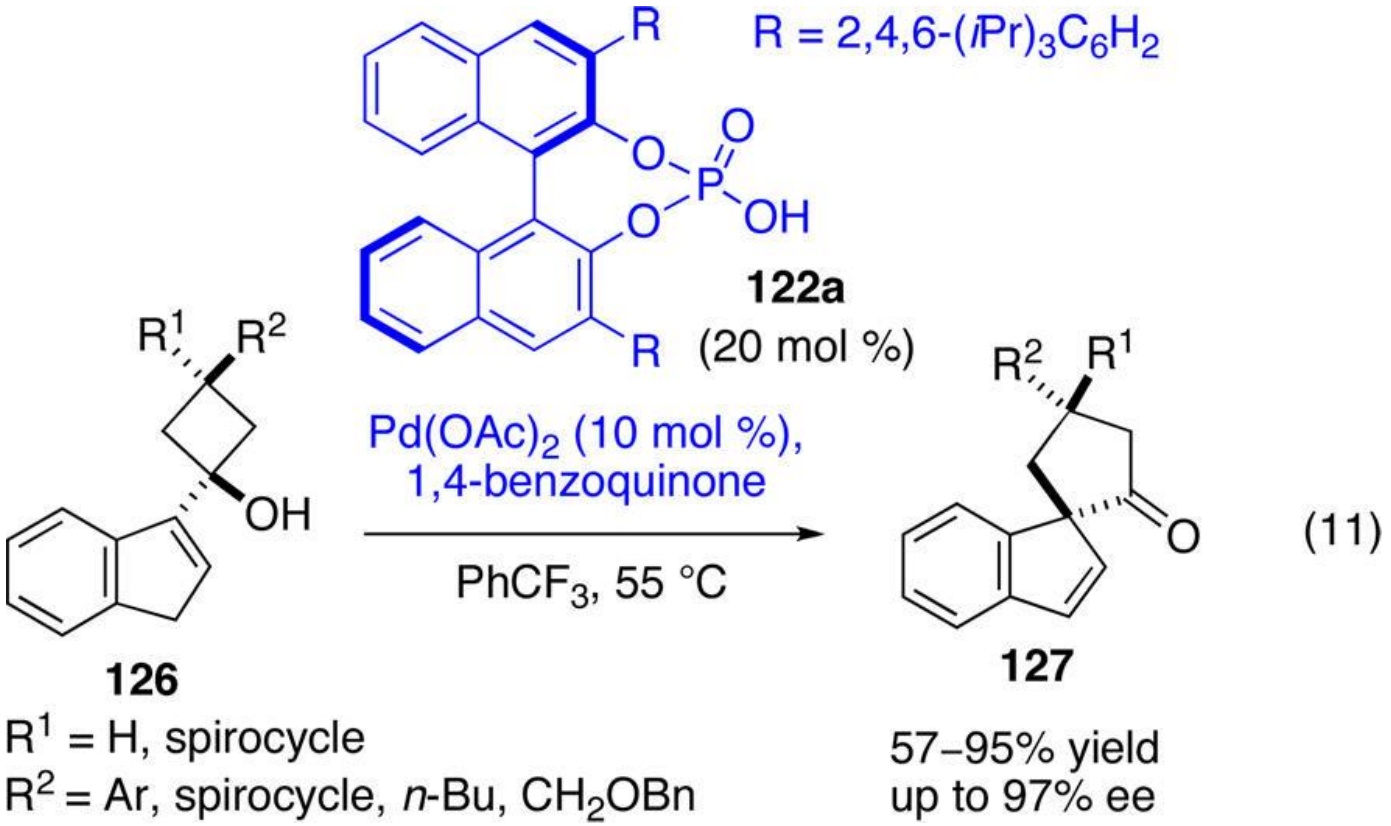
Franz, A. K.; Hanhan, N. V.; Ball-Jones, N.R. *ACS Catal.* **2013**, *3*, 540

Trienamine/DA approach Strategy



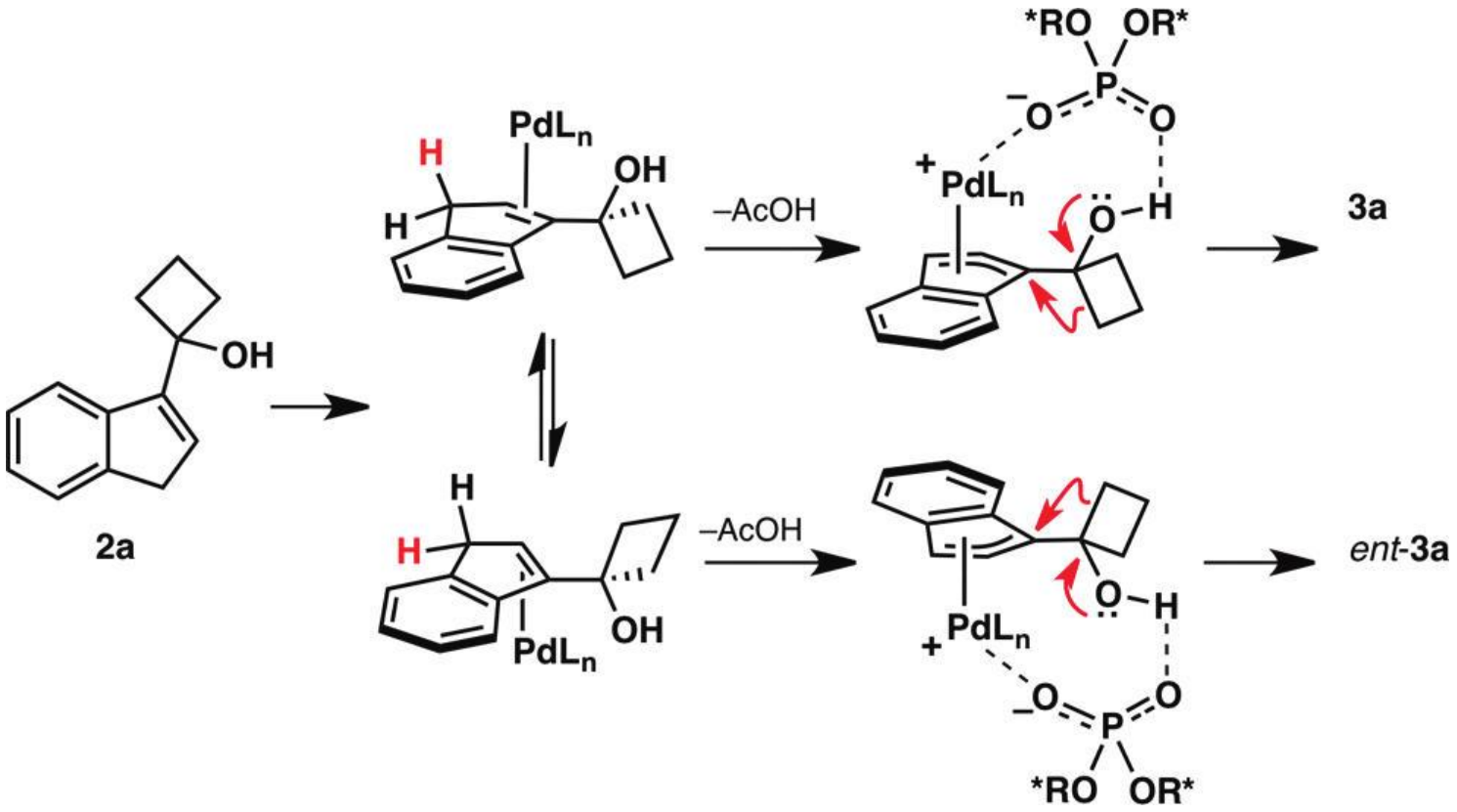
Franz, A. K.; Hanhan, N. V.; Ball-Jones, N.R. *ACS Catal.* **2013**, *3*, 540
Jorgensen, K. A.; *J. Am. Chem. Soc.* **2011**, *133*, 5053

Ring Expansion Approach



Franz, A. K.; Hanhan, N. V.; Ball-Jones, N.R. *ACS Catal.* **2013**, *3*, 540
Chai, Z.; Rainey, T.J. *J. Am. Chem. Soc.* **2012**, *134*, 3615

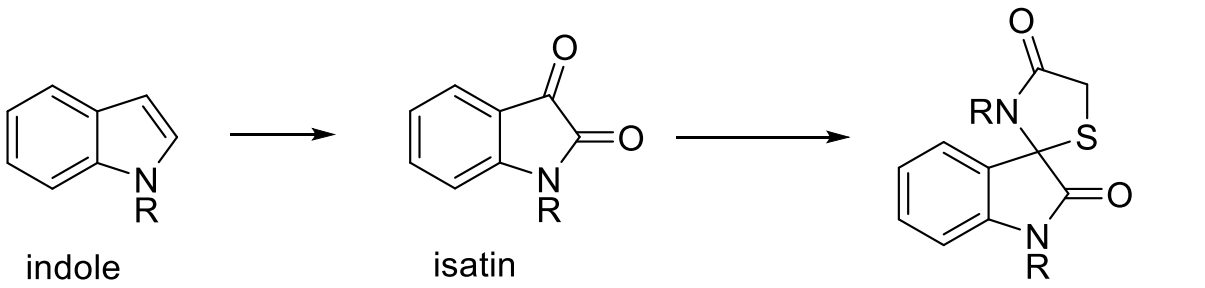
Proposed reaction mechanism



Chai, Z.; Rainey, T.J. *J. Am. Chem. Soc.* **2012**, *134*, 3615

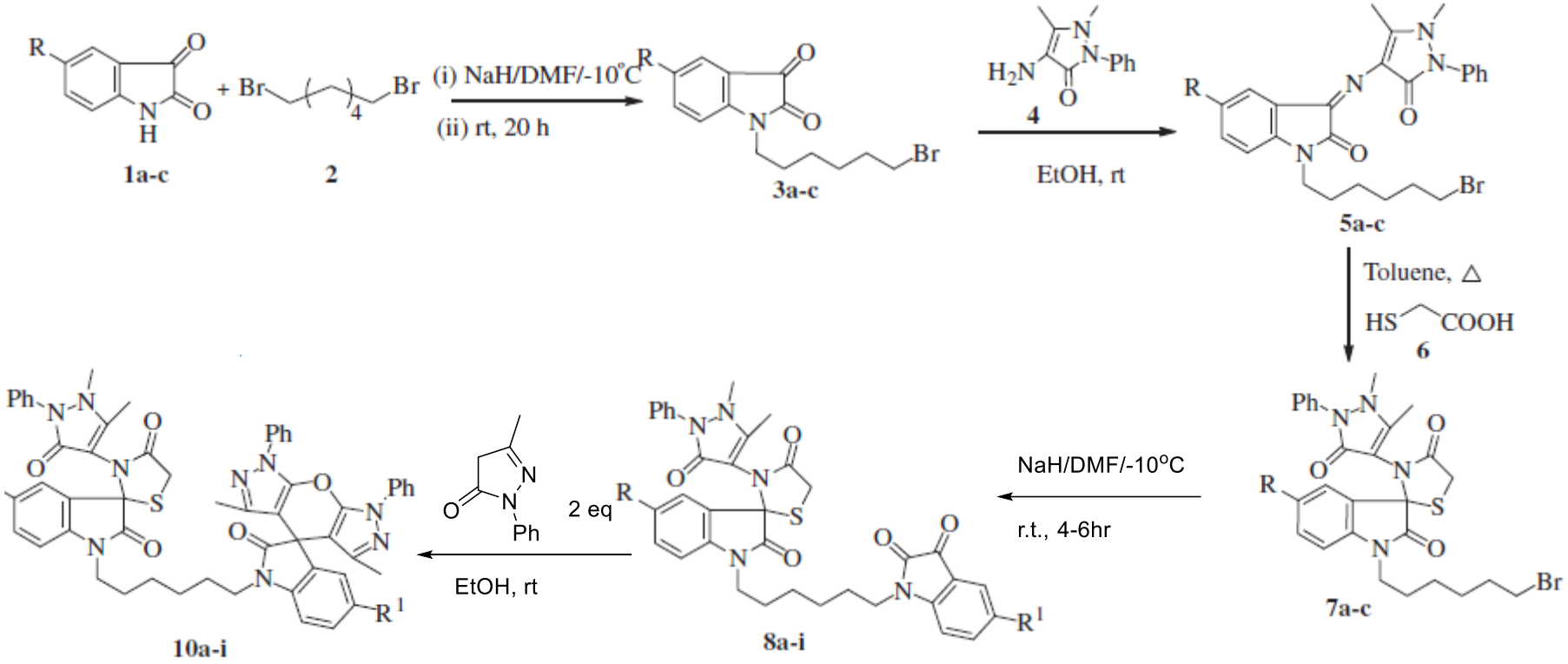
Thiazolidines

- Indoles and its analogous (such as isatin) are good pharmacophores for designing chemotherapeutic
- Spiro[indole-thiazolidines] have broad spectrum of pharmacological properties



R. Sakhuja *et al. Bioorg. Med. Chem. Lett.* **2011**, *21*, 5465

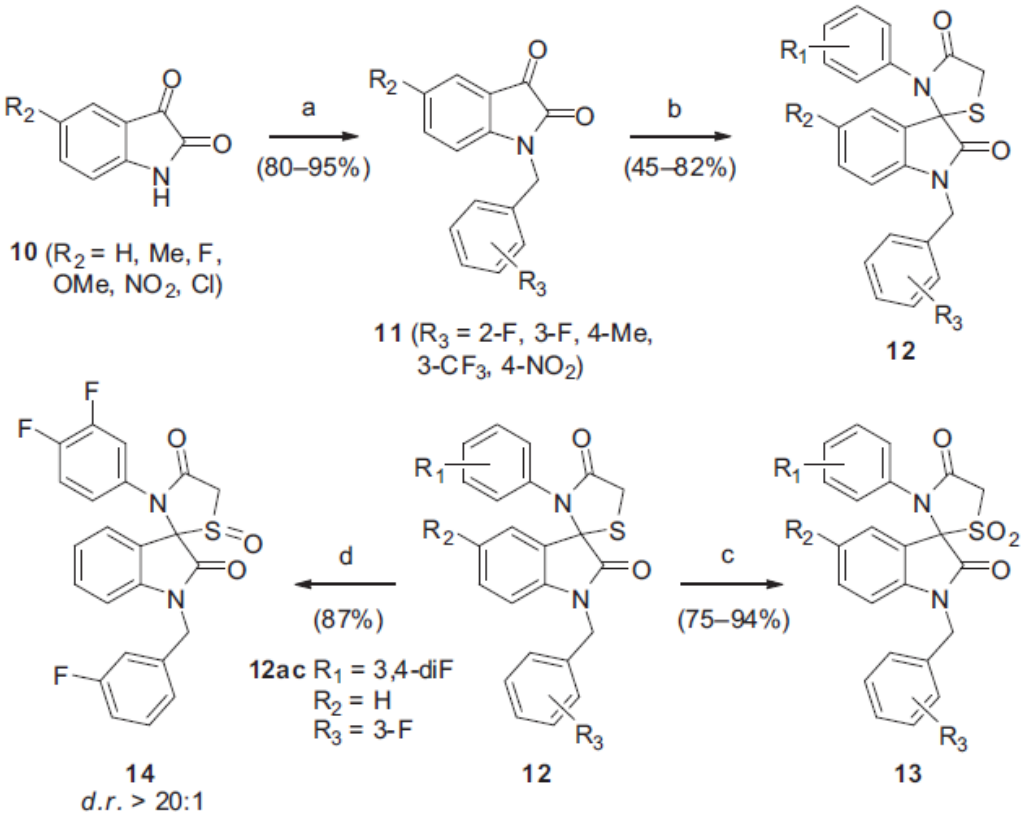
Example synthesis of a thiazolidine



racemate of one
ntantiomeric pair

R. Sakhuja *et al. Bioorg. Med. Chem. Lett.* **2011**, *21*, 5465

Another example of thiazolidine



(a) NaH, DMF, 0°C then $ArCH_2Br$ or Alk-X; (b) (1) $Ar'NH_2$, EtOH, reflux, 6 h; (2) mercaptoacetic (aka thioglycolic acid) acid, toluene, reflux, 16 h; (c) mCPBA (5 equiv), $CHCl_3$, rt, 24h (d) mCPBA (1.1 equiv), $CHCl_3$, 0°C, 1 h

