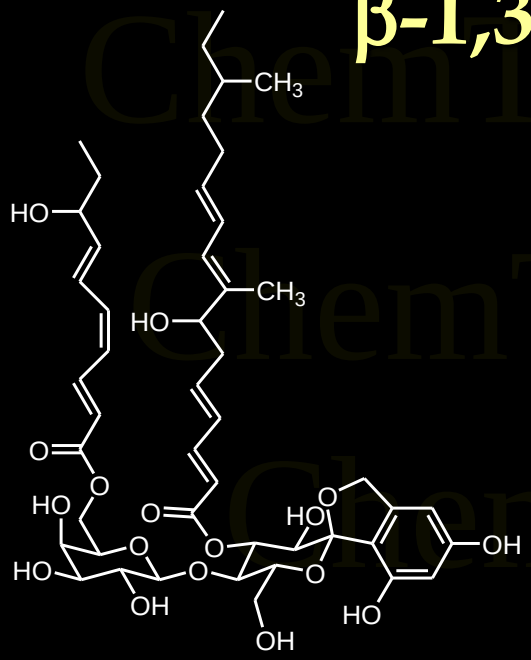
Natural Products in Drug Discovery

The Next Generation
β-1,3-Glucan Synthase Inhibitor

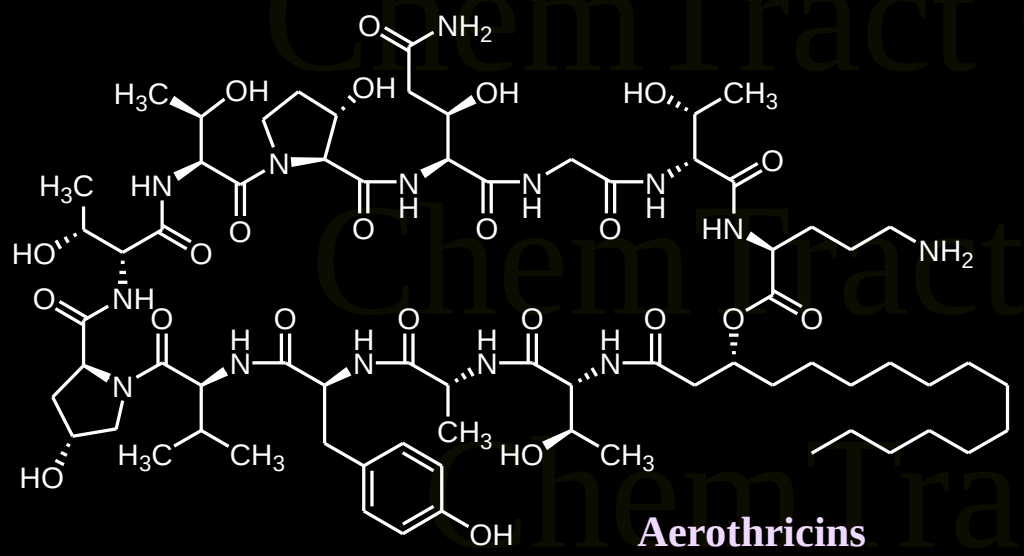
James M. Balkovec
ChemTract Consulting

β-1,3-Glucan Synthesis Inhibitors

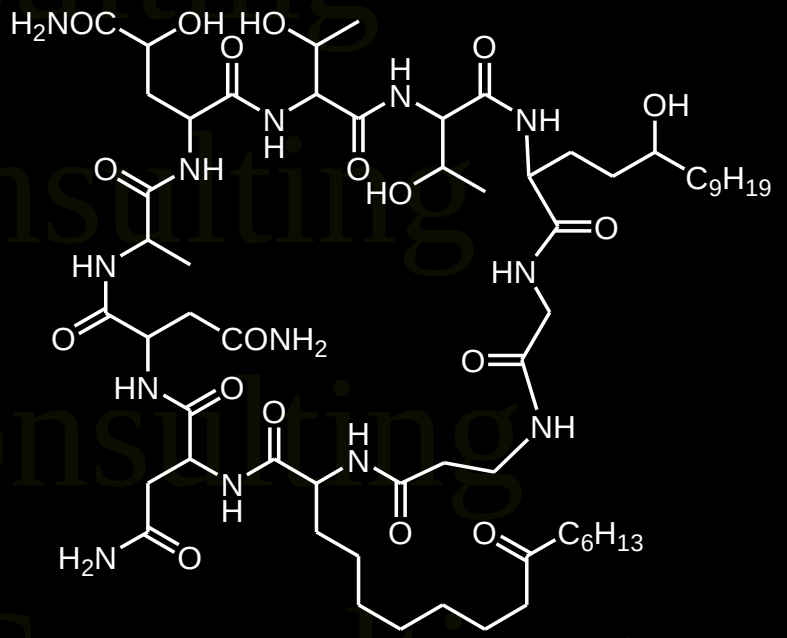


Papulacandins

Echinocandins

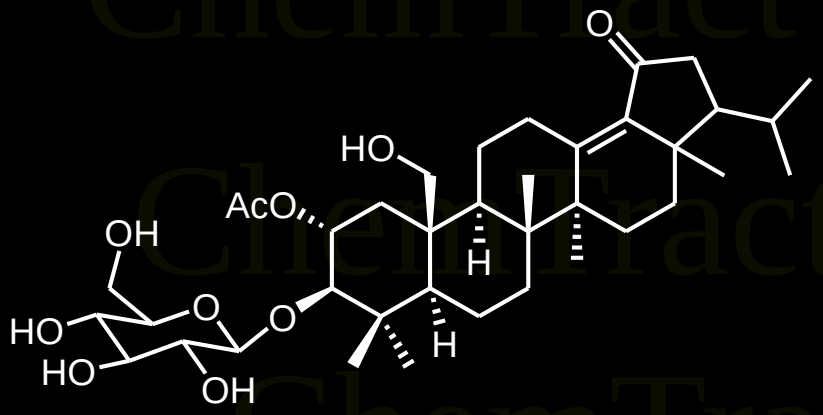


Aerothricins

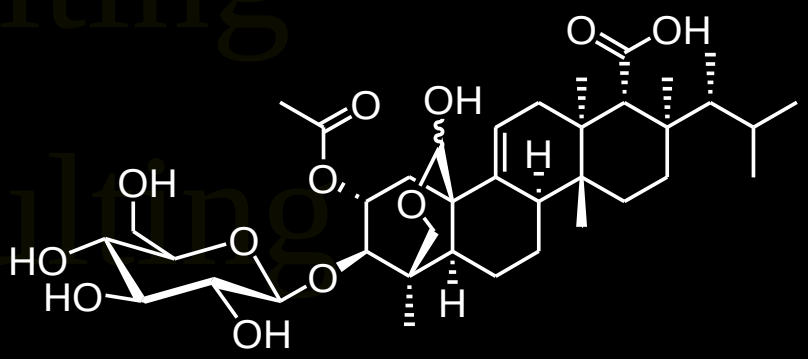


Sankyo Lipopeptides

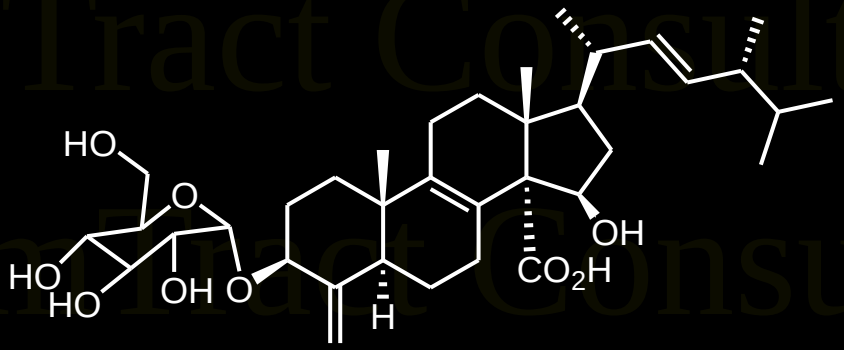
β-1,3-Glucan Synthesis Inhibitors



Hyalodendrosides

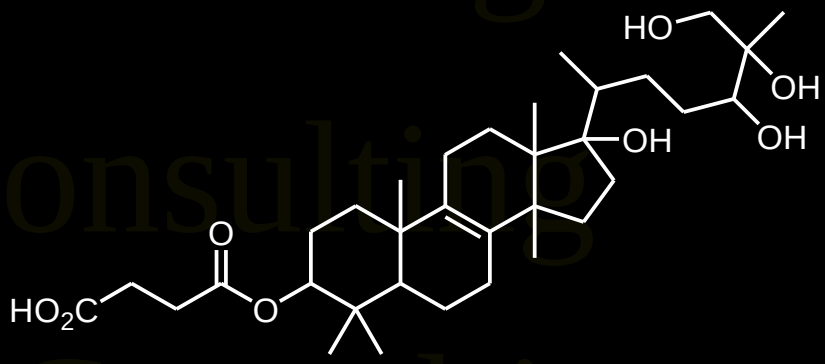


Enfumafungin



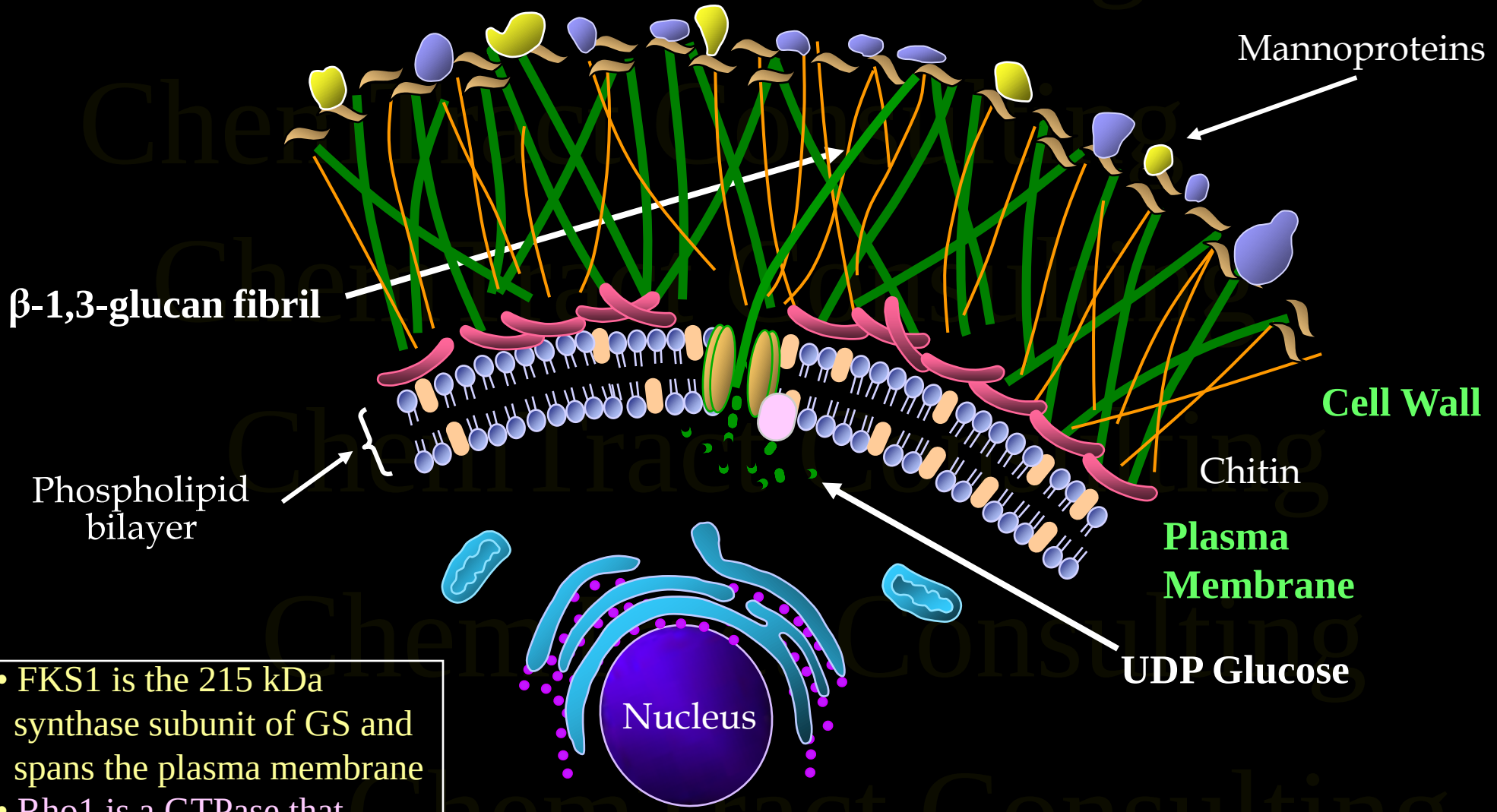
Ascosteroside

Ergokinins



Arundifungins

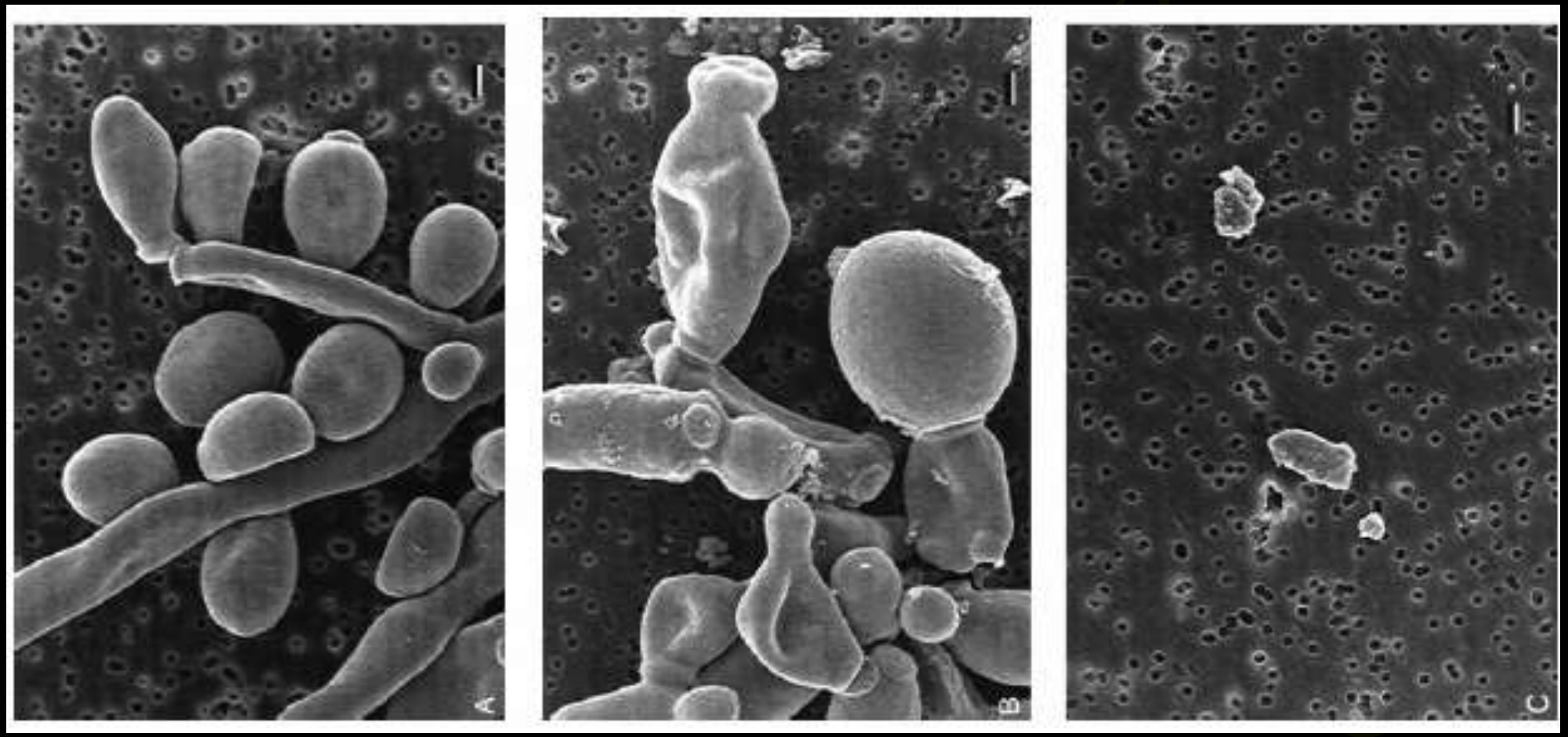
