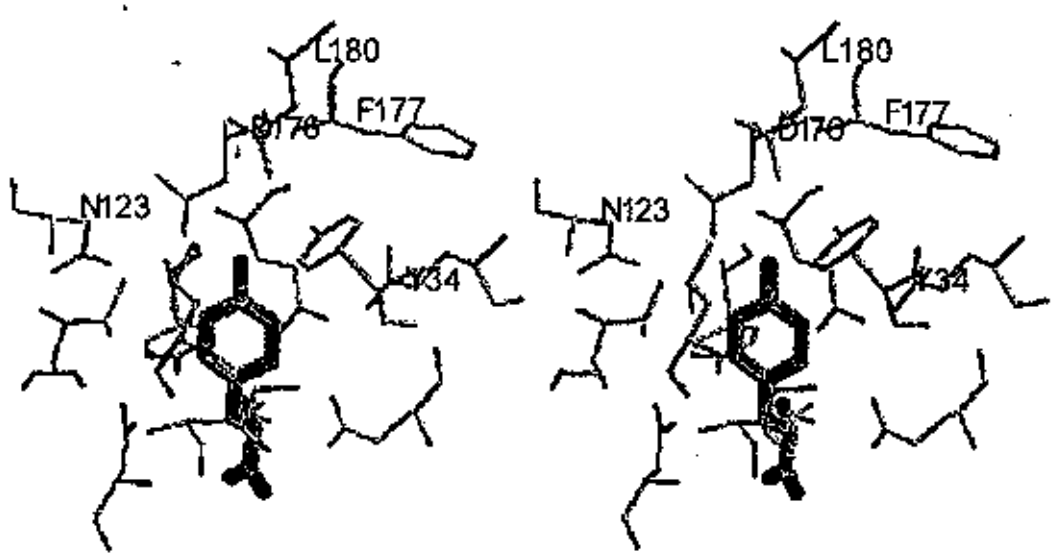


Fig. 1

Fig. 2A

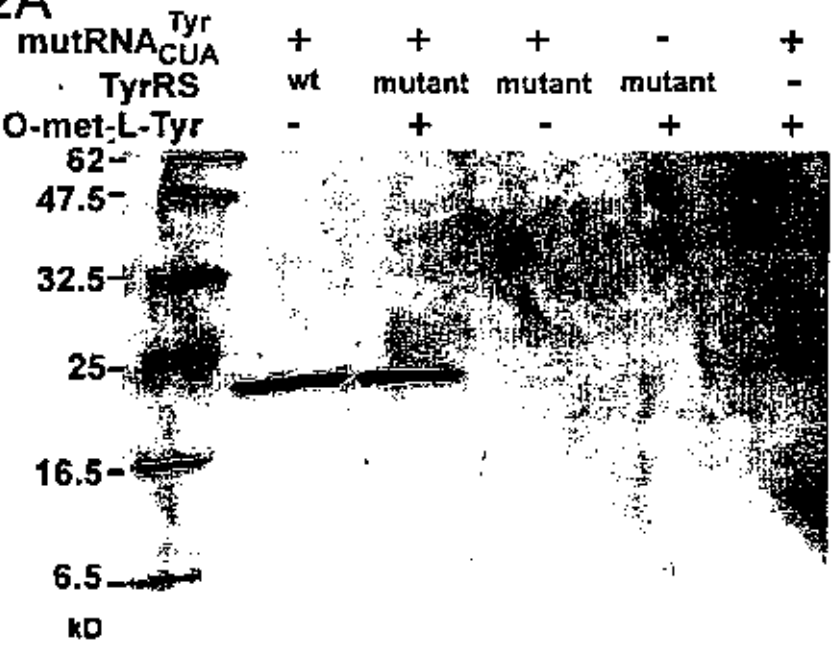


Fig. 2B



Fig. 2

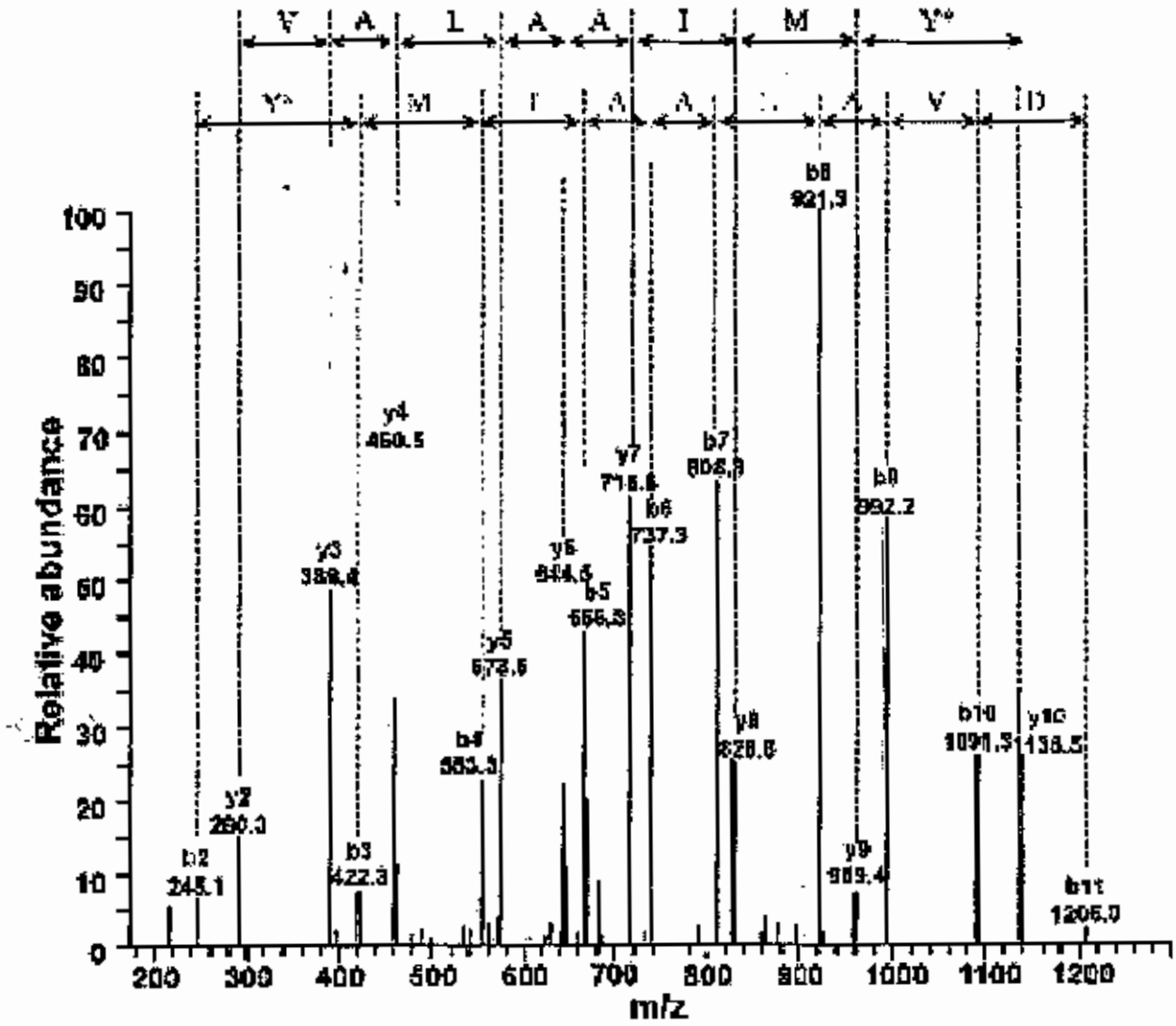

Relative abundance
m/z
100
90
80
70
60
50
40
30
20
10
0
200
300
400
500
600
700
800
900
1000
1100
1200
b2
245.1
y2
290.3
y4
460.5
b3
422.3
y3
388.4
b4
553.3
y5
572.5
y6
644.5
b5
656.3
y7
716.6
b6
737.3
b7
808.3
y8
828.6
b8
921.3
b9
992.2
y9
989.4
b10
1091.3
y10
1138.5
b11
1206.0

