

Figure 1. Capped-stick and space-filling models of **5** from X-ray data.

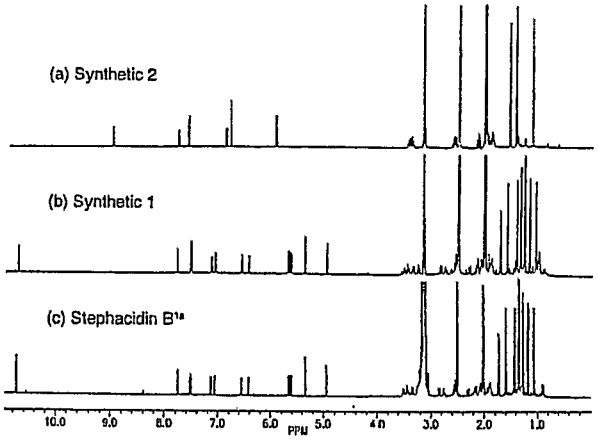


Figure 2. ^{1}H NMR spectra at 23 °C (500 MHz, 1:1 DMSO-d_6–CD_3CN) of (a) synthetic "avrainvillamide" (**2**), (b) synthetic stephacidin B (**1**), (c) stephacidin B from a fungal source

^{1}H (500 MHz) and ^{13}C (100 MHz) NMR Spectra of Synthetic Avrainvillamide (2) ($CDCl_3$)

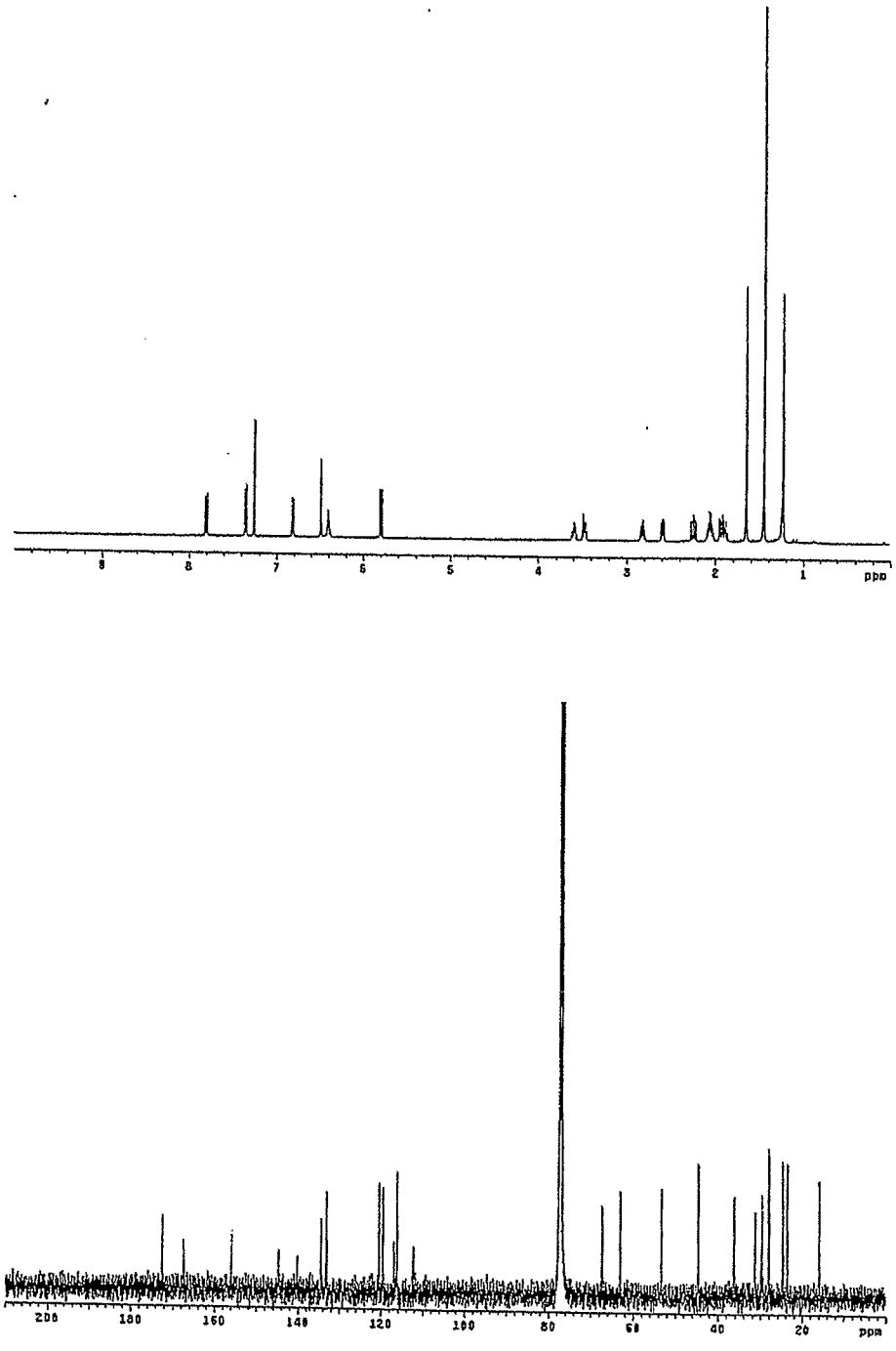


Fig. 3

Authentic stephacidin B (500 MHz, 50% d_6-DMSO-CD_3CN)

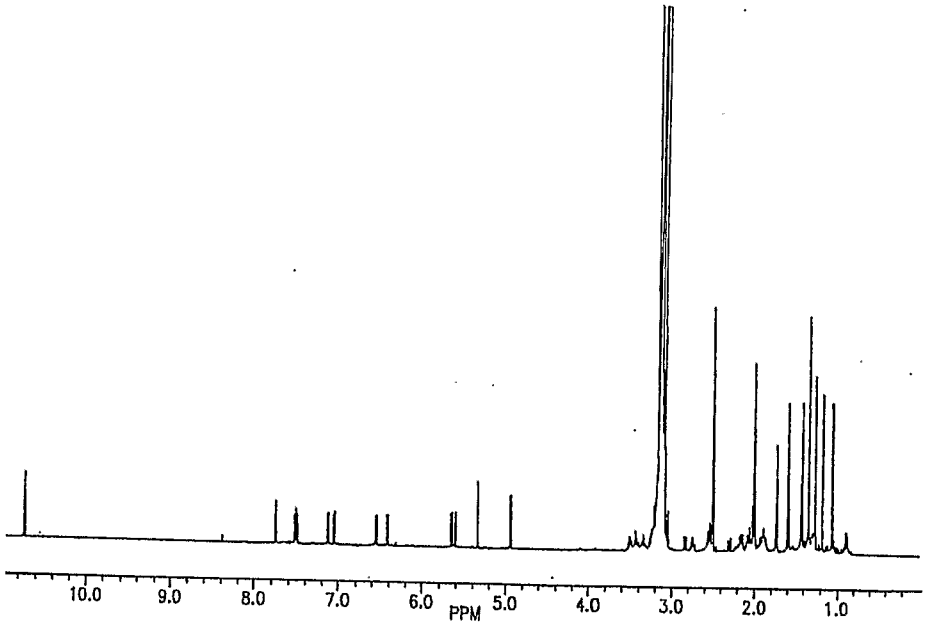


Fig. 4A

Synthetic stephacidin B (500 MHz, 50% d_6-DMSO-CD_3CN)

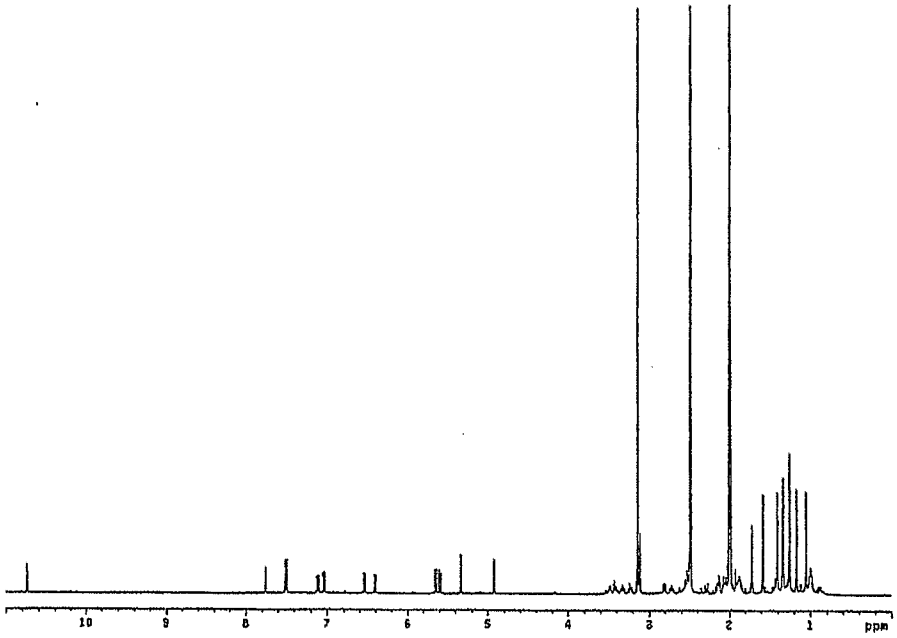
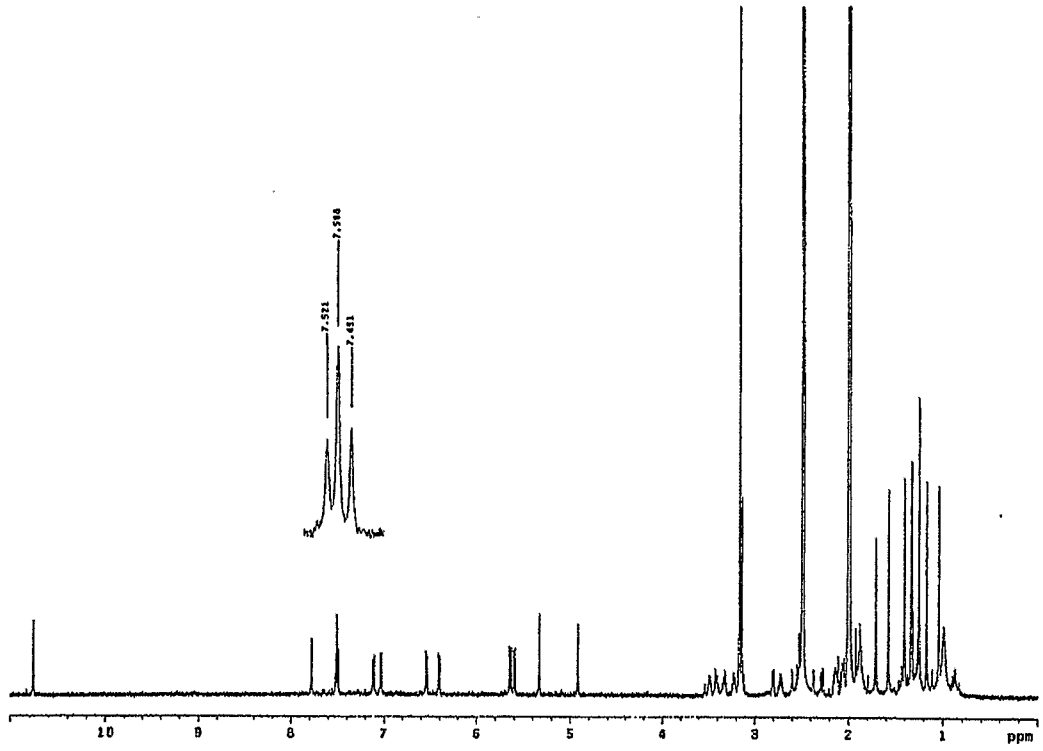