V.V. Vintonyak *et al. Tetrahedron* **2011**, *67*, 6713

Thiazolidine synthesis w/ sulfactant

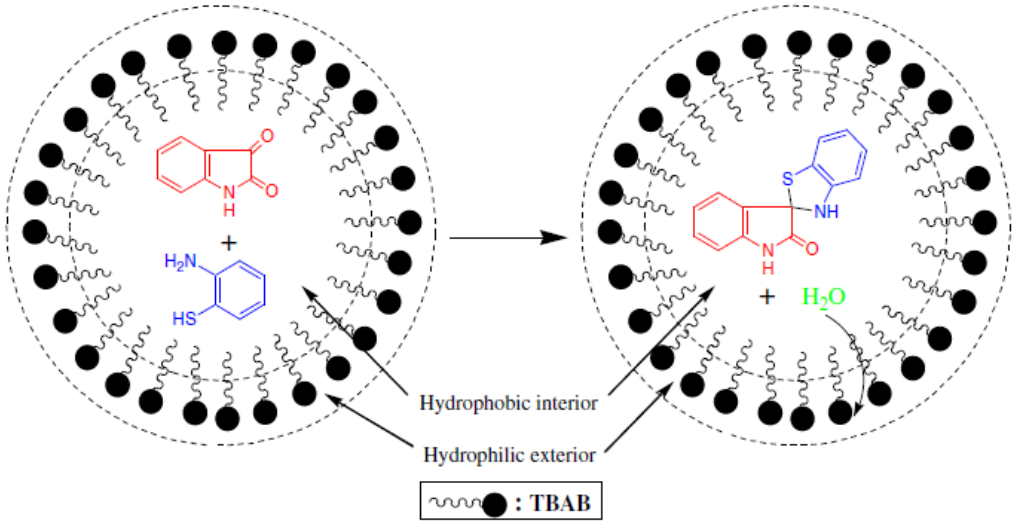
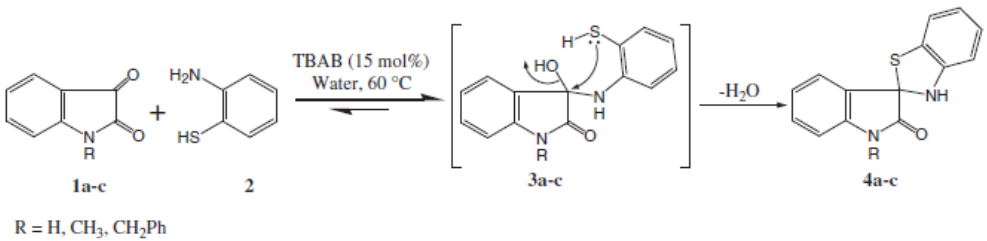


Figure 3. Micelles-promoted green synthesis of 3′*H*-Spiro[indole-3,2′-[1,3]benzothiazole]-2(1*H*)-one.



Scheme 1. Synthesis of 3′*H*-spiro[indole-3,2′-[1,3]benzothiazole]-2(1*H*)-ones **4a–c**.

R. Jain *et al. Tetrahedron Letters* **2012**, *53*, 6236

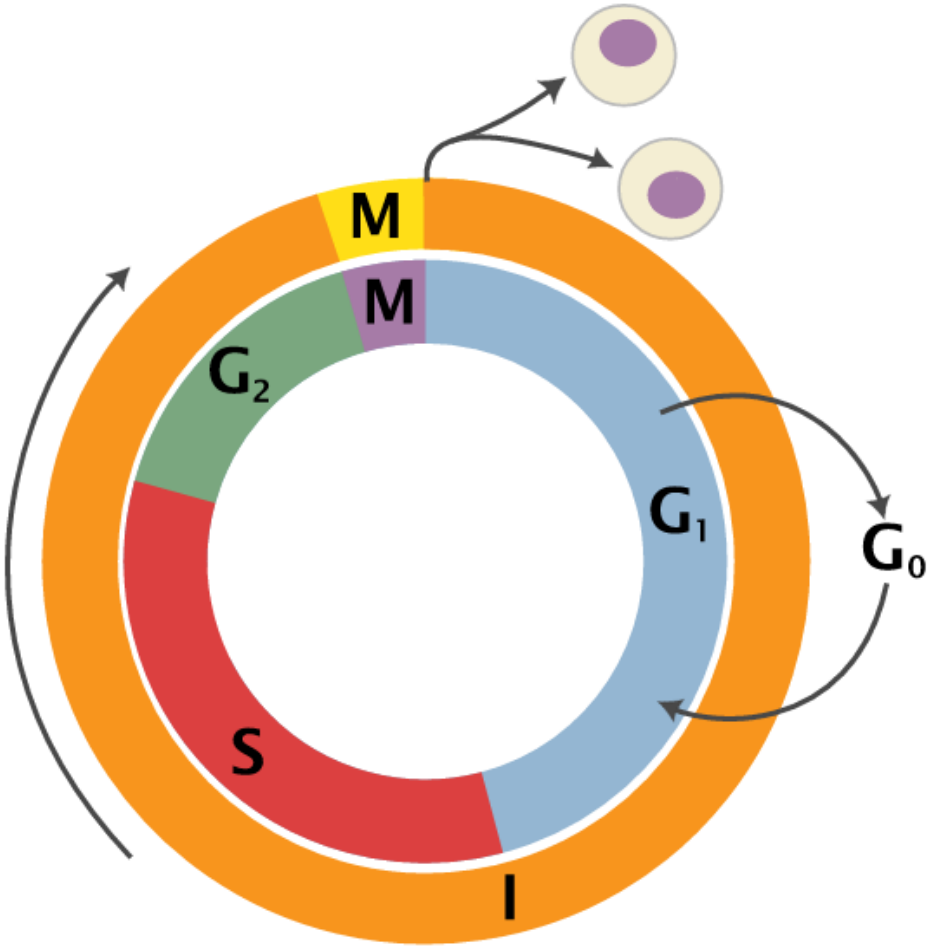
Biological Aside

Before specific case study

"I try to show the public that chemistry, biology, physics, astrophysics is life. It is not some separate subject that you have to be pulled into a corner to be taught about." -Neil deGrasse Tyson

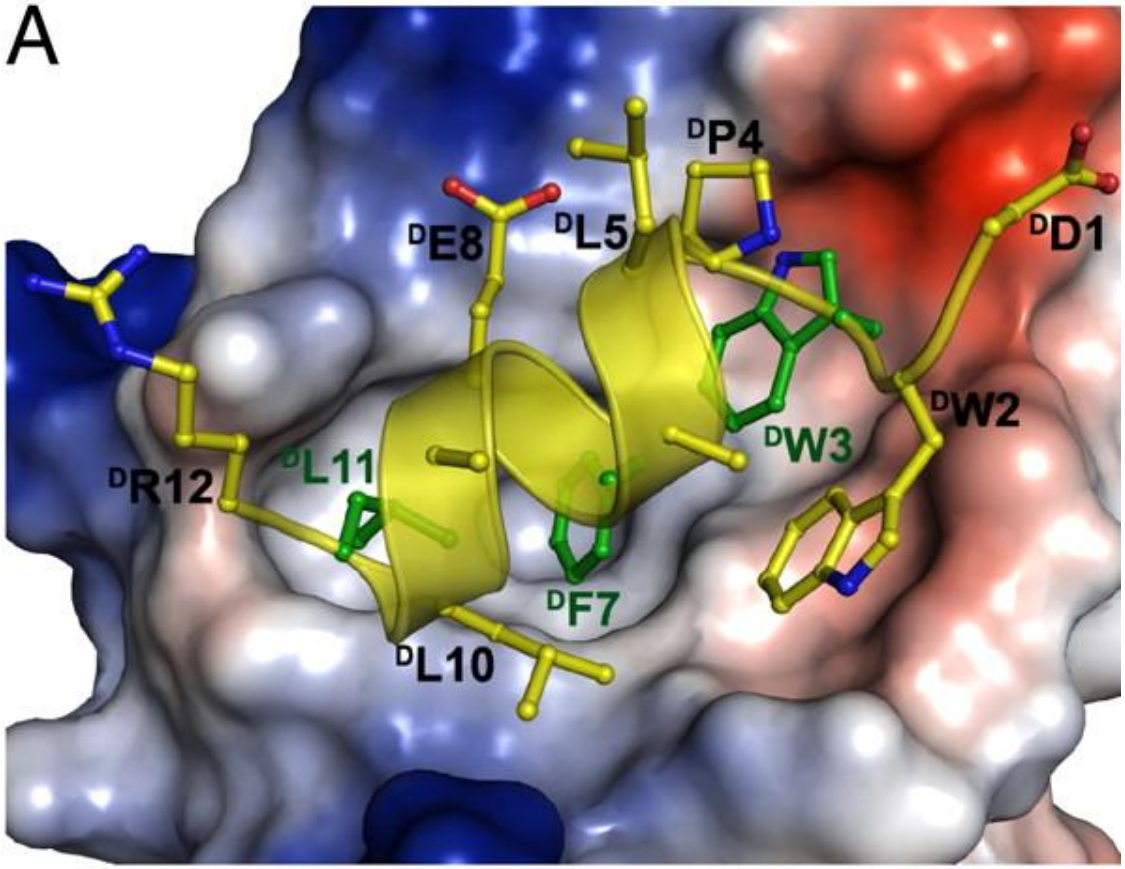
- 1) cell cycle
- 2) p53 and MDM2
- 3) Western Blot

Cell Cycle



Phase	Abbreviation	Description
Interphase	I	Includes G_o, S, and G_1
Gap 1	G_1	cell growth, protein and organelles accumulation
Synthesis	S	DNA replication
Gap 2	G_2	Cell growth
Mitosis	M	Cell division
Gap 0	G_0	Quiescent/ senescent

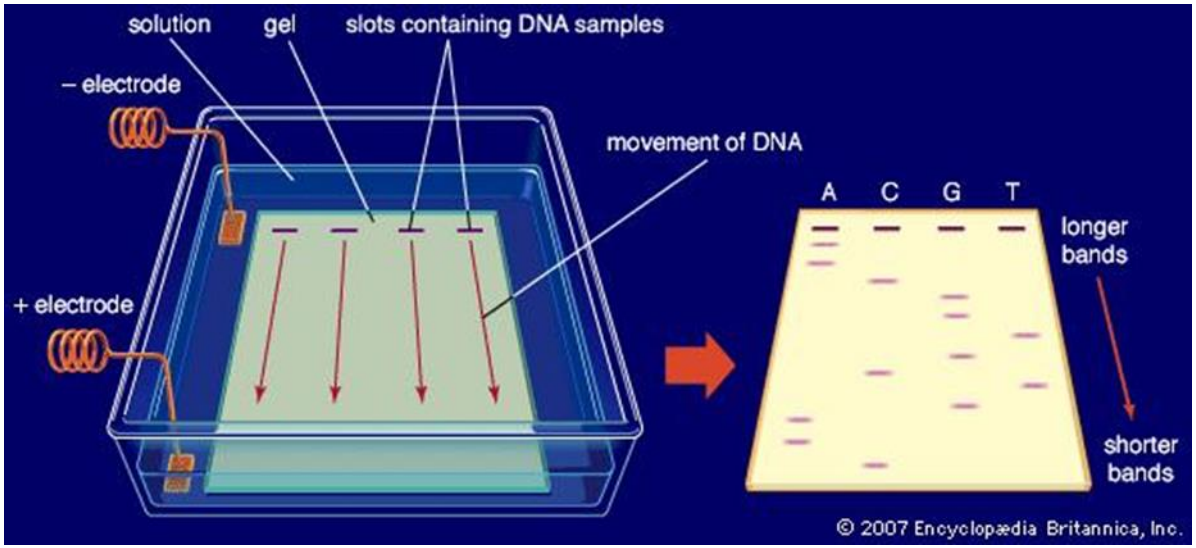
P53-MDM2



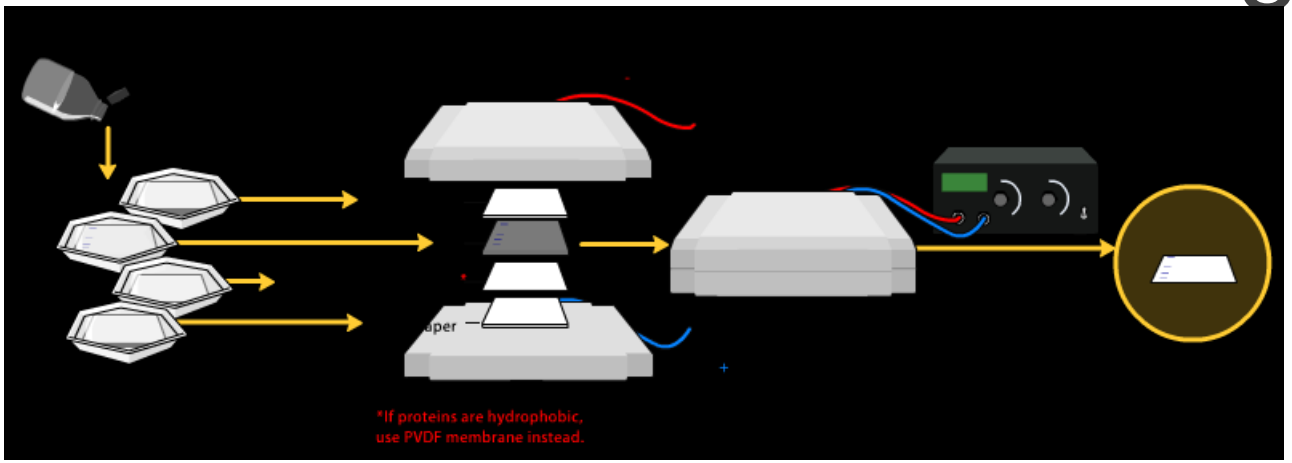
Crystal structure of the p53-binding domain of MDM2

Western Blotting

1) Cell lysis and separation to extract protein
2) Gel Electrophoresis
   - Charged molecules are separated according to physical properties



