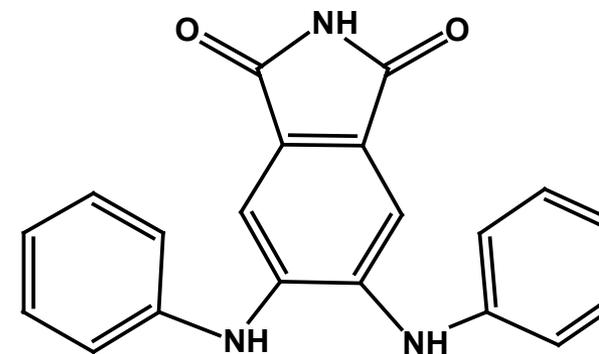
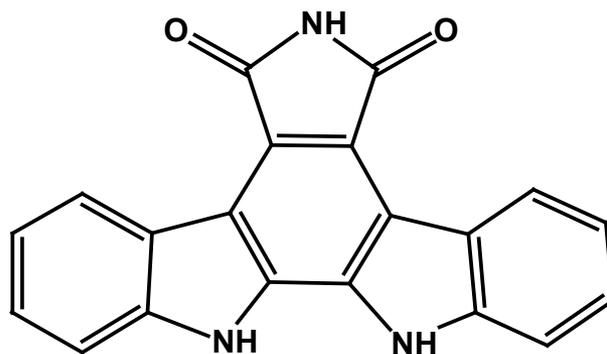
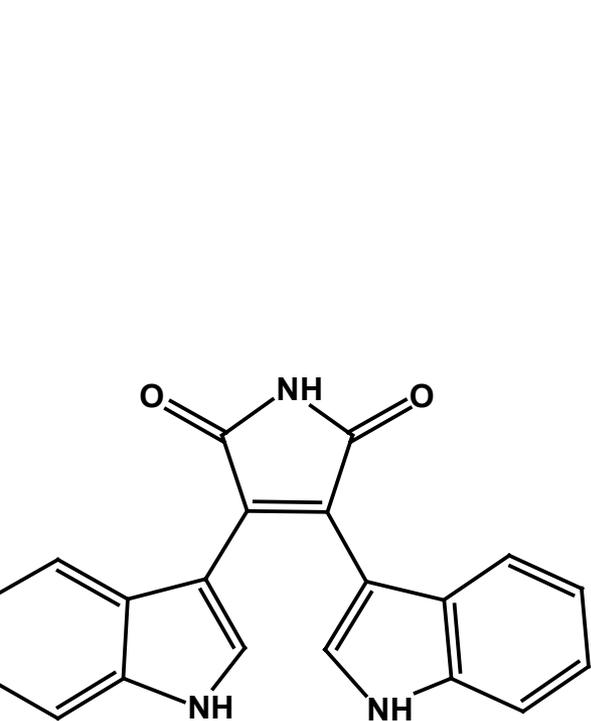


The Staurosporin Nucleus; A Molecule for All Diseases



David J. Newman

Natural Products Branch

Developmental Therapeutics Program, NCI

Staurosporins

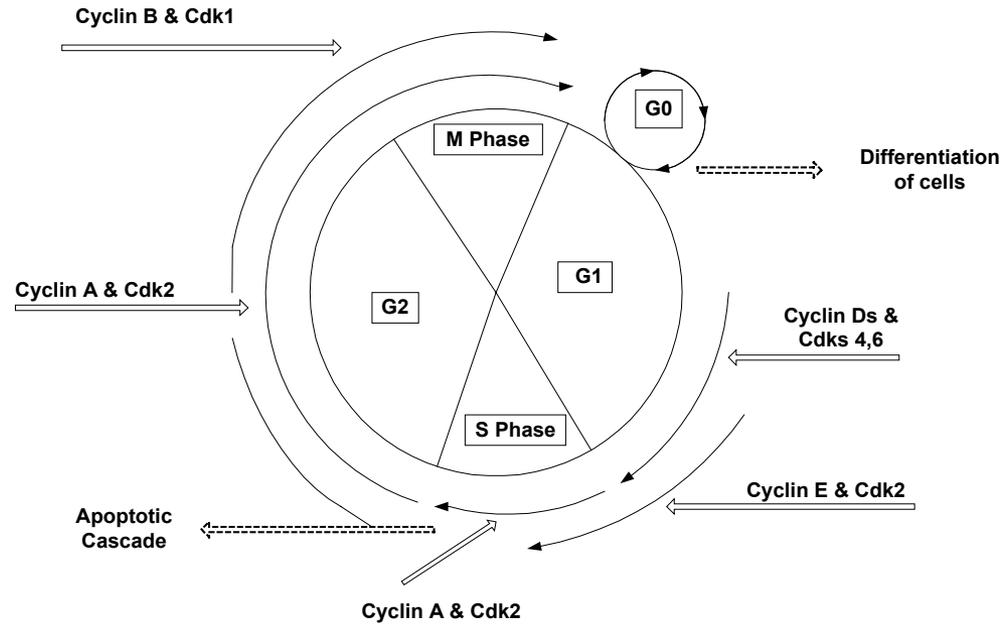
Originally isolated from a *Streptomyces* in 1977 by Omura's group as an antifungal agent and then reisolated as a PKC inhibitor in 1986 with nanomolar activity.

This discovery led to the synthesis of a significant number of compounds, both as the ring-closed planar molecules of the parent structure and of the bis-indolyl derivatives minus the bridging C-C bond.

Two of the latter type, Ro31-8220 & Go 6850 (GF109203X) were used as biological probes but were then found to be pan-PK inhibitors.

Meanwhile, in 1987, Kyowa Hakko scientists isolated UCN-01 (and the isomer, UCN-02) from a *Streptomyces* using a PKC inhibition assay with an IC₅₀ in the 4 nM range.

Cell Cycle (idealized)



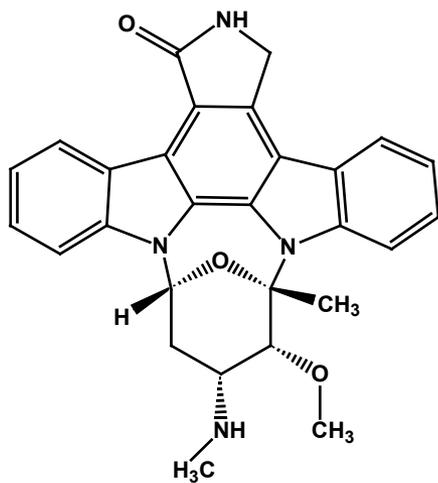
Activities

Cdk Inhibitors
 Hsps
 ChkPt Inhibitors
 Topoisomerase I
 Topoisomerase II
 Tubulin Interactives
 Actin Inhibitors
 Phosphatase Inhibitors (Cdc25's)
 Phosphokinase C Inhibitors
 PI3K Inhibitors

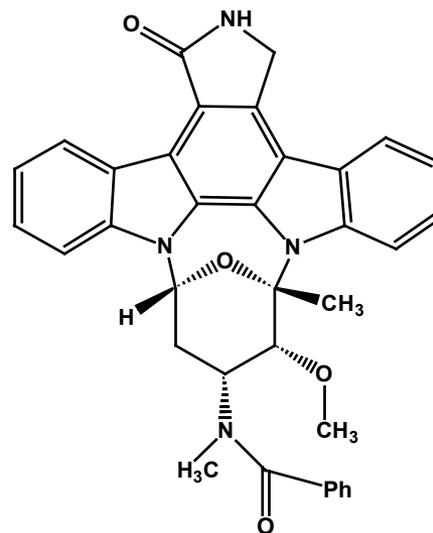
Sites

G1, S, G2
 G1
 G1, G2
 S
 S, G2
 M
 M
 G1, S, G2
 G1, G2
 G1, S?, G2?