Fig. 3

Fig. 4A

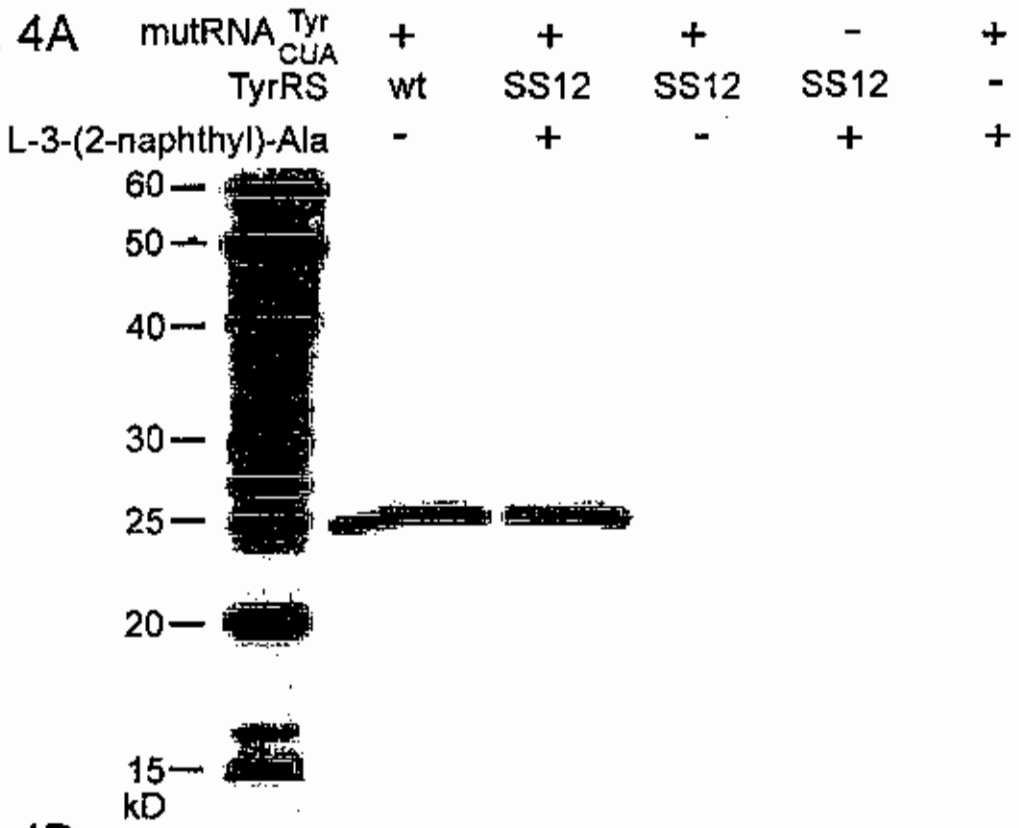

mutRNA Tyr CUA + + + - +
TyrRS wt SS12 SS12 SS12 -
L-3-(2-naphthyl)-Ala - + - + +
60
50
40
30
25
20
15
kD