Transfer Method: Blotting

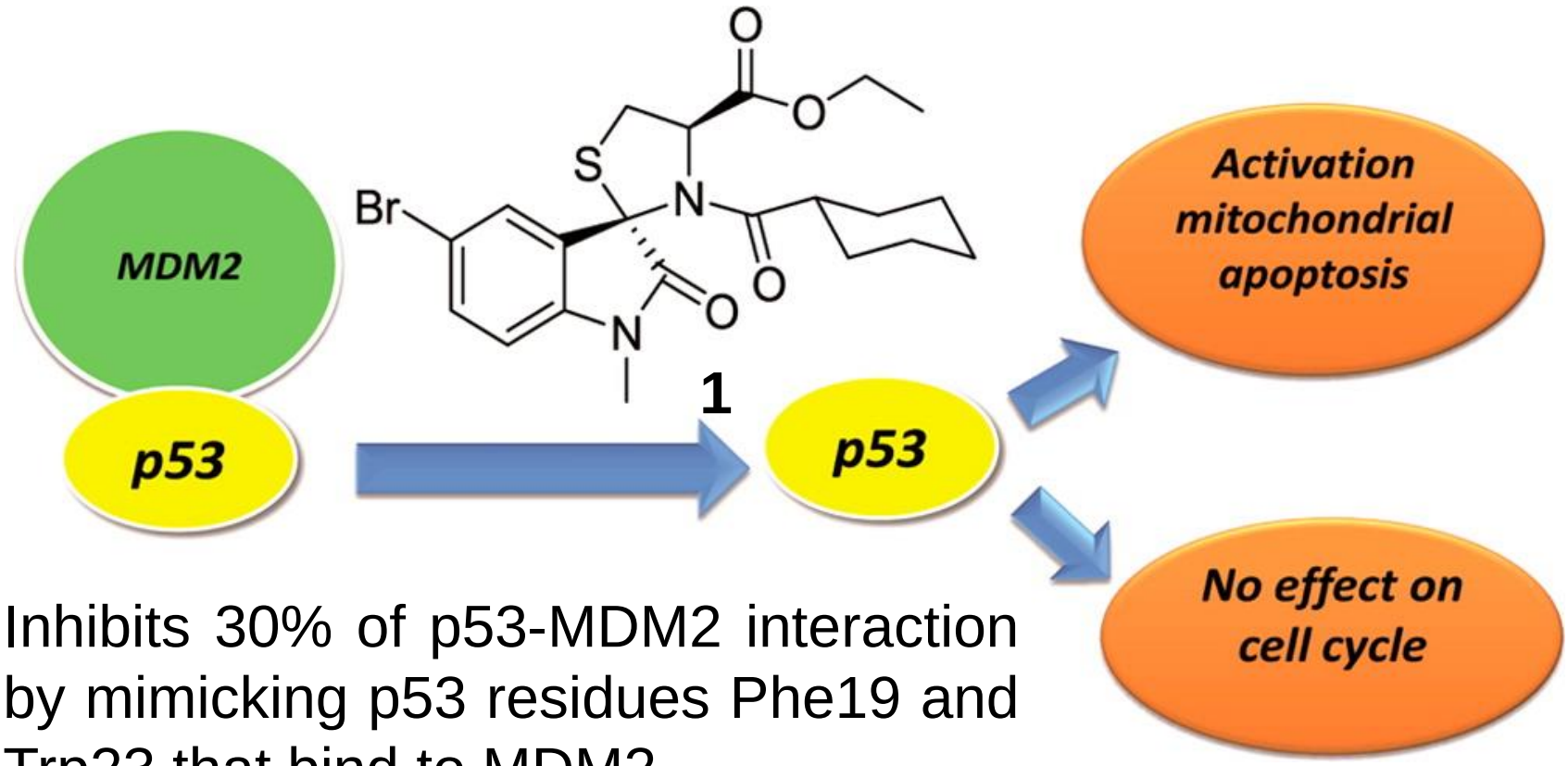


3) Blotting

- proteins are transferred to a membrane via electroelution.
- Nonspecific sites of the remaining membrane surface are blocked
- Proteins are labeled for detection

Specific Case Study

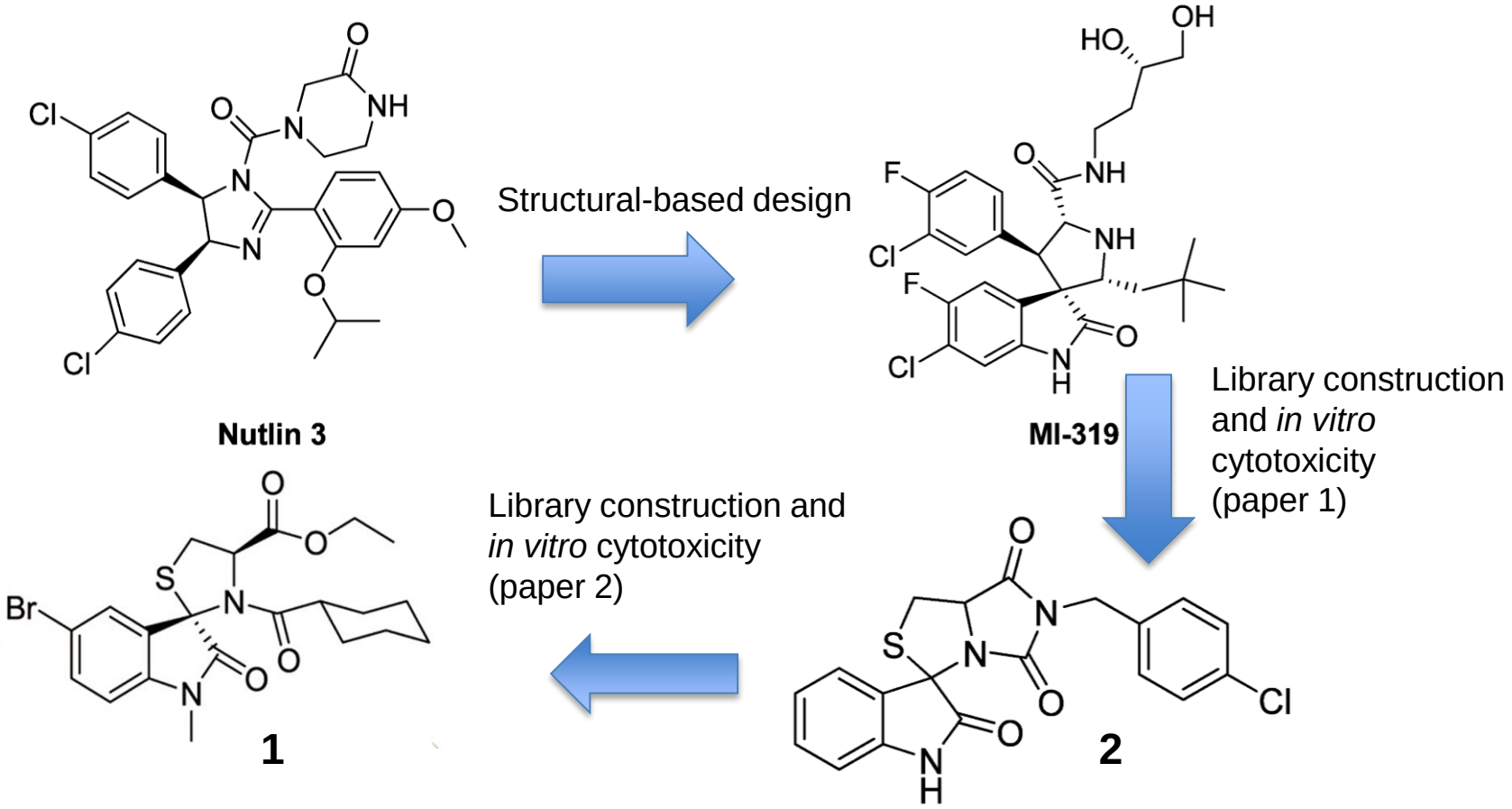
Development of a MDM2 antagonist



Inhibits 30% of p53-MDM2 interaction by mimicking p53 residues Phe19 and Trp23 that bind to MDM2

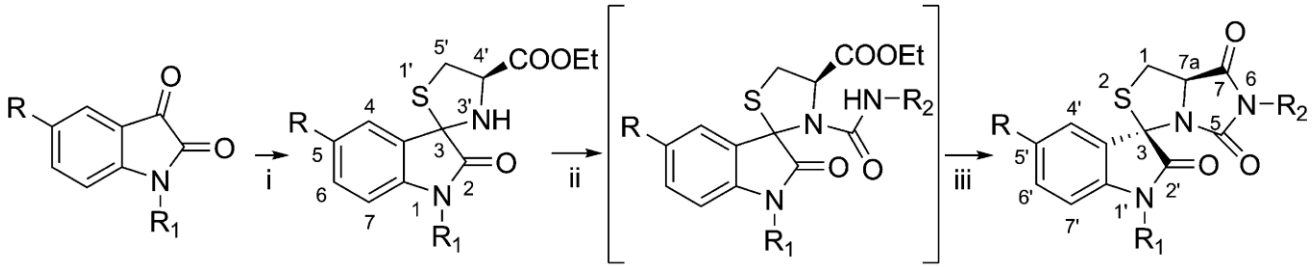
Bertamino, A. *et al*. *J. Med. Chem.,* **2013**, *56,* 5407

Outline of MDM2 antagonist Discovery

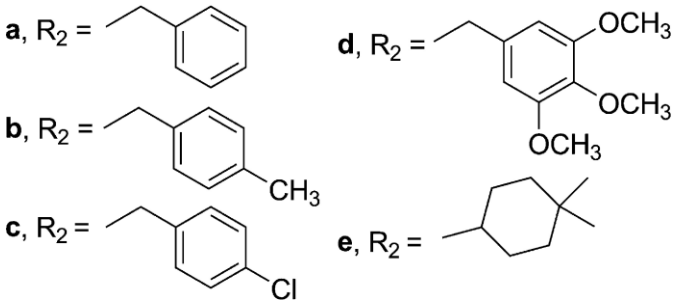


Gomez-Monterrey. *et al*. *J. Med. Chem.*, **2010**, *53,* 8319

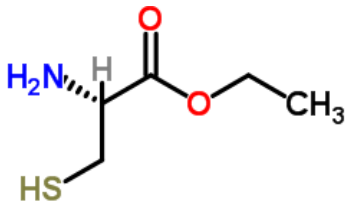
Synthesis of derivatives



1, 5. 9 R = H, R_1 = H
2, 6.10 R =CH_3, R_1 = H
3, 7. 11 R =Br, R_1 = H
4,8. 12 R = H, R_1 = CH_3



(i) Cys-OEt, $NaHCO_3$ in MeOH, MW; (ii) triphosgene TEA, THF, rt, 10 min, then R_2-NH_2; (iii) MeOH, TEA, reflux, 1-3 hr

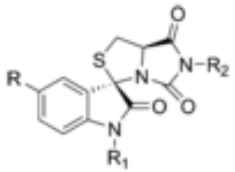


Cysteine ethyl ester

Gomez-Monterrey. *et al*. *J. Med. Chem.,* **2010**, *53,* 8319

Cytotoxic activity of derivatives

Table 1. Cytotoxic Activity of Spiro[imidazo[1,5-*c*]thiazole-3,3′-indoline]-2′,5,7(6*H*,7a*H*)-trione Derivatives **9a−e**/**12a−e**



9-12

compd	R	R_1	R_2	$IC_{50} \pm SD^a$ (μM) HEK[b]	M14[c]	U937[d]
9a	H	H	$-CH_2C_6H_5$	4.80 ± 0.15	10.64 ± 0.04	3.90 ± 0.01
9b	H	H	$-CH_2C_6H_4(4\text{-}CH_3)$	4.53 ± 0.15	13.27 ± 0.03	5.91 ± 0.02
9c	H	H	$-CH_2C_6H_4(4\text{-Cl})$	0.44 ± 0.01	0.53 ± 0.01	0.87 ± 0.01
9d	H	H	$-CH_2C_6H_2(3,4,5\text{-}OCH_3)$	3.80 ± 0.08	4.73 ± 0.02	2.51 ± 0.05
9e	H	H	4-dimethylcyclohexyl	4.22 ± 0.07	7.07 ± 0.01	2.61 ± 0.05
10a	CH_3	H	$-CH_2C_6H_5$	3.30 ± 0.07	3.88 ± 0.02	2.09 ± 0.04
10b	CH_3	H	$-CH_2C_6H_4(4\text{-}CH_3)$	3.01 ± 0.06	3.39 ± 0.03	2.77 ± 0.02
10c	CH_3	H	$-CH_2C_6H_4(4\text{-Cl})$	3.88 ± 0.05	6.65 ± 0.02	3.31 ± 0.04
10d	CH_3	H	$-CH_2C_6H_2(3,4,5\text{-}OCH_3)$	2.04 ± 0.03	2.40 ± 0.02	2.06 ± 0.04
10e	CH_3	H	4-dimethylcyclohexyl	16.01 ± 0.05	19.12 ± 0.07	12.48 ± 0.05

Cell Lines: HEK, transformed human embryonic kidney cell line; M14, human melanoma cell line; U937, Human leukemia monocyte lymphoma

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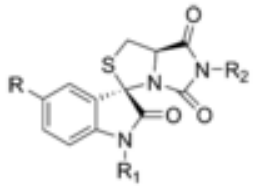
Cytotoxic activity of derivatives

Table 1. Cytotoxic Activity of Spiro[imidazo[1,5-*c*]thiazole-3,3′-indoline]-2′,5,7(6*H*,7a*H*)-trione Derivatives **9a−e**/**12a−e**



9-12

compd	R	R_1	R_2	$IC_{50} \pm SD^a$ (μM) HEK[b]	M14[c]	U937[d]
11a	Br	H	$-CH_2C_6H_5$	8.48 ± 0.09	12.04 ± 0.03	7.58 ± 0.05
11b	Br	H	$-CH_2C_6H_4(4\text{-}CH_3)$	7.61 ± 0.10	10.24 ± 0.10	6.23 ± 0.14
11c	Br	H	$-CH_2C_6H_4(4\text{-}Cl)$	7.13 ± 0.06	7.06 ± 0.05	5.01 ± 0.03
11d	Br	H	$-CH_2C_6H_2(3,4,5\text{-}OCH_3)$	9.31 ± 0.09	11.04 ± 0.02	5.02 ± 0.03
11e	Br	H	4-dimethylcyclohexyl	> 40	> 40	> 40
12a	H	CH_3	$-CH_2C_6H_5$	3.98 ± 0.05	6.37 ± 0.04	2.89 ± 0.03
12b	H	CH_3	$-CH_2C_6H_4(4\text{-}CH_3)$	10.71 ± 0.10	31.79 ± 0.04	16.75 ± 0.02
12c	H	CH_3	$-CH_2C_6H_4(4\text{-}Cl)$	2.11 ± 0.05	2.47 ± 0.02	2.91 ± 0.01
12d	H	CH_3	$-CH_2C_6H_2(3,4,5\text{-}OCH_3)$	6.01 ± 0.06	7.66 ± 0.23	5.70 ± 0.05
12e	H	CH_3	4-dimethylcyclohexyl	> 40	> 40	> 40
doxorubicin				0.9 ± 0.08	1.0 ± 0.05	0.8 ± 0.01

Cell Lines: HEK, transformed human embryonic kidney cell line; M14, human melanoma cell line; U937, Human leukemia monocyte lymphoma

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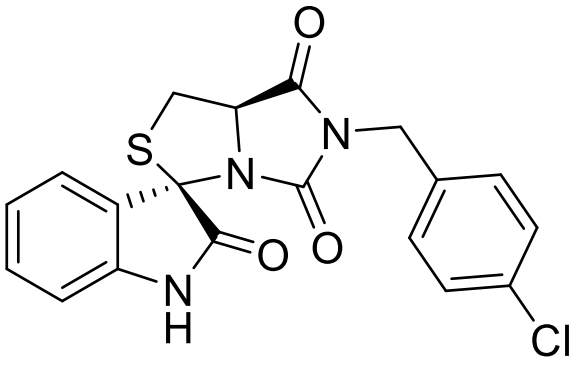
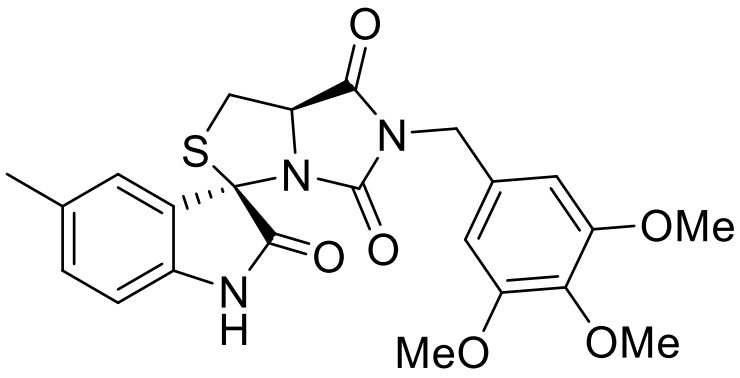
Hit compounds **9c** and **10d**