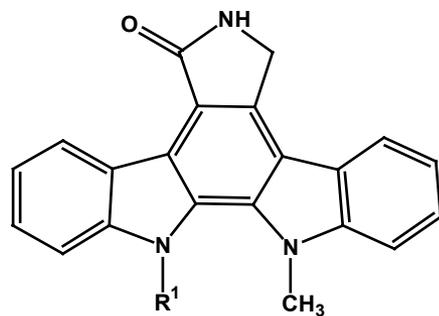
PKC Inhibitors



Staurosporin

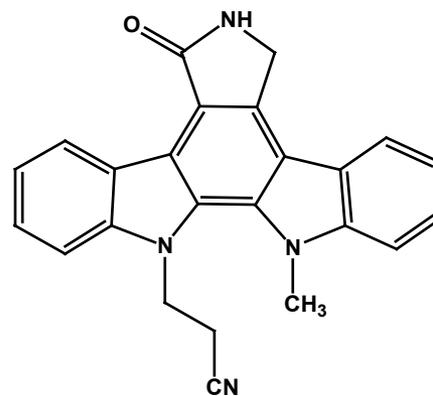


CGP41251



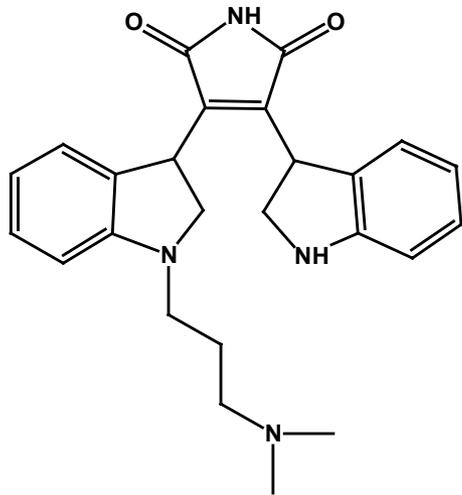
Go 7612 R¹ = -CH₂CH₂CH₂CN

Go 7874 R¹ = -CH₂CH₂CHOHCH₂N(CH₃)₂

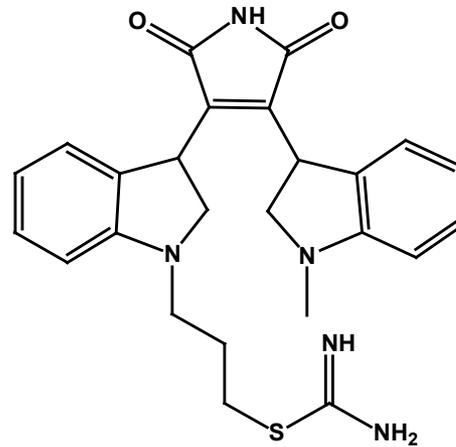


Go 6976

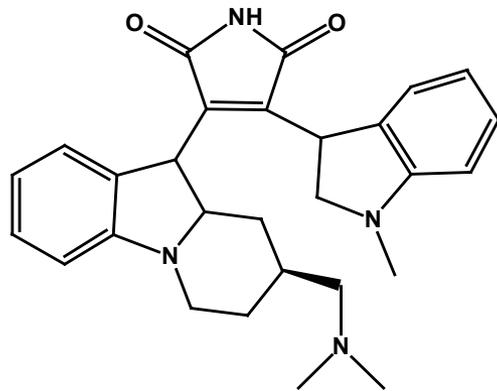
Bis-Indolyl Derivatives



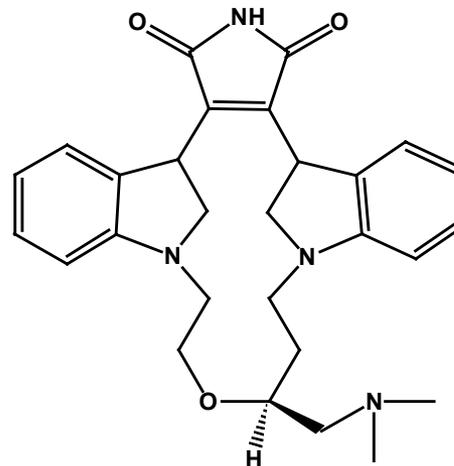
GF 109203x



Ro31-8220

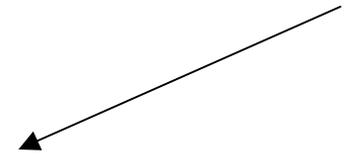


Ro32-0432



LY 333531

Phase III Diabetic retinopathy

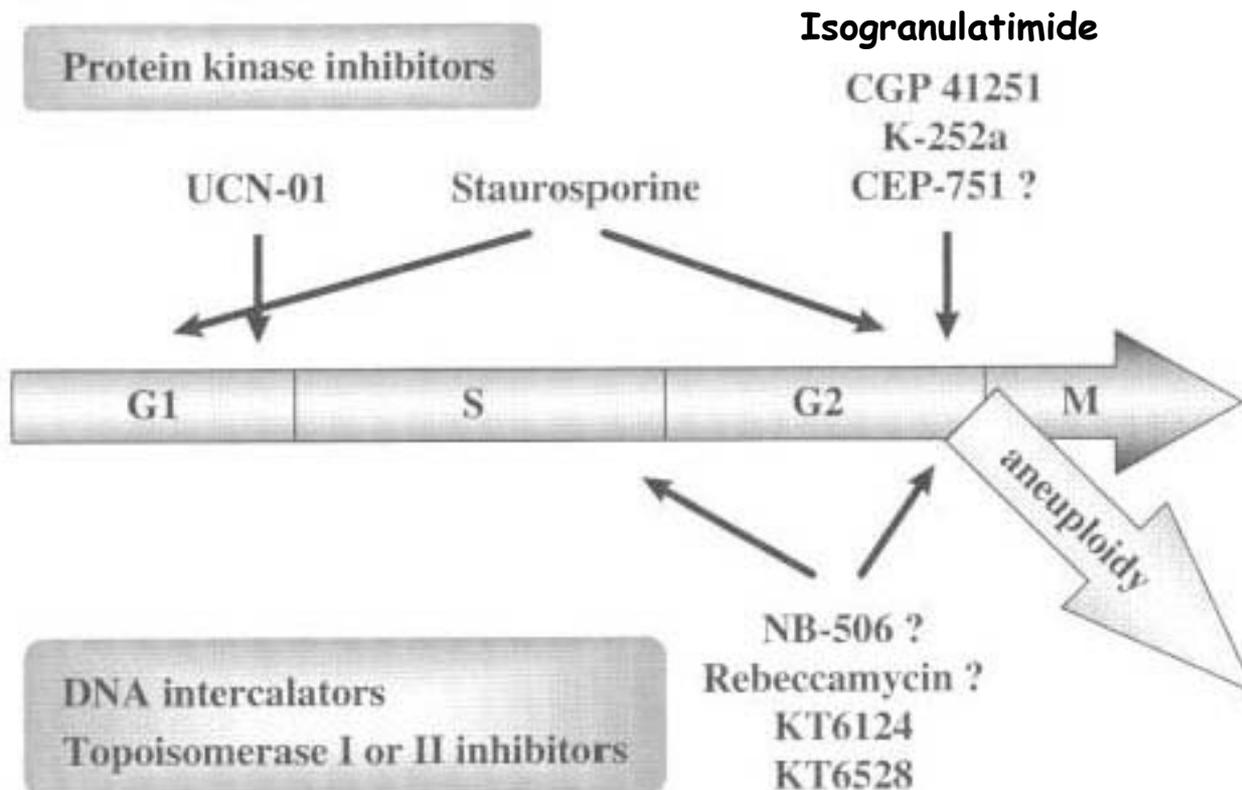


Protein Kinase Inhibition Data

PK	Stauro	CGP41251	Go 6976	Go 7612	Go 7874	Ro 31-8220	Ro 32-0432	GF-109203X	LY333531
PKC Mixed	9	50	20	2	4	23	21	30	6-10000
PKA	40	2400	>10000	400	500	1500	22375	2000	>100000
PK	3	48					15625		
S6K	5	5000				15		100	
PKC A	3		2.3			24	28	14	360
PKC B1	9		6.2			24	28	18	4.7
PKC D	27		>10000					210	250