Fig. 4B

Synthetic stephacidin B (500 MHz, 50% d_6-DMSO-CD_3CN,



5

Fig. 4C

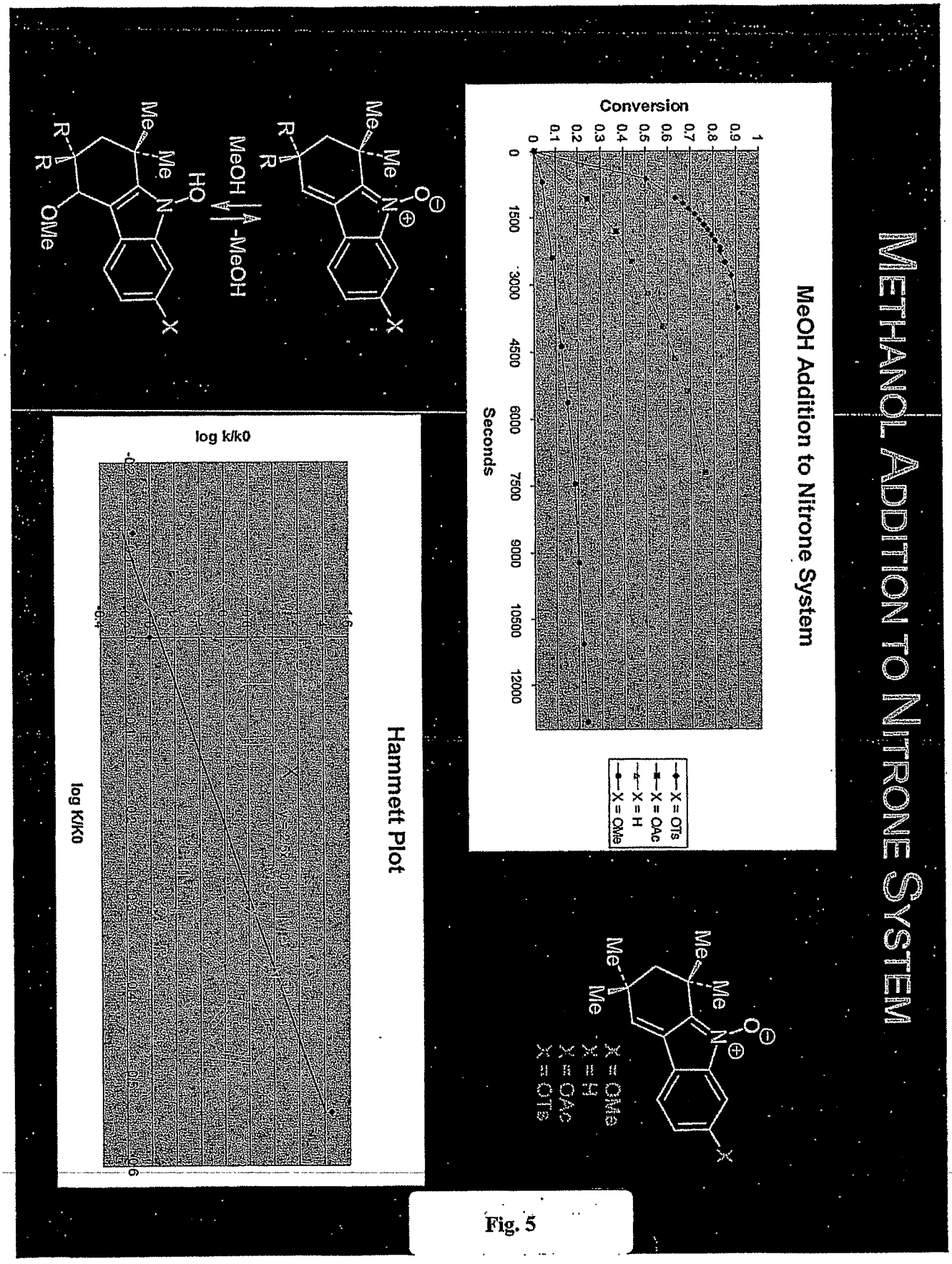

METHANOL ADDITION TO NITRONE SYSTEM
MeOH Addition to Nitrone System
Conversion
Seconds
X = OTs
X = OAc
X = H
X = OMe
MeOH
-MeOH
X = OMe
X = H
X = OAc
X = OTs
Hammett Plot
log k/k0
log K/K0

Fig. 5

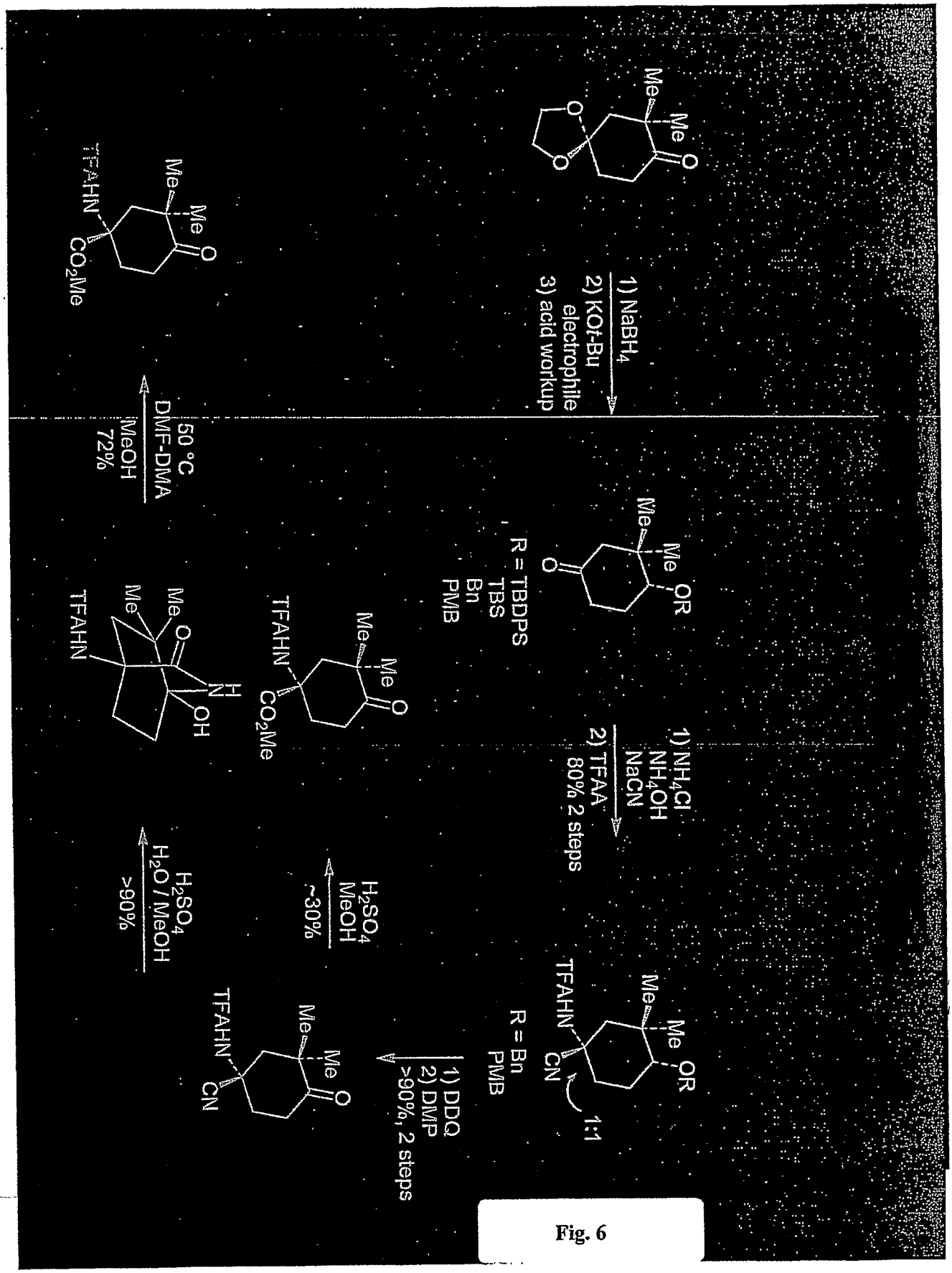

1) NaBH4
2) KOt-Bu
electrophile
3) acid workup
1) NH4Cl
NH4OH
NaCN
2) TFAA
80% 2 steps
R = TBDPS
TBS
Bn
PMB
R = Bn
PMB
1:1
1) DDQ
2) DMP
>90%, 2 steps
50 °C
DMF-DMA
MeOH
72%
H2SO4
MeOH
~30%
H2SO4
H2O / MeOH
>90%
TFAHN
CO2Me
CN
OH
OR
Me

Fig. 6

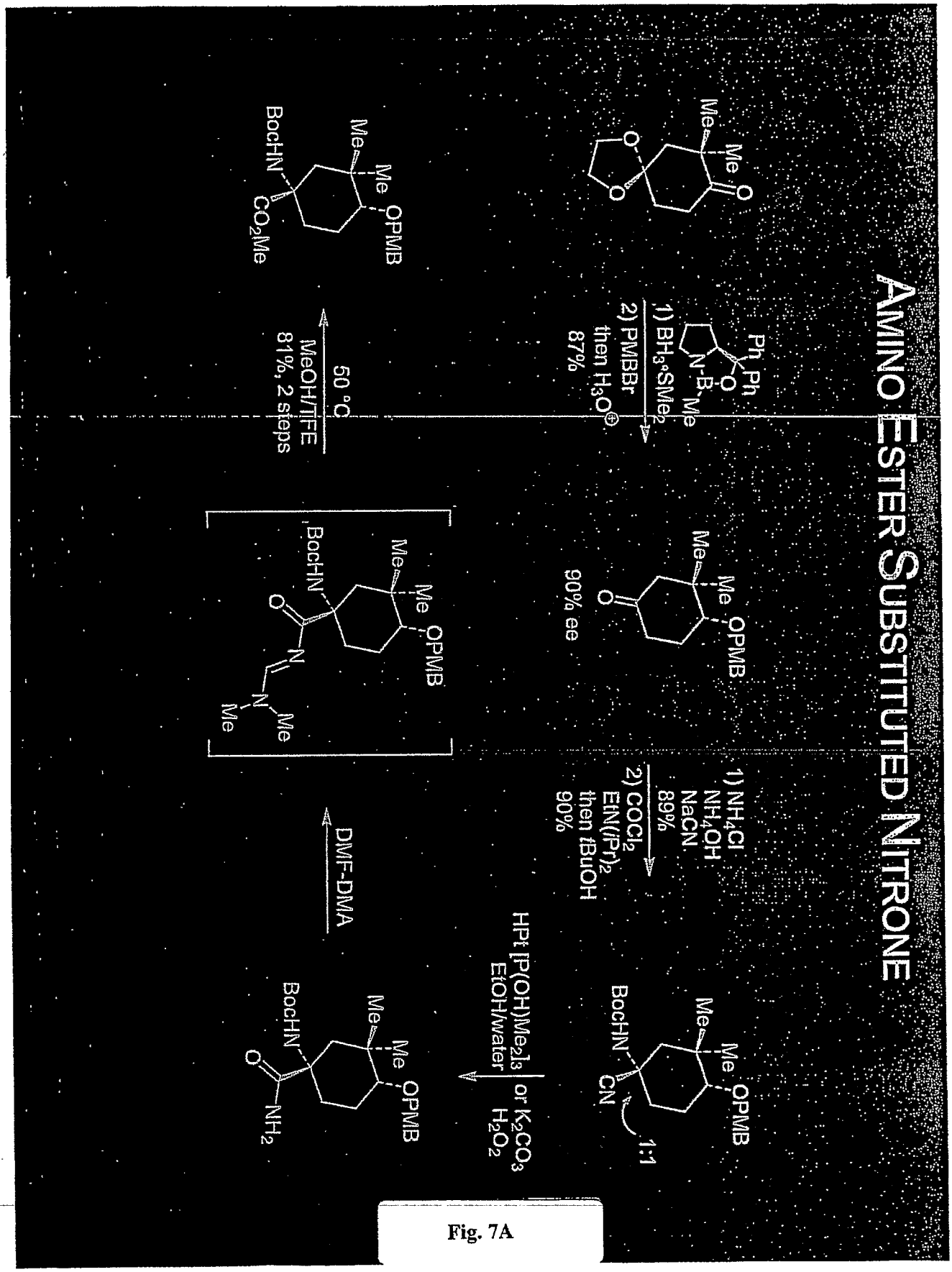

AMINO ESTER SUBSTITUTED NITRONE
1) NH4Cl NH4OH NaCN 89%
2) COCl2 EtN(iPr)2 then tBuOH 90%
90% ee
1:1
HPt[P(OH)Me2]3 EtOH/water
or K2CO3 H2O2
DMF-DMA
1) BH3•SMe2
2) PMBBr then H3O⊕ 87%
50 °C MeOH/TFE 81%, 2 steps
BocHN
CN
NH2
CO2Me
OPMB
Me
Ph
N-B-Me
O
N