β-1,3-Glucan As A Target Of The Cell Wall



- FKS1 is the 215 kDa synthase subunit of GS and spans the plasma membrane
- Rho1 is a GTPase that upregulates glucan synthesis

spatial and temporal orchestration of cell structure

Morphological Effects of GS Inhibitor MK-0991 on *C. albicans*

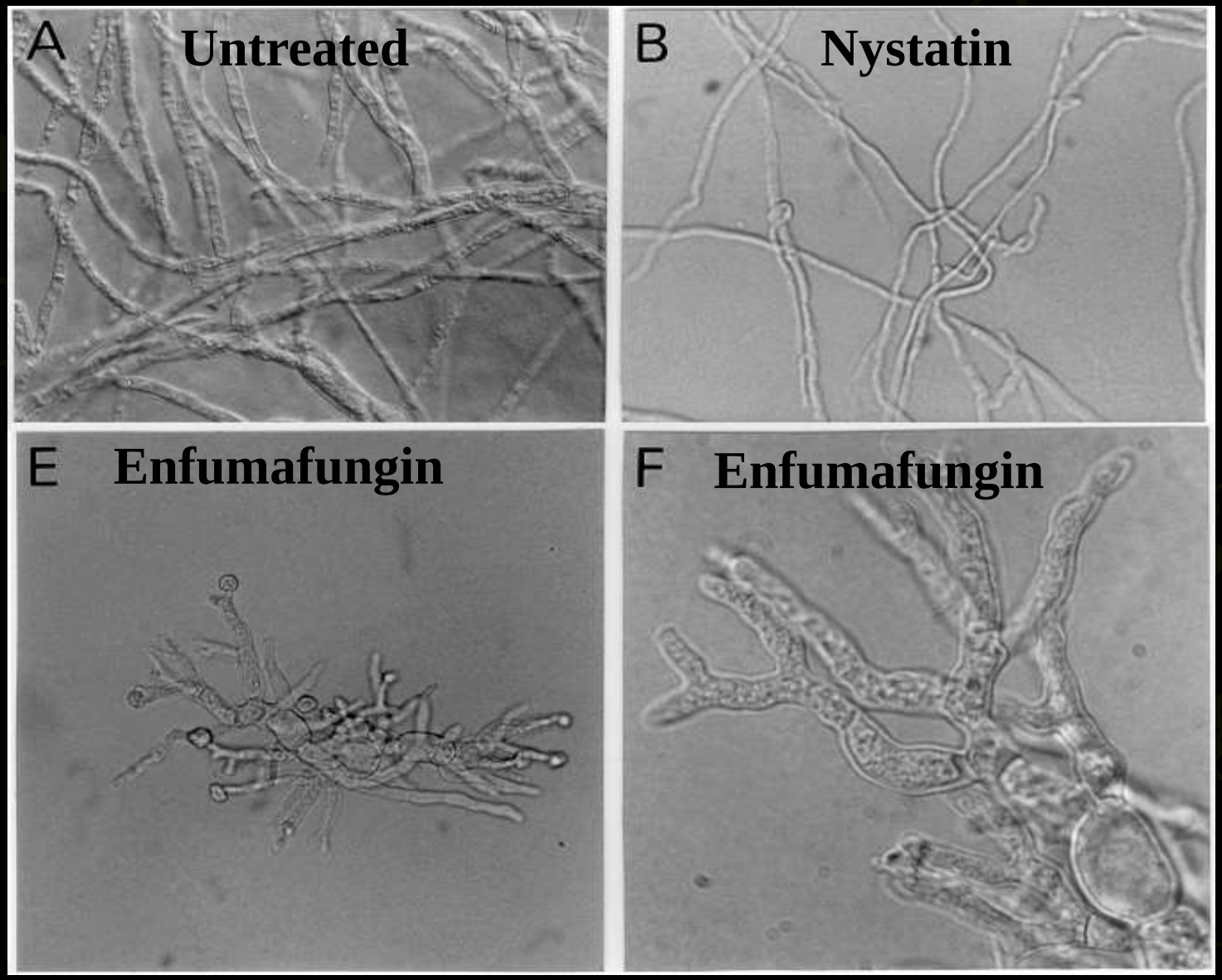


no inhibitor **MIC_{80}** **MIC_{100}**

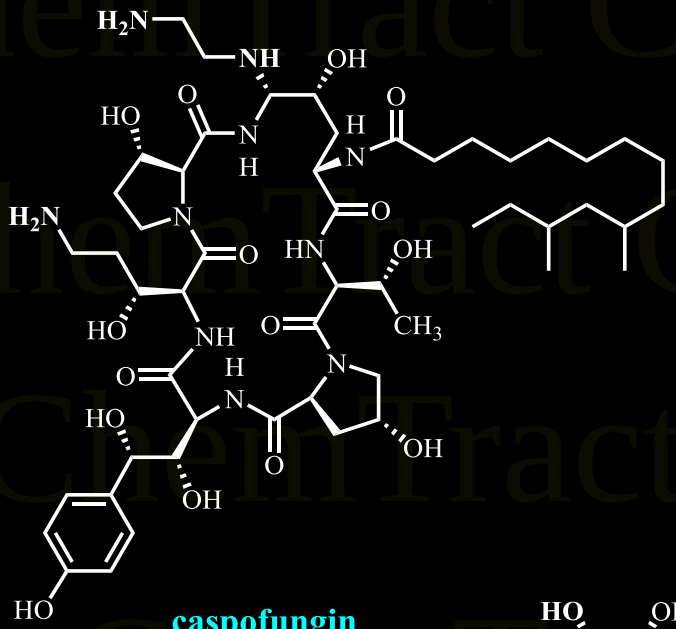
[| = 1 µm]

E. Ernst, *et al.* Diagn Microbiol. Infect. Dis. **1999**, 33: 75-80

Morphological Effects of GS Inhibitor Enfumafungin on *Aspergillus fumigatus*



Commercial β-1,3-Glucan Synthase Inhibitors



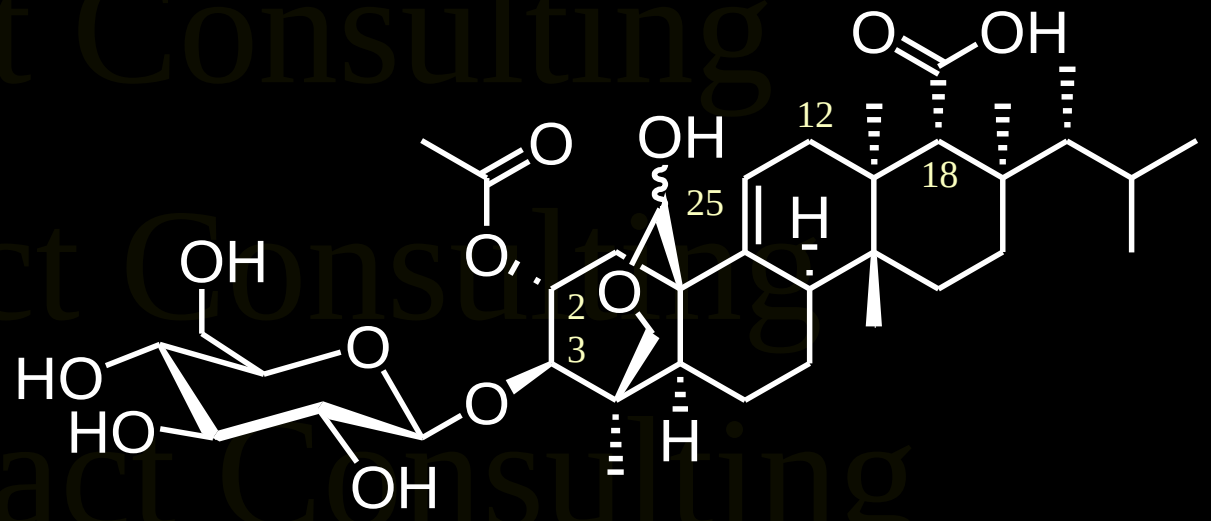
caspofungin
(Merck)

anidulafungin
(Pfizer)

micafungin
(Astellas)