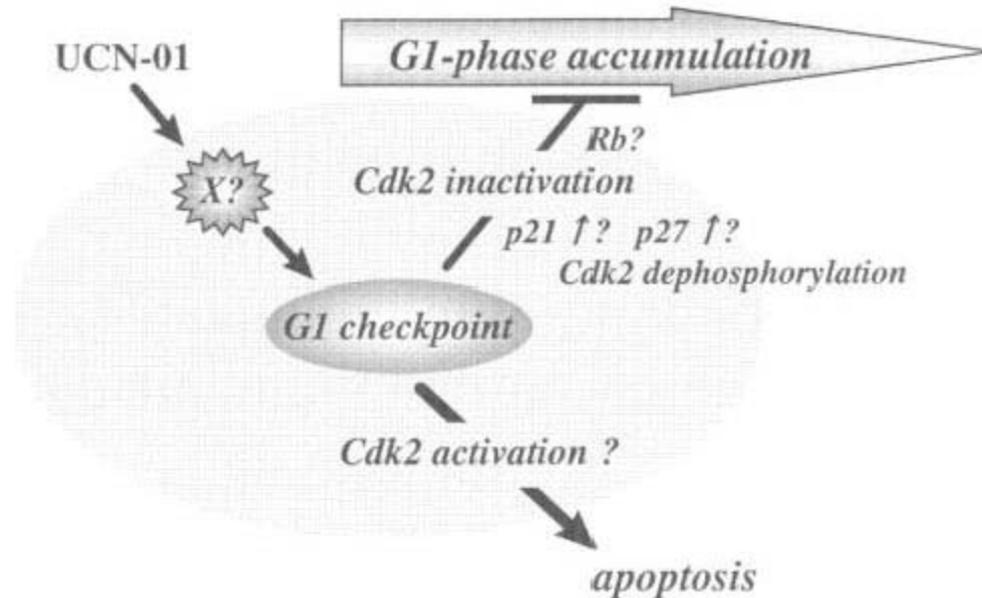
All figures are IC50s in nM

Potential "Action Sites" in Cell Cycle



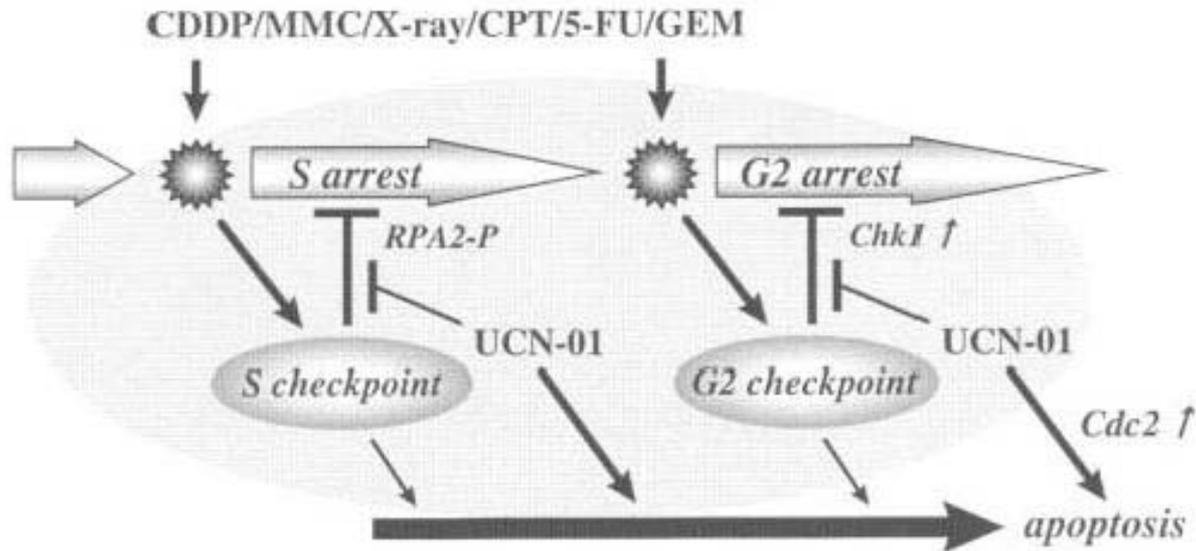
Action points of indolocarbazole compounds on the cell cycle.

UCN-01 As A Single Agent



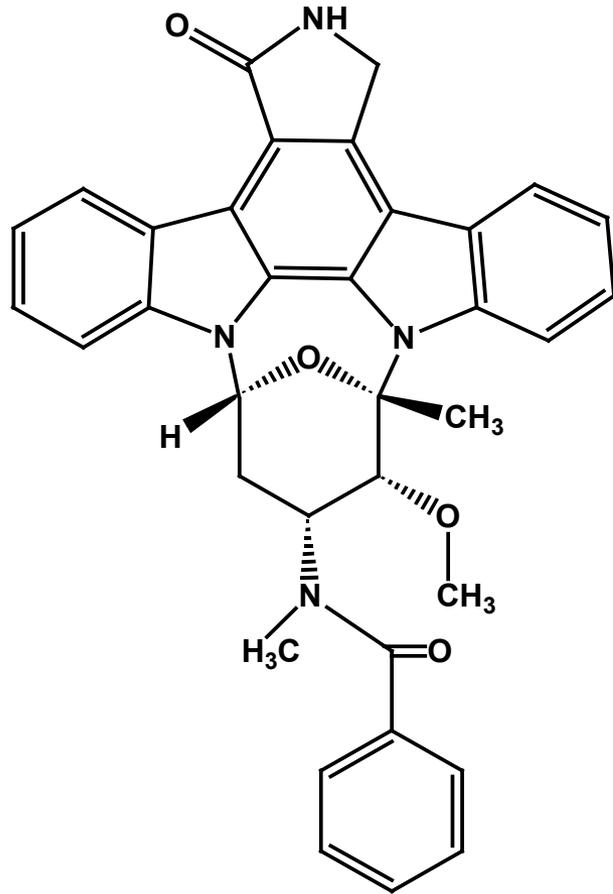
Putative mode of action of UCN-01 as a single agent. If the cellular G₁ checkpoint function is normal or the dose of UCN-01 is low, UCN-01-treated cells will arrest at the G₁ phase. If the cellular G₁ checkpoint function is abrogated or the dose of UCN-01 is high, UCN-01-treated cells will override the G₁ checkpoint and undergo apoptosis.

UCN-01 As A "Sensitizer"



Putative mode of action of UCN-01 as a sensitizer for DNA-damaging agents and anti-metabolite drugs. When cells are exposed to a DNA-damaging agent or an anti-metabolite drug, the S or G₂ checkpoint function will be activated and the damage will be repaired by pausing the cell cycle at the S or G₂ phase. UCN-01 abrogates this S or G₂ arrest through putative inhibition of checkpoint kinase(s), thus leading the cells to cell death or apoptosis.