Fig. 7A

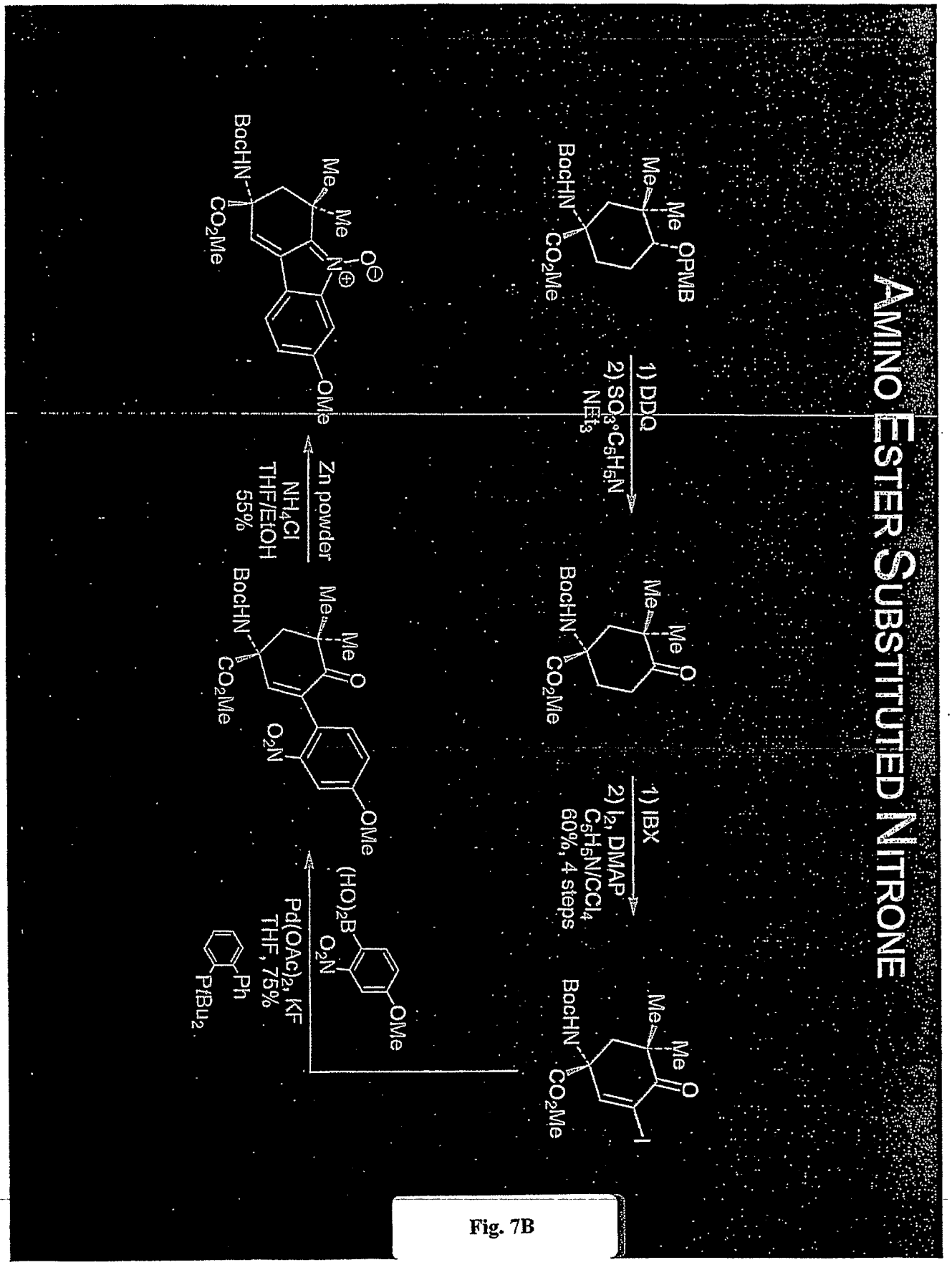

AMINO ESTER SUBSTITUTED NITRONE
BocHN
CO2Me
Me
Me
OPMB
1) DDQ
2) SO3•C5H5N
NEt3
1) IBX
2) I2, DMAP
C5H5N/CCl4
60%, 4 steps
O
I
Zn powder
NH4Cl
THF/EtOH
55%
O2N
OMe
(HO)2B
Pd(OAc)2, KF
THF, 75%
Ph
PtBu2

Fig. 7B

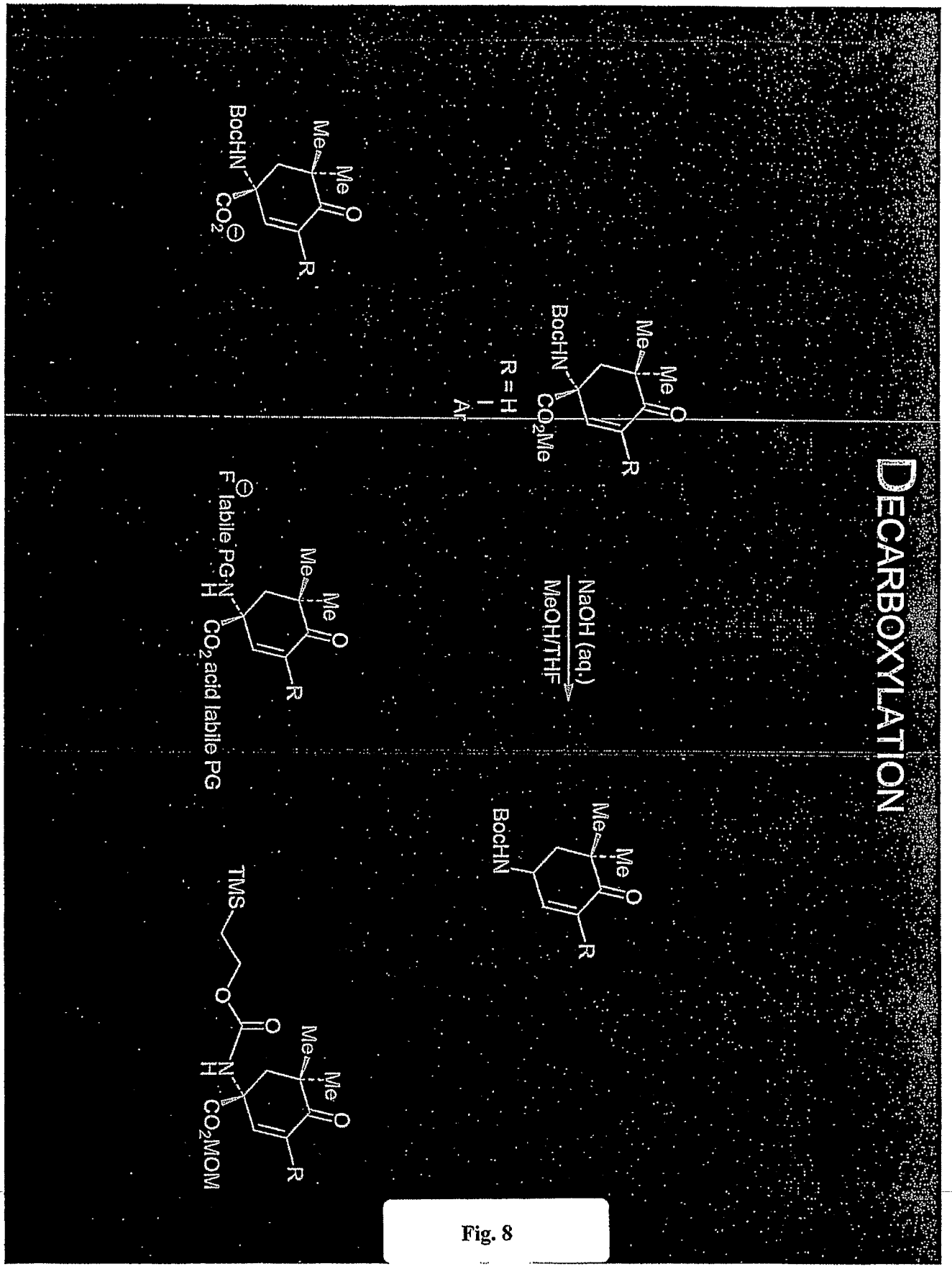

DECARBOXYLATION
NaOH (aq.)
MeOH/THF
BocHN
Me
Me
O
R
CO2Me
R = H
Ar
CO2⊖
F⊖ labile PG
N
H
CO2 acid labile PG
TMS
O
CO2MOM

Fig. 8

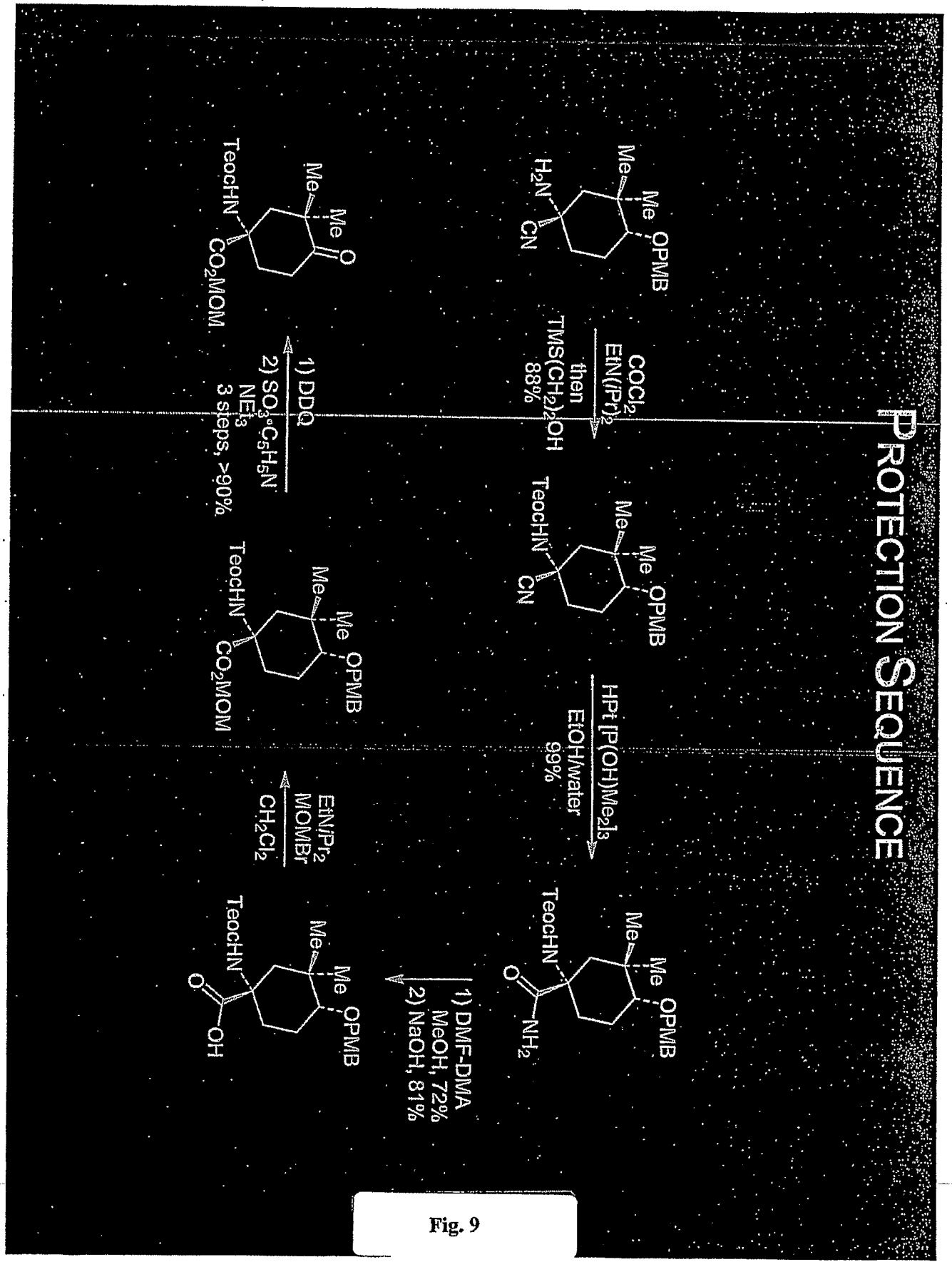

PROTECTION SEQUENCE
COCl2
EtN(iPr)2
then
TMS(CH2)2OH
88%
1) DDQ
2) SO3•C5H5N
NEt3
3 steps, >90%
HPt[P(OH)Me2]3
EtOH/water
99%
1) DMF-DMA
MeOH, 72%
2) NaOH, 81%
EtN/Pr2
MOMBr
CH2Cl2
Me
OPMB
TeocHN
CN
CO2MOM
NH2
OH
O