Fig. 4B



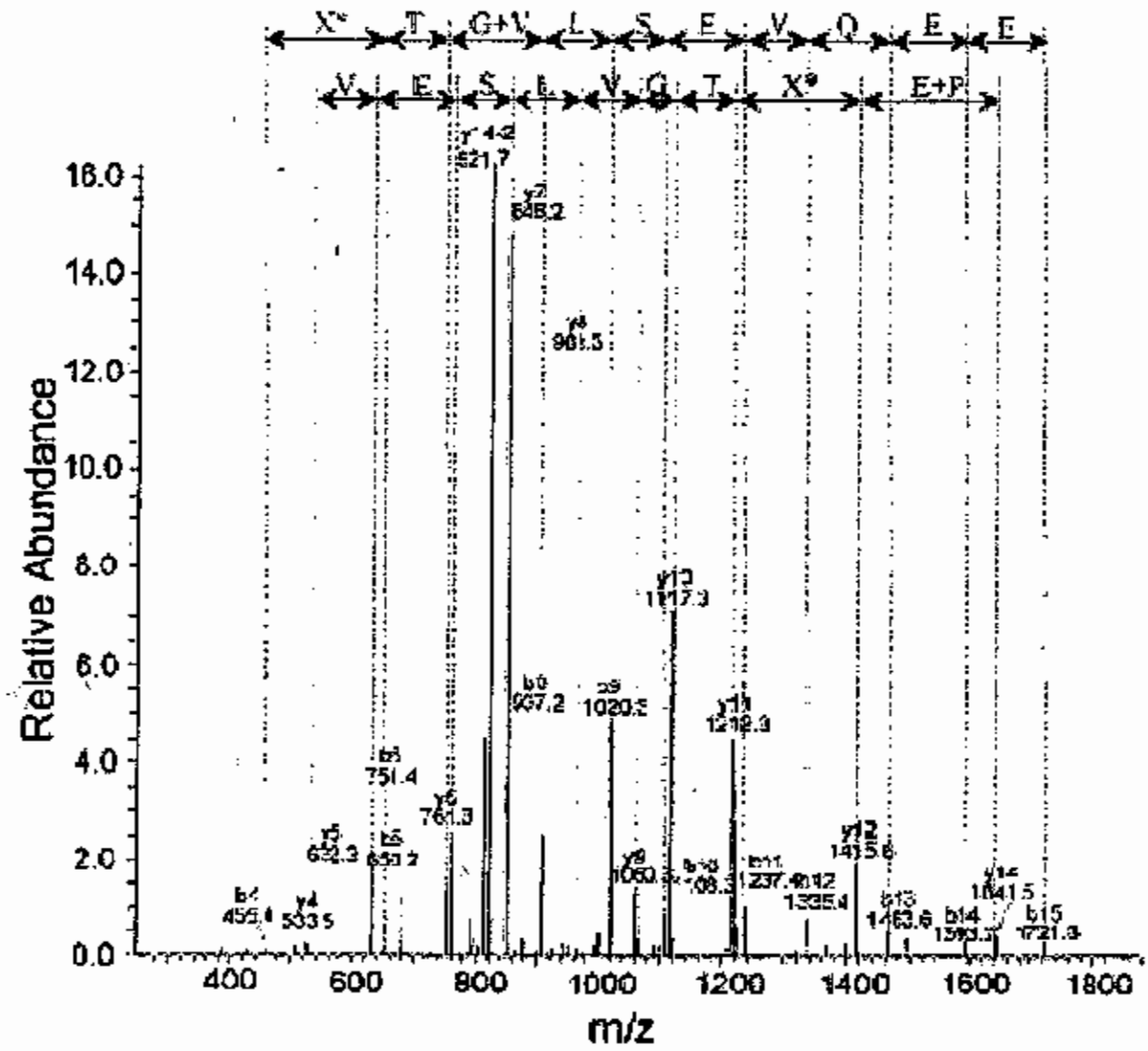

X* T G+V L S E V Q E E
V E S L V Q T X* E+P
Relative Abundance
m/z
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
400
600
800
1000
1200
1400
1600
1800