IV administration only

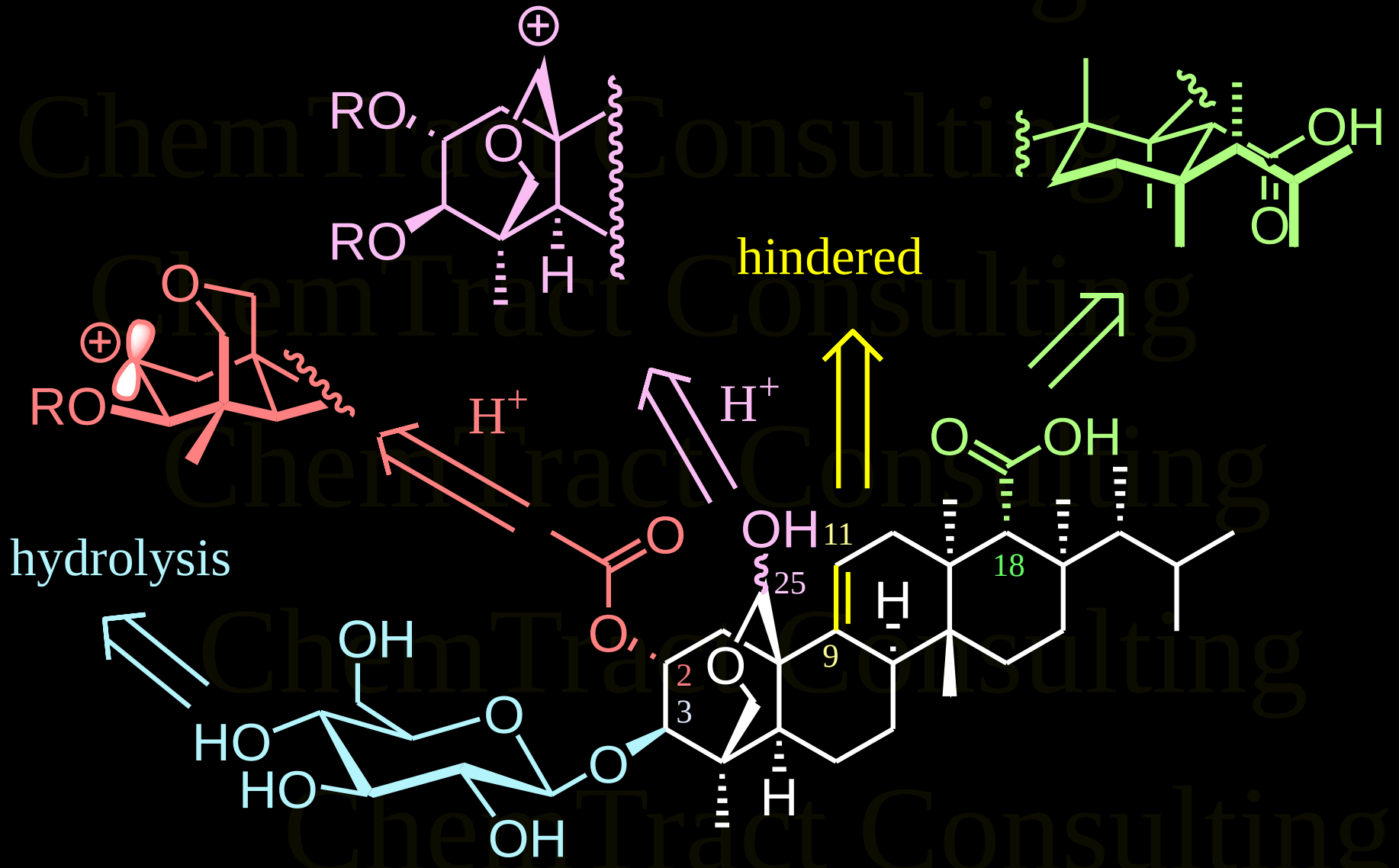
1996 - Enfumafungin



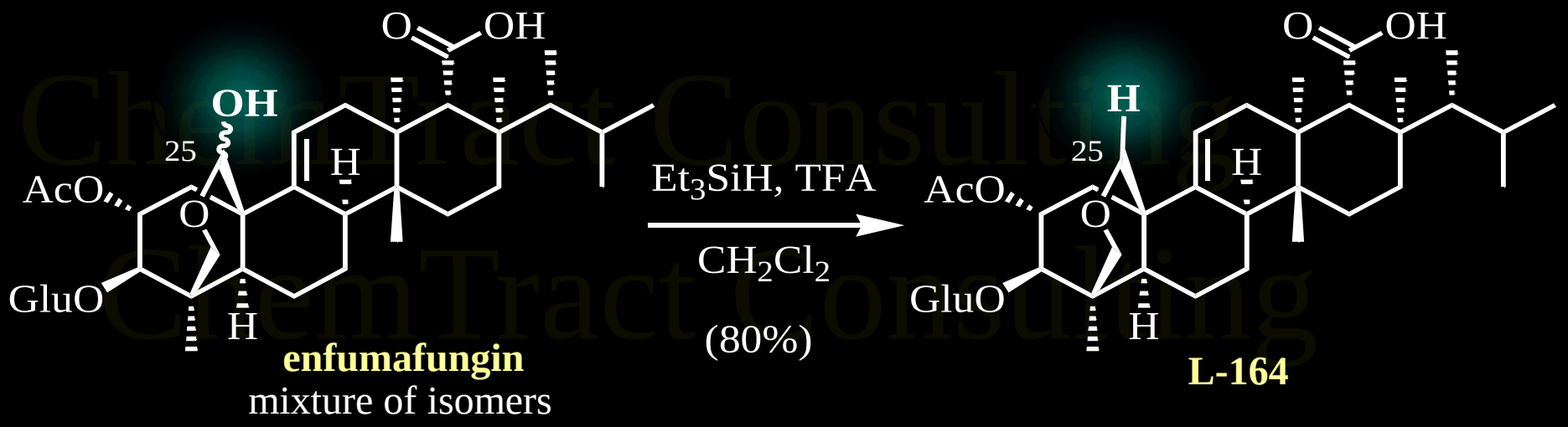
- Triterpene isolated from *Hormonema carpetanum*
  - titer 100 mg/L; Isolation yield 65%
- Inhibitor of β-1,3-glucan synthesis
- Broad spectrum: *Candida* and *Aspergillus* spp.
- Mixture of isomers
- Weak *in vivo* activity
- Potential for oral activity

F. Pelaez, *et al. Sys. Appl. Microbiol.* **2000**, 23: 333-43
R. Schwartz, *et al. JACS* **2000**, 122: 4882-86
J. Onishi, et al. AAC **2000**, 44: 368-77

Chemistry Highlights



Deoxygenated Analog



enfumafungin
mixture of isomers

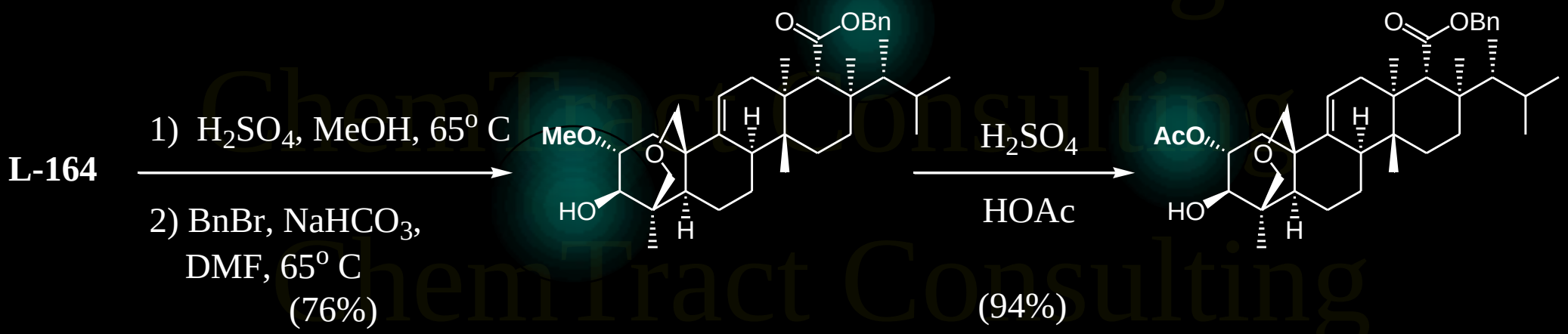
L-164

GS IC_{50}:	0.045	0.06	µg/mL
C. albicans (+ser)	0.25 (4)	0.06 (8)	µg/mL
A. fumigatus	0.04	<0.003	µg/mL

- **Solves the isomer issue**
- **Comparable broad spectrum activity**
- **Not orally bioavailable**
- **Short half life**
- **Weak in vivo activity**

F. A. Bouffard

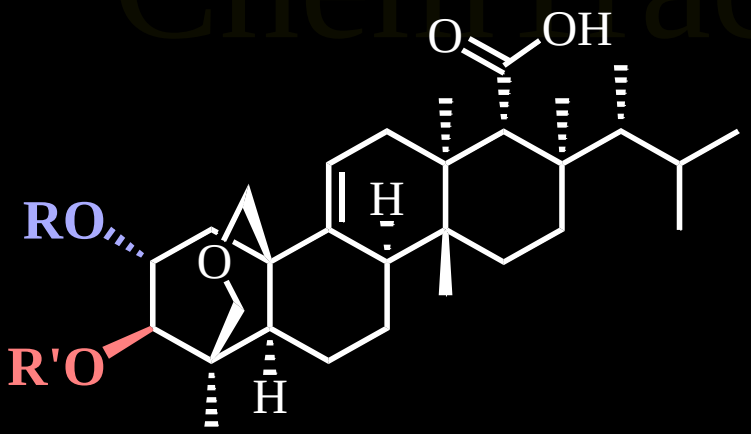
Synthesis of Key Intermediate

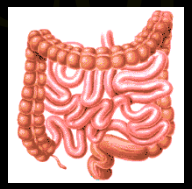


Deglycosylation could also be achieved enzymatically:
A. Shafiee, *et al. J. Molecular Catalysis B* **2001**, *16*, 27-32