PKC412; A Clinically-active Kinase Inhibitor



PKC 412 or CGP41251

Potentiates clinically active cytotoxins

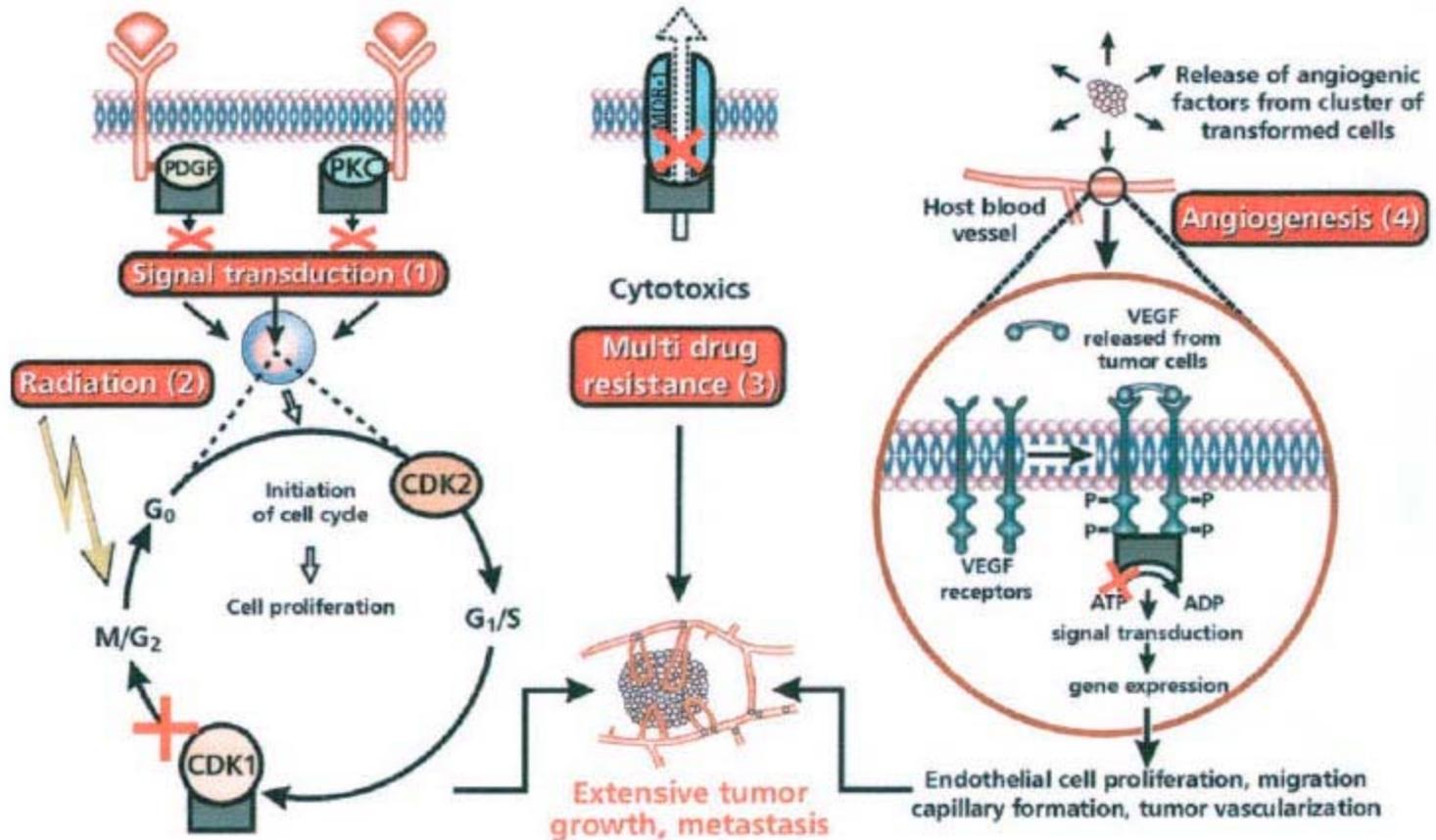
May inhibit VEGF-dependent angiogenesis

Orally active as an inhibitor of retinal neovascularization

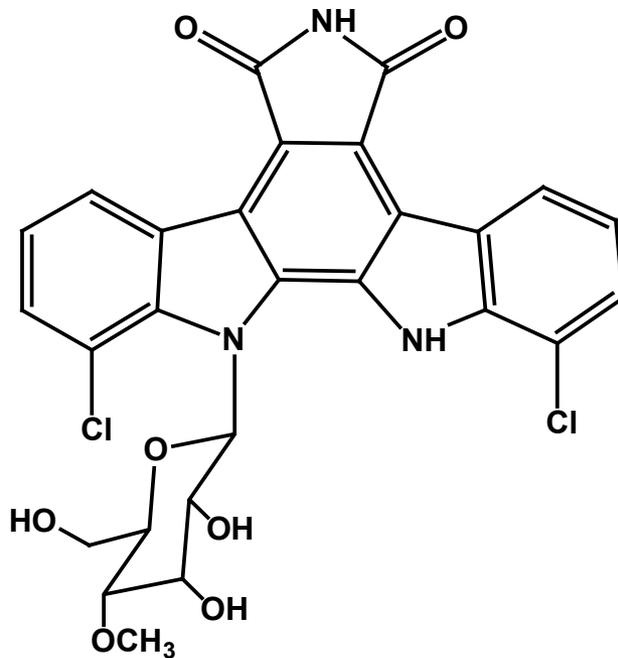
Treatment potential for ischemic retinopathy

In Phase I clinical trials with Novartis

PKC 412 Possible Mechanisms



Rebeccamycin; A "Model" for Topoisomerase Inhibitors

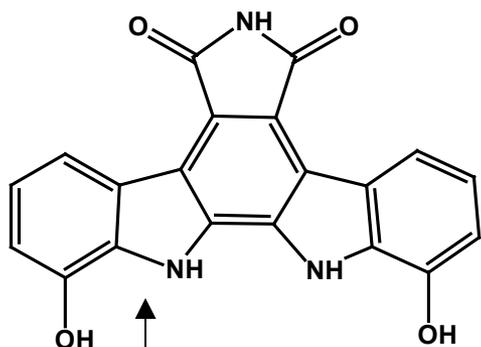


Isolated from *Saccharothrix aerocolonigenes* from Panama and structure published in 1985.

Antitumor active and activity is partially due to its capacity to inhibit Topoisomerase I.

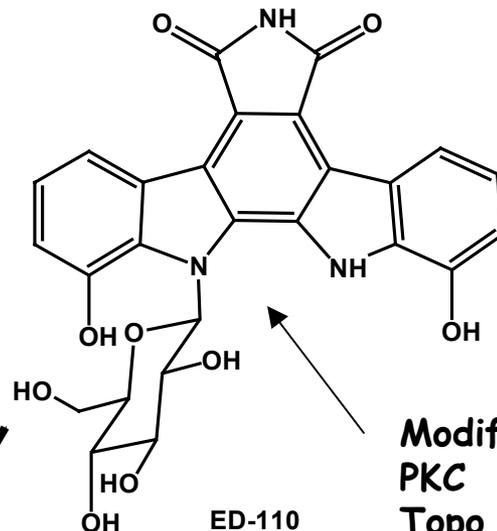
No distal site activity found in animals so BMS and other groups in Europe and Japan used structure as a basis for development.

Evolution of NB-506; the First "Active" Topo I Inhibitor



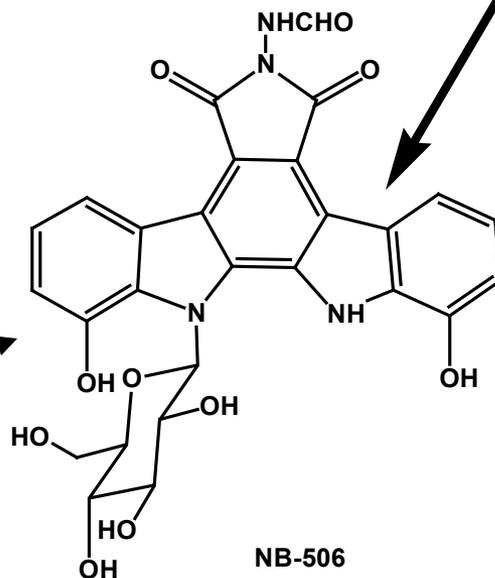
BE-13793C

Natural Product
 PKC 40 μ M
 Topo I >3 μ M
 Topo II >50 μ M



ED-110

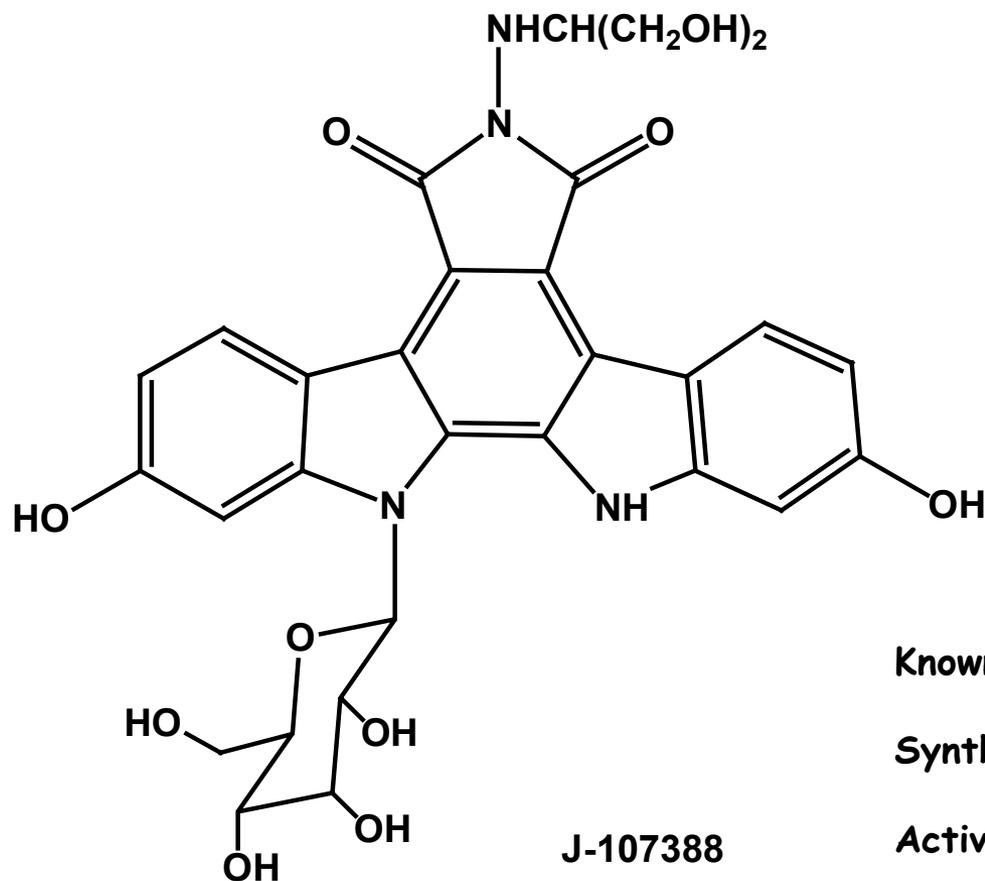
Modified NP
 PKC 4 μ M
 Topo I 3 μ M
 Topo II >50 μ M