Fig. 5

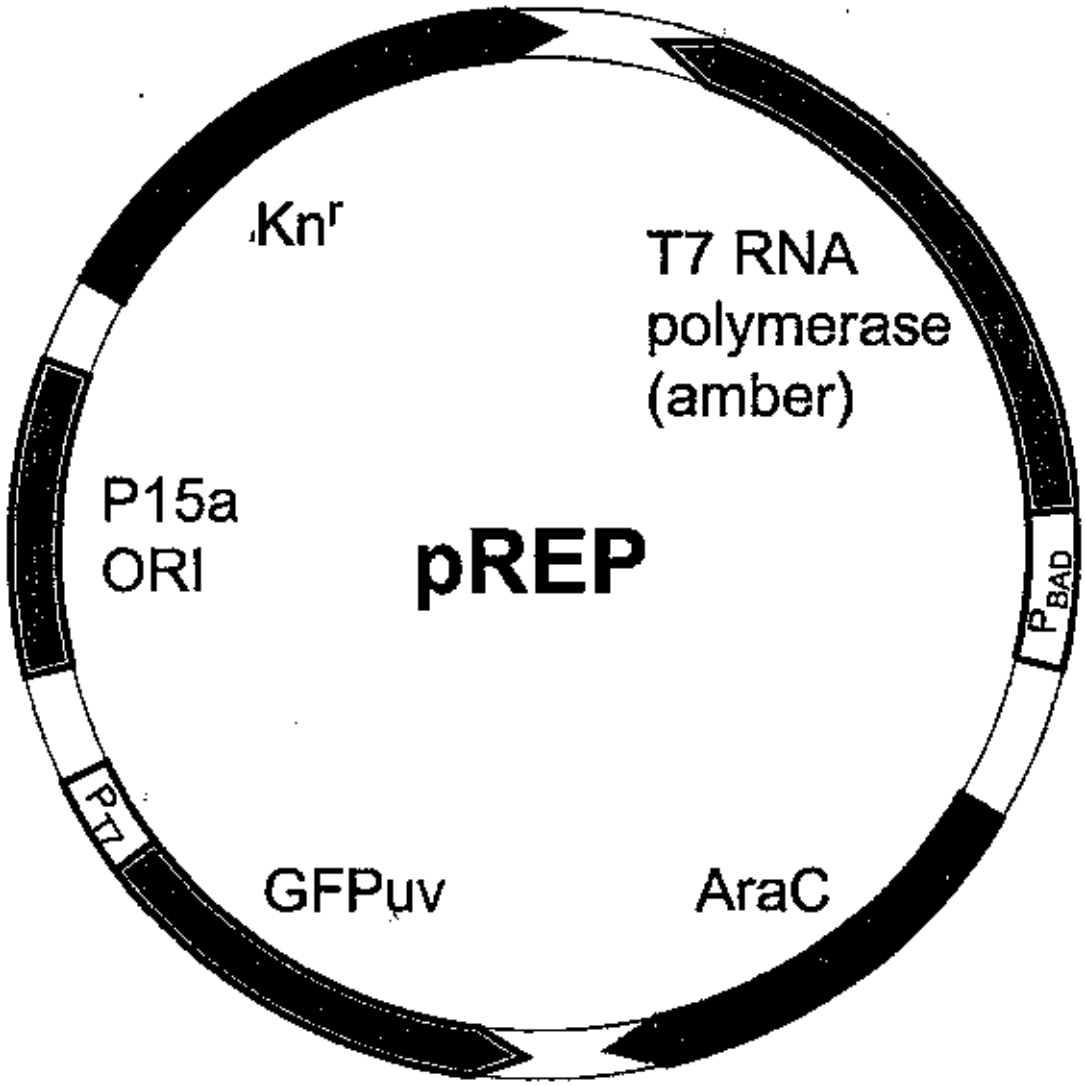

Kn^r
T7 RNA
polymerase
(amber)
P15a
ORI
pREP
P_BAD
P_T7
GFPuv
AraC

Figure 6A

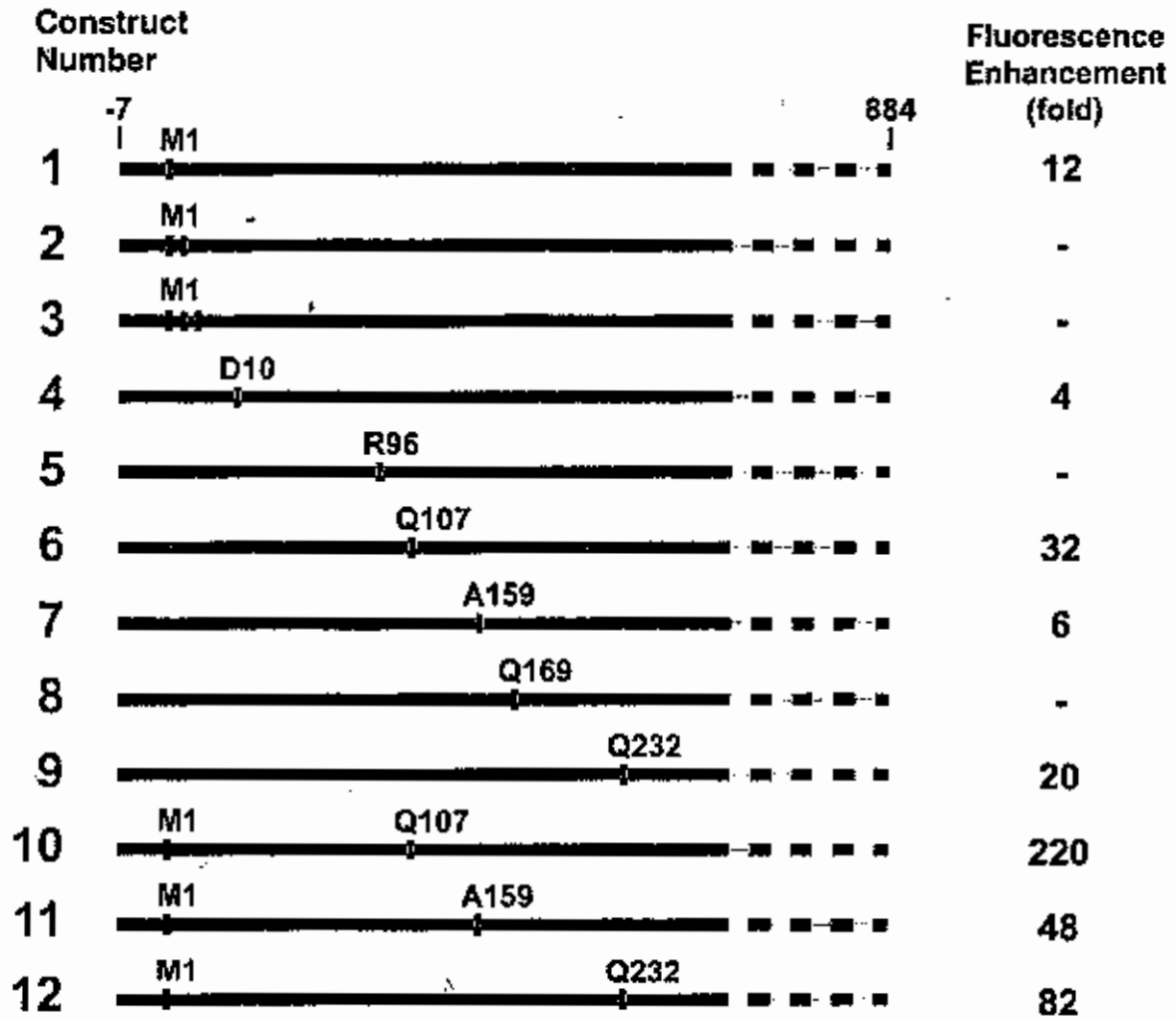

Construct Number
Fluorescence Enhancement (fold)
-7
884
1 M1 12
2 M1 -
3 M1 -
4 D10 4
5 R96 -
6 Q107 32
7 A159 6
8 Q169 -
9 Q232 20
10 M1 Q107 220
11 M1 A159 48
12 M1 Q232 82