Fig. 9

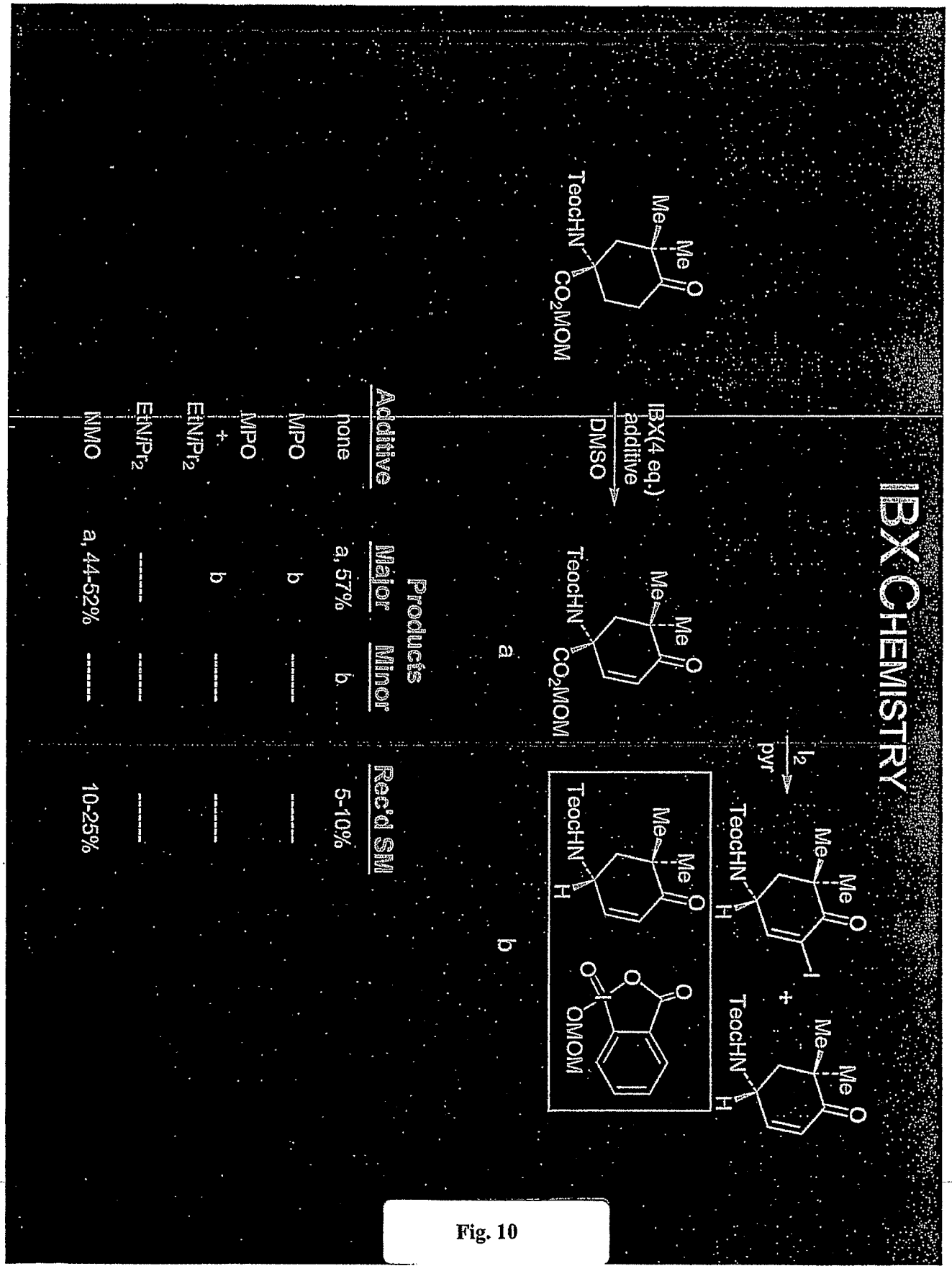

IBX CHEMISTRY
I2
pyr
TeocHN
Me
Me
O
I
H
OMOM
IBX(4 eq.)
additive
DMSO
CO2MOM
a
b
Products
Additive
Major
Minor
Rec'd SM
none
a, 57%
b
5-10%
MPO
b
MPO + EtNiPr2
b
EtNiPr2
NMO
a, 44-52%
10-25%

Fig. 10

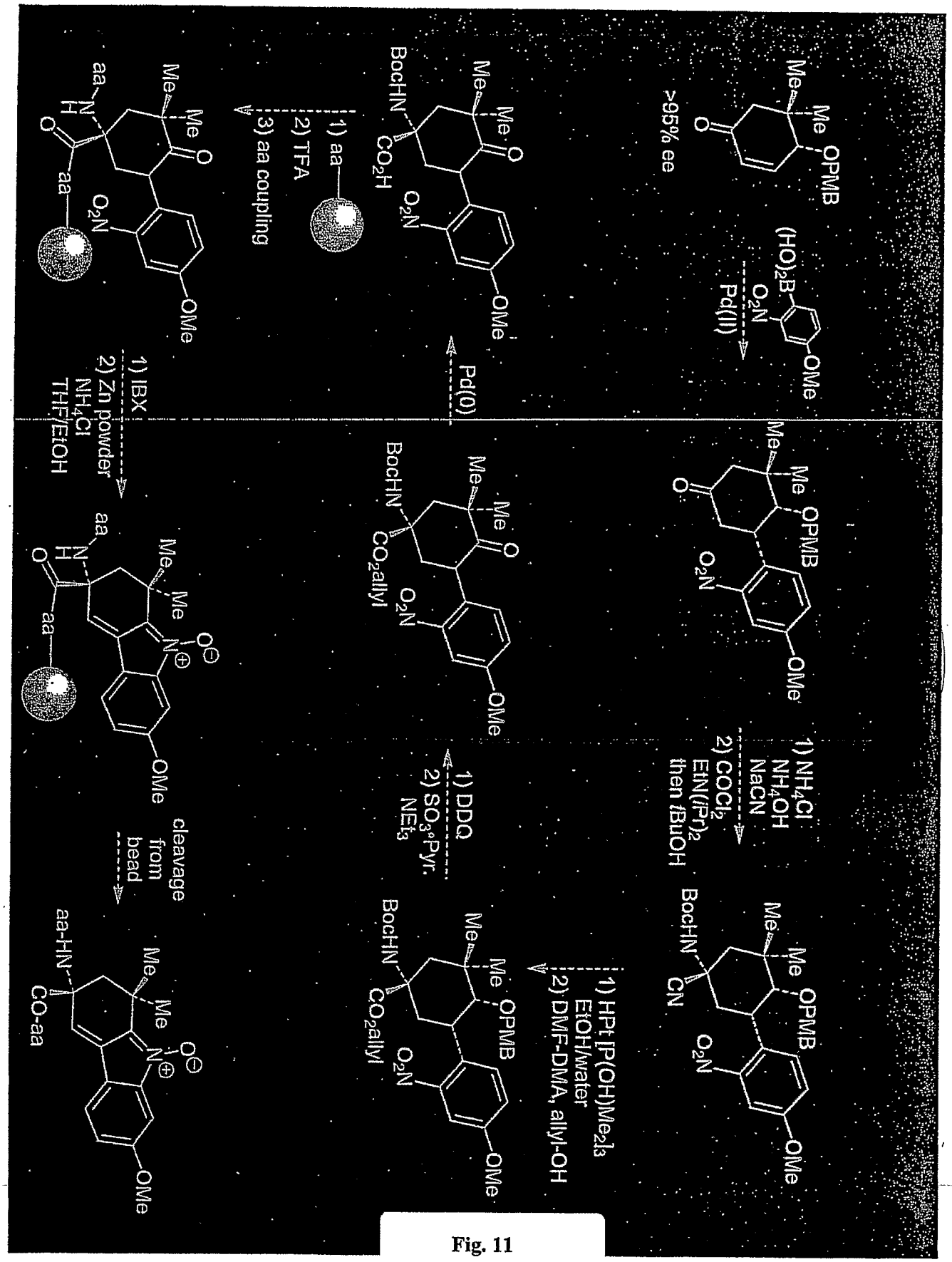
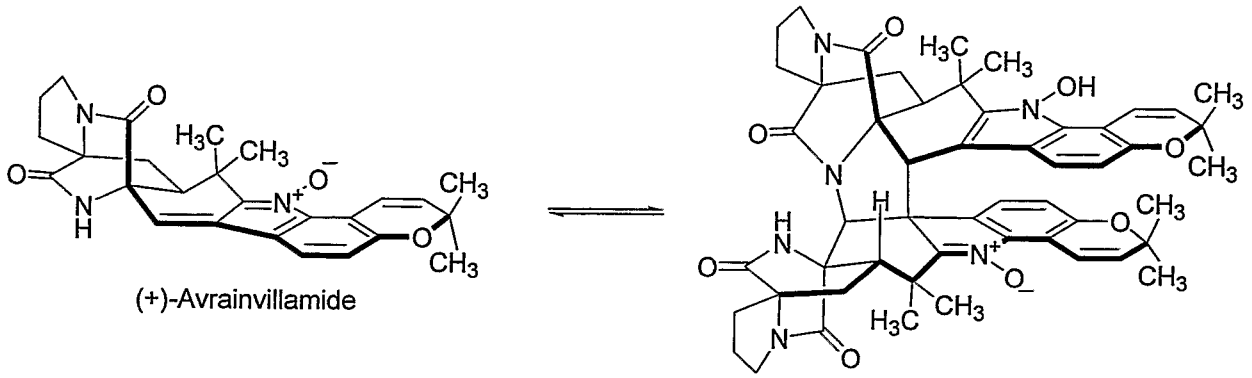

>95% ee
Pd(II)
Pd(0)
1) aa
2) TFA
3) aa coupling
1) IBX
2) Zn powder
NH4Cl
THF/EtOH
1) NH4Cl
NH4OH
NaCN
2) COCl2
EtN(iPr)2
then tBuOH
1) HPt[P(OH)Me2]3
EtOH/water
2) DMF-DMA, allyl-OH
1) DDQ
2) SO3•Pyr.
NEt3
cleavage
from
bead

Fig. 11



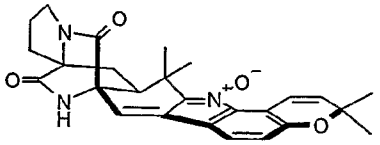
GI50 Data for Avrainvillamide & Stephacidin B: "Method A"

	"Natural" Enantiomer		"Unnatural" Enantiomer		Literature Value	
	(+)-Avrainvillamide	(-)-Stephacidin B	(-)-Avrainvillamide	(+)-Stephacidin B	Avrainvillamide	Stephacidin B
BT-549	621 nM (531 nM – 710 nM)	346 nM (315 nM – 377 nM)	786 nM (703 nM – 869 nM)	550 nM (475 nM – 624 nM)	34 nM	not reported
T-47D	205 nM (88 nM – 321 nM)	91 nM (16 nM – 165 nM)	1485 nM (1267 nM – 1703 nM)	942 nM (508 nM – 1376 nM)	72 nM	not reported
MALME-3M	406 nM (160 nM – 653 nM)	289 nM (67 nM – 511 nM)	1854 nM (1729 nM – 1978 nM)	987 nM (673 nM – 1301 nM)	53 nM	not reported
LnCAP	241 nM (140 nM – 342 nM)	135 nM (18 nM – 253 nM)	1514 nM (1186 nM – 1843 nM)	952 nM (572 nM – 1332 nM)	not reported	60 nM

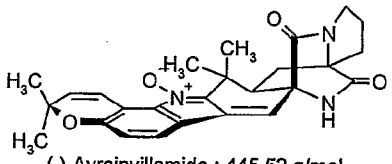
values in parentheses represent 95% confidence intervals

Fig. 12

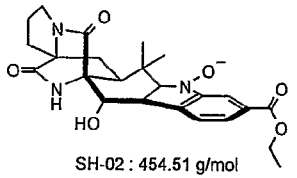
GI50 Data for Avrainvillamide Analogues: "Method C"