NB-506

2nd Generation Modification
 PKC 200 μ M
 Topo I 0.7 μ M
 Topo II >50 μ M

J-107088; Clinically Active Topoisomerase 1 Inhibitor



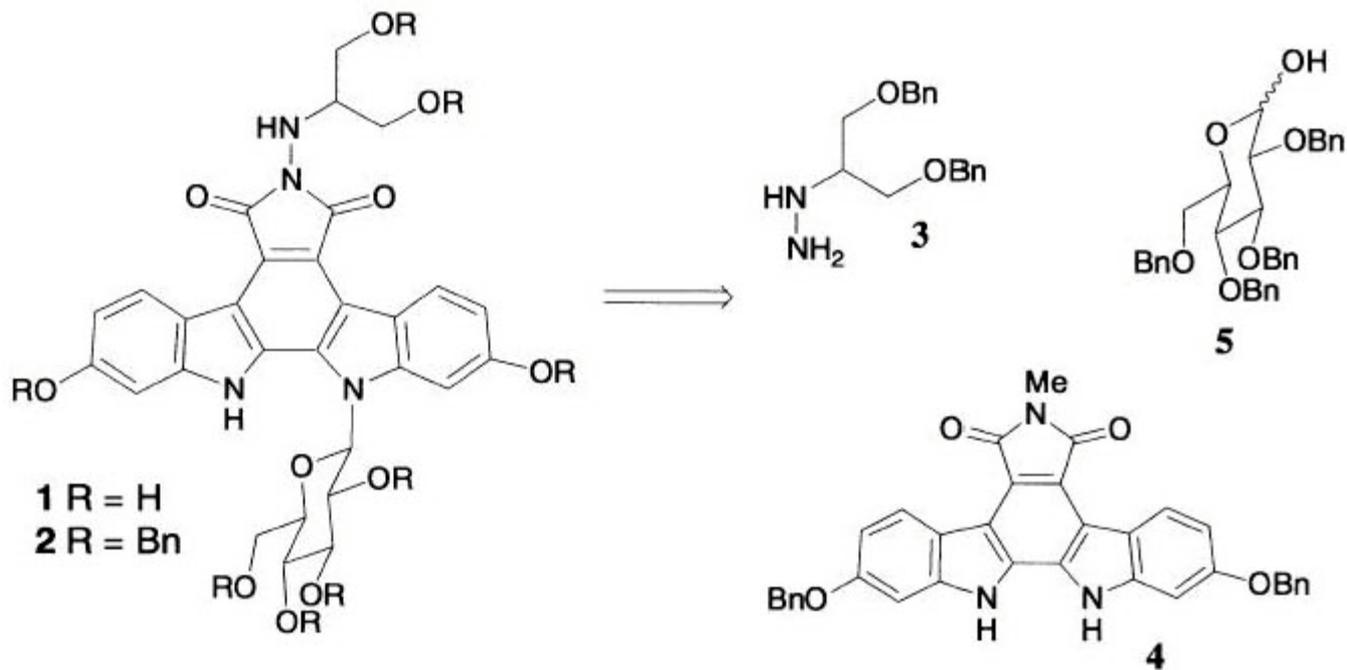
J-107388

Known as ED-749

Synthetic but based on NP

Active against CNS Tumors

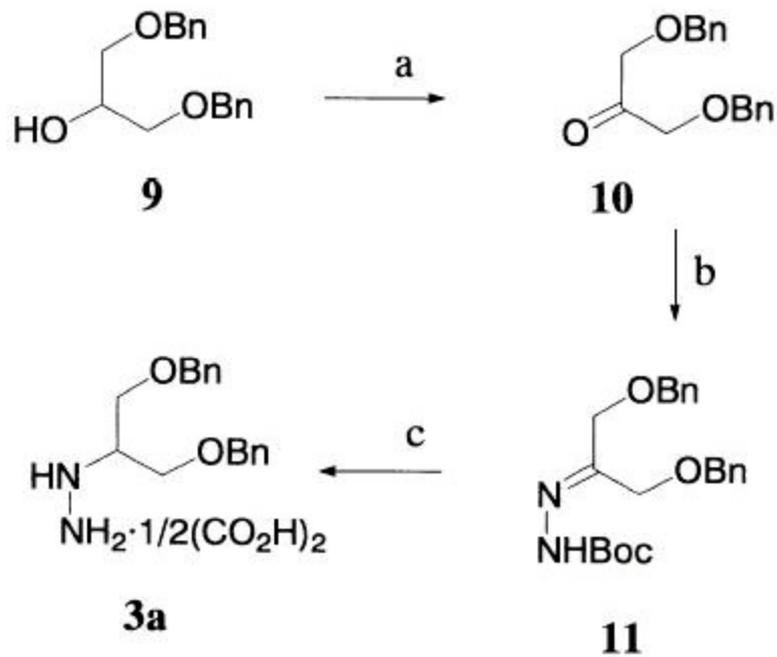
Synthetic Schema for J-107088 (1)



Scheme 1.

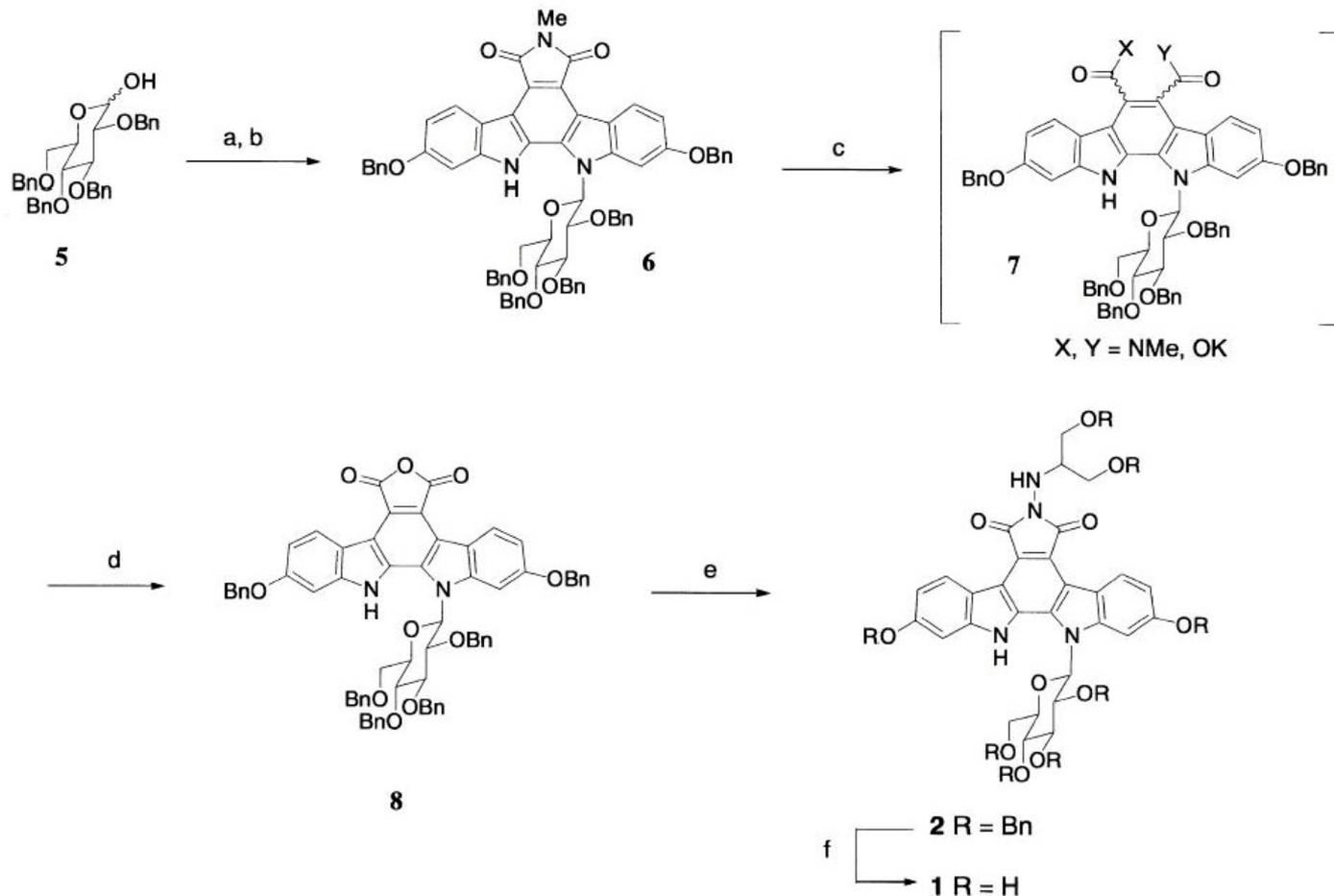
Retrosynthetic schema on perbenzoylated molecule