Figure 6B

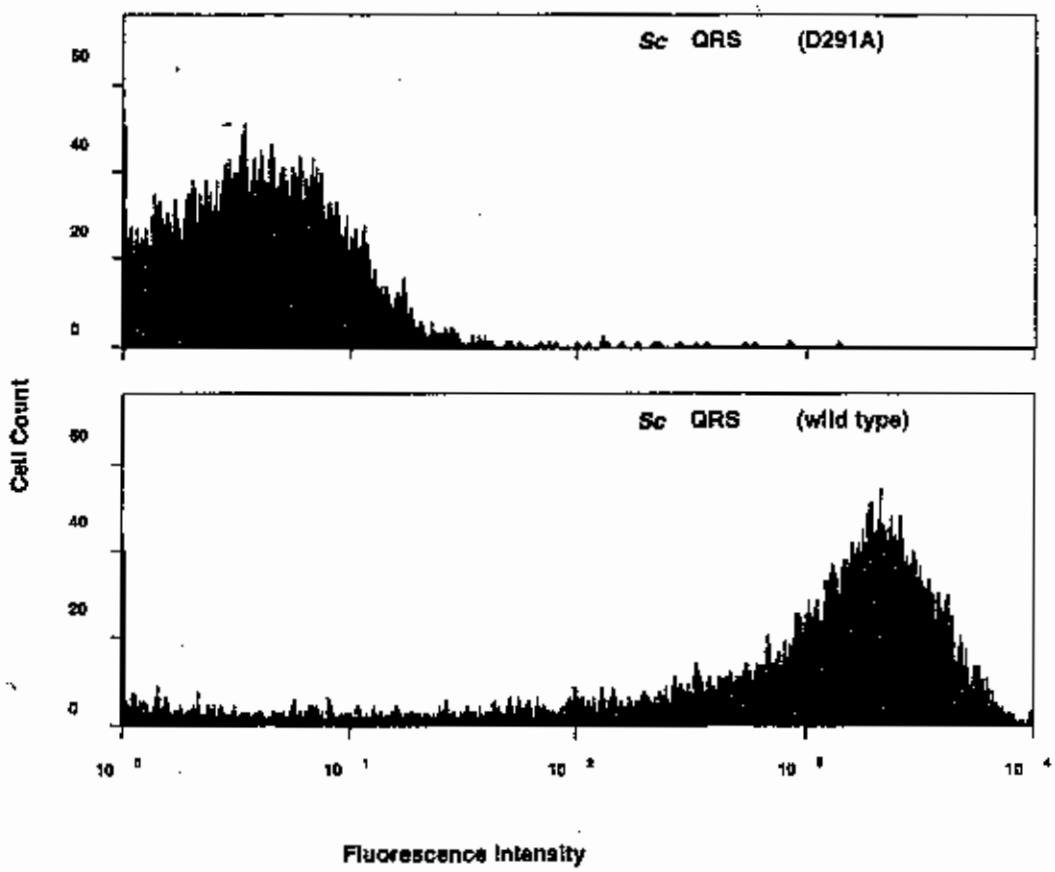

Sc QRS (D291A)
Sc QRS (wild type)
Cell Count
Fluorescence Intensity

Figure 6C

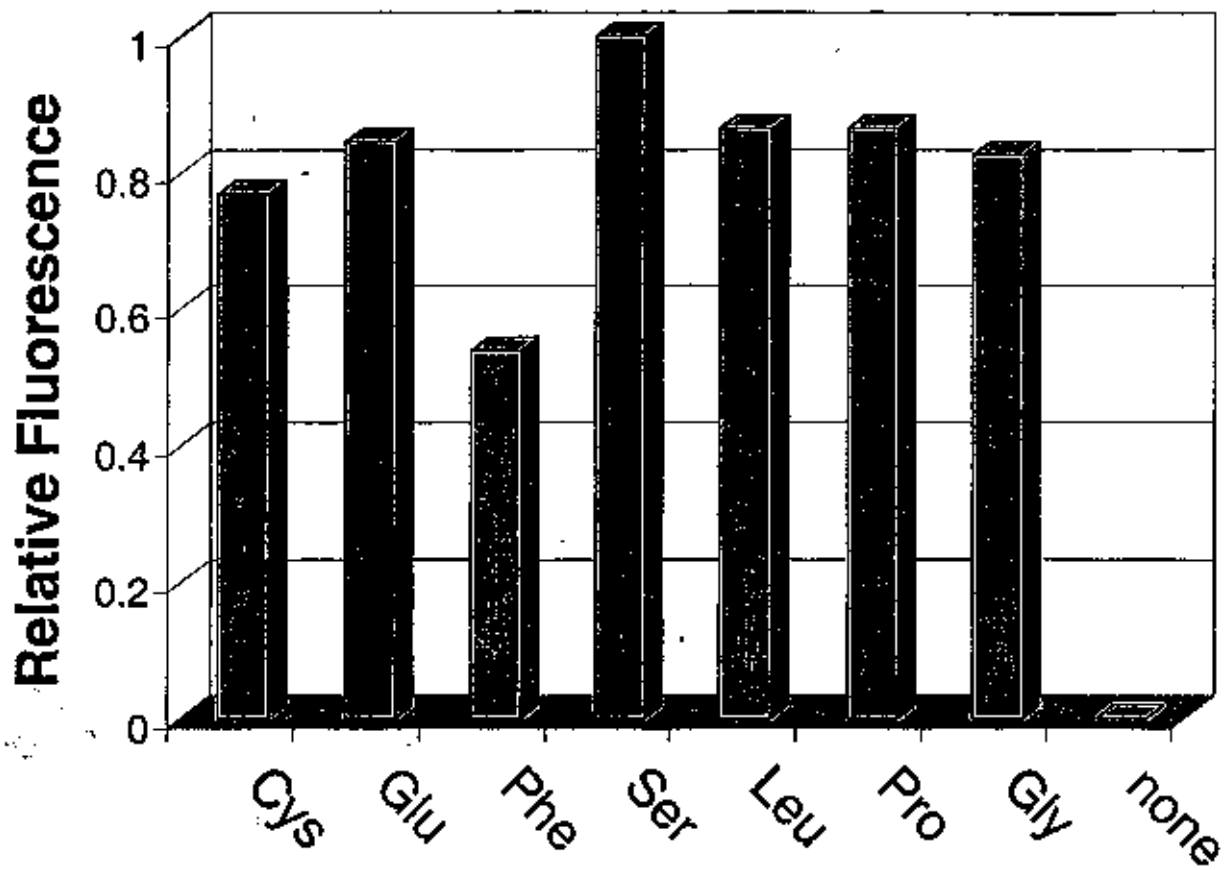