(+)-Avrainvillamide : 445.52 g/mol

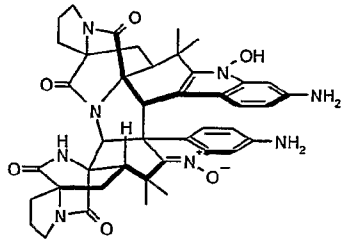


(-)-Avrainvillamide : 445.52 g/mol



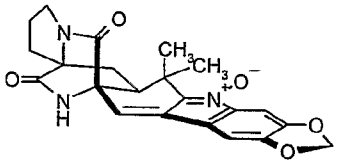
SH-02 : 454.51 g/mol

GI50 values:	**LnCAP**	**0.3 μM**	**2.7 μM**	**> 20 μM**
	T-47D	**0.4 μM**	**1.9 μM**	**> 20 μM**



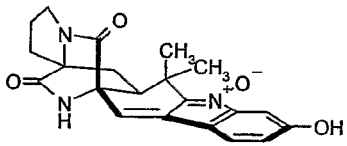
Stephacidin-amine

> 20 μM
> 20 μM



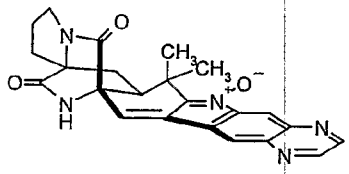
Avrain-methylenedioxy: 407.43 g/mol

9.9 μM
7.4 μM



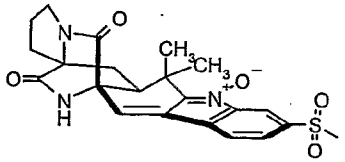
Avrain-phenol : 379.42 g/mol

> 20 μM
> 20 μM



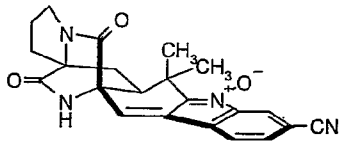
JS-II-303-2: 415.44 g/mol

> 20 μM
> 20 μM



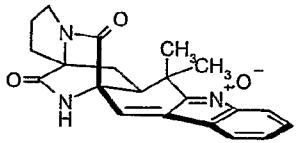
Avrain-sulfone

10 μM
5.9 μM



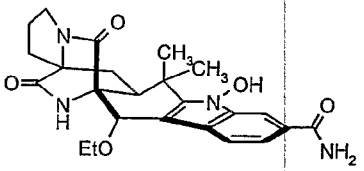
Avrain-nitrile

> 20 μM
> 20 μM



Avrain-H : 363.42 g/mol

10 μM
8.1 μM



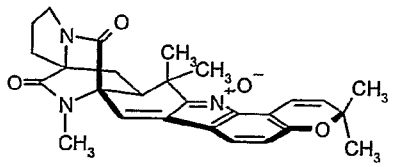
Avrain-Amide : 451.51 g/mol

> 20 μM
> 20 μM

values are based on at least four duplicate analyses (ie: 8 assays in total) over a minimum of two weeks

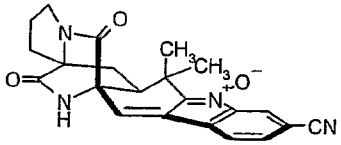
Fig. 13A

GI50 Data for Avrainvillamide Analogues Continued: "Method C"



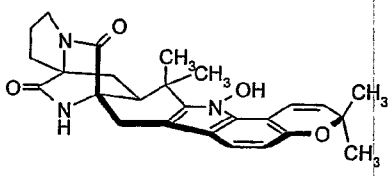
N-Me Avrainvillamide

> 20 μM
> 20 μM



Avrain-nitrile

> 20 μM
> 20 μM



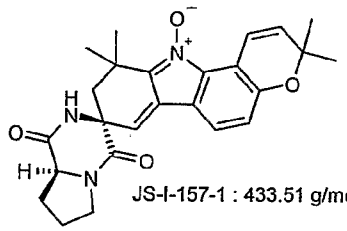
Reduced Avrainvillamide

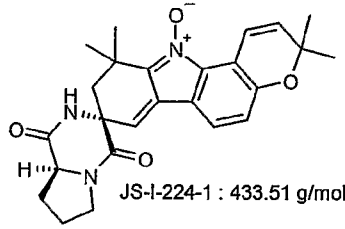
1.7 μM*
0.7 μM*

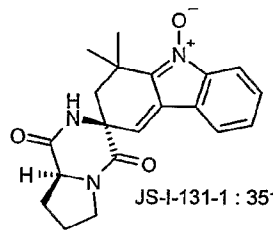
values are based on at least four duplicate analyses (ie: 8 assays in total) over a minimum of two weeks
* this compound got more active each week - becoming oxidized?

Fig. 13B

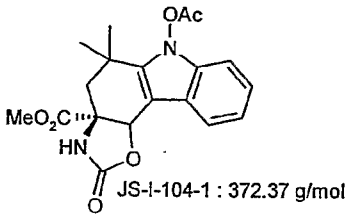
GI50 Data for Amino-Ester Analogues: "Method C"

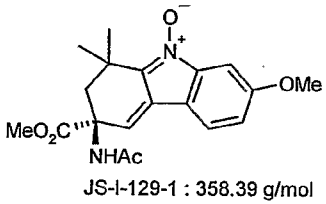


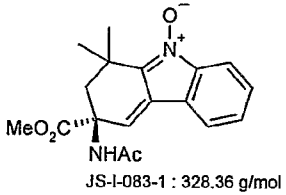


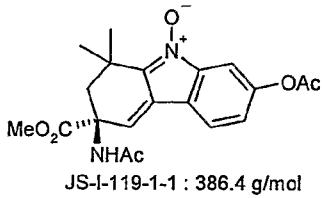


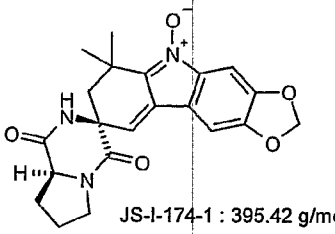
GI50 values:	LnCAP	3.8 μM	3.8 μM	> 20 μM*
	T-47D	2.6 μM	3.1 μM	> 20 μM*











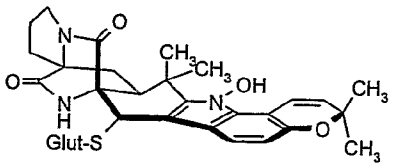
12 μM	10 μM*	> 20 μM*	> 20 μM*	19 μM
6.2 μM	8.1 μM*	15 μM*	> 20 μM*	13 μM

values are based on at least four duplicate analyses (ie: 8 assays in total) over a minimum of two weeks

* these (mostly inactive) compounds have not been re-run in the three-day assay

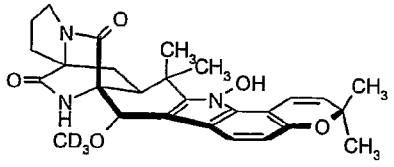
Fig. 14

GI50 Data for Nucleophile Adducts: "Method C"



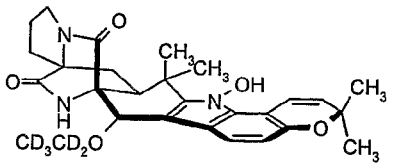
Avrainvillamide Glutathione Adduct:

772 nM
552 nM



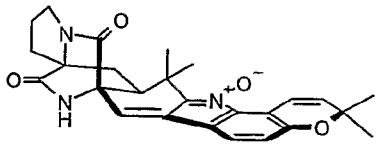
Methanol-d_3 adduct: 480.59 g/mol

528 nM
718 nM



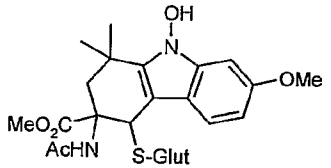
Ethanol-d_5 adduct: 496.62 g/mol

380 nM
670 nM



(+)-Avrainvillamide : 445.52 g/mol

496 nM
476 nM
(on the same day)



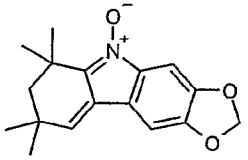
Amino-Ester Glutathione Adduct:

20 μM
9.2 μM

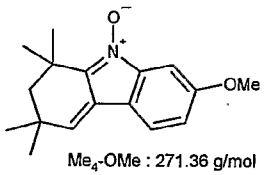
Fig. 15

values are based on at least four duplicate analyses (ie: 8 assays in total) over a minimum of two weeks

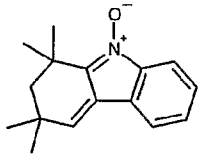
GI50 Data for Tetramethyl-Substituted Analogues: "Method C"



Me_4-O_2Me : 285.35 g/mol

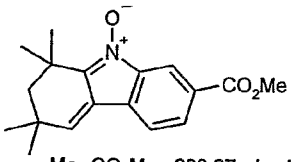


Me_4-OMe : 271.36 g/mol



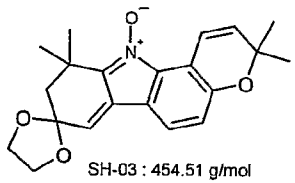
Me_4-H : 241.34 g/mol

GI50 values:				
	LnCAP	6.1 μM	7.3 μM	4.2 μM
	T-47D	1.7 μM	7.1 μM	1.4 μM



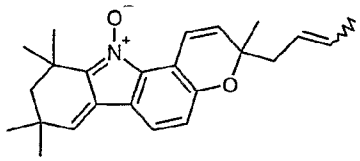
Me_4-CO_2Me : 299.37 g/mol

4.0 μM
4.6 μM



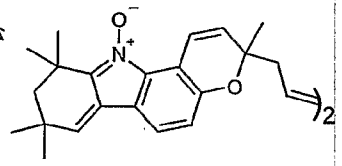
SH-03 : 454.51 g/mol

5.2 μM*
4.0 μM*



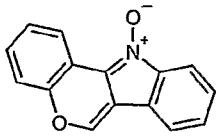
JW-I-161 : 363.50 g/mol

7.6 μM
4.6 μM



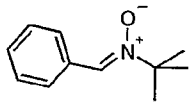
JS-I-190 : 670.90 g/mol

> 20 μM
7.5 μM



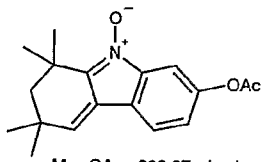
JS-II-316-1: 235.24 g/mol

> 20 μM
> 20 μM



PBN : 241.34 g/mol

> 20 μM
> 20 μM



Me_4-OAc : 299.37 g/mol

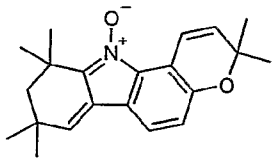
11 μM*
4.4 μM*

values are based on at least four duplicate analyses (ie: 8 assays in total) over a minimum of two weeks

* these (mostly inactive) compounds have not been re-run in the three-day assay

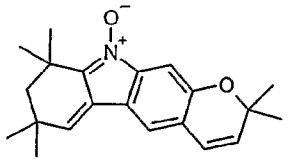
Fig. 16A

GI50 Data for Tetramethyl-Substituted Analogues Continued: "Method C"



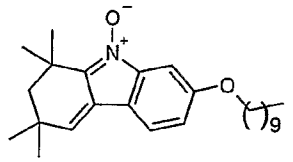
Romain_01 : 323.44 g/mol

5.2 μM
8.4 μM



Romain_02 : 323.44 g/mol

2.2 μM
2.3 μM



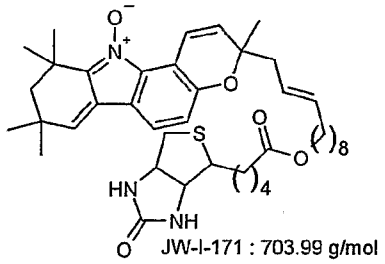
Romain_03 : 285.3807 g/mol

not run
8.2 μM

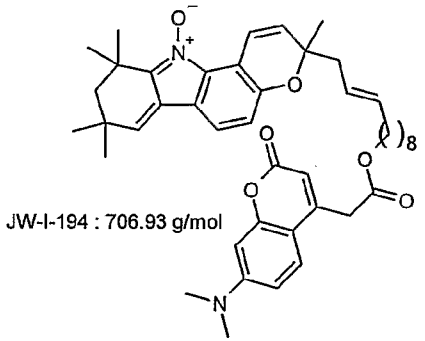
Fig. 16B

values are based on at least four duplicate analyses (ie: 8 assays in total) over a minimum of two weeks