9c



10c

To undergo additional *in vitro* testing and derivatization

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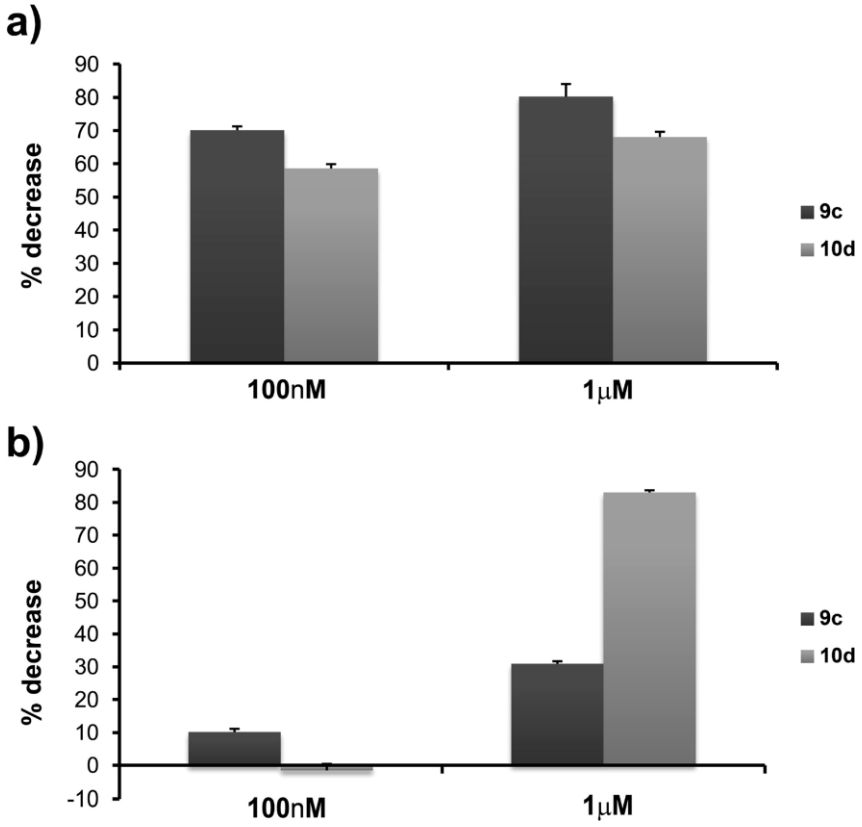
Derivatives **9c** and **10d** exhibit good cell selectivity



a) Human papillary thyroid carcinoma TPC1

b) Normal thyroid TAD-2 cell

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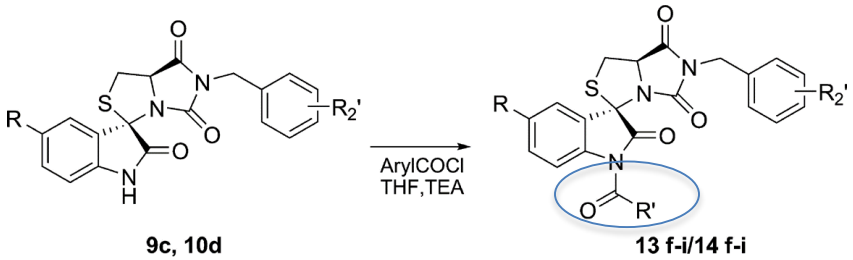
Derivatives of **9c** and **10d**

Table 2. Cytotoxic Activity of 1′-Acylspiro[(dihydroimidazo[1,5-*c*]-thiazolo-5,7-dione)-3,3′-(dehydroindol-2-one)] Derivatives **13f–i/14f–i**



9c, 10d **13 f-i/14 f-i**

acylated series
less potent

compd	R	R_1'	R_2'	$IC_{50} \pm SD^a$ (μM) HEK	M14	U937
9c	H		4-Cl	0.44 ± 0.01	0.53 ± 0.01	0.87 ± 0.01
13f	H	$-C_6H_5$	4-Cl	3.50 ± 0.02	3.25 ± 0.36	3.12 ± 0.07
13g	H	$-C_6H_4(4-CH_3)$	4-Cl	2.50 ± 0.10	2.08 ± 0.10	2.01 ± 0.15
13h	H	$-C_6H_4(4-Cl)$	4-Cl	5.01 ± 0.15	3.37 ± 0.37	2.61 ± 0.05
13i	H	$-CH_2CH_2CH_3$	4-Cl	2.01 ± 0.05	2.04 ± 0.34	2.10 ± 0.09
10d	CH_3		3,4,5-OCH_3	2.04 ± 0.03	2.40 ± 0.02	2.06 ± 0.04
14f	CH_3	$-C_6H_5$	3,4,5-OMe	4.22 ± 0.14	7.84 ± 0.02	4.31 ± 0.02
14g	CH_3	$-C_6H_4(4-CH_3)$	3,4,5-OMe	3.81 ± 0.05	5.95 ± 0.01	2.42 ± 0.04
14h	CH_3	$-C_6H_4(4-Cl)$	3,4,5-OMe	4.71 ± 0.15	8.14 ± 0.02	3.11 ± 0.01
14i	CH_3	$-CH_2CH_2CH_3$	3,4,5-OMe	4.42 ± 0.16	7.40 ± 0.0126	4.10 ± 0.02

[a] Data represent mean values (SD) of three independent determinations.

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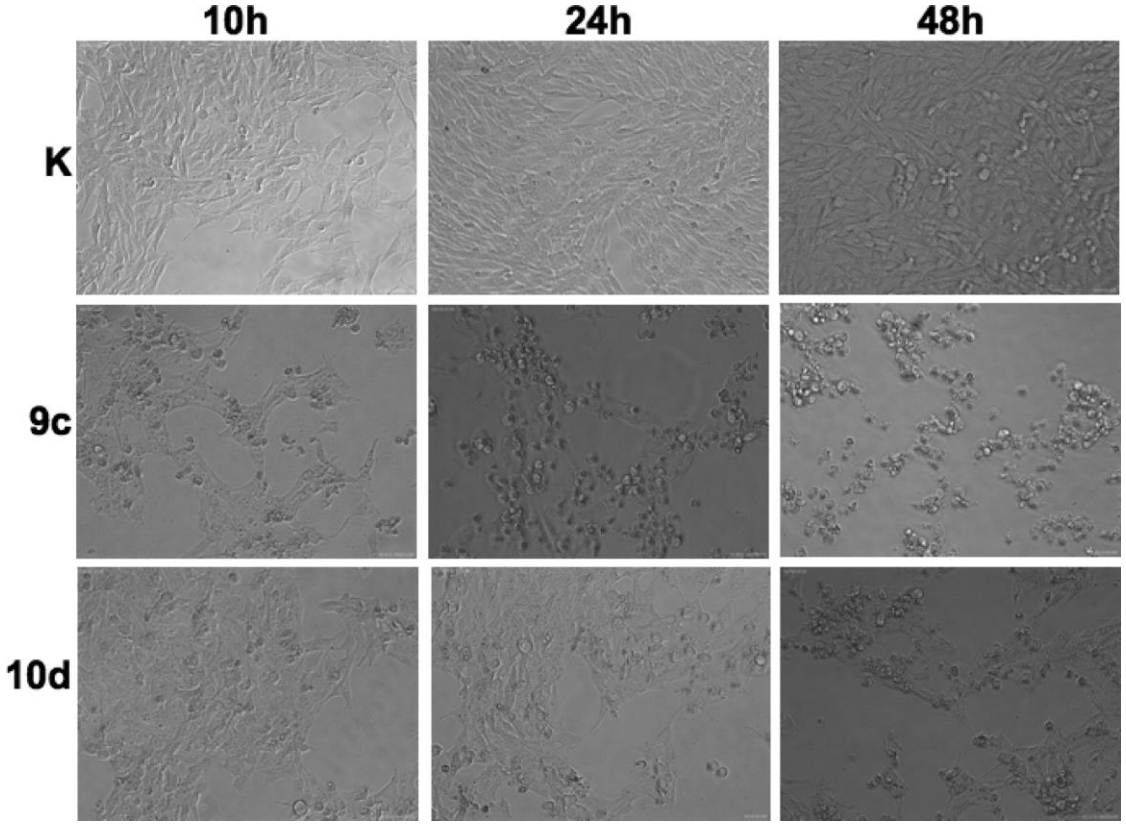
Video Time Lapse Microscopy



treated cells experienced reduced cell division, but what was the cause of the reduced cell division?

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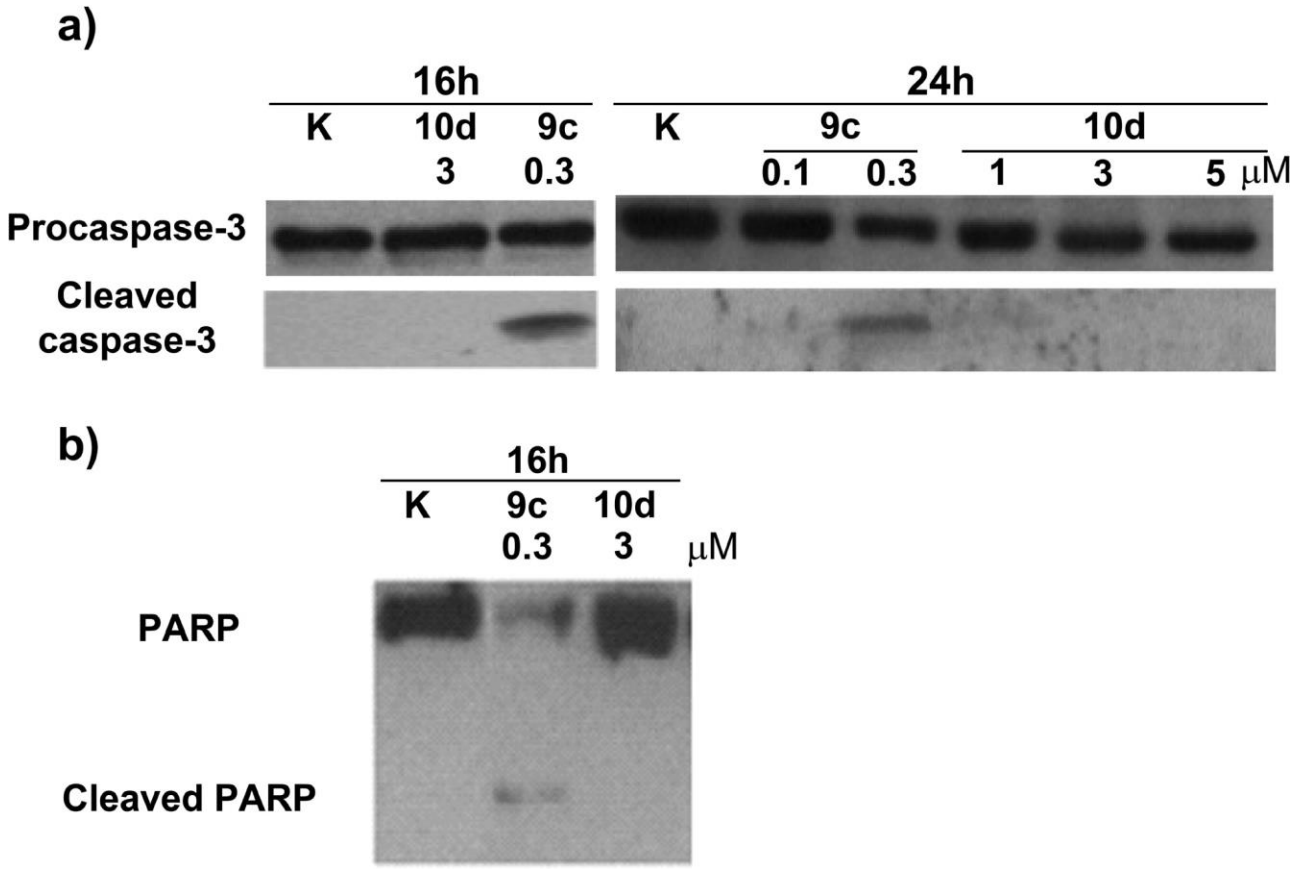
Determining mode of Cytotoxicity



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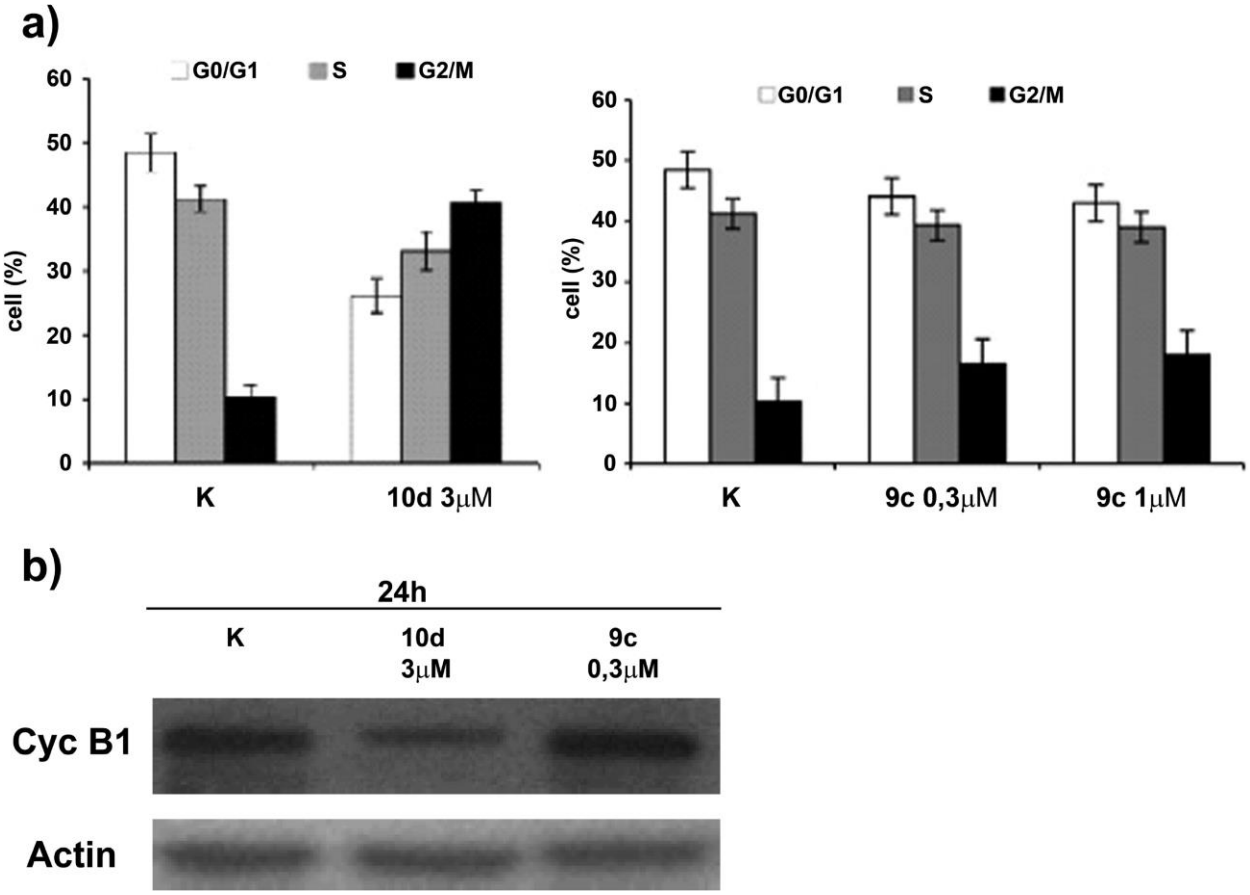
Determining mode of Cytotoxicity