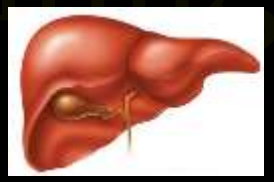
F. A. Bouffard
J. F. Dropinski

Oral Absorption in Rats



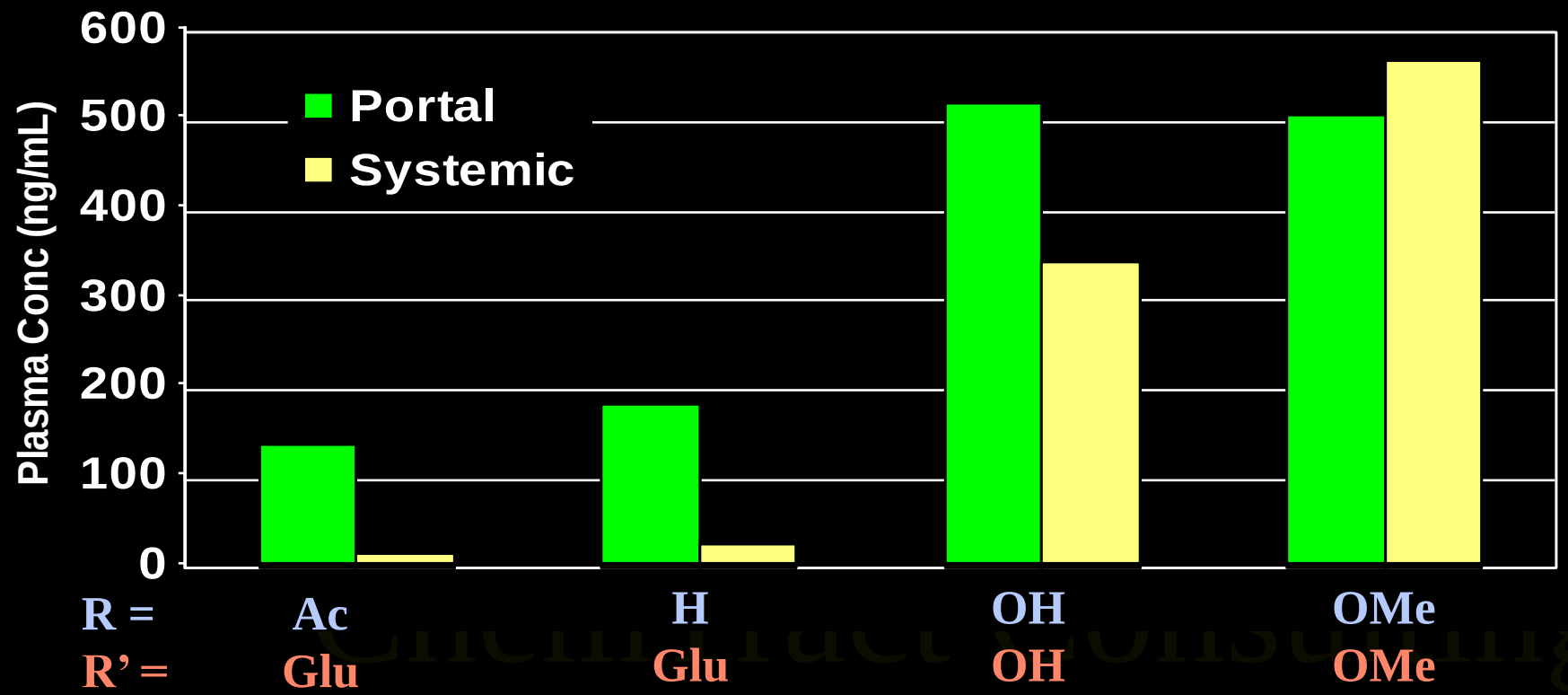


portal vein →

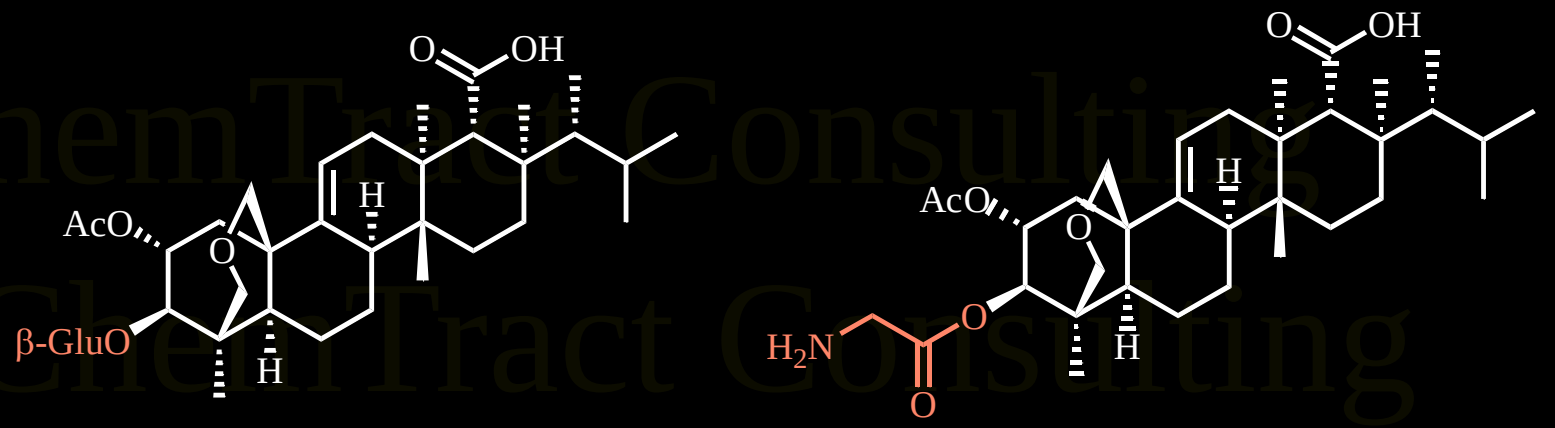


→ systemic circulation

Rats PO 3mg/kg
Drug levels @ 2 h



Glucose Can Be Replaced By Polar Groups

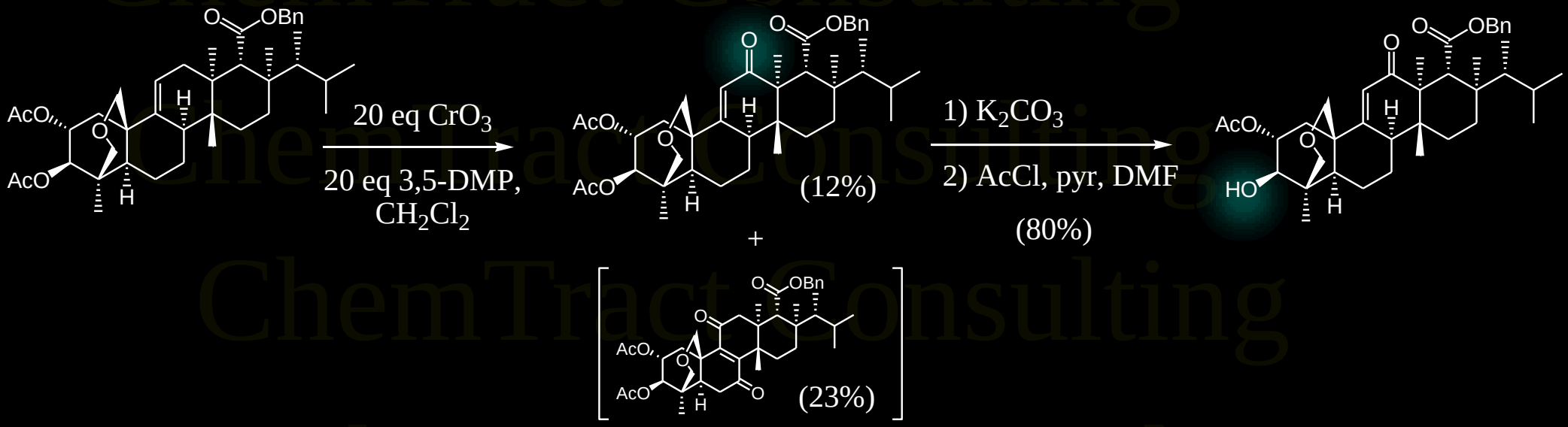


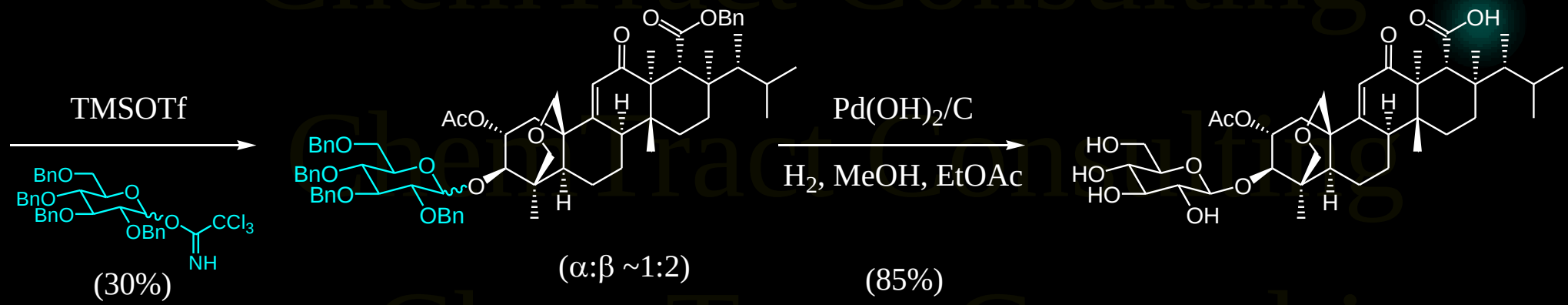
GS IC_{50}:	0.06	0.095	µg/mL
C. alb (+ser)	0.06 (8)	0.5 (4)	µg/mL
A. fum	<0.003	0.063	µg/mL

- **Orally bioavailable in a rat (24%)**
- **Improved PK ($t_{1/2}$ = 1.7 h)**
- **Similar broad spectrum of activity**
- **No in vivo activity**
- **More acutely toxic**

F. A. Bouffard

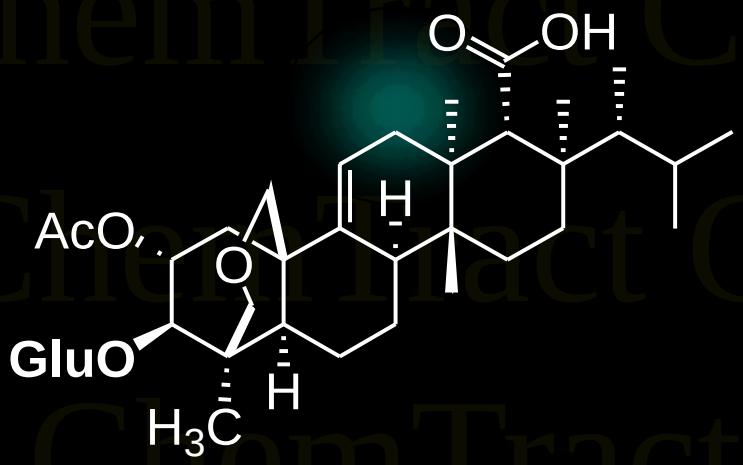
Synthesis of 12-Oxo Derivative

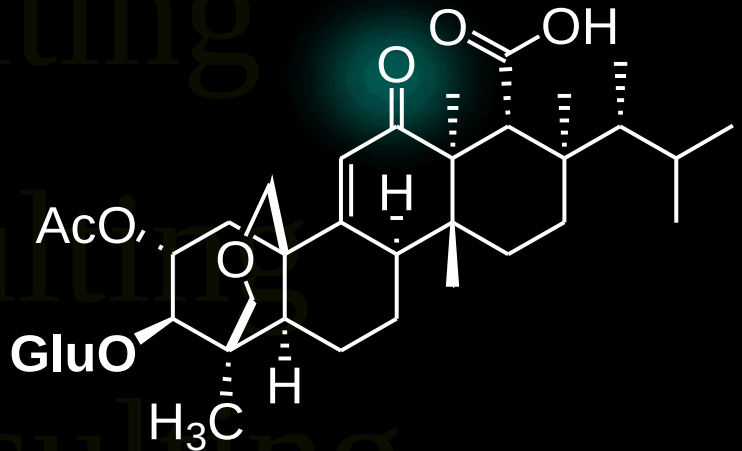




P. Siliphaivanh

12-Oxo Derivative

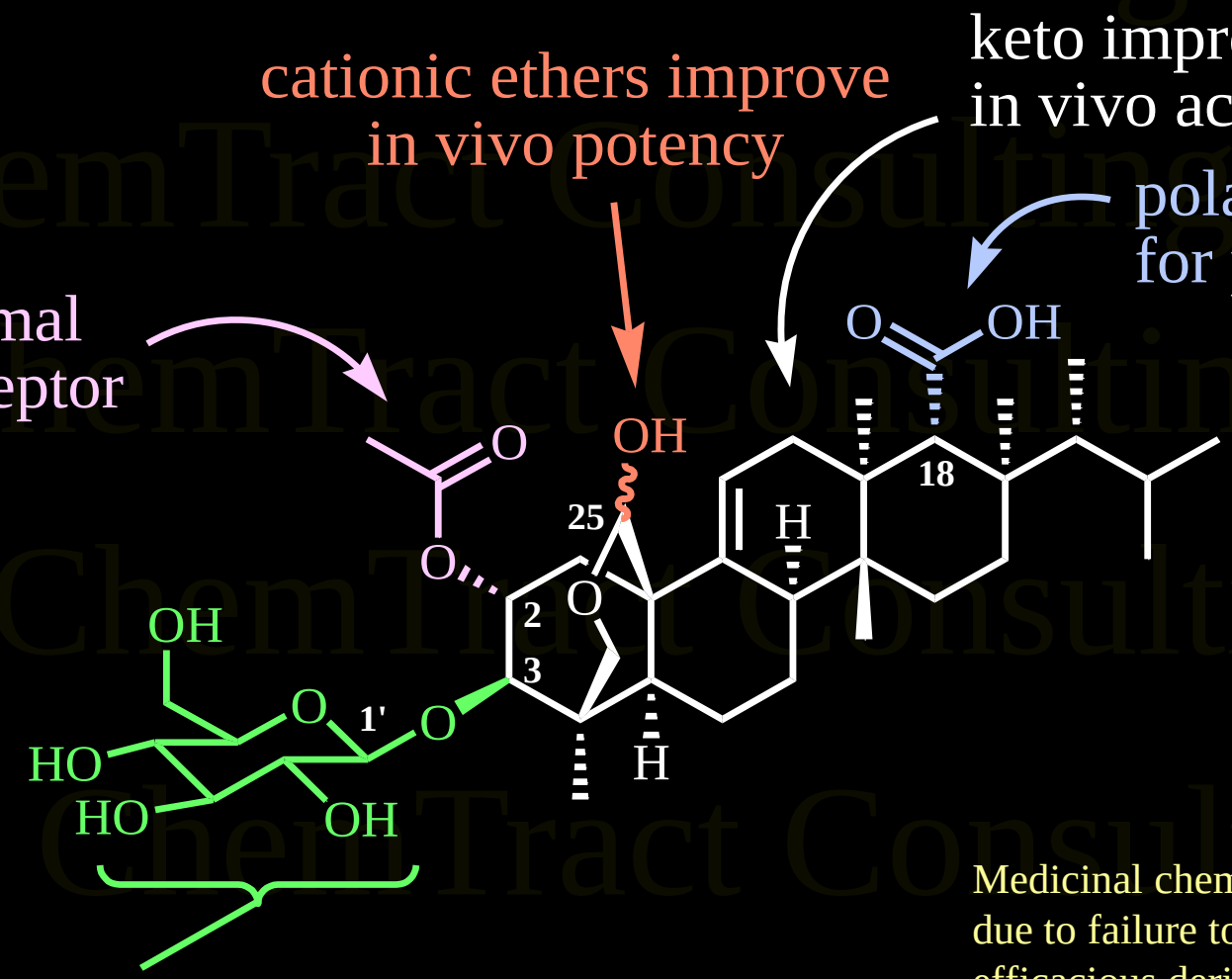




GS IC_{50}:	0.06	0.095	µg/mL
C. alb (+ser)	0.06 (8)	0.125 (1)	µg/mL
A. fum	<0.003	NT	µg/mL
TOKA (IP ED_{99})	>100	<50 (-2.8 log)	mg/kg

- **Similar broad spectrum of activity**
- **Improved MFC in presence of serum**
- **Modest in vivo activity**
- **Challenging synthesis**
- **Not orally active**

SAR Summary (1997-8)

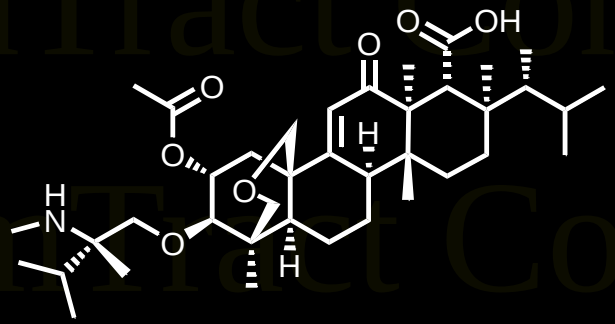


Medicinal chemistry program halted due to failure to identify an orally efficacious derivative

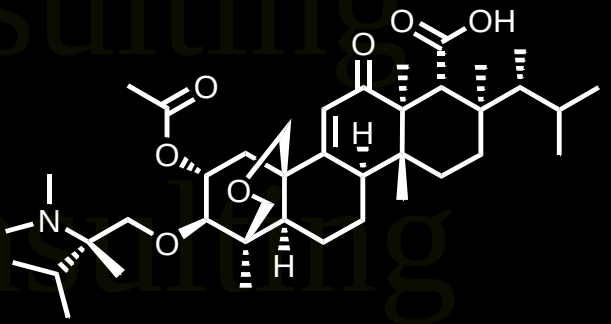
Reinitiation of Oral GS Program

2002

1st Development Compounds



MK-7166



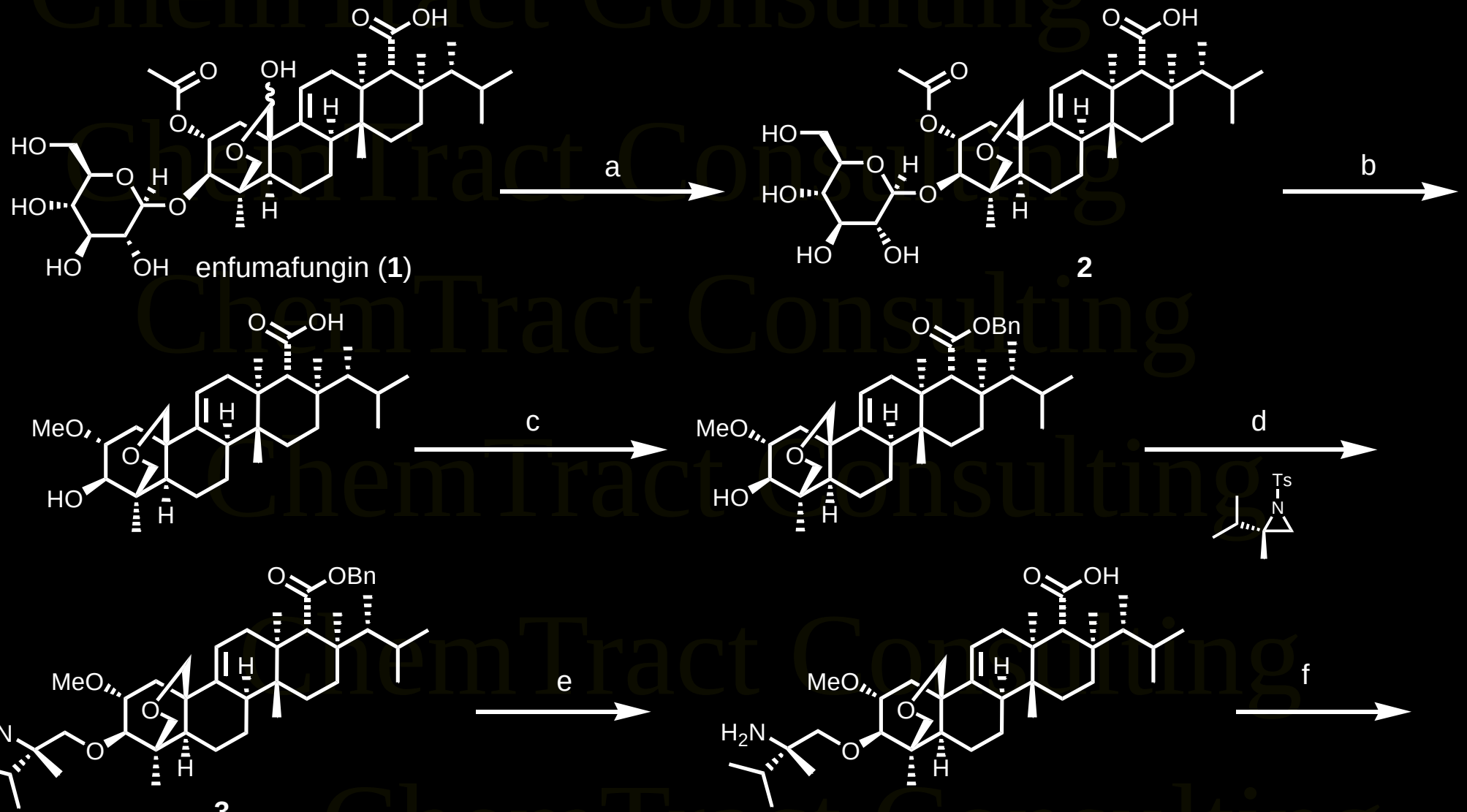
MK-6038

GS IC_{50}:	0.013	0.018	µg/mL
C. alb (+ser)	0.25 (4)	0. 5 (4)	µg/mL
A. fum (+ser)	0.125 (0.5)	0.25 (1)	µg/mL
7d TOKA (***Δlog @ 25 mpk PO***)	-4.4 (0%)	-4.1 (60%)	mg/kg