IC50 & GI50 Data for Probe Compounds: "Method B"

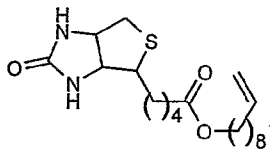


JW-I-171 : 703.99 g/mol

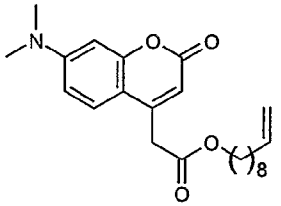


JW-I-194 : 706.93 g/mol

GI50 values:	**LnCAP**	**5.6 μM**	**> 20 μM**
	T-47D	**7.0 μM**	**16 μM**



JW-I-159 : 382.99 g/mol



JW-I-191 : 385.51 g/mol

> 20 μM	**> 20 μM**	
> 20 μM	**> 20 μM**	

Fig. 17

values are based on at least four duplicate analyses (ie: 8 assays in total) over a minimum of two weeks

Characterization: Decomposition of Stephacidin B

In DMSO and Cell Culture Media at 22.5 °C -- Monitored by HPLC

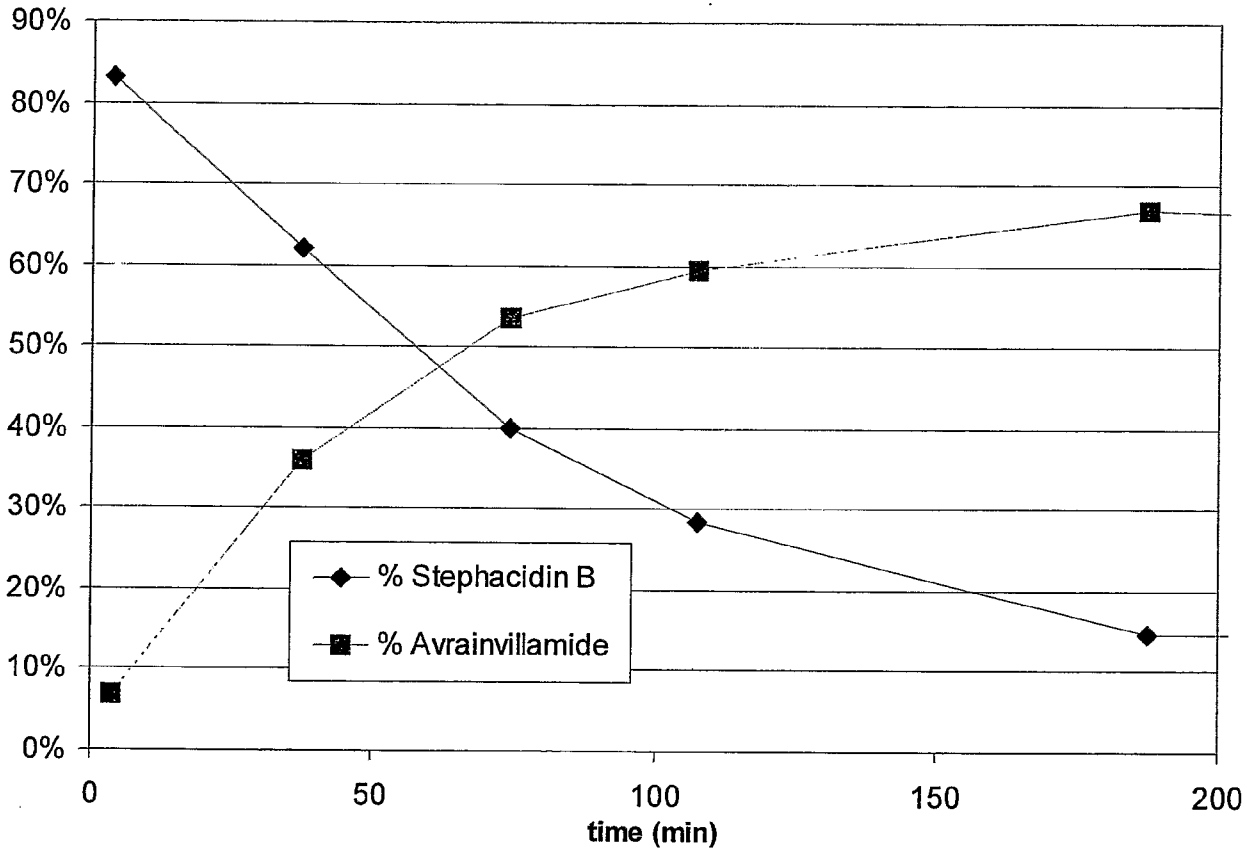


Fig. 18

Half Life at 22.5 °C: 52 minutes

Half Life at 37 °C: ~ 10 minutes

1 mmol stephacidin B produces

~1.7 mmol avrainvillamide

➢ Avrainvillamide is the major species present under physiological conditions.

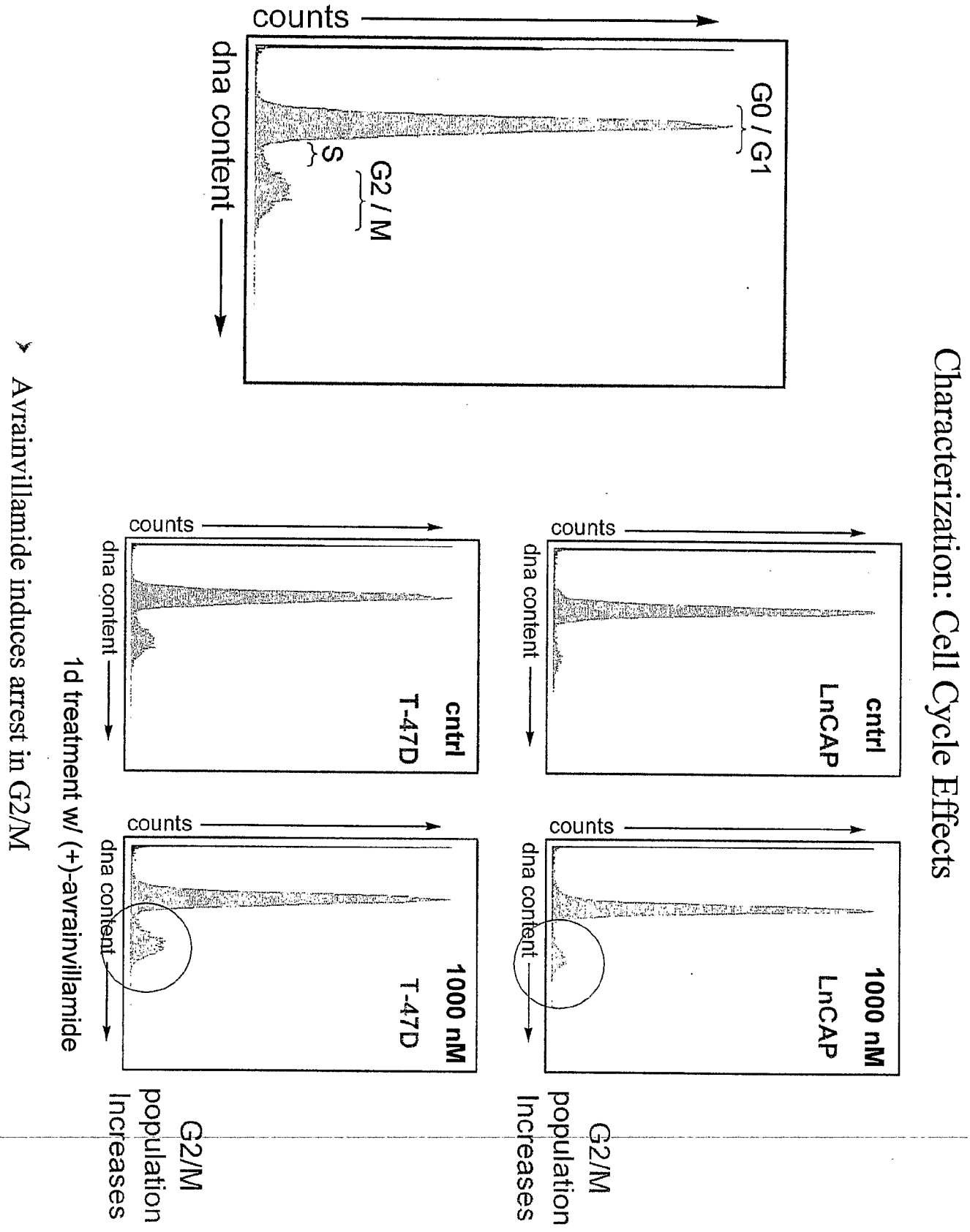
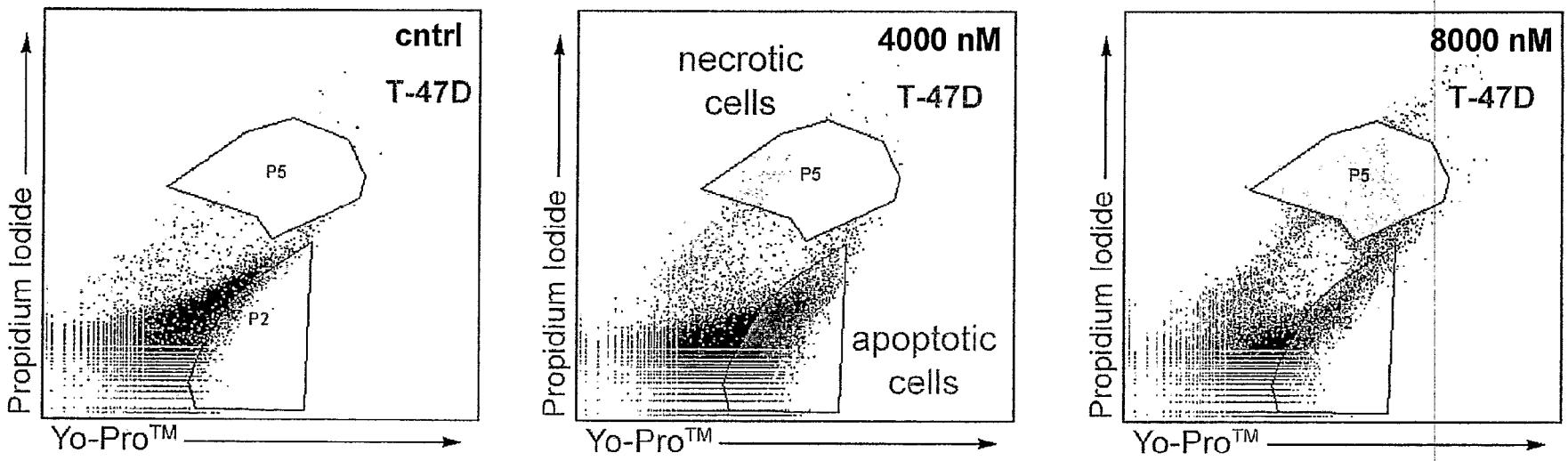
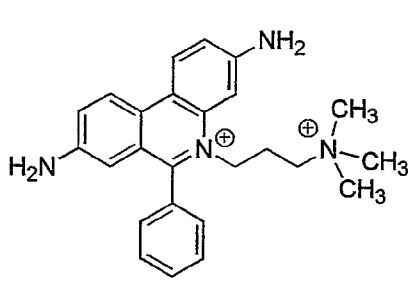

counts
dna content
G0 / G1
S
G2 / M
Characterization: Cell Cycle Effects
counts
dna content
cntrl
LnCAP
1000 nM
LnCAP
G2/M
population
Increases
cntrl
T-47D
1000 nM
T-47D
G2/M
population
Increases
1d treatment w/ (+)-avrainvillamide
Avrainvillamide induces arrest in G2/M