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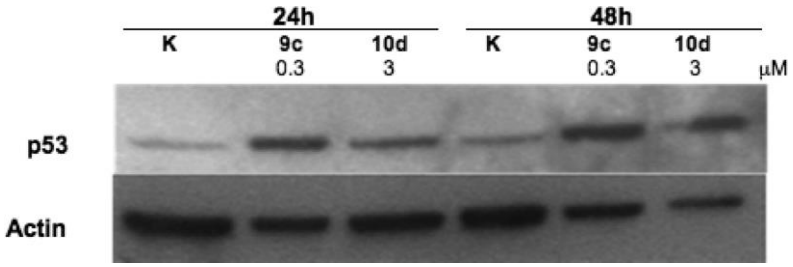
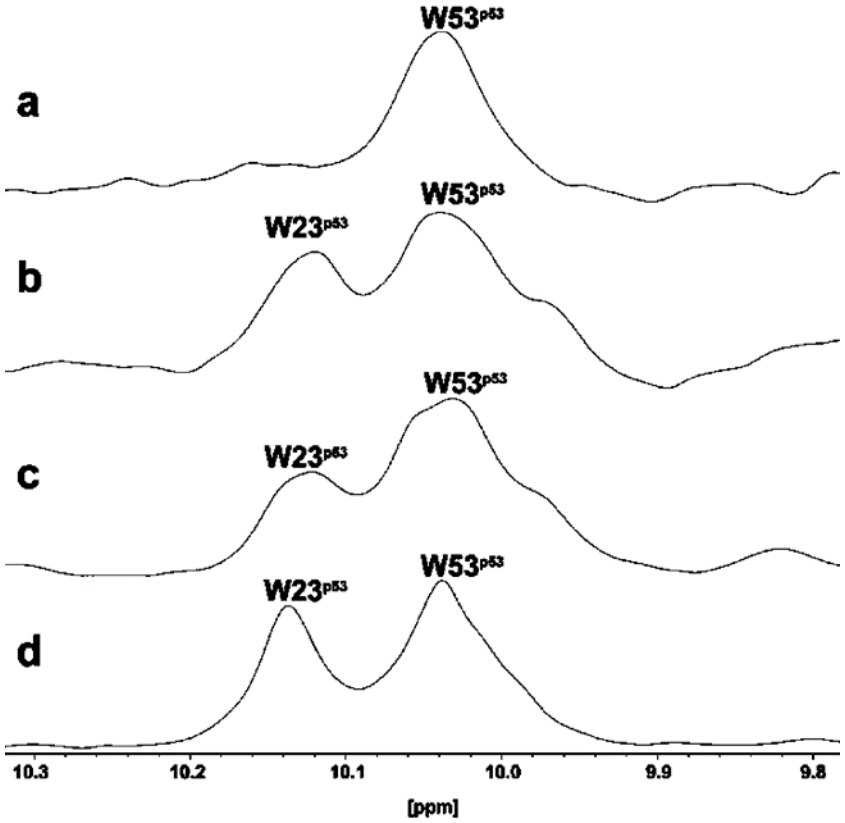
Determining mode of Cytotoxicity



Both treated cells exhibit and increase in p53 concentration



a) p53-MDM2 complex and b) **9c** c) **10d** d) nutlin-3 treated

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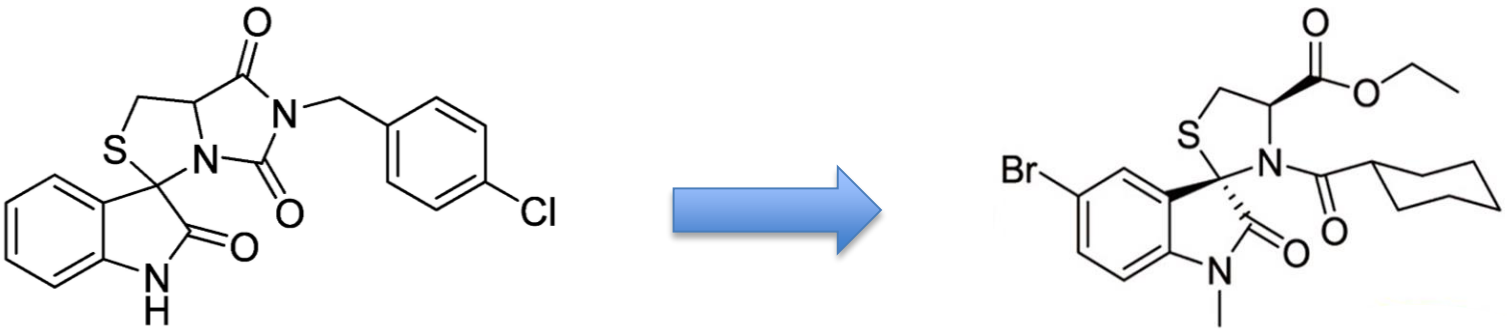
Further Development of **9c**



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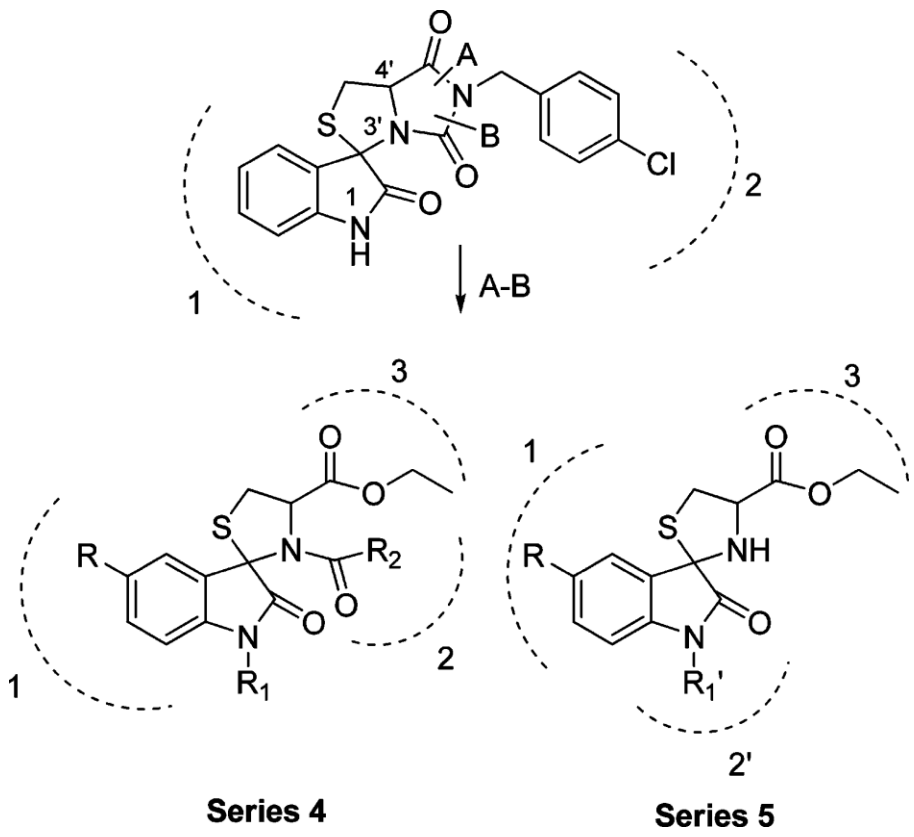
Derivatization



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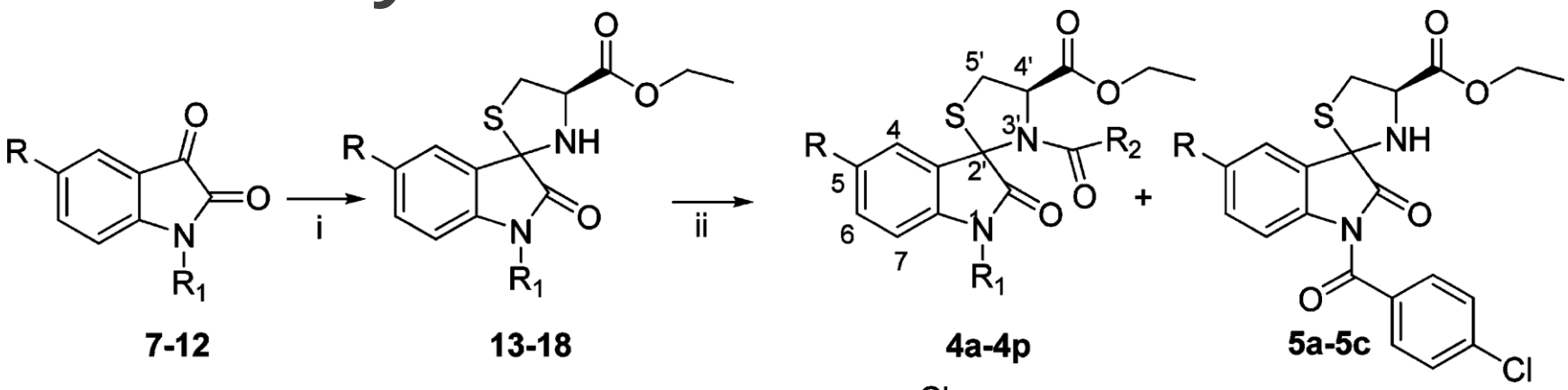
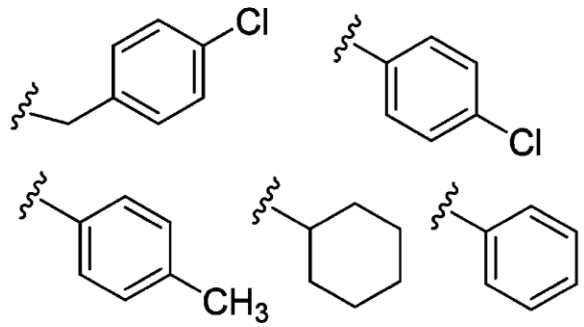
Synthesis of Series 4



7, 13	R = H	R_1=H
8, 14	R = CH_3	R_1=H
9, 15	R = Br	R_1=H
10, 16	R = H	R_1=CH_3
11, 17	R = CH_3	R_1=CH_3
12, 18	R = Br	R_1=CH_3

R_2 =



(i) Cys-OEt, $NaHCO_3$ in EtOH; (ii) R_2-COCl, TEA, THF, 2 h, rt

What about stereoselectivity? => lets look at a similar system

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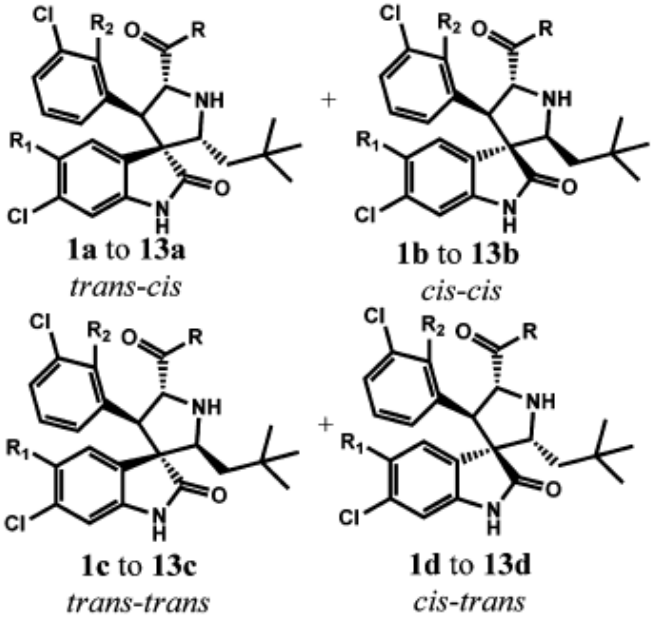
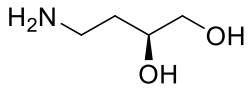
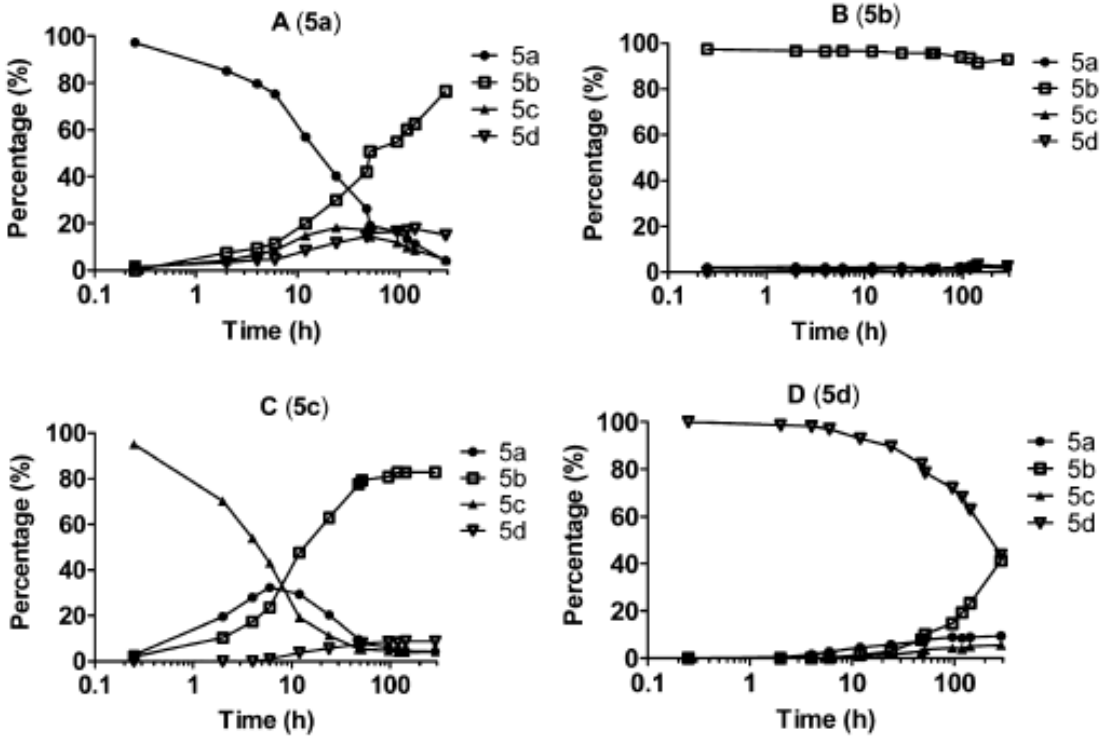
Facile Ring Opening of Spirooxindoles

a look at a similar system



compound **5**: R_1=H R_2=F R_3= 



Yujun Zhao *et al*. *J. Am. Chem. Soc.* **2013**, *135*, 7223 and *J. Med. Chem.* **2013**, *56*, 5553