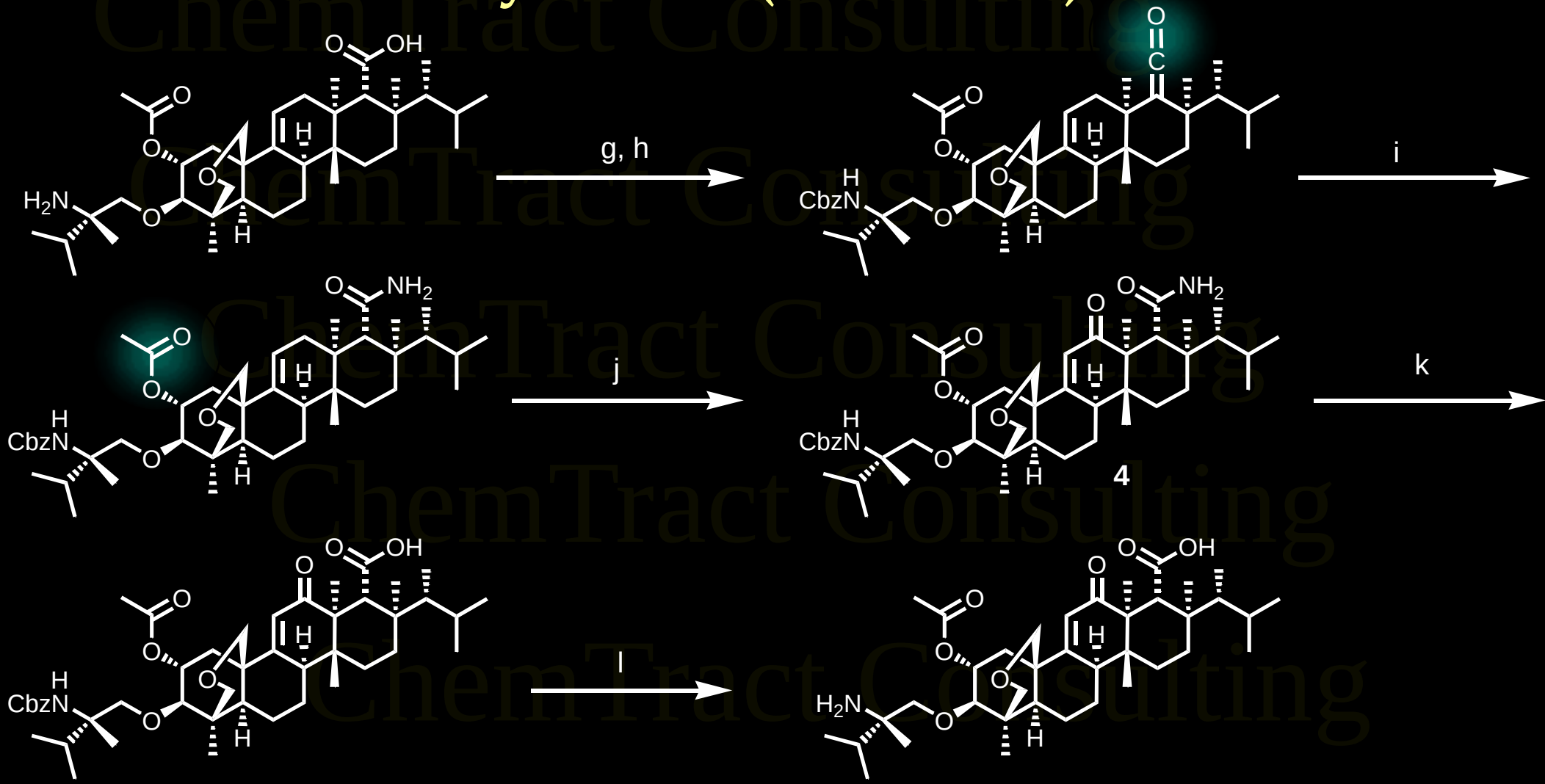
- **Similar broad spectrum of activity**
- **Good oral activity**
- **Challenging synthesis**
- **Compounds demethylate in vivo to give active metabolites**

J-L Zhong, et al. J. Org. Chem., 2012, 77 , 3297–3310

Synthesis

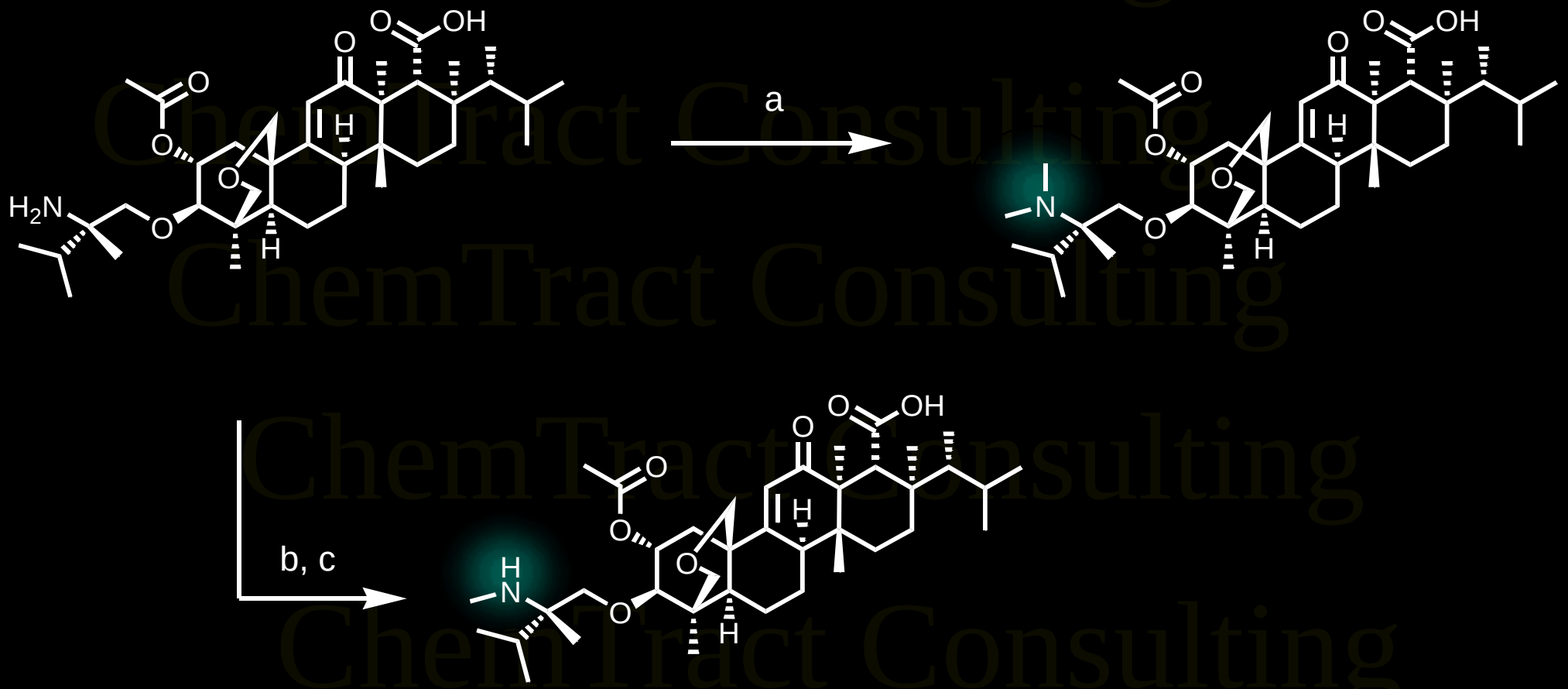


Synthesis (continued)



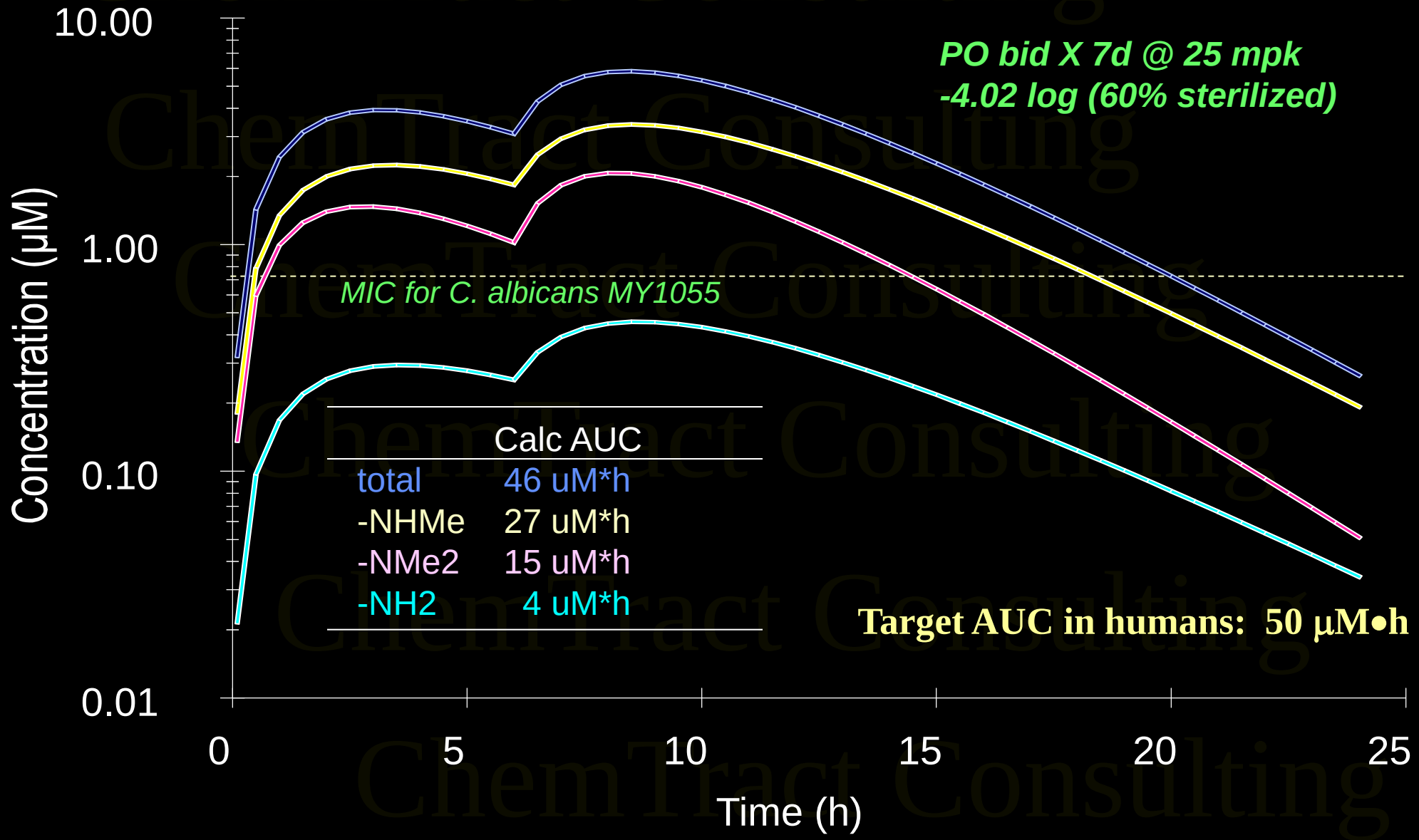
g) CbzOSucc, $NaHCO_3$, H_2O, acetone (73%) h) EDCI, CH_2Cl_2 i) NH_3, CH_2Cl_2, 100 psi, RT (80%, 2 steps) j) CrO_3, 3,5-DMP, CH_2Cl_2 -20° C (85%) k) t-BuONO, KF, 1% H_2O, CH_3CN (80%) l) H_2, $Pd(OH)_2/C$ (wet), HOAc, MeOH, EtOAc (90%)