Synthetic Schema for J-107088 (2)



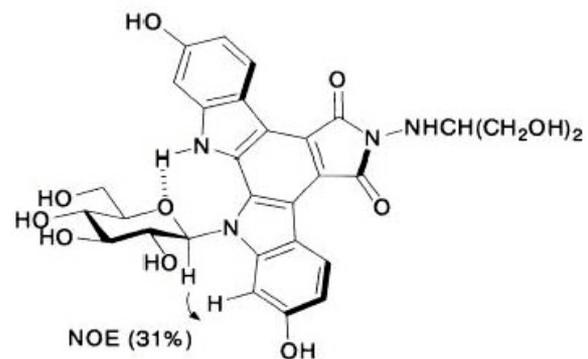
Reagents and conditions: (a) aqueous NaOCl, cat. TEMPO, MeCN, 5°C/1 h; (b) Boc-NH-NH₂, heptane/toluene, 70°C/1 h (86% from **9**); (c) (i) NaBH₄, BF₃-etherate, THF, 0°C/1 h; (ii) 6N HCl, 60°C/4 h; (iii) 0.5 equiv. oxalic acid, MTBE, EtOH, 20–40°C/12 h (79% overall).

Synthetic Schema for J-107088 (3)

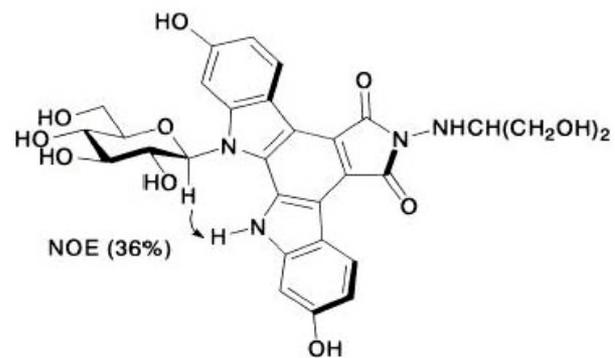


Reagents and conditions: (a) thionyl chloride, DMF 5–25°C/2 h (>95%); (b) **4**, 48% aqueous KOH, Aliquat 336, MTBE, rt/3 h (83%); (c) 48% KOH, EtOH, toluene, 30°C/16 h; (d) Aqueous citric acid to pH 8, 25°C/6 h (82%); (e) **3a**, TEA, DMA, 65°C/3 h (99%); (f) H₂ (40 psi), 10% Pd/C, THF, IPA, aqueous HCl, 40°C/14 h (82%).

Synthetic Schema for J-107088 (4)

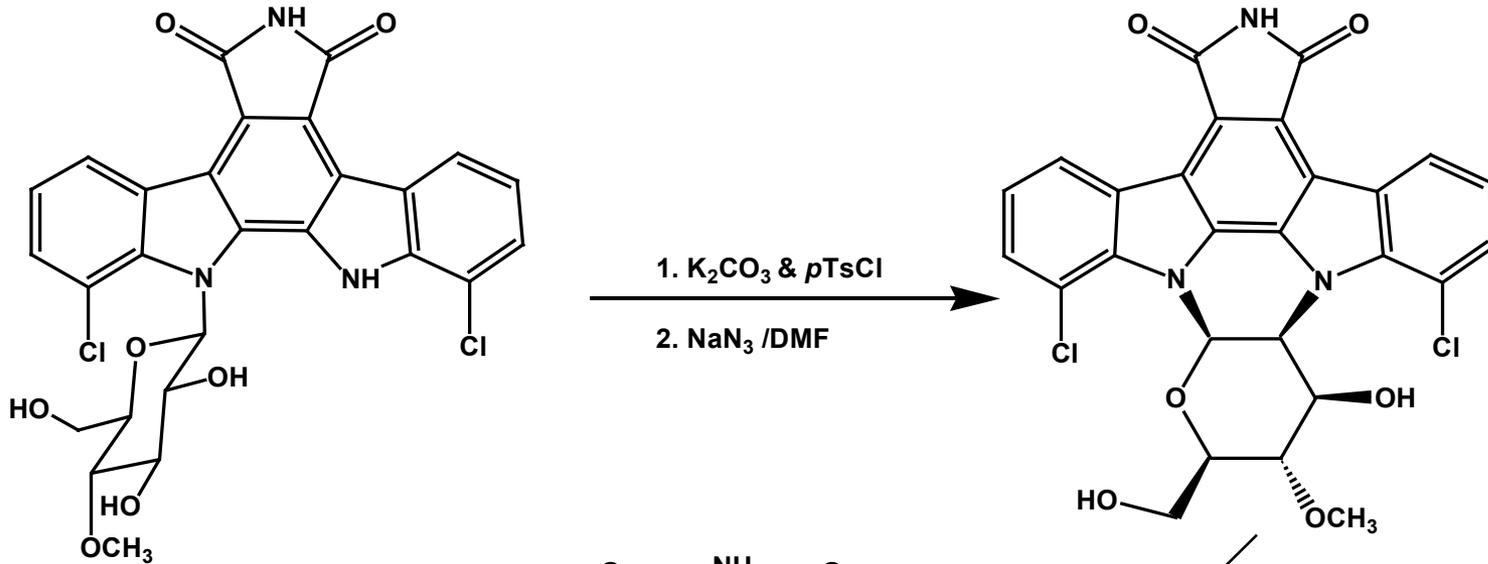


1 (major rotamer)



1' (minor rotamer)

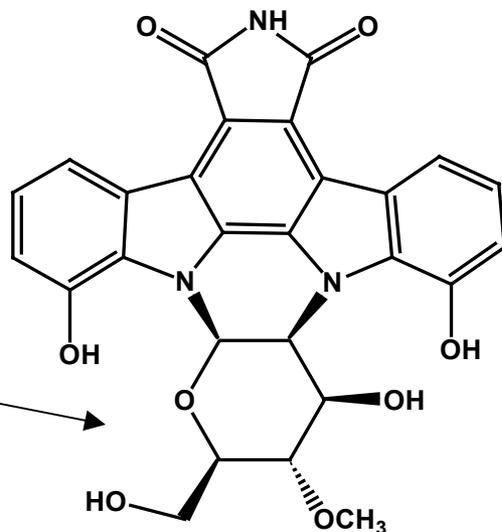
Synthesis of a Topoisomerase I Molecule with No PKC Activity