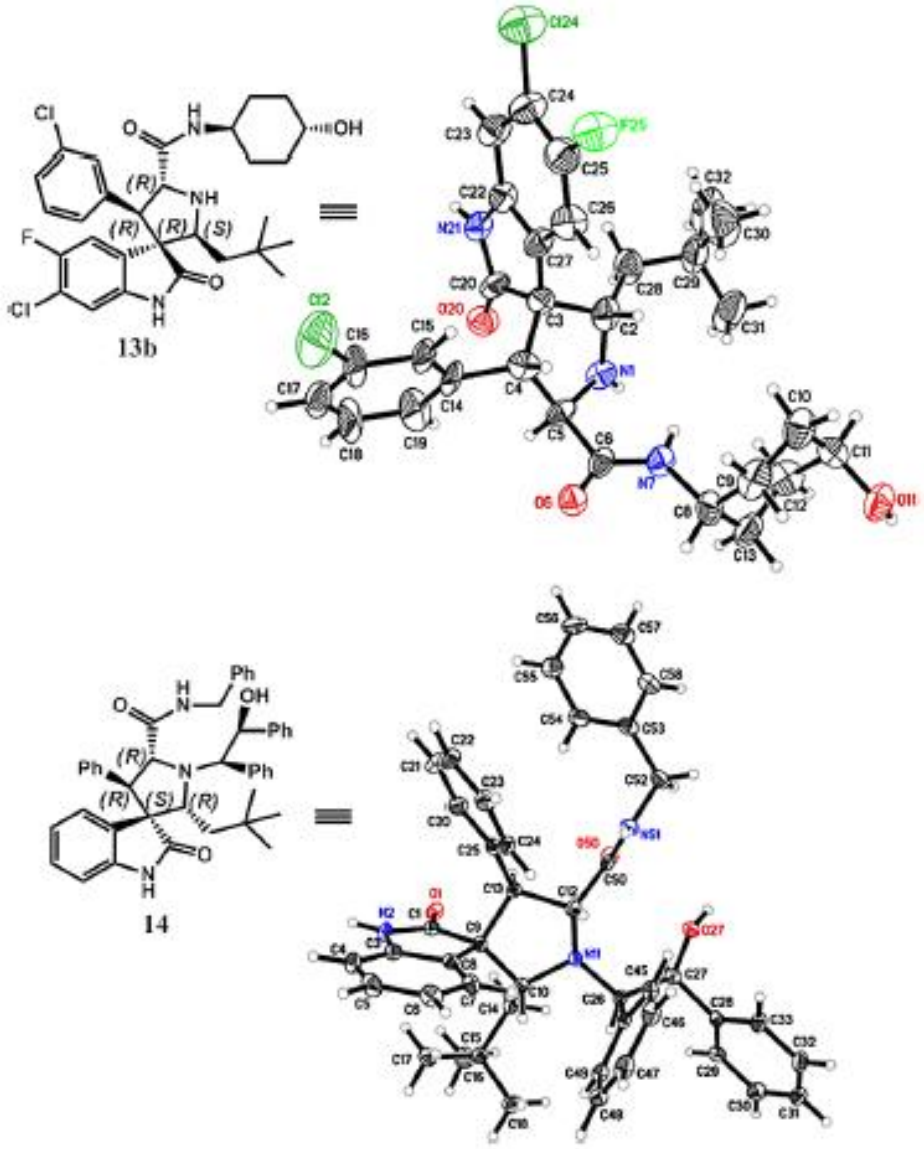


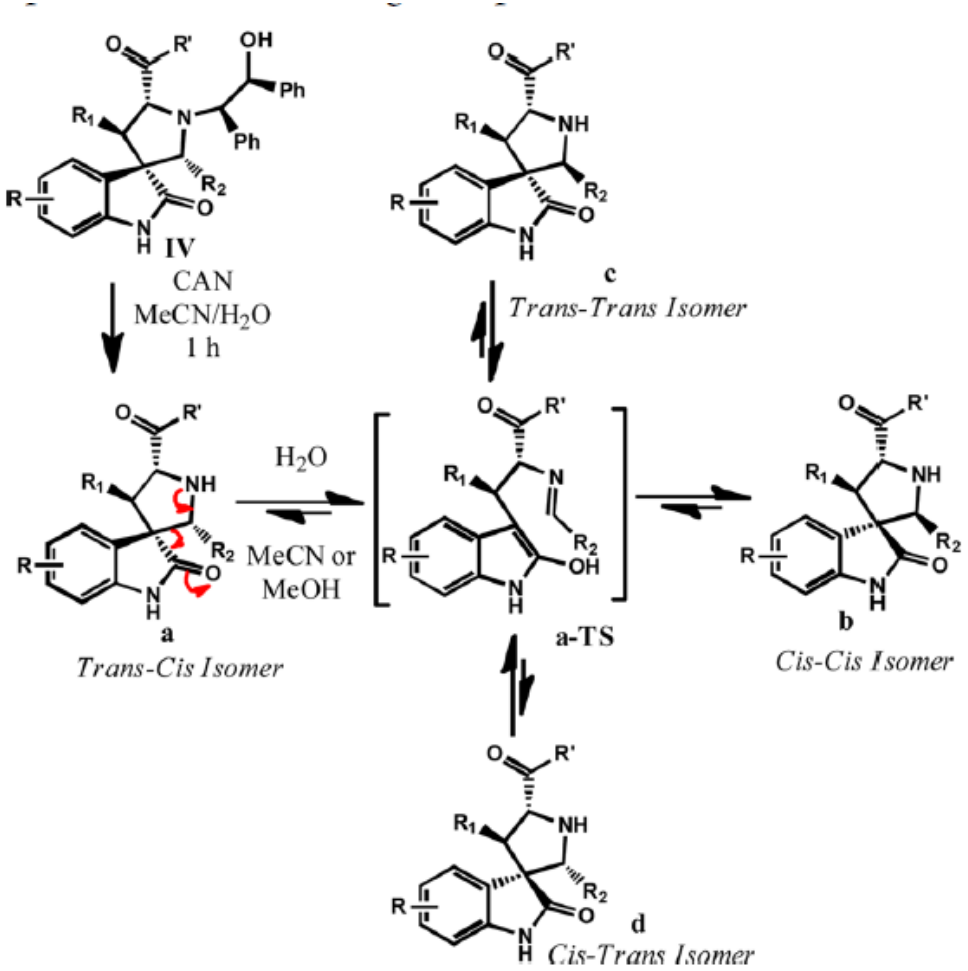
Figure 3. Absolute stereochemistry from X-ray crystallography.

Stable cis-cis isomer

Open ring intermediate

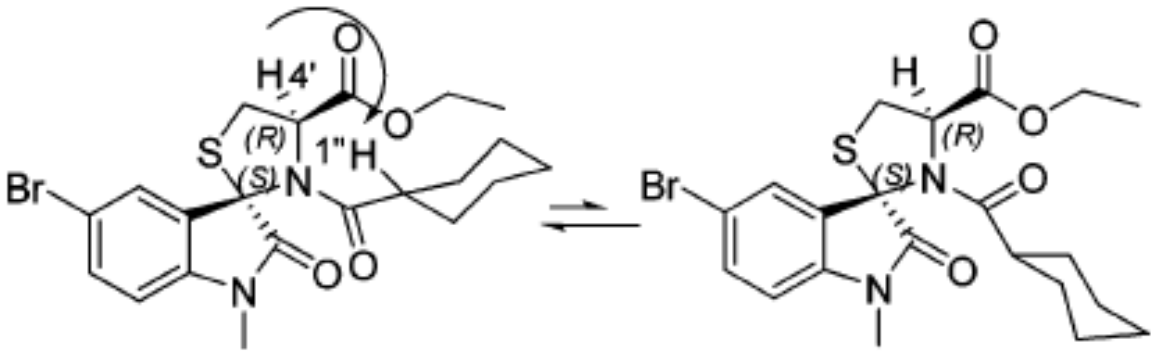
Isomerization Mechanism

a look at a similar system



Yujun Zhao *et al*. *J. Am. Chem. Soc.* **2013**, *135*, 7223 and *J. Med. Chem.* **2013**, *56*, 5553

Stereochemistry



cis isomer
major

trans isomer
minor

Figure 3. ROE interaction observed between H-4′ and H-1″ in the ROESY spectrum of compound **4n**.

Question: Please provide a mechanistic rational for the observed isomerization.

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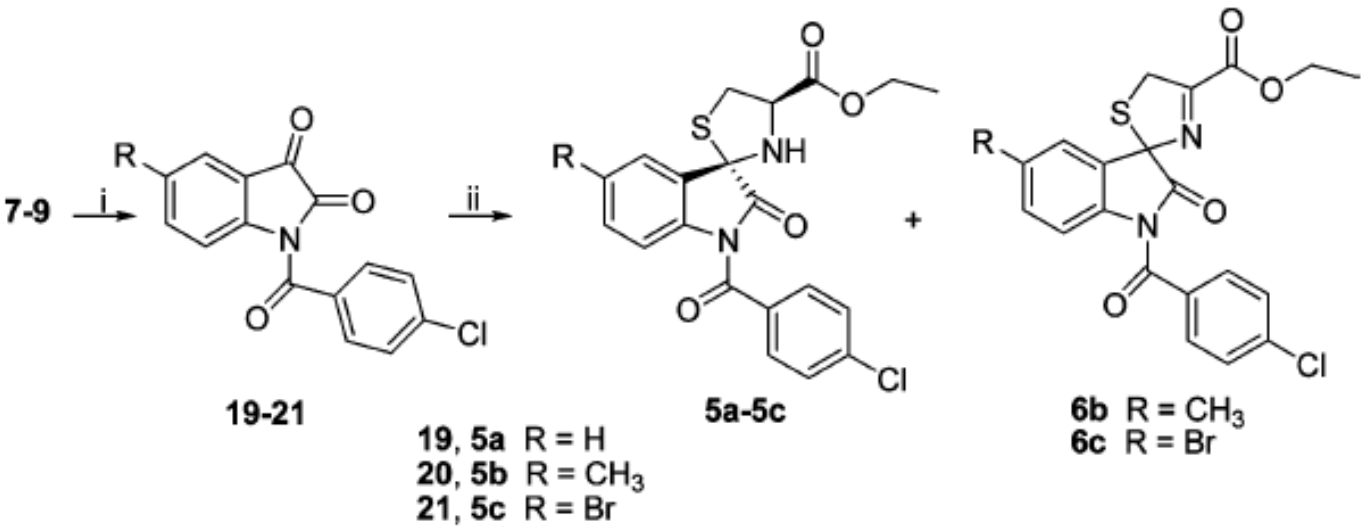
Synthesis of Series 5

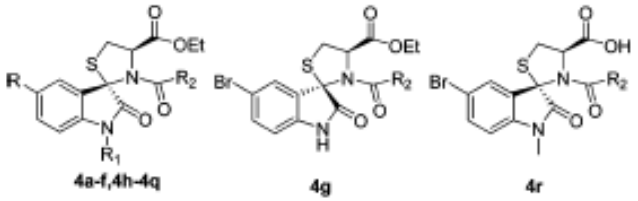


(i) 4-Cl-C_6H_4COCl, TEA, THF, 2h, rt; (ii) Cys-OEt, $NaHCO_3$, EtOH

Bertamino, A. *et al*. *J. Med. Chem.,* **2013**, *56,* 5407

Biological effects

Table 1. Antiproliferative Activity of Spiro[indoline-3,2′-thiazolidine] (4 and 5) and Spiro[indoline-3,2′-thiazole] (6) Derivatives

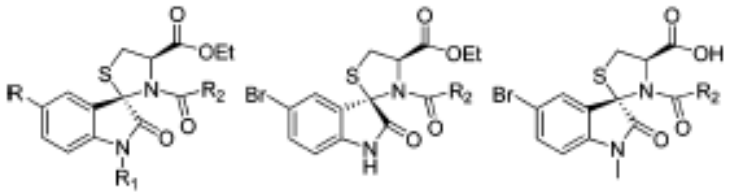


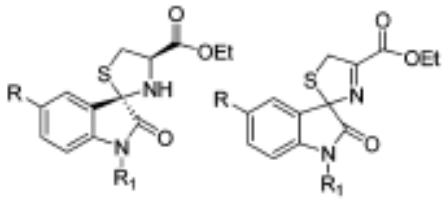
compd	R	R_1	R_2	$IC_{50} \pm SD$ (μM)[a] MCF-7[b]	HT29[c]
3	H	H		1.21 ± 0.6	1.60 ± 0.4
4a	H	H	$CH_2C_6H_4$(4-Cl)	>5	1.00 ± 0.2
4b	CH_3	H	$CH_2C_6H_4$(4-Cl)	4.81 ± 1.0	0.78 ± 0.2
4c	Br	H	$CH_2C_6H_4$(4-Cl)	2.90 ± 0.8	0.66 ± 0.1
4d	H	H	C_6H_4(4-Cl)	2.15 ± 0.7	3.69 ± 0.9
4e	CH_3	H	C_6H_4(4-Cl)	2.12 ± 0.7	1.09 ± 0.6
4f	Br	H	C_6H_4(4-Cl)	0.90 ± 0.2	0.11 ± 0.09
4g	Br	H	C_6H_4(4-Cl)	3.00 ± 0.2	2.00 ± 0.8
4h	H	CH_3	C_6H_4(4-Cl)	4.52 ± 1.1	0.18 ± 0.09
4i	CH_3	CH_3	C_6H_4(4-Cl)	1.23 ± 0.4	0.12 ± 0.07
4j	Br	CH_3	C_6H_4(4-Cl)	0.52 ± 0.3	0.08 ± 0.01
4k	Br	CH_3	$CH_2C_6H_4$(4-Cl)	0.27 ± 0.1	0.36 ± 0.09
4l	Br	CH_3	C_6H_5	0.31 ± 0.1	0.21 ± 0.2
4m	Br	CH_3	C_6H_4(4-CH_3)	0.06 ± 0.05	0.09 ± 0.05
4n	Br	CH_3	cydohexyl	0.04 ± 0.01	0.07 ± 0.01
4o	CH_3	CH_3	cydohexyl	1.20 ± 0.6	1.10 ± 0.6
4p	H	CH_3	cydohexyl	2.30 ± 0.8	1.90 ± 0.8
4q	Br	H	cydohexyl	0.22 ± 0.1	0.56 ± 0.1
4r	Br	CH_3	cydohexyl	2.01 ± 0.9	1.90 ± 0.7

MCF-7: Human breast adenocarcinoma cell. HT29: Human colon carcinoma cell

Biological effects

Table 1. Antiproliferative Activity of Spiro[indoline-3,2′-thiazolidine] (4 and 5) and Spiro[indoline-3,2′-thiazole] (6) Derivatives





compd	R	R_1	R_2	$IC_{50} \pm SD$ (μM)[a] MCF-7[b]	HT29[c]
3	H	H		1.21 ± 0.6	1.60 ± 0.4
5a	H	COC_6H_4(4-Cl)	H	1.01 ± 0.6	1.03 ± 0.8
5b	CH_3	COC_6H_4(4-Cl)	H	3.46 ± 0.9	0.23 ± 0.1
5c	Br	COC_6H_4(4-Cl)	H	0.15 ± 0.1	0.02 ± 0.01
5d	Br	cyclohexyl	H	2.08 ± 0.8	1.40 ± 0.8
6b	CH_3	COC_6H_4(4-Cl)		2.78 ± 0.9	0.21 ± 0.1
6c	Br	COC_6H_4(4-Cl)		0.86 ± 0.4	0.20 ± 0.1
6d	Br	cyclohexyl		1.63 ± 0.6	0.85 ± 0.4

[a]Data represent mean values (±SD) of three independent determinations. [b]Human breast adenocarcinoma cell line. [c]Human colon carcinoma cell line.

MCF-7: Human breast adenocarcinoma cell. HT29: Human colon carcinoma cell

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Hit compound **4n**

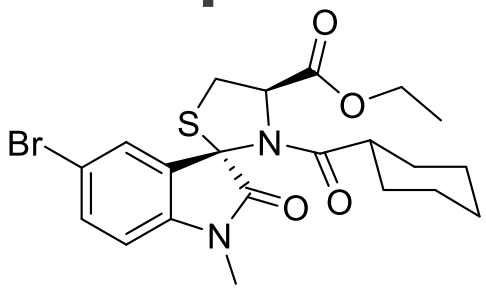

Table 2. Antiproliferative Activity of 4n on Multiple Human Tumor Cell Lines and One Normal Cell Line

		$IC_{50} \pm SD$ (μM)[a]		
	cell line	**4n**	nutlin-3	Dox
origin tumor				
breast	MCF-7	0.04 ± 0.01	2.9 ± 0.31[27a]	0.02 ± 0.01
prostate	PC3	0.41 ± 0.21	30.3 ± 2.9[27b]	0.75 ± 0.10
leukemia	U937	0.07 ± 0.01	15.6 ± 1.9	0.12 ± 0.03
lung	Calu	0.10 ± 0.06	27.2 ± 5.3	1.81 ± 0.33
liver	HEPG2	0.14 ± 0.06	10.2 ± 5.1[27c]	0.08 ± 0.01
anaplastic thyroid	C643	0.55 ± 0.08	23 ± 11.2	0.07 ± 0.01
origin normal				
human gingival fibroblast	HGF	1.60 ± 0.15	1.40 ± 3.6	0.50 ± 0.15

[a]Data represent mean values (±SD) of three independent determinations at 24 h.