Conversion to Final Compounds

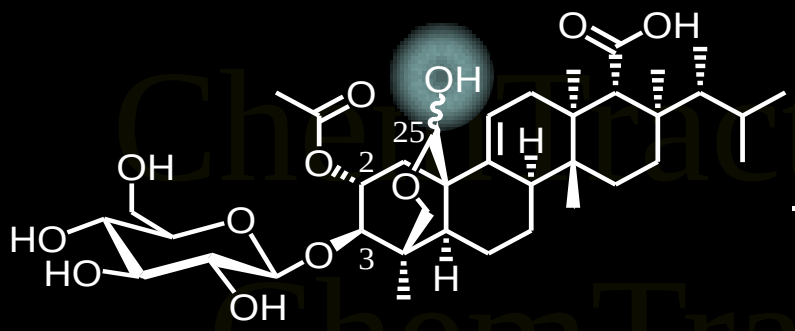


a) formalin, $NaCNBH_3$ (90%) b) PhCHO, $NaCNBH_3$, then formalin, $NaCNBH_3$ (64%) c) H_2, $Pd(OH)_2/C$, MeOH, HOAc (99%)

Calculated Plasma Levels After Oral Dosing of MK-6038 In Mice @ 25 MPK BID Based on Single Dose PK



Synthesis of Intermediates

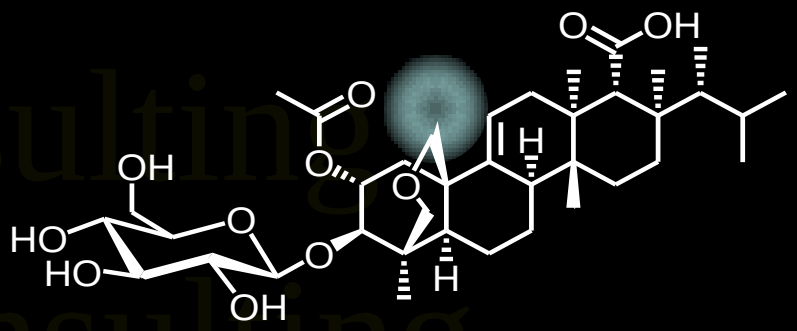


enfumafungin (**1**)
(~1:1 mixture)

Et_3SiH

TFA, CH_2Cl_2

(80%)

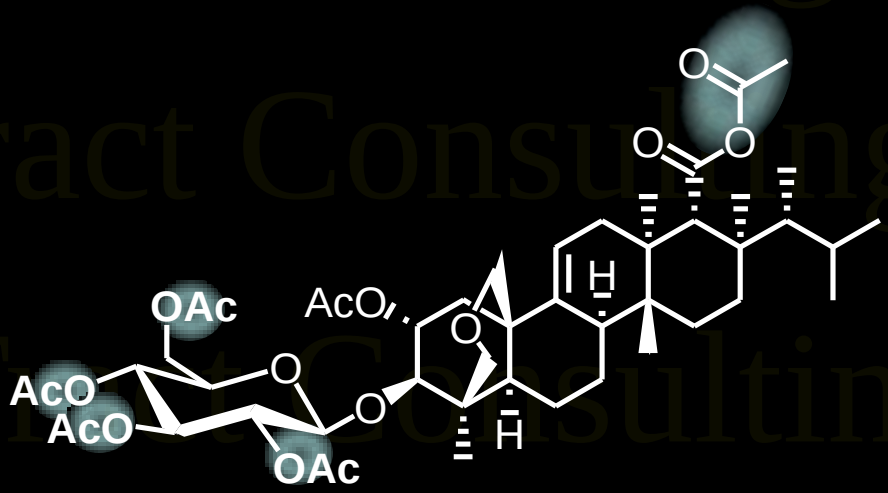


25-deoxyenfumafungin **(2)**

(2)

Ac_2O, TEA

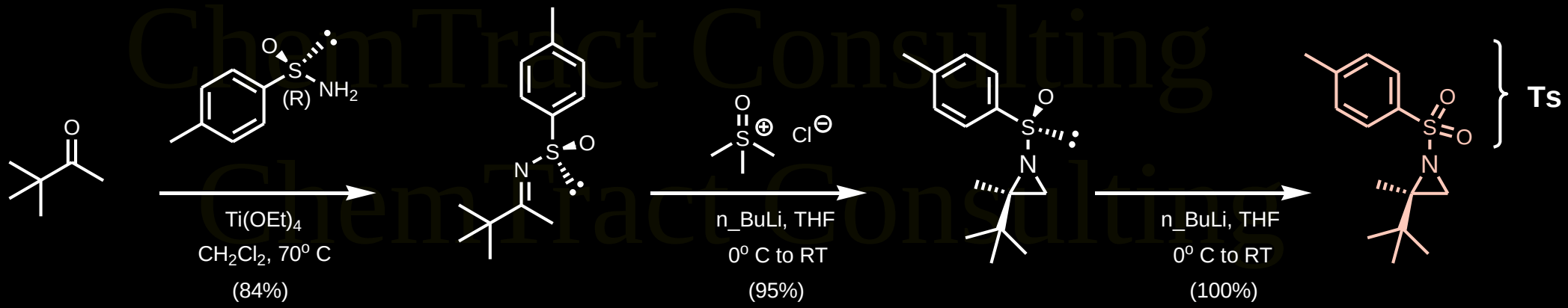
CH_2Cl_2 RT

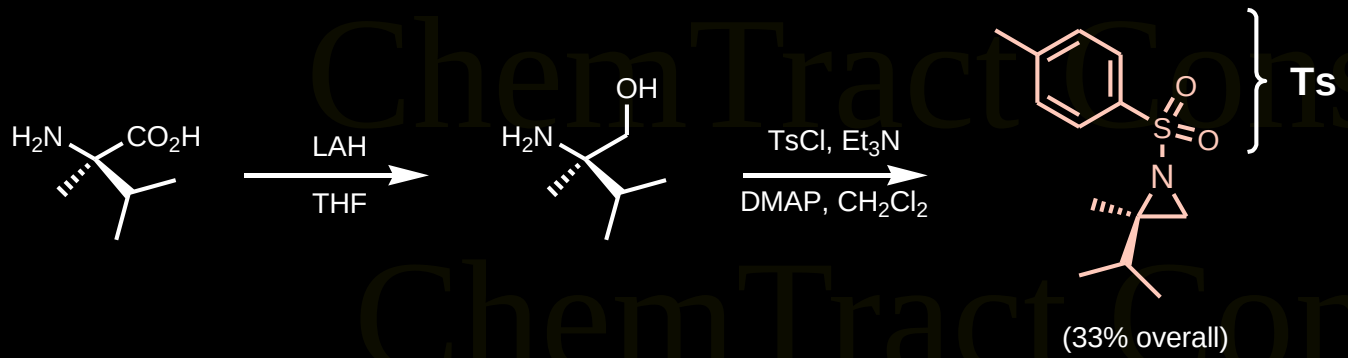


per-acetylated 25-deoxyenfumafungin **(3)**

R. Wilkening

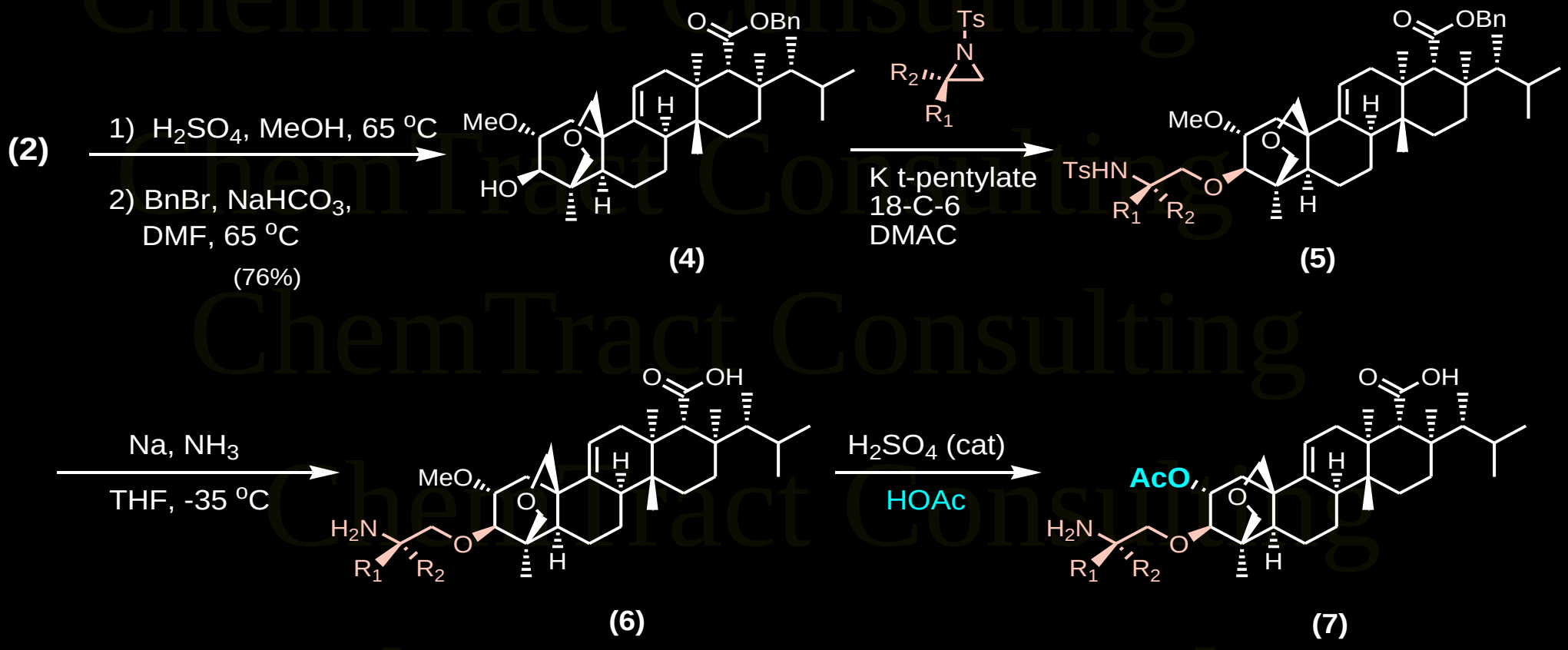
Synthesis of Enantiomerically Pure Aziridines



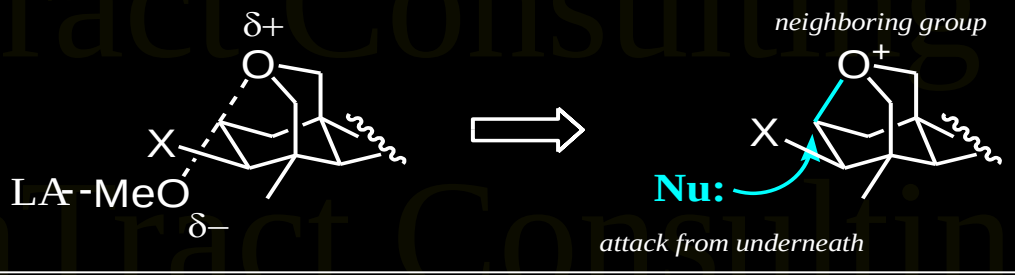


(33% overall)

Synthesis of 3-Aminoethers



retention of configuration:



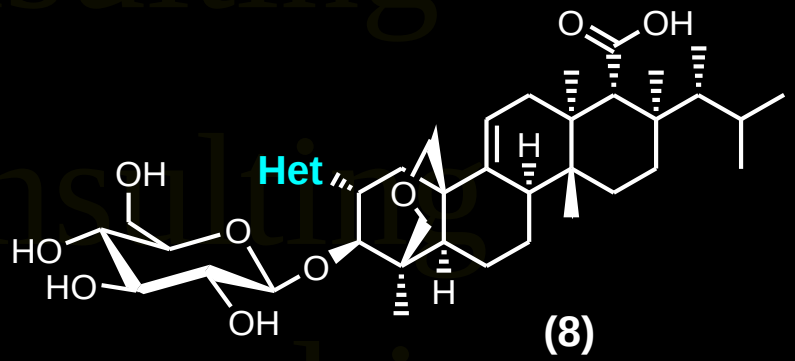
Synthesis of 2-Heterocyclic Derivatives

peracetylated

(3)

1) **Heterocycle**
$BF_3 \bullet OEt_2$
CH_2Cl_2, 50 °C

2) 2.0 M NH_3 in MeOH



(8)

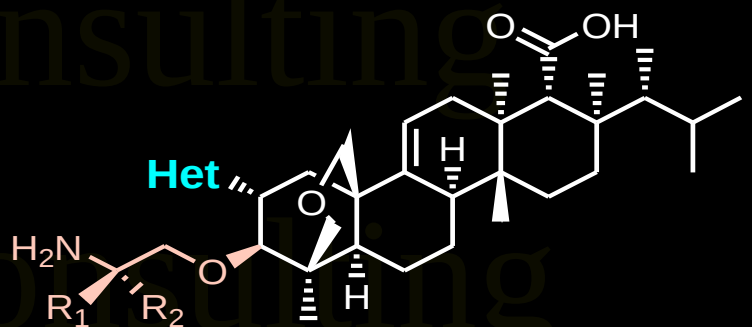
regioisomers were separated by HPLC

(6)

Heterocycle

$BF_3 \bullet OEt_2$

CH_2Cl_2, 50 °C

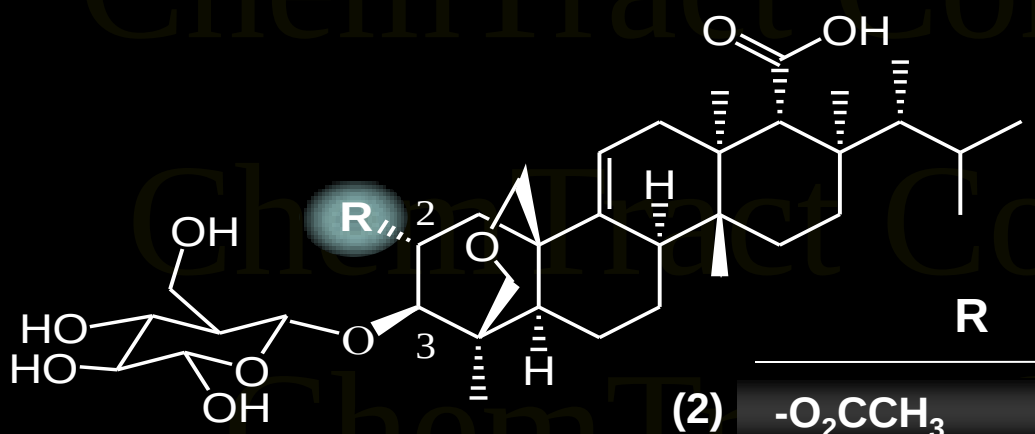


(9)