Fig. 19

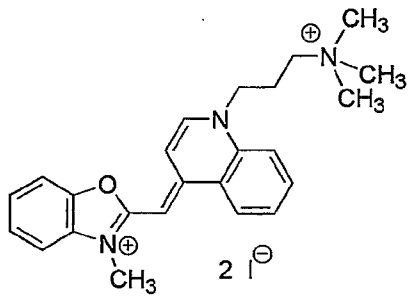
Characterization: Apoptosis



Plated 1d; incubated 1d w/ (+)-avrainvillamide



Propidium Iodide
permeates necrotic cells only



Yo-Pro™
permeates apoptotic and necrotic cells

Fig. 20

➢ Avrainvillamide induces apoptosis at 1 - 4 μM, necrosis at 8 μM

In-Cellulo Affinity-Based Probe (Pulldown) Experiments

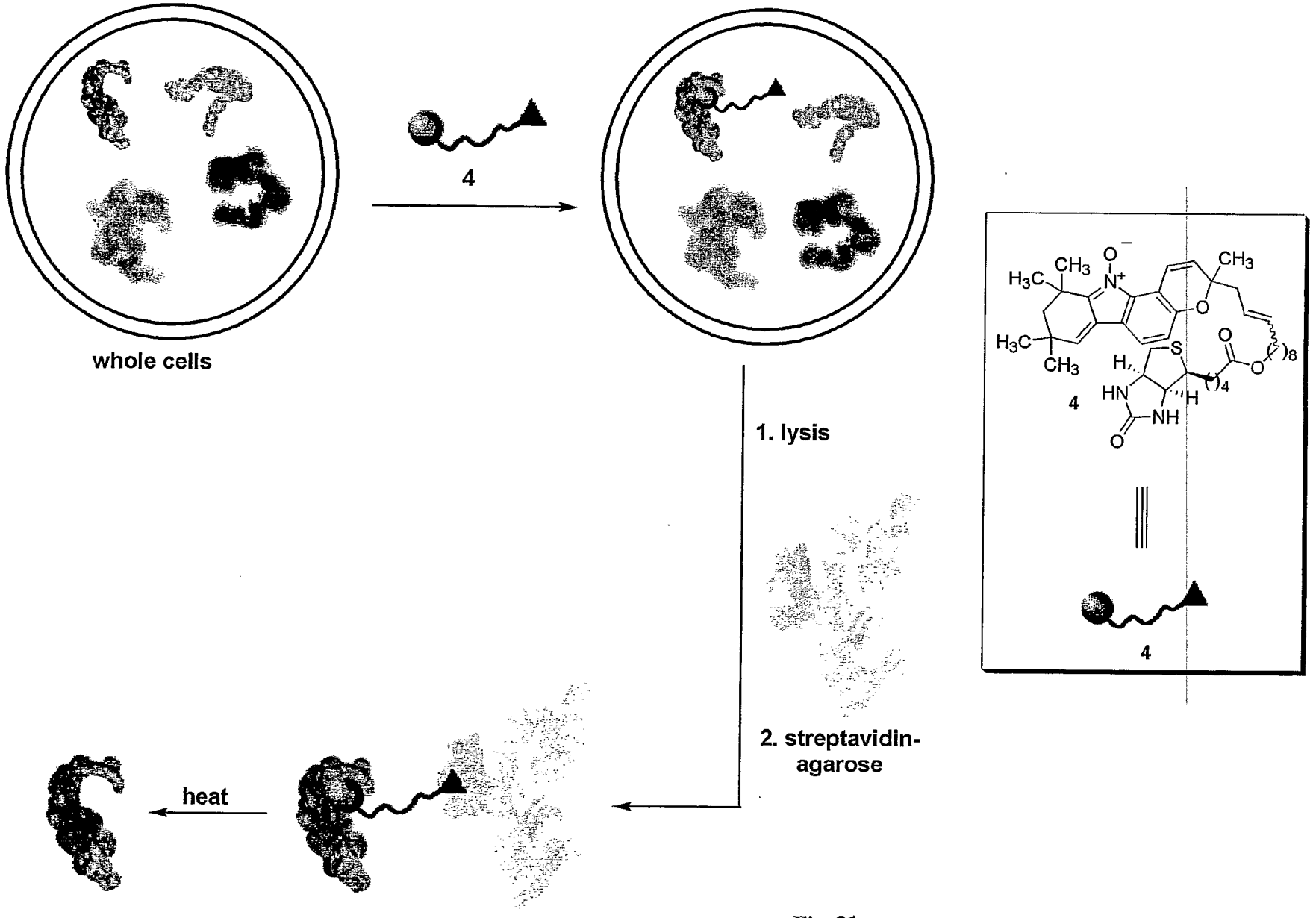
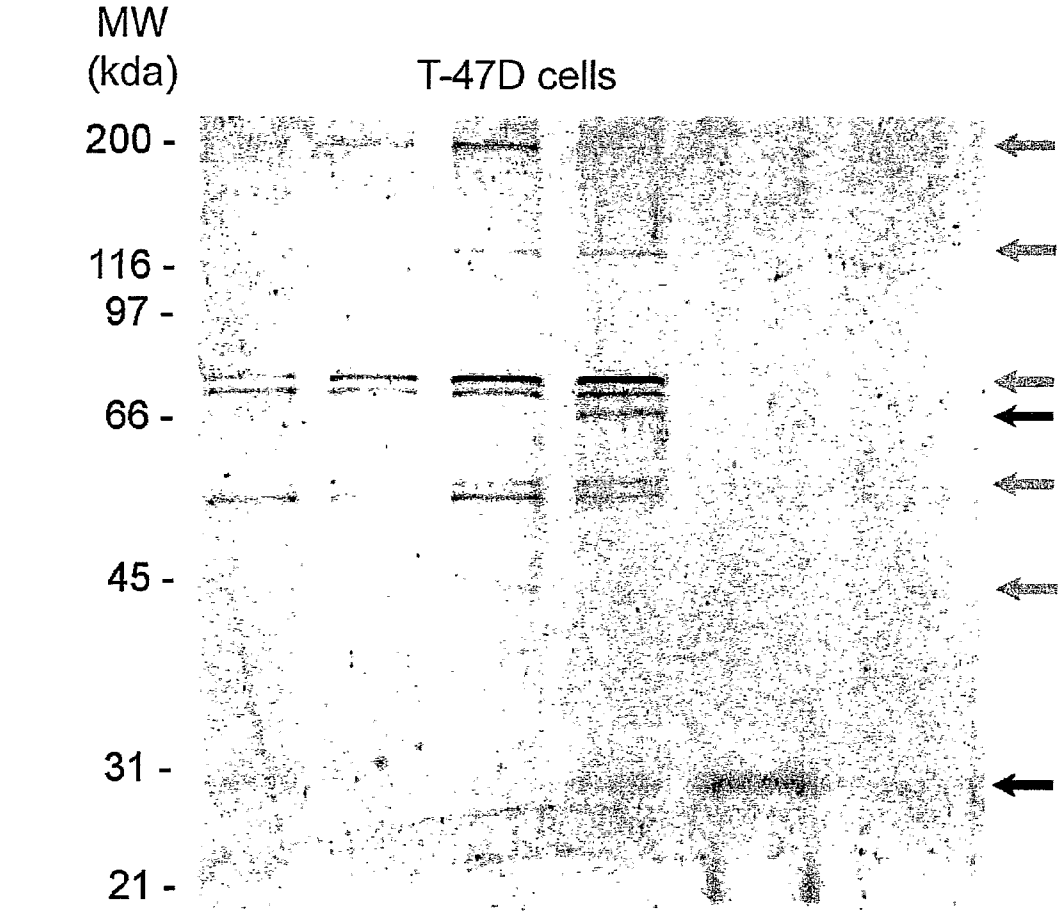


Fig. 21

In-Cellulo Affinity-Based Probe (Pulldown) Experiments



Legend: possible target
probable biotinylated protein
apoptotic protein (nonspecific)

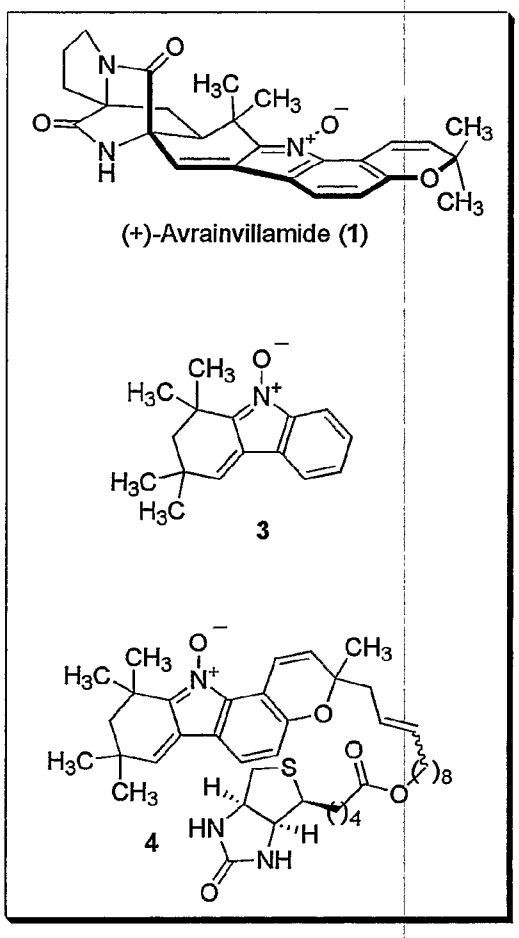


Fig. 22

In-Cellulo Affinity-Based Probe (Pulldown) Experiments
MW (kda)
T-47D cells
200 -
116 -
97 -
66 -
45 -
31 -
21 -
myosin 9 / 10 (226 / 229 kda)
pyruvate carboxylase (129 kda)
PCCA (77 / 75 kda)
CLIMP-63 (66 kda)
PCCB (58 / 56 kda)
actin proteins
probably caspases
[4] (μM): 0 3 6 9 6 0
[3] (μM): 0 0 0 0 6 6
Legend: possible target
probable biotinylated protein
apoptotic protein (nonspecific)

Fig. 23

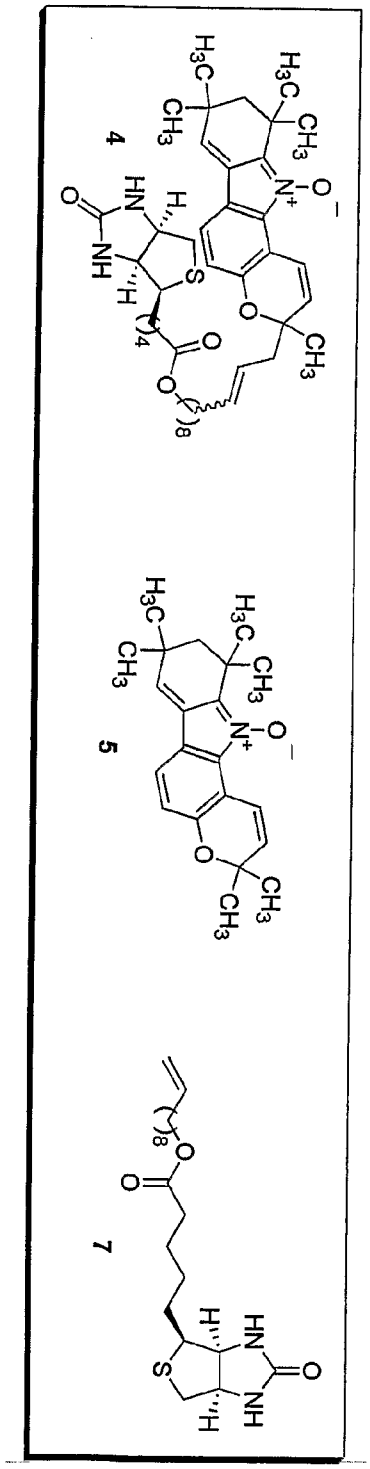

Confirmation of CLIMP-63 by Western Blot
lane: 1 2 3 4 5 6
+
190 -
120 -
85 -
MW (kda) 60 -
50 -
40 -
25 -
20 -
15 -
CLIMP-63 (66 kda)
4 : - 3 μM 6 μM 9 μM - -
5 : - - - - 9 μM -
7 : - - - - - 9 μM
4
5
7