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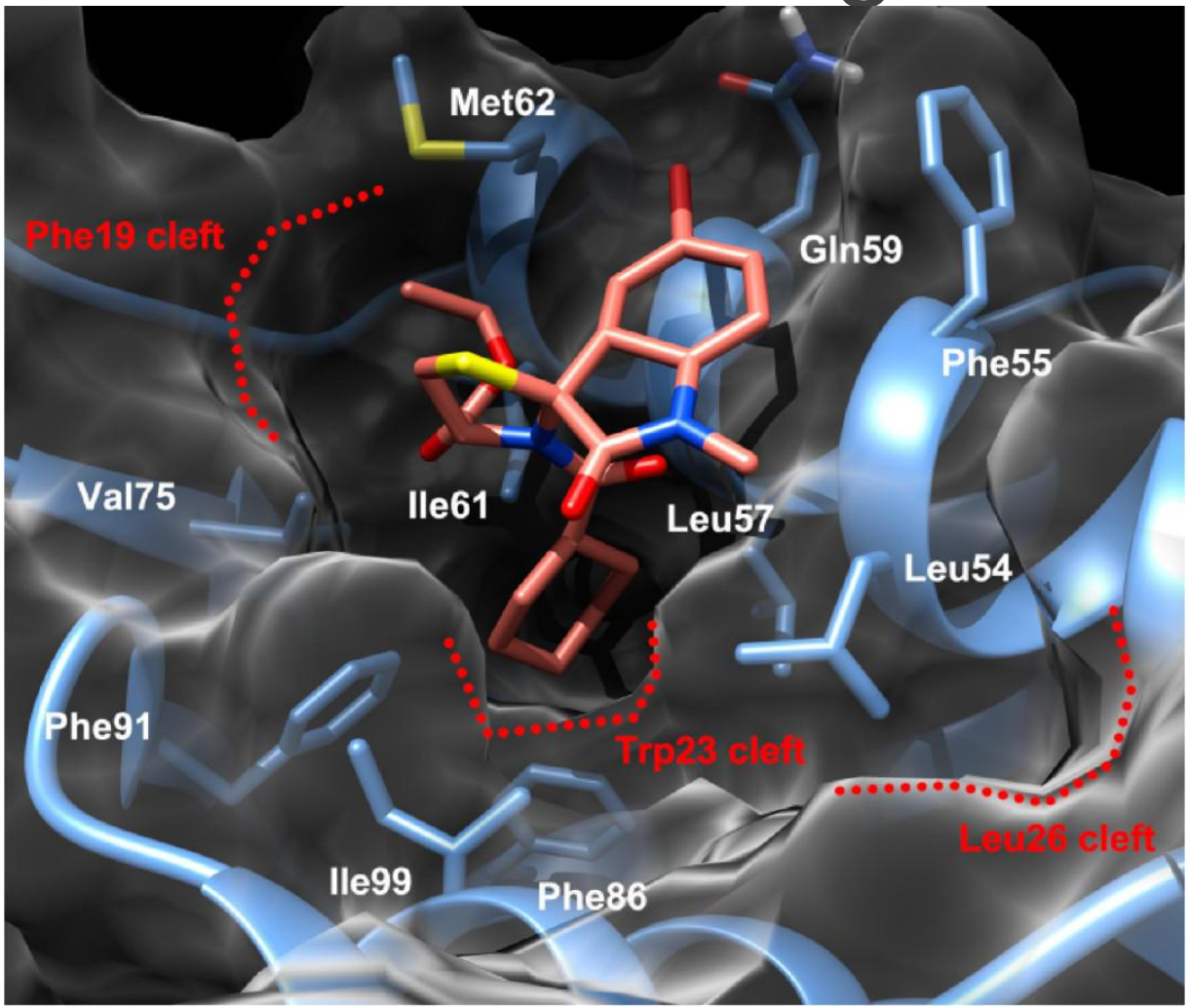
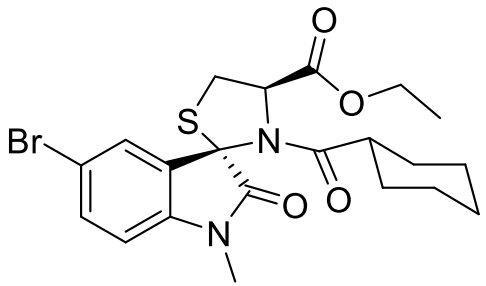
Binding Model





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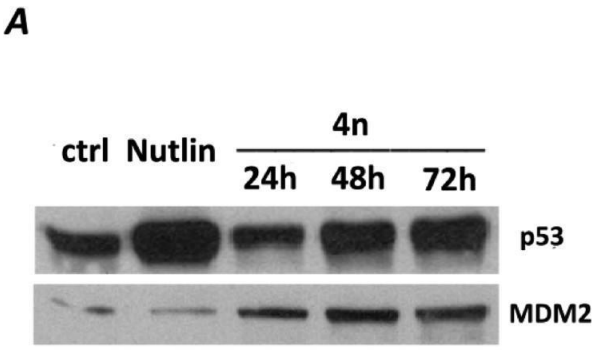
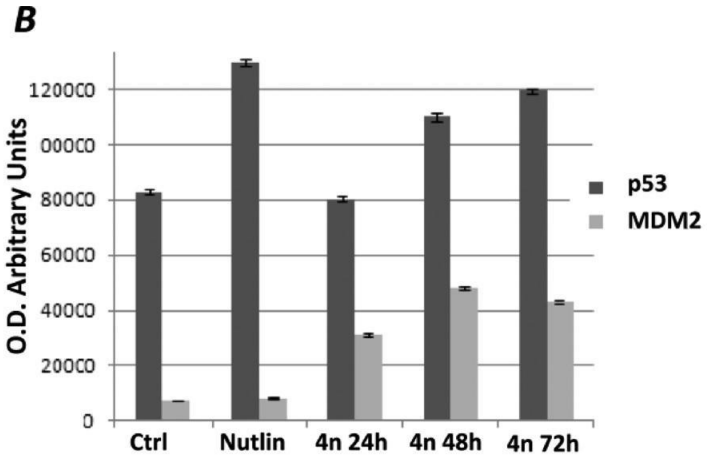
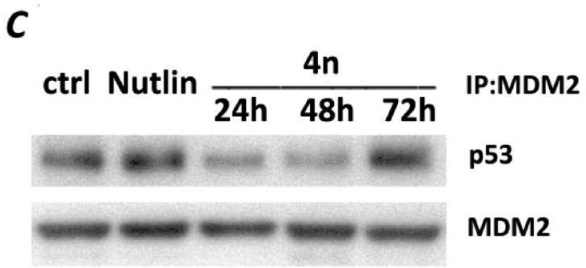
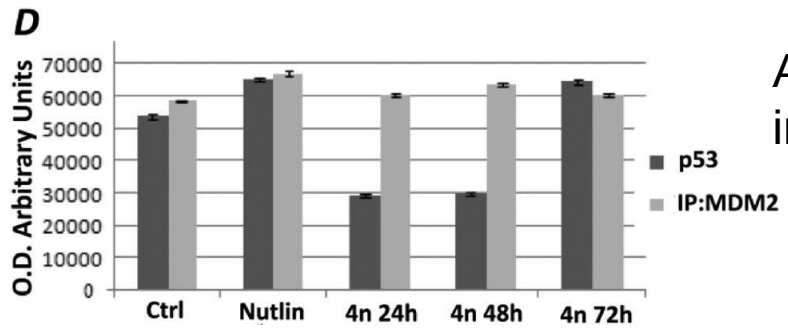
Determination of p53 levels





Total cell lysates





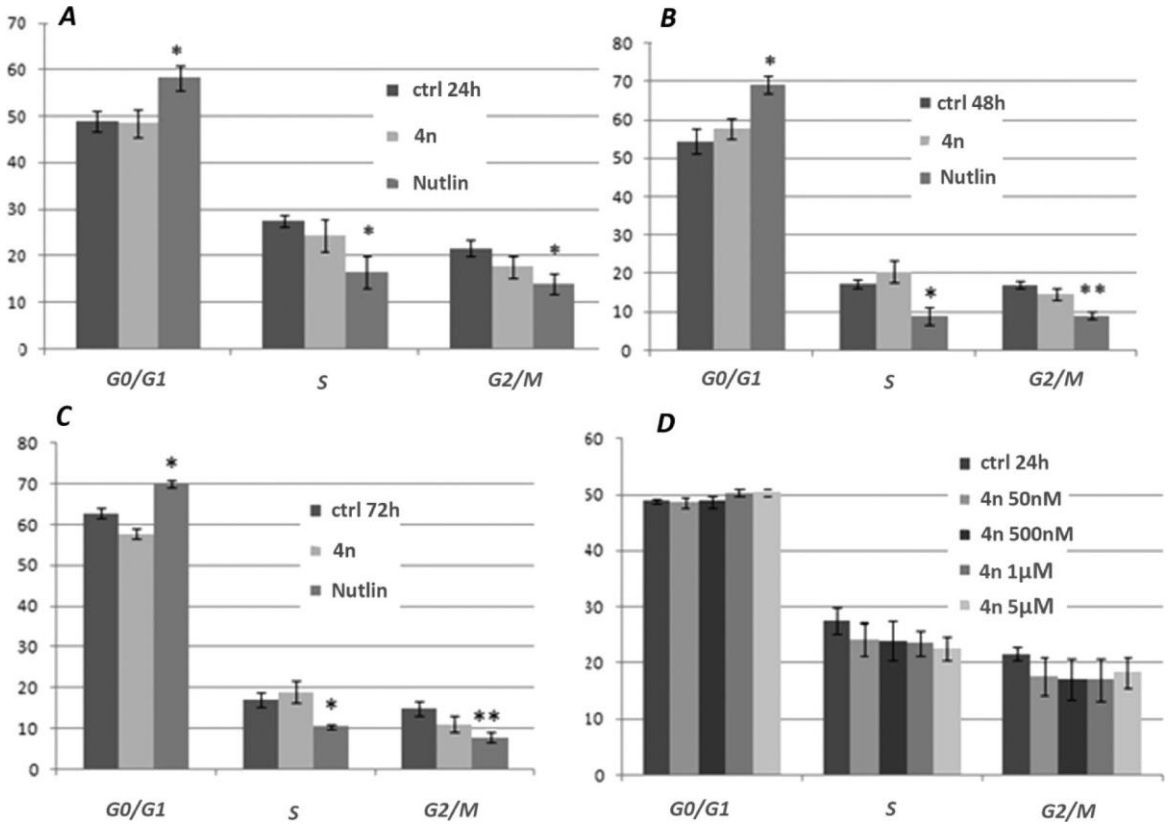
After immunoprecipitation

Western blot

Quantification of immunoblots

Bertamino, A. *et al*. *J. Med. Chem.*, **2013**, *56,* 5407

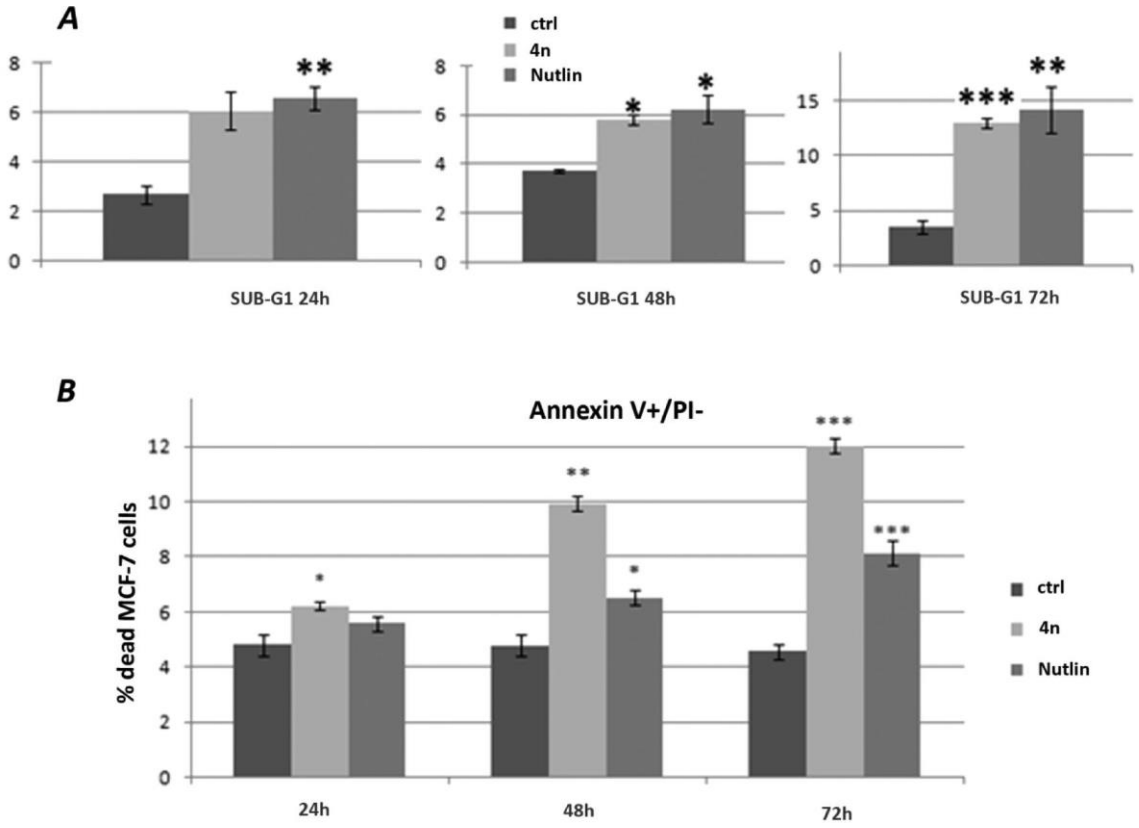
Cell cycle progression



a) 24 hr b) 48hrs c)72 hrs d) 24 hr and varying concentration

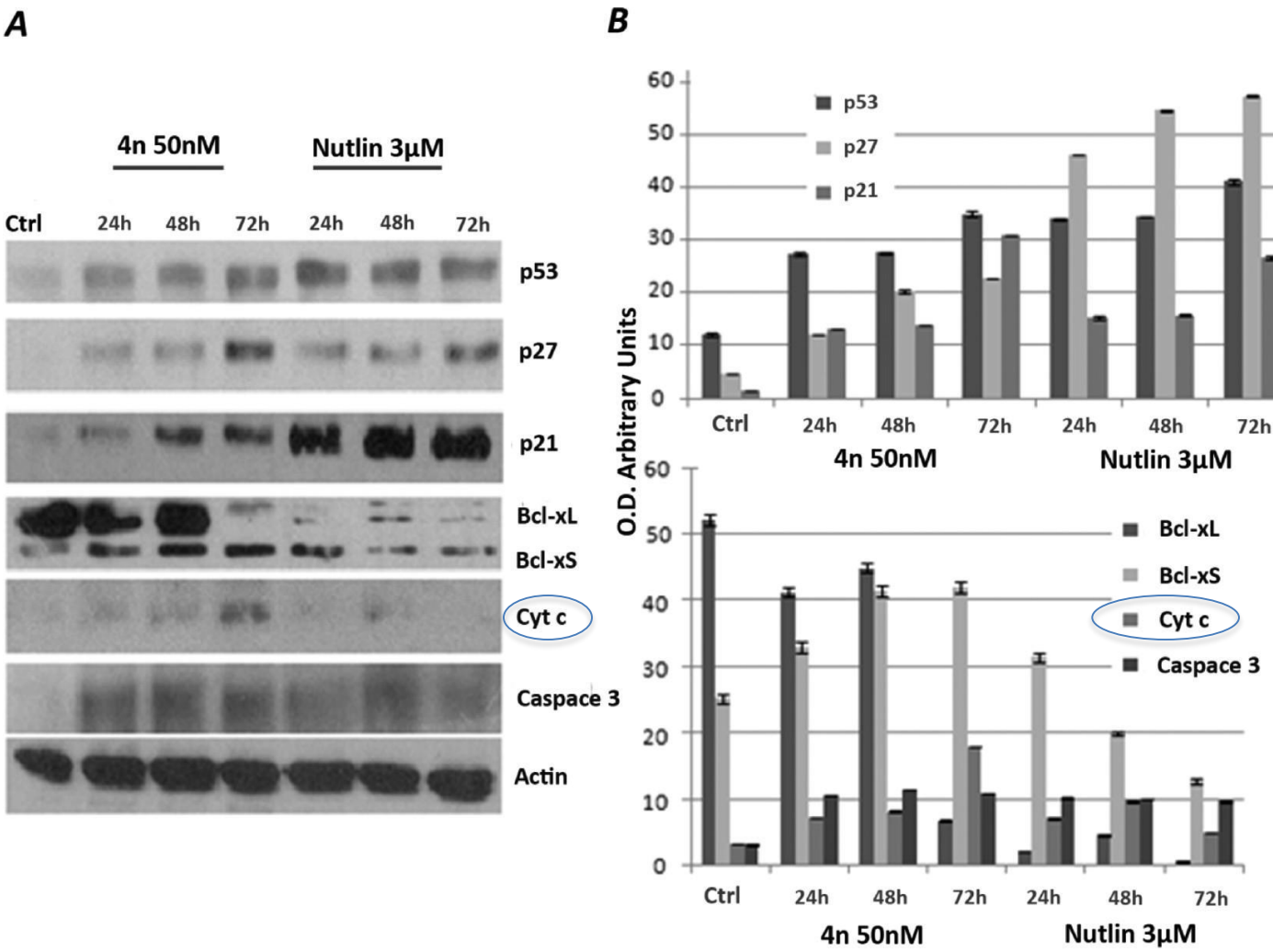
Bertamino, A. *et al*. *J. Med. Chem.,* **2013**, *56,* 5407