R. Wilkening, J. Apgar

B. Heasley Bioorg Med Chem Lett 22, 6811–6 (2012)

Antifungal Activity of C2-Derivatives



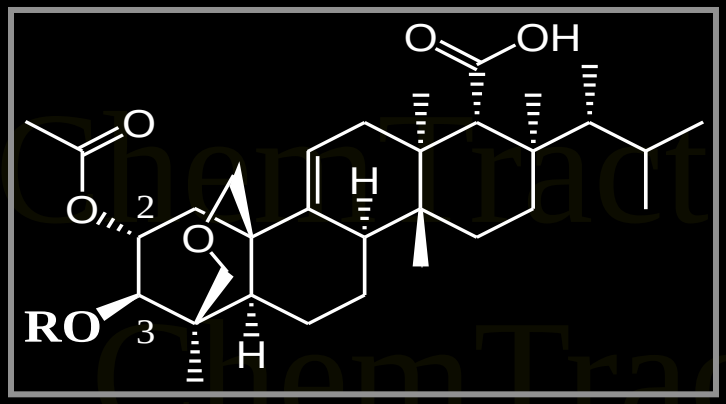
all concentrations in μg/mL

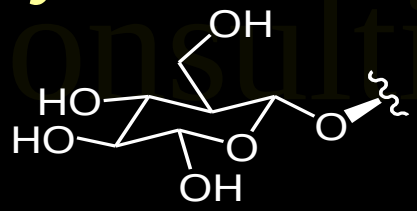
	R	IC_{50} GS	MIC *C. albicans*	MEC *A. fumigatus*
(2)	**$-O_2CCH_3$**	**0.06**	**0.25**	**<0.03**
	$-OC_6H_5$	0.42	2	0.125
	p-$(OCH_3)C_6H_4$	0.47	2	0.25
	1-imidazolone	1.04	1	<0.03
	1-indole	1.06	>32	4
	1-indazole	>1	32	8
	1-pyrazole	0.57	2	0.06
(8a)	1-tetrazole	0.20	1	<0.03
	1-[1,2,3-triazole]	>10	>32	2
(8b)	1-[1,2,4-triazole]	0.24	2	0.125
	4-[1,2,4-triazole]	NT	4	1

- In 3β-glycoside series, neither GS nor AF activity was improved upon replacement of the 2-acetoxy group with a variety of heterocycles

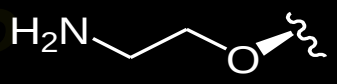
R. Wilkening, J. Apgar

Antifungal Activity of C3-Aminoethers





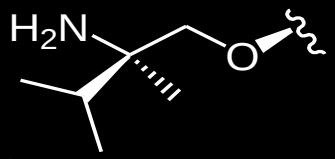
(2)



(10)

(11)

(12)



(13)

all concentrations in μg/mL

Mouse PK and PO Efficacy

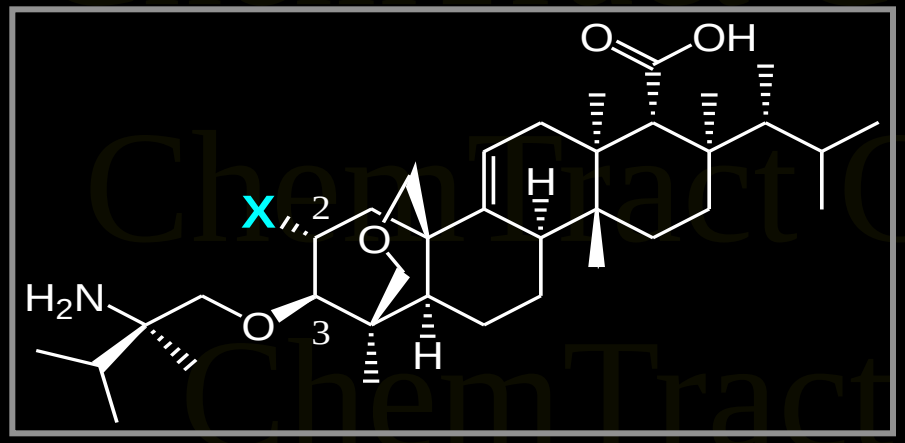
Compound	IC_{50} GS	MIC (+ser) *C. albicans*	MEC *A. fumigatus*	$t_{1/2}$ (h)	F_{oral}	TOKA ED_{99} (mg/kg) (Δlog CFUs)[a]
2	0.06	0.25 (8)	<0.03	--	--	>50
10	0.046	1 (32)	<0.03	4.7	24%	>25
11	0.045	1 (32)	<0.03	7.3	37%	**>25 (-1.4)**
12	0.014	0.25 (16)	<0.03	5.6	31%	**>25 (-1.9)**
13	0.006	0.5 (>32)	0.06	8.3	39%	**>25 (-2.2)**

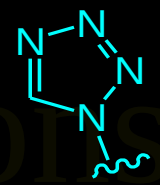
[a] *25 mg/kg, b.i.d. X 4d.*

- In 2-acetoxy series, aminoalkyl ether substitution gave improved GS activity but higher MICs. Geminal substitution provided weak activity in the TOKA

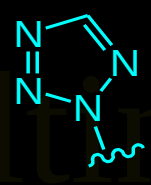
J. Apgar, D. Meng, D. Parker, R. Wilkening

C2-Tetrazole Derivatives





(9a)



(9b)

(9c)

(9d)

all concentrations in μg/mL

Mouse PK and PO Efficacy

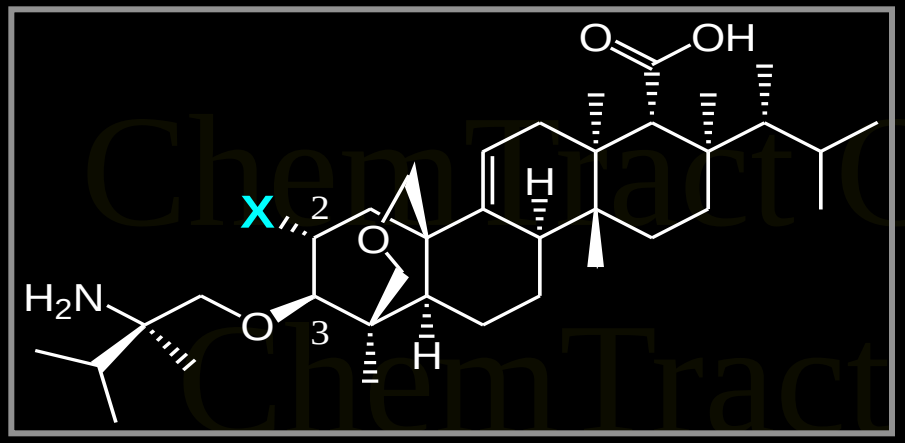
Compound	IC_{50} GS	MIC (+ser) *C. albicans*	MEC *A. fumigatus*	$t_{1/2}$ (h)	F_{oral}	TOKA ED_{99} (mg/kg) (Δlog CFUs)[a]
9a	0.05	16 (>32)	0.125	--	--	--
9b	0.005	0.25 (16)	<0.03	1.9	7%	**>25 (-1.3)**
9c	0.016	1 (>32)	0.5	--	--	**--**
9d	0.002	<0.03 (2)	<0.03	6.3	14%	**>25 (-2.2)**

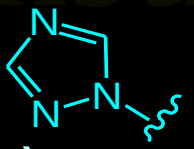
[a] *25 mg/kg, b.i.d. X 4d.*

- 2-Substituted tetrazole more active than 1-substituted tetrazole while amine substitution provided superior potency

J. Apgar, R. Wilkening

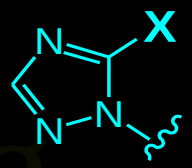
C2-Triazole Derivatives





(9e)

(9f)



(9g) X = phenyl
(9h) X = 3-pyridyl
(9i) X = 4-pyridyl

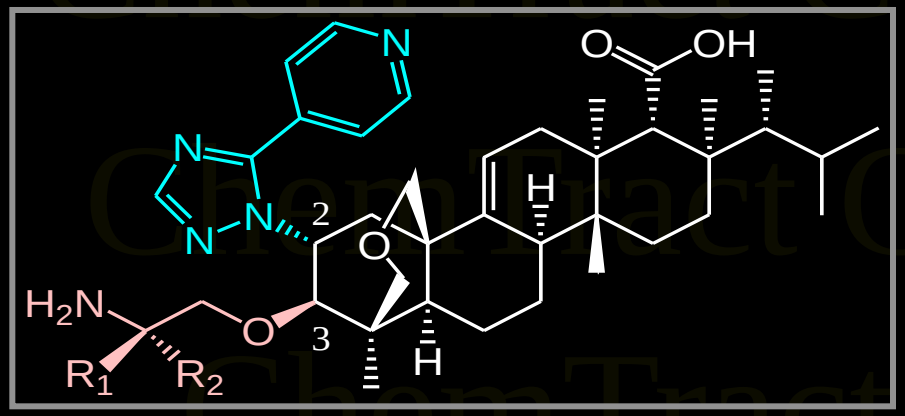
all concentrations in μg/mL

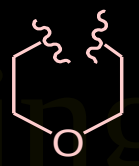
Mouse PK and PO Efficacy

Compound	IC_{50} GS	MIC (+ser) *C. albicans*	MEC *A. fumigatus*	$t_{1/2}$ (h)	F_{oral}	TOKA ED_{99} (mg/kg) (Δlog CFUs)[a]
9e	0.006	0.25 (4)	<0.03	--	--	--
9f	0.006	0.25 (8)	<0.03	7.5	1%	--
9g	0.003	0.25 (4)	<0.03	7.0	27%	**15 (-2.4)**
9h	0.004	0.06 (1)	<0.03	4.5	11%	**9.3 (-3.1)**
9i	0.002	<0.03 (0.5)	<0.03	4.9	30%	**5.9 (-4.5[b])**

[a] *25 mg/kg, b.i.d. X 4d.* [b] *clearance of kidneys observed in 75% of mice*

3-Aminoether Optimization



R_1 = CH_3	Et		t-Bu	t-Bu
R_2 = i-Pr	Et		H	Me
(9j)	**(9k)**	**(9l)**	**(9m)**	**(9n)**

all concentrations in μg/mL

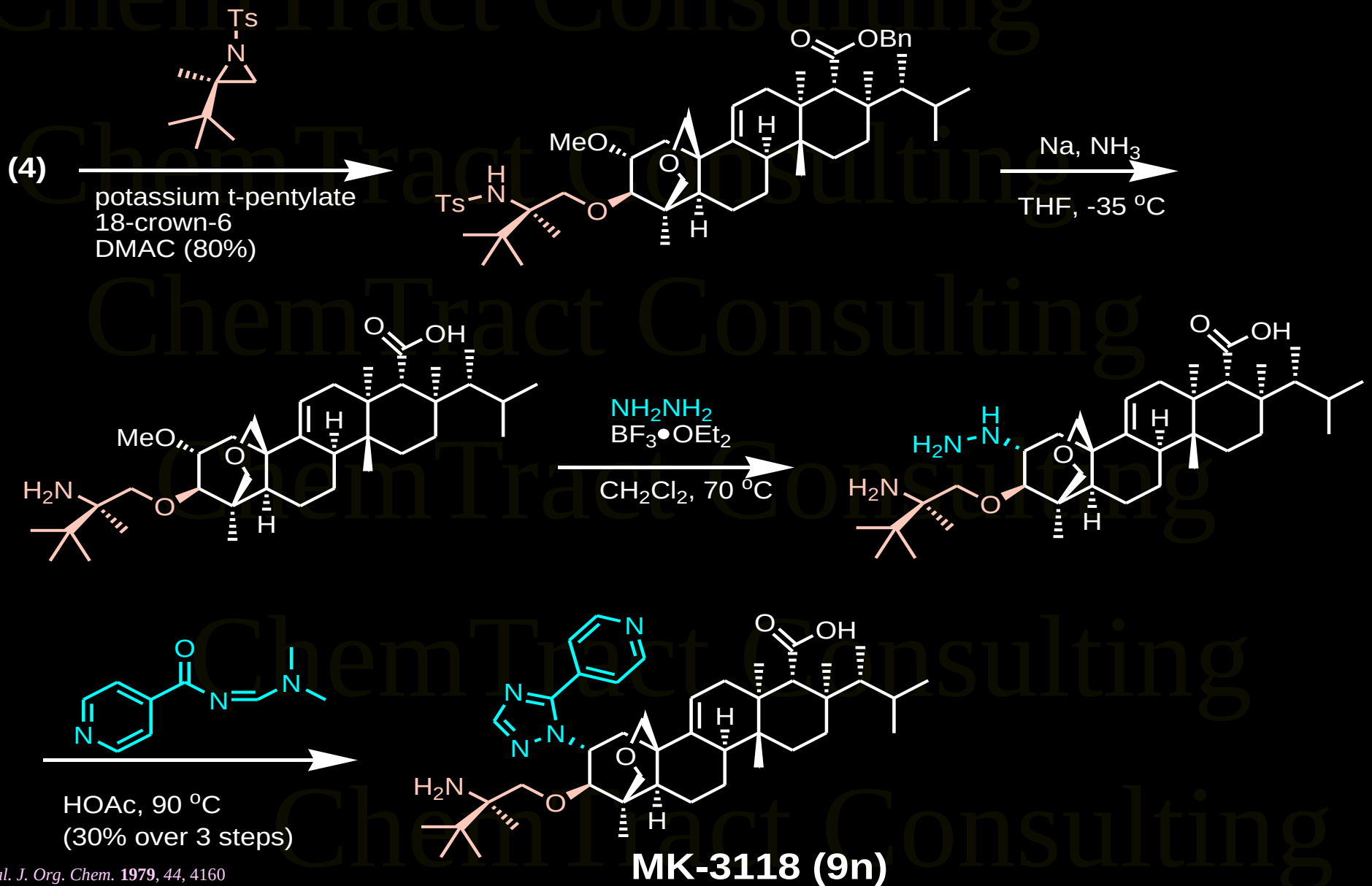
Mouse PK and PO Efficacy

Compound	IC_{50} GS	MIC (+ser) *C. albicans*	MEC *A. fumigatus*	$t_{1/2}$ (h)	F_{oral}	TOKA ED_{99} (mg/kg) (Δlog CFUs)[a]
9j	0.004	0.25 (2)	<0.03	--	--	**13 (-2.6)**
9k	0.001	0.06 (1)	<0.03	--	--	**10 (-3.3)**
9l	0.002	<0.03 (0.25)	<0.03	2.1	13%	**4 (-3.9)**
9m	0.007	0.25 (2)	<0.03	--	--	**11 (-2.6)**
9n	0.0006	0.06 (0.5)	<0.03	4.4	34%	**6.3 (-4.6[b])**