Relative Fluorescence
1
0.8
0.6
0.4
0.2
0
Cys
Glu
Phe
Ser
Leu
Pro
Gly
none

Figure 6D

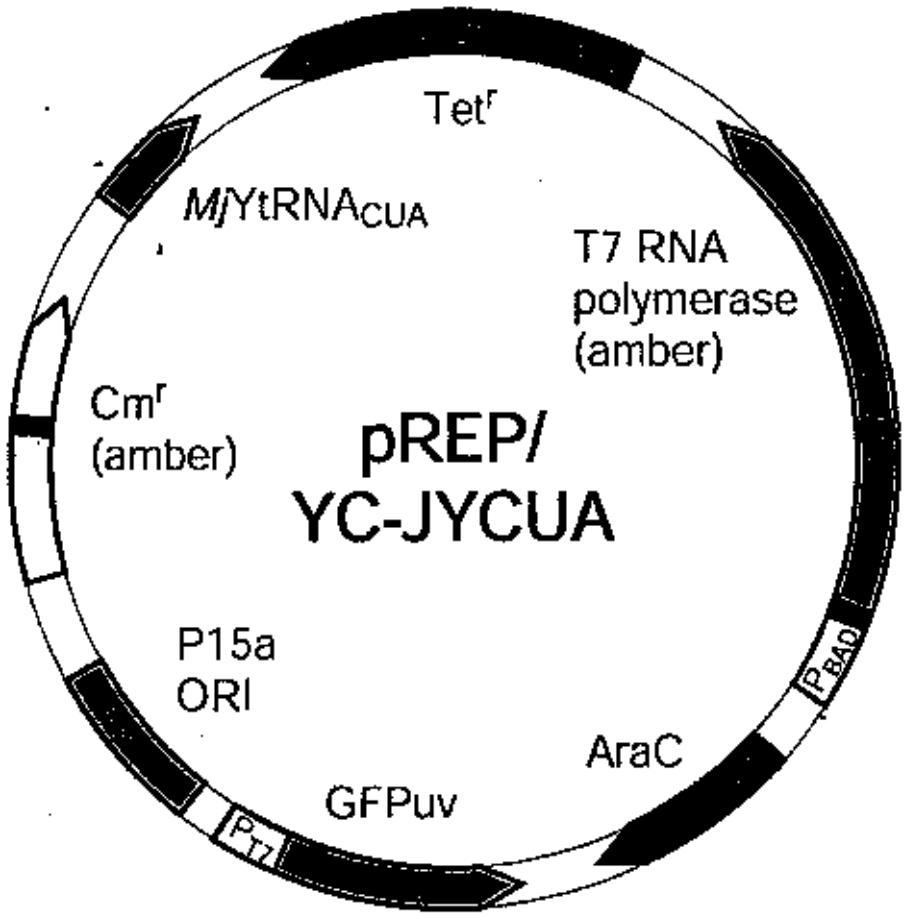

Tet^r
*Mj*YtRNA$_{CUA}$