Apoptotic cell death



Bertamino, A. *et al*. *J. Med. Chem.,* **2013**, *56,* 5407

Mechanism of apoptosis



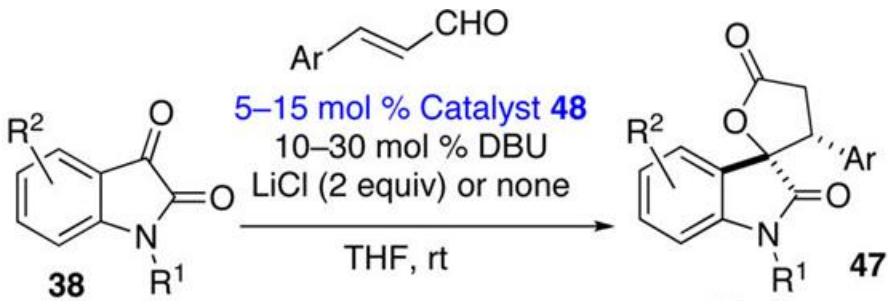
Bertamino, A. *et al*. *J. Med. Chem.,* **2013**, *56,* 5407

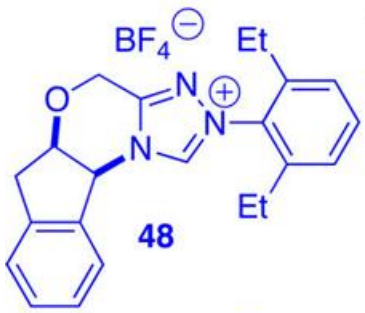
Summary

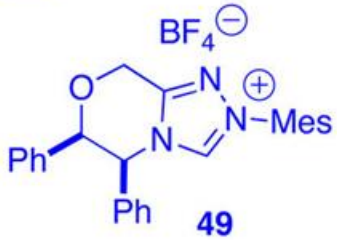
- Examples of spirocycle formation/synthesis
- Examples of thiazolidine formation/synthesis; a reoccurring and potent moiety
- Biological Aside
  - western blot, cell cycle, and P53-MDM2 complex
- Specific thiazolidine drug example
  - 4n high efficiency in breast, colon, lung, and leukemia cancer cell lines
  - Docking studies allowed to predict bind mode
  - 4n induces apoptosis

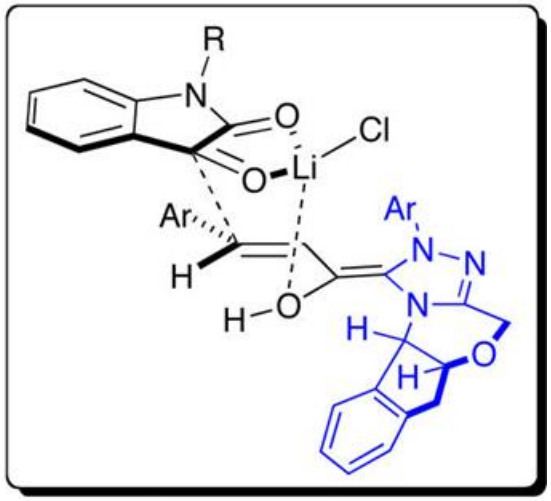
THANK YOU

Question 1





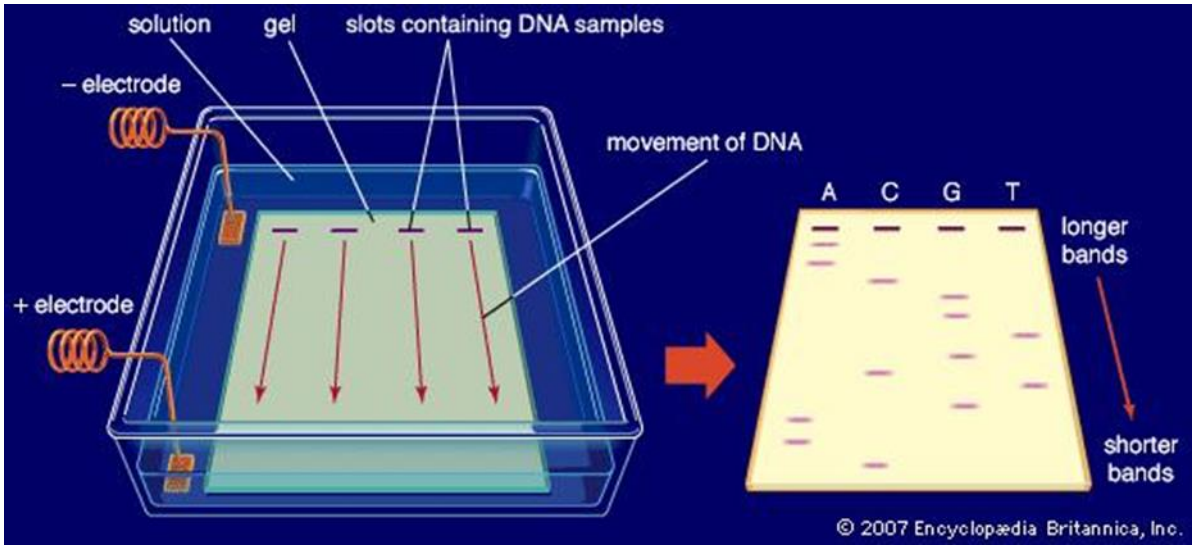




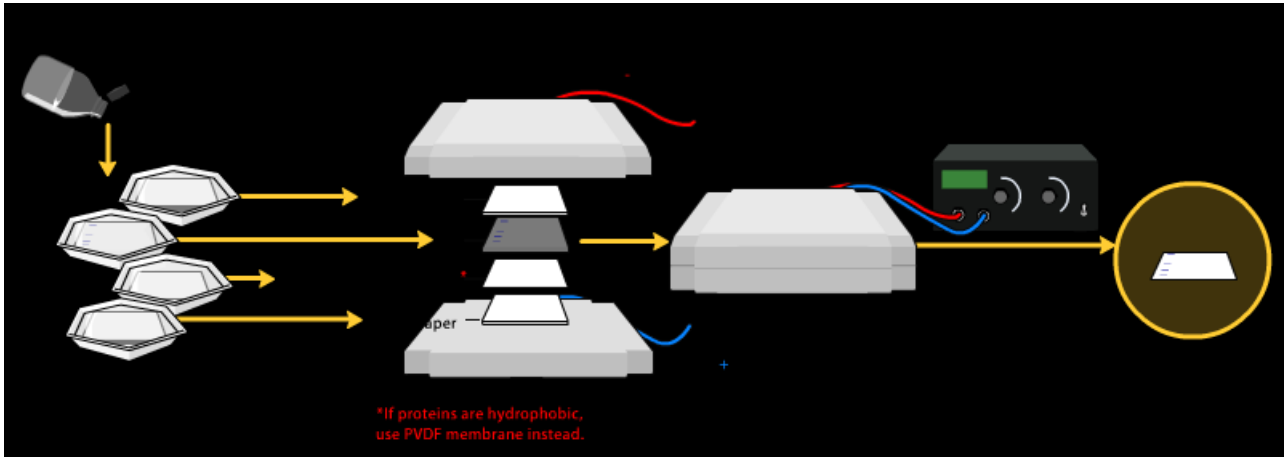
Stereochemical model giving rise to major diastereomer

Question 2: Western Blotting

1) Cell lysis and separation to extract protein
2) Gel Electrophoresis
   - Charged molecules are separated according to physical properties



Question 2: Transfer Method: Blotting

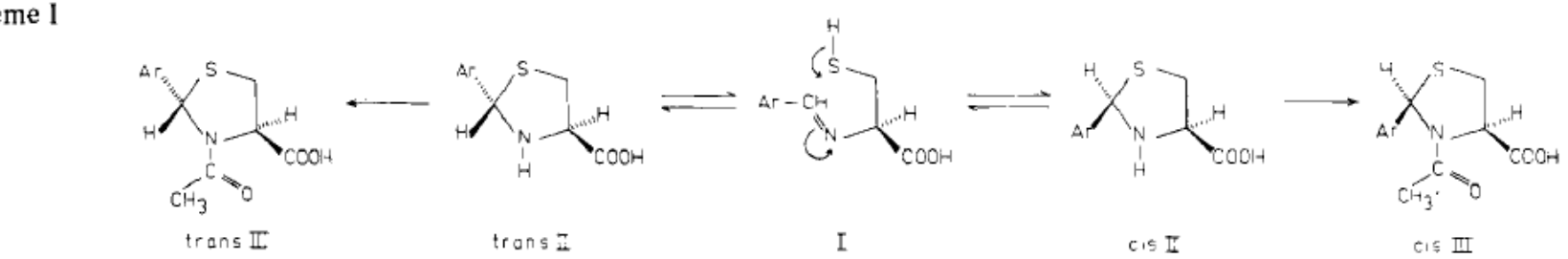


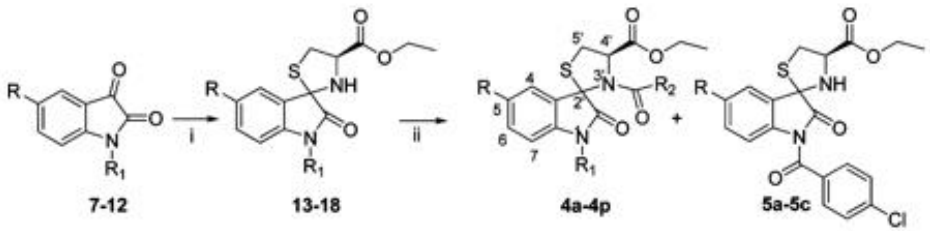
3) Blotting

- proteins are transferred to a membrane via electroelution.
- Nonspecific sites of the remaining membrane surface are blocked
- Proteins are labeled for detection

Question 3

Scheme I





(i) Cys-OEt, $NaHCO_3$ in EtOH; (ii) R_2-COCl, TEA, THF, 2 h, rt

Observed isomerization:

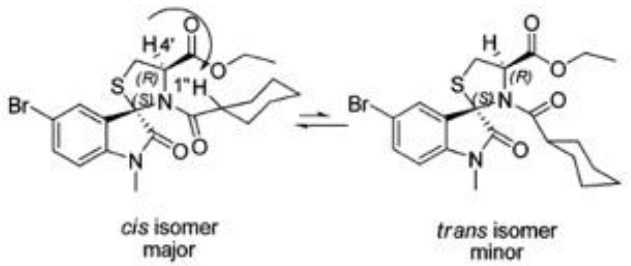
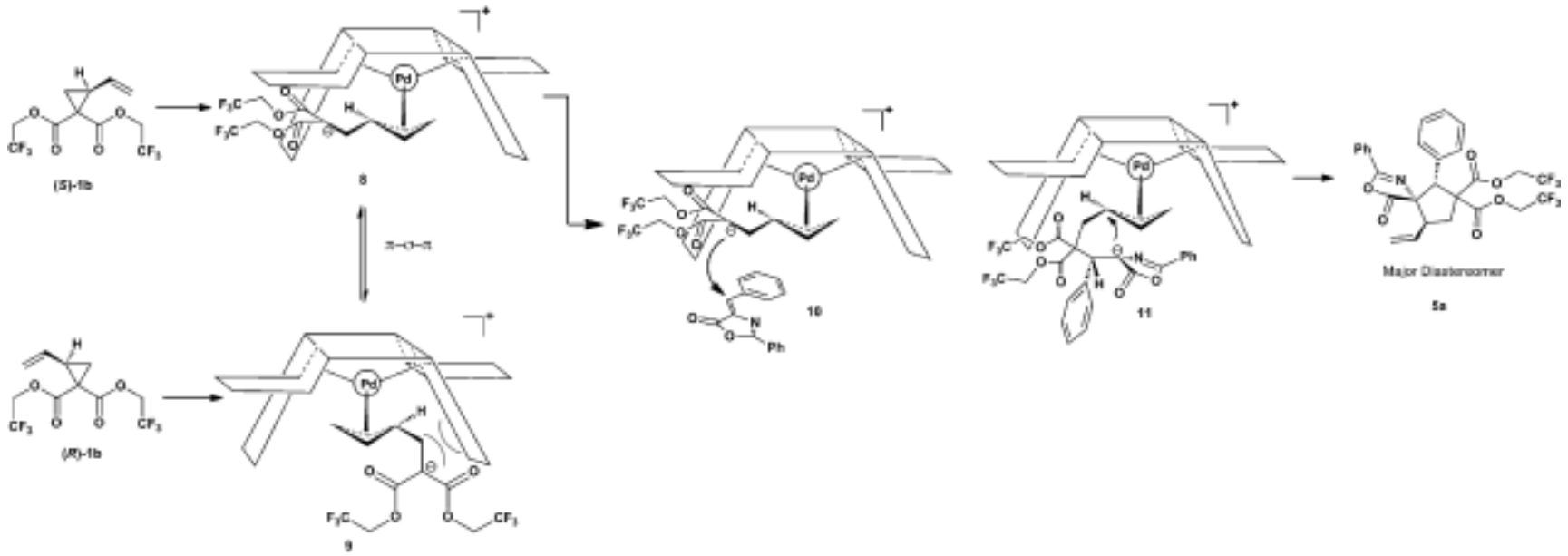


Figure 3. ROE interaction observed between H-4′ and H-1″ in the ROESY spectrum of compound 4n.

"Base-catalyzed isomerization of thiazolidines through Schiff Base intermediates (I) is a well-known process"

Trost, B.M. *et al*. *Angew. Chem. Int. Ed.,* **2011**, *50,* 6167

Wall and Flap Model



Trost, B.M. *et al*. *Angew. Chem. Int. Ed.,* **2011**, *50,* 6167