Fig. 24

MS/MS identified CLIMP-63

CLIMP-63 → MPSAKQRGSK GGHGAASPSE KGAHPSGGAD DVAKKPPPAP QQPPPPPAPH PQQHPQQHPQ

NQAHGKGGHR GGGGGGGKSS SSSSASAAAA AAAASSSASC SRRLGRALNF LFYLALVAAA

AFSGWCVHHV LEEVQQVRRS HQDFSRQREE LGQGLQGVEQ KVQSLQATFG TFESILRSSQ

HKQDLTEKAV KQGESEVSRI SEVLQKLQNE ILKDLSDGIH VVKDARERDF TSLENTVEER

LTELTKSIND NIAIFTEVQK RSQKEINDMK AKVASLEESE GNKQDLKALK EAVKEIQTSA

KSREWDMEAL RSTLQTMESD TYTEVRELVS LKQEQQAFKE AADTERLALQ ALTEKLLRSE

ESVSRLPEEI RRLEEELRQL KSDSHGPKED GGFRHSEAFE ALQQKSQGLD SRLQHVEDGV

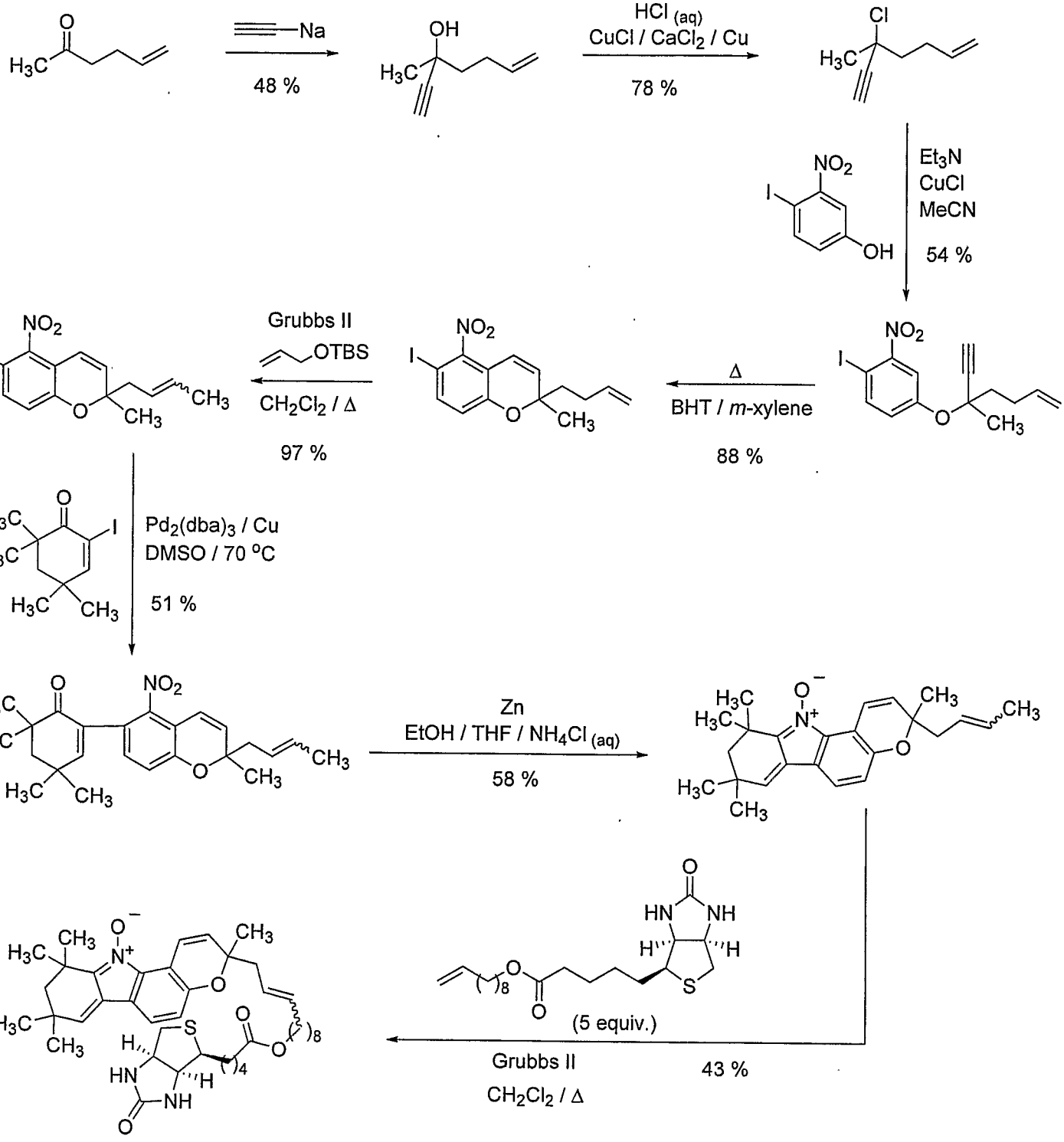
LSMQVASARQ TESLESLLSK SQEHEQRLAA LQGRLEGLGS SEADQDGLAS TVRSLGETQL

VLYGDVEELK RSVGELPSTV ESLQKVQEQV HTLLSQDQAQ AARLPPQDFL DRLSSLDNLK

ASVSQVEADL KMLRTAVDSL VAYSVKIETN ENNLESAKGL LDDLRNDLDR LFVKVEKIHE KV

Fig. 25

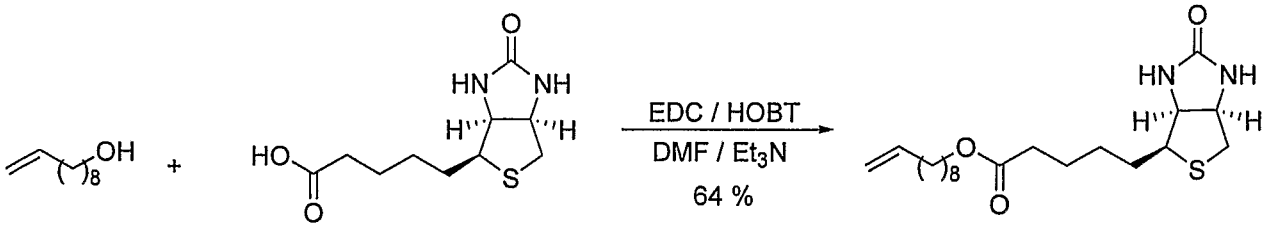
Synthesis of the Probe:



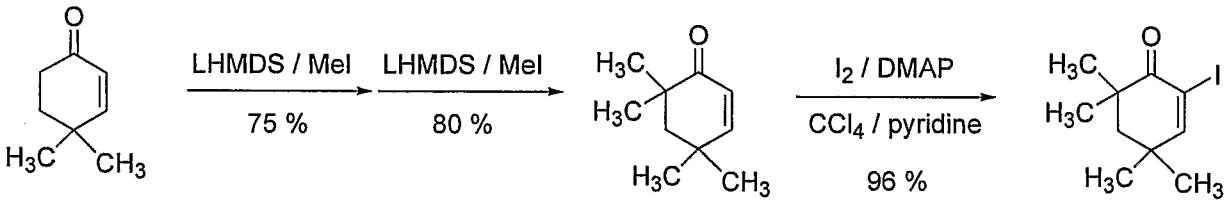
**Grubbs II = Grubbs' second generation catalyst, benzylidene[1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(tricyclohexylphsophine)ruthenium

Fig. 26A

Preparation of the biotin portion of the probe:



Preparation of the "left half" of the probe and several analogues:



Preparation of the "right half" of the natural product and several analogues:

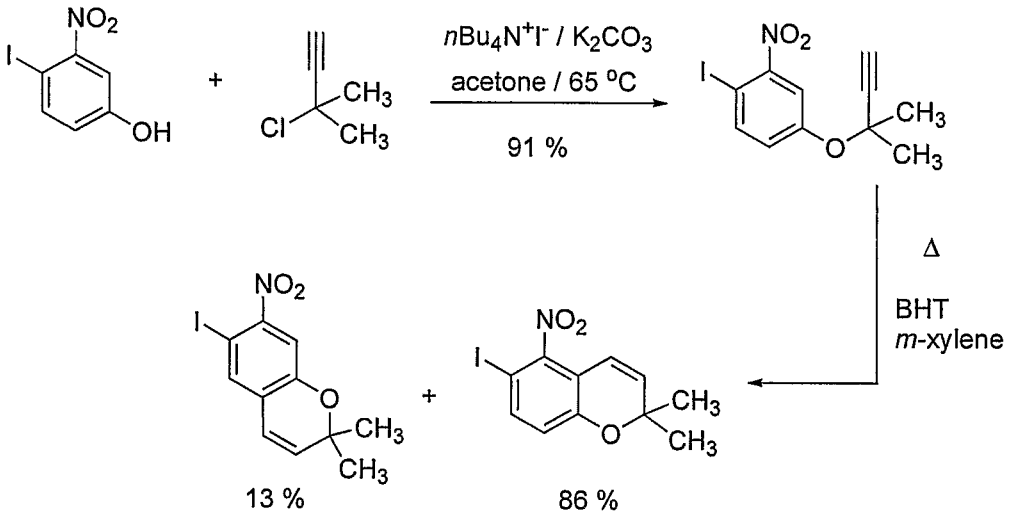


Fig. 26B

Ways of Coupling the Left and Right Halves:

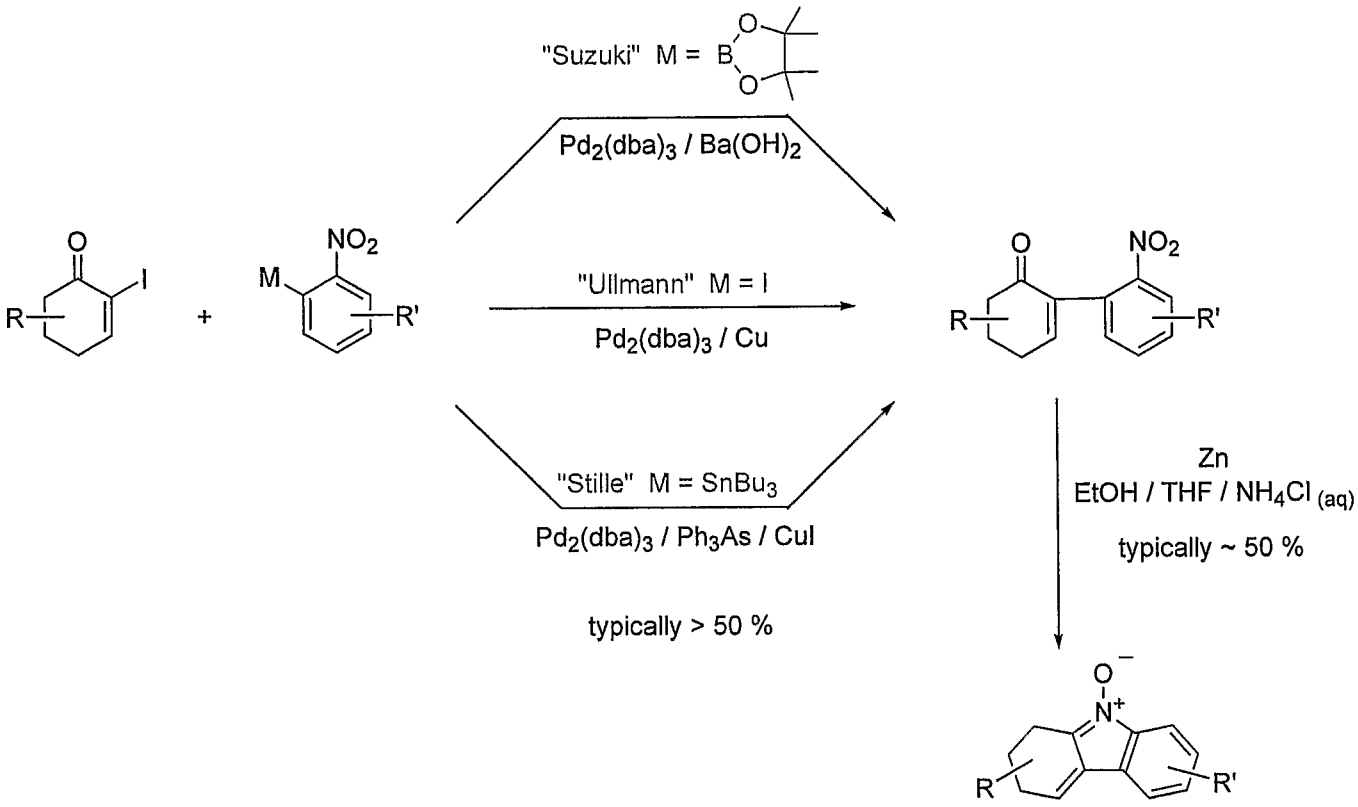


Fig. 27