Rebecamycin

HCO_2NH_4

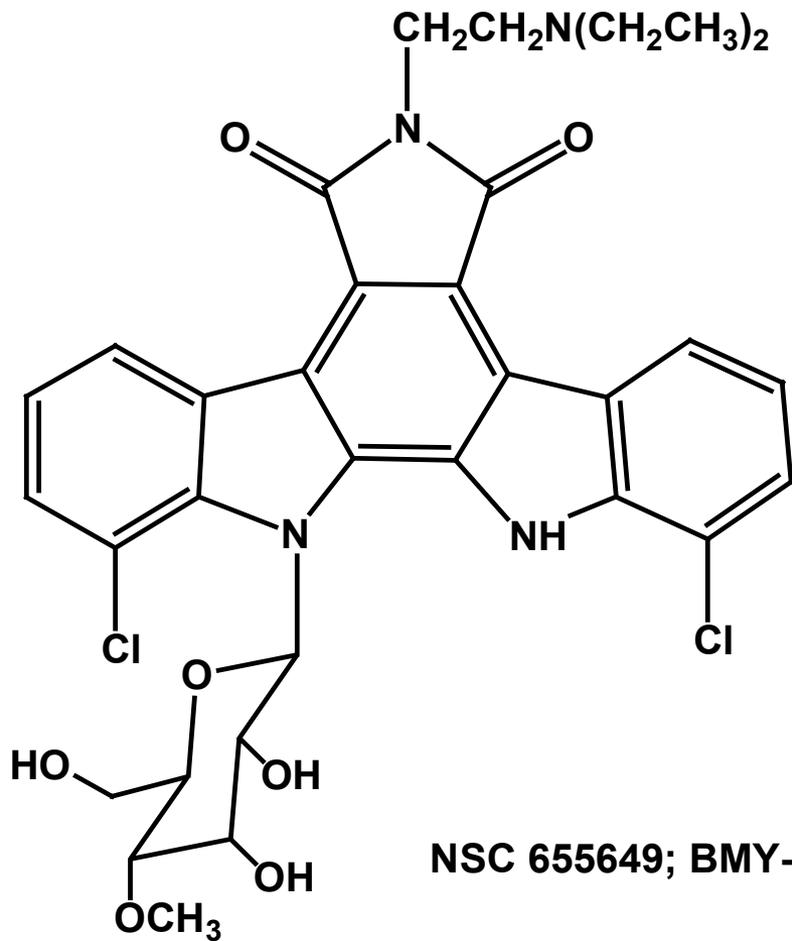
$Pd/C, CH_3OH$



IC_{50} >207 micromolar
 IC_{50} 2 micromolar

Topoisomerase I

NSC 655649; Topoisomerase II

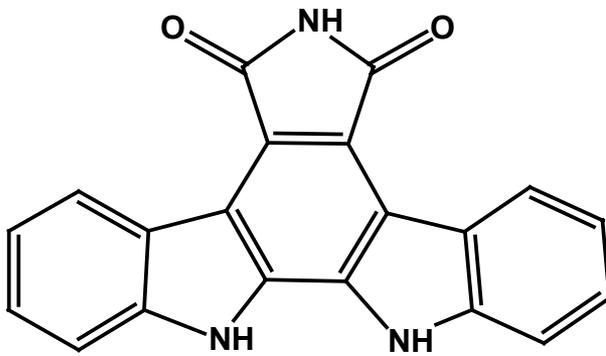


Topoisomerase II inhibitor

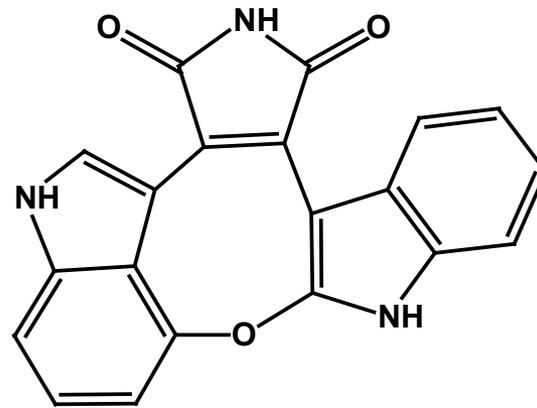
Now in Phase II Clinical Trials

NSC 655649; BMY-27557-14

Aglycones

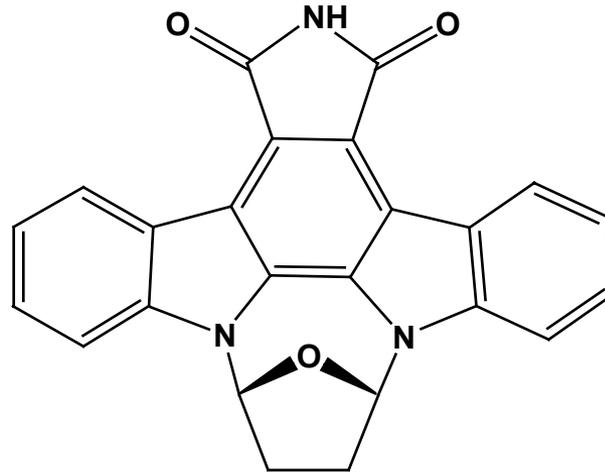


Arcyriaflavine A



Arcyroxocin A

Checkpoint 1 Inhibition



SB-218078

Tetrahydrofuran ring in place of pyranose sugar

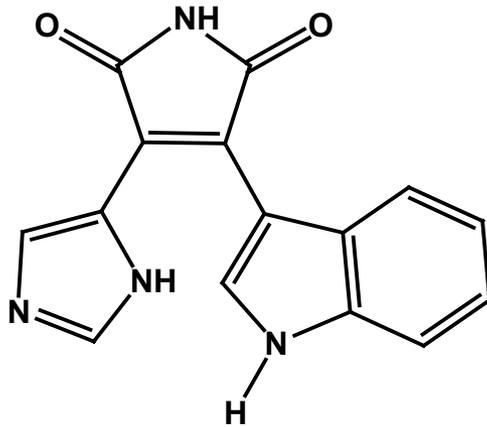
Maleimide of Didemnides in place of reduced pyrrolidone

SB Chk1, IC50 15 nM; Cdk1, IC50 250 nM; PKC, IC50 1000 nM

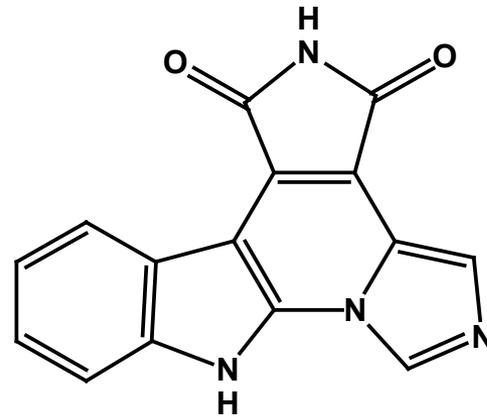
Stauro 5-8 5-8 5-8

UCN-01 7 10 4

G2 Blockers and Similar Structures



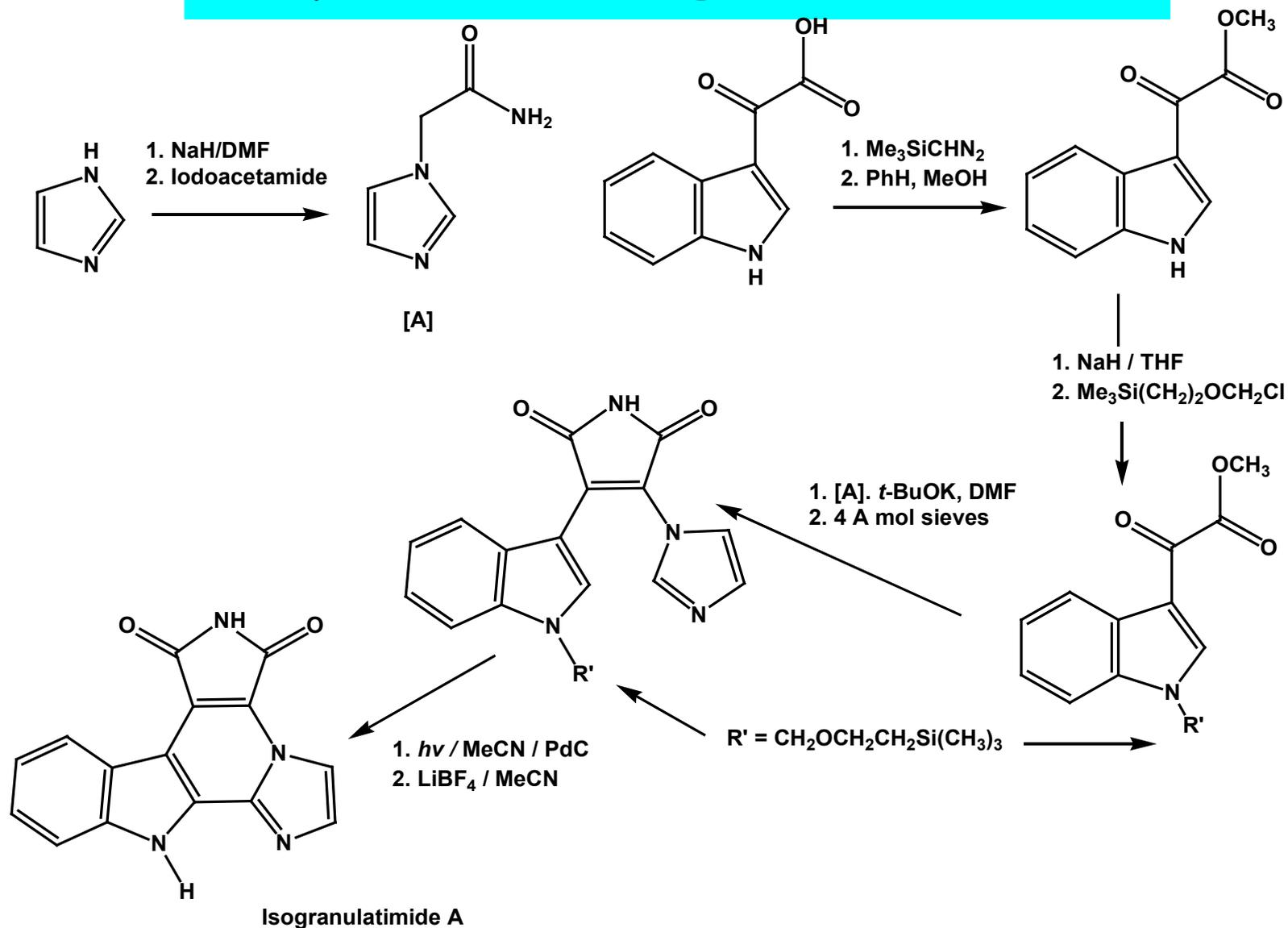
Didemnimide A



Isogranulatimide

The imidazole ring can be altered as far as the C-bond to the indole is concerned giving A, B & C isomers; all have been isolated from nature.