[a] *25 mg/kg, b.i.d. X 4d.* [b] *clearance of kidneys observed in 50% of mice*

- The *tert*-butyl, methyl aminoalkyl ether gave superior potency and PK behavior. **9n** was evaluated more fully as a potential development candidate (MK-3118)

Synthesis of MK-3118



MK-3118 (9n)

Y. Lin, *et al. J. Org. Chem.* **1979**, *44*, 4160

In Vitro Activity Comparison

(all values are in **(μg/mL)**	**MK-3118**	**Caspofungin**
***Glucan Synthase* (IC_{50})**		
C. albicans	0.6	0.6
A. fumigatus	1.7	0.5
***Candida* spp. (MIC_{90})**		
C. albicans (32)	0.015	0.015
C. glabrata (15)	0.25	0.125
C. krusei (18)	1	0.125
C. parapsilosis (22)	0.125	0.5
C. tropicalis (55)	0.5	<0.03
C. guilliermondii (18)	1	0.5
C. lusitaniae (5)	0.5 - 4	0.06 - 0.25
***Aspergillus* spp. (MEC_{90})**		
A. fumigatus (14)	0.008	0.015
A. flavus (13)	0.015	0.03
A. niger (10)	0.015	0.03
A. terreus (3)	0.008	0.015

Minimum Inhibitory Concentration (MIC) is the concentration that results in prominent inhibition of growth of *Candida* spp.
Minimum effective concentration (MEC) is the concentration that results in altered morphology of *Aspergillus* spp.

M. Motyl, et al. ICAAC F1-847 (2010)

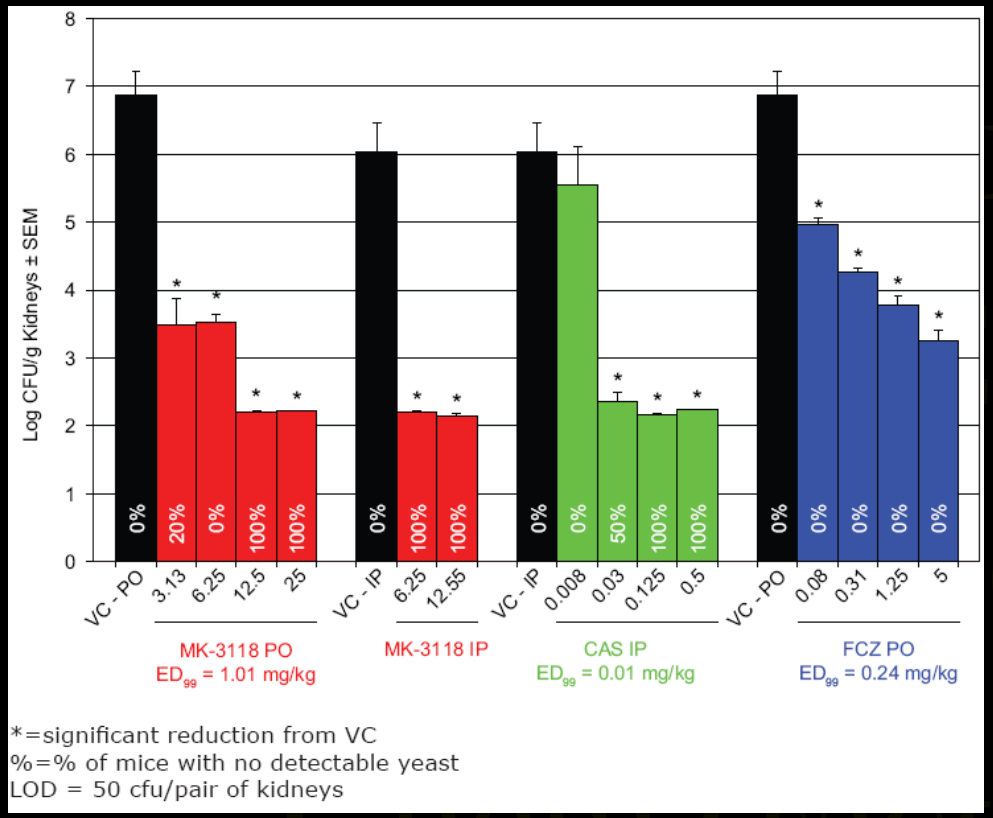
Activity Against Caspofungin Resistant Isolates

	Genotype	MK-3118	Caspofungin
C. albicans			
CLY16996	S645F/S645F	<0.03	1
CLY16997	S645F/S645P	0.125	4
CLY724	D648Y/D648Y	<0.03	4
CLY16376	R1361H/R1361H	0.125	0.5
CLY18559	S645Y/S645Y	<0.03	2
CLY19231	S645F/S645F	<0.03	2
CLY18600	WT/S645F (R1361H)	<0.03	0.5
CLY24738	S645F/S645F	<0.03	0.5
CLY719	F641L/F641L	<0.03	0.25
CLY22916	F641S/S641F	2	2
C. krusei			
CLY16038	R1361G/R1361G	0.25	8

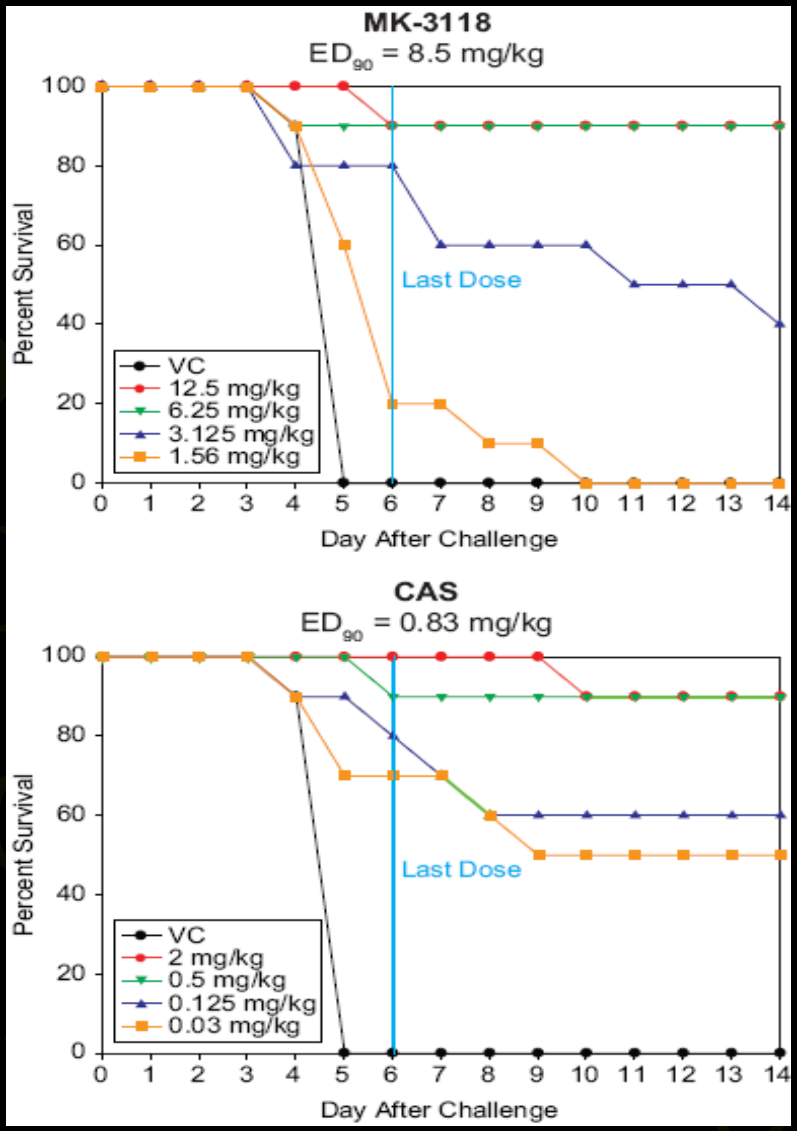
[a] MICs based on CLSI Method 27-A2 and are expressed in μg/mL

In Vivo Antifungal Activity of MK-3118

Mouse Candidiasis Model



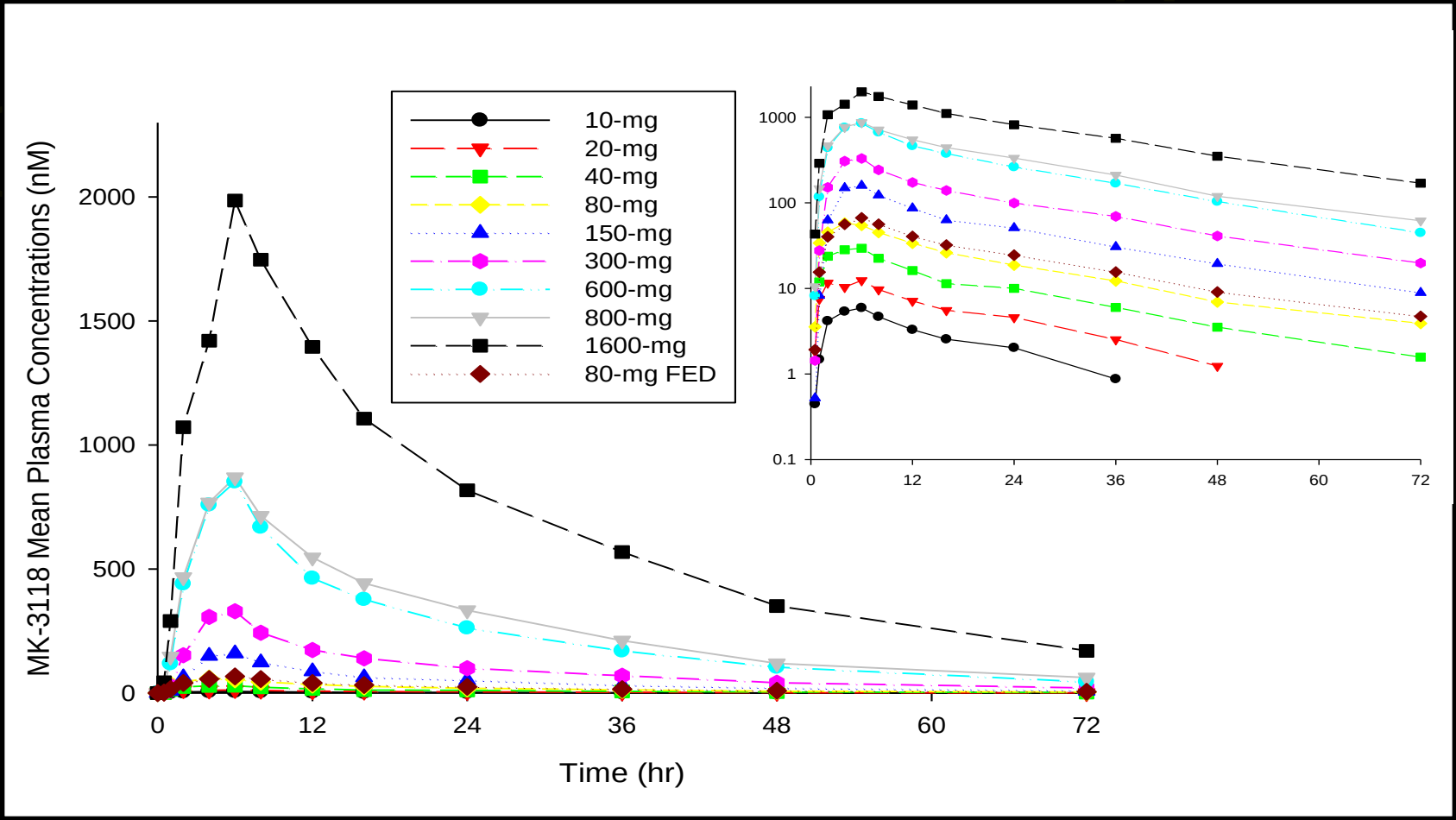
Mouse Aspergillosis Model



Drugs were administered orally (PO) or intraperitoneally (IP) twice daily for 7 days after challenge with either *C. albicans* (MY1055) or *A. fumigatus* (MF5668)

J. Apgar, D. Meng, D. Parker, R. Wilkening

Mean Plasma Concentration Profiles for Subjects Administered Oral Doses of MK-3118



- MK-3118 was generally well tolerated at doses up to 1600 mg
- Harmonic mean terminal half life was ~ 20 hours consistent with once daily dosing
- No significant food effect

ChemTract Consulting

ChemTract Consulting

ChemTract Consulting

ChemTract Consulting

ChemTract Consulting

ChemTract Consulting