Synthesis of Isogranulatimide A

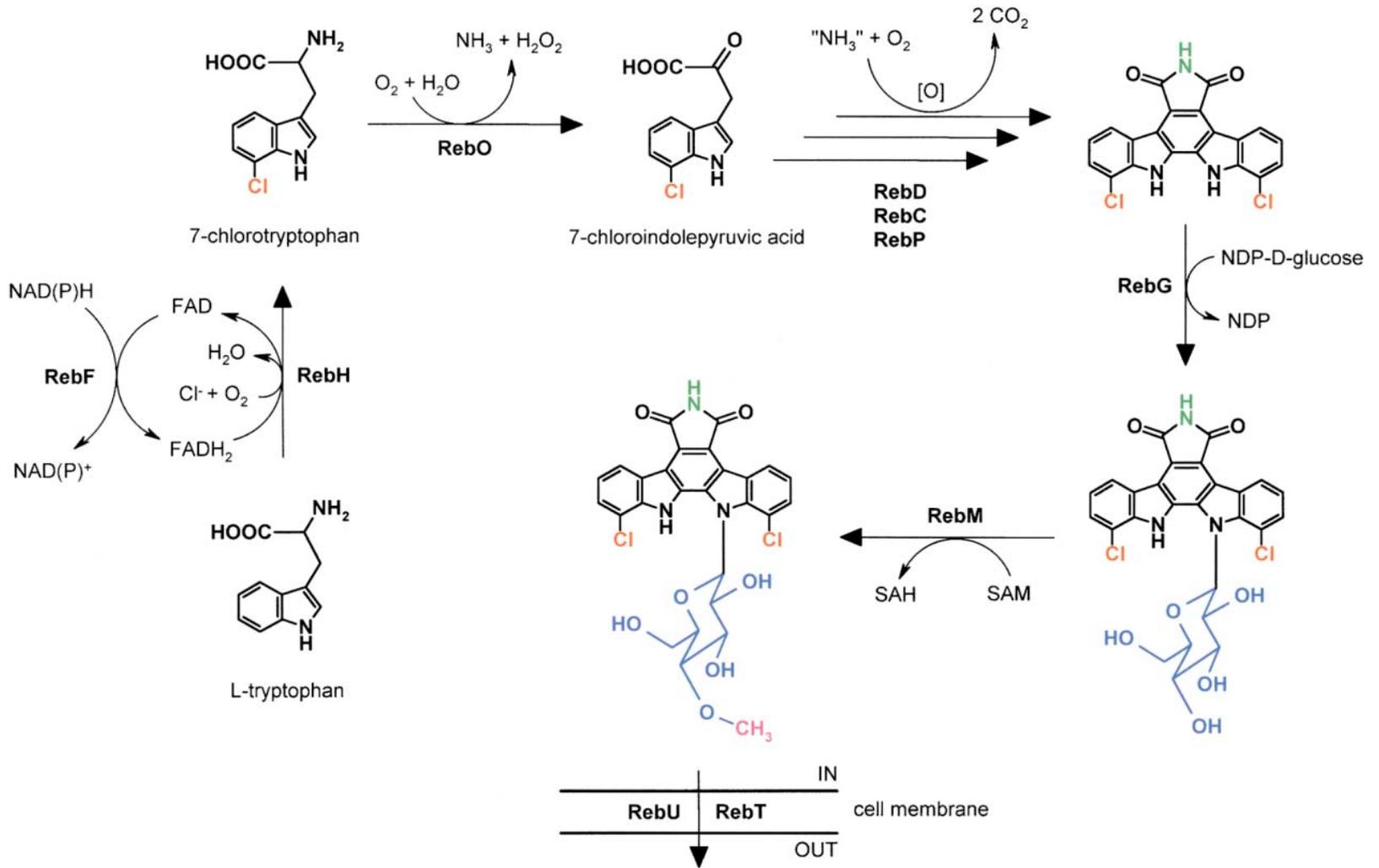


"Different Methods of Synthesis"

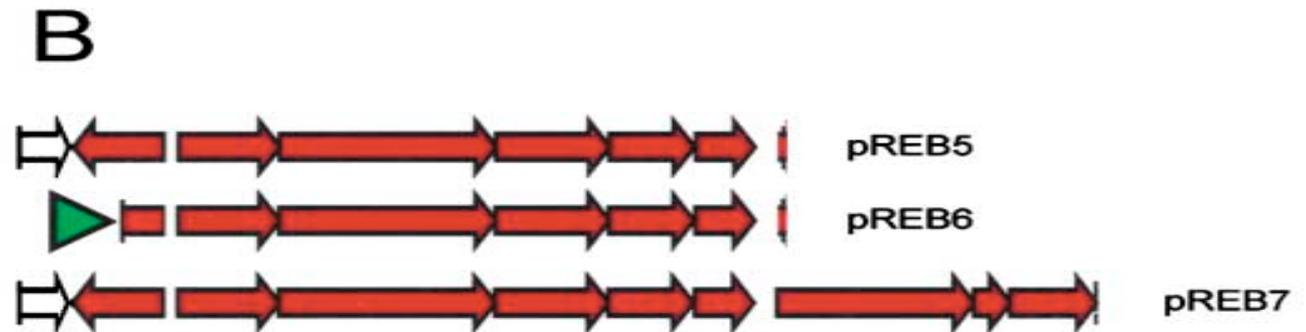
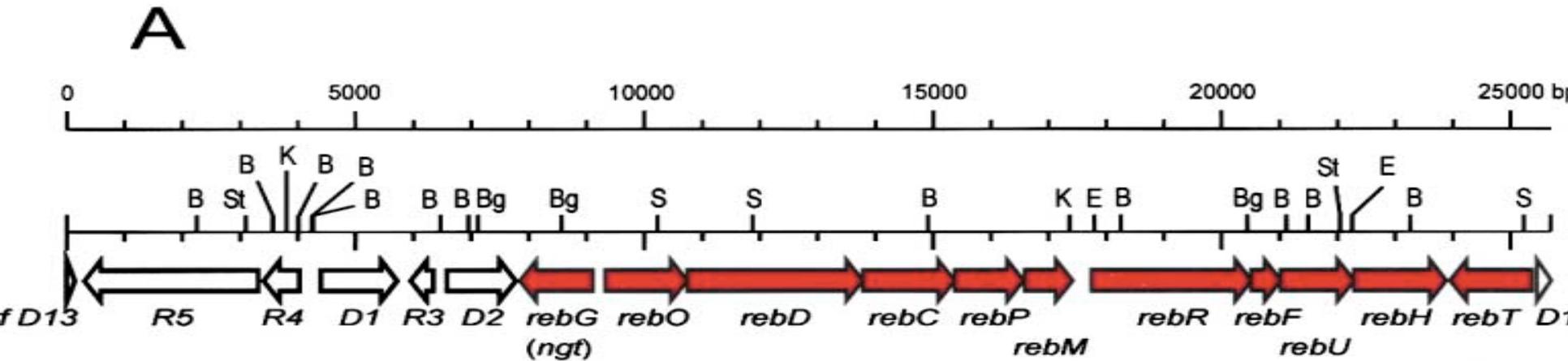
As the biochemical information on the biosynthesis of these molecules becomes available then rather than classical approaches to synthetic modifications, why not utilize the resources of "Mother Nature" to produce "different" molecules using techniques that give 100% fidelity in enantiomeric insertions.

Thus "ENZYMES.....ENZYMES.....ENZYMES" is the mantra of the 21st Century

Suggested Biosynthesis of Rebeccamycin



Rebeccamycin Gene Cluster



Expressed gene set from *Saccharothrix aerocolonigenes* in *Streptomyces albus*

Where could Mass Spectroscopy be utilized ?

With the advent of molecules of similar basic structures but entirely different binding characteristics it is tempting to ask whether MS techniques could be used to determine binding sites or number of molecules bound per protein ?

Could modifications be made to the basic structures that would permit photo-activatable ligands (azido for example) to be bound and then the sites of interaction determined by "high resolution" MALDI-TOF ?

Protein-protein interactions that are possibly modulated by these types of molecule may be amenable to MS techniques.

MORAL "Start thinking obliquely" [Dr. Brian Hartley, 1966]