T7 RNA polymerase (amber)
Cm^r (amber)
pREP/ YC-JYCUA
P15a ORI
P$_{BAD}$
AraC
GFPuv
P$_{T7}$

Figure 7A

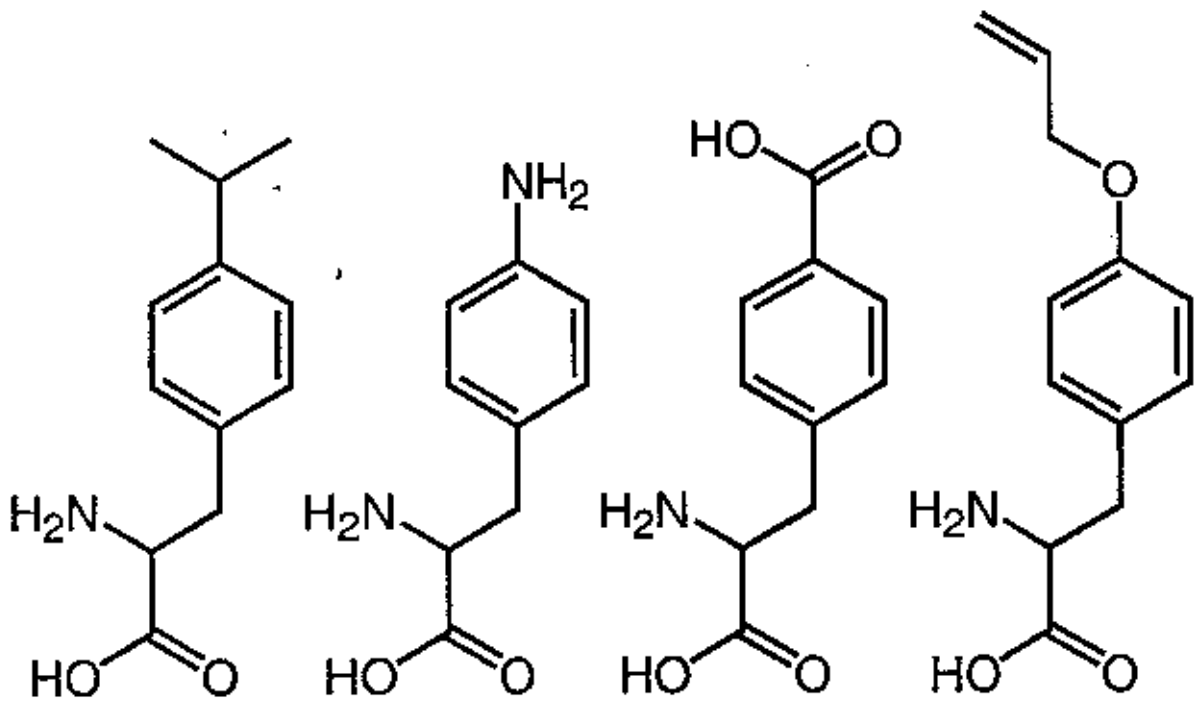


Figure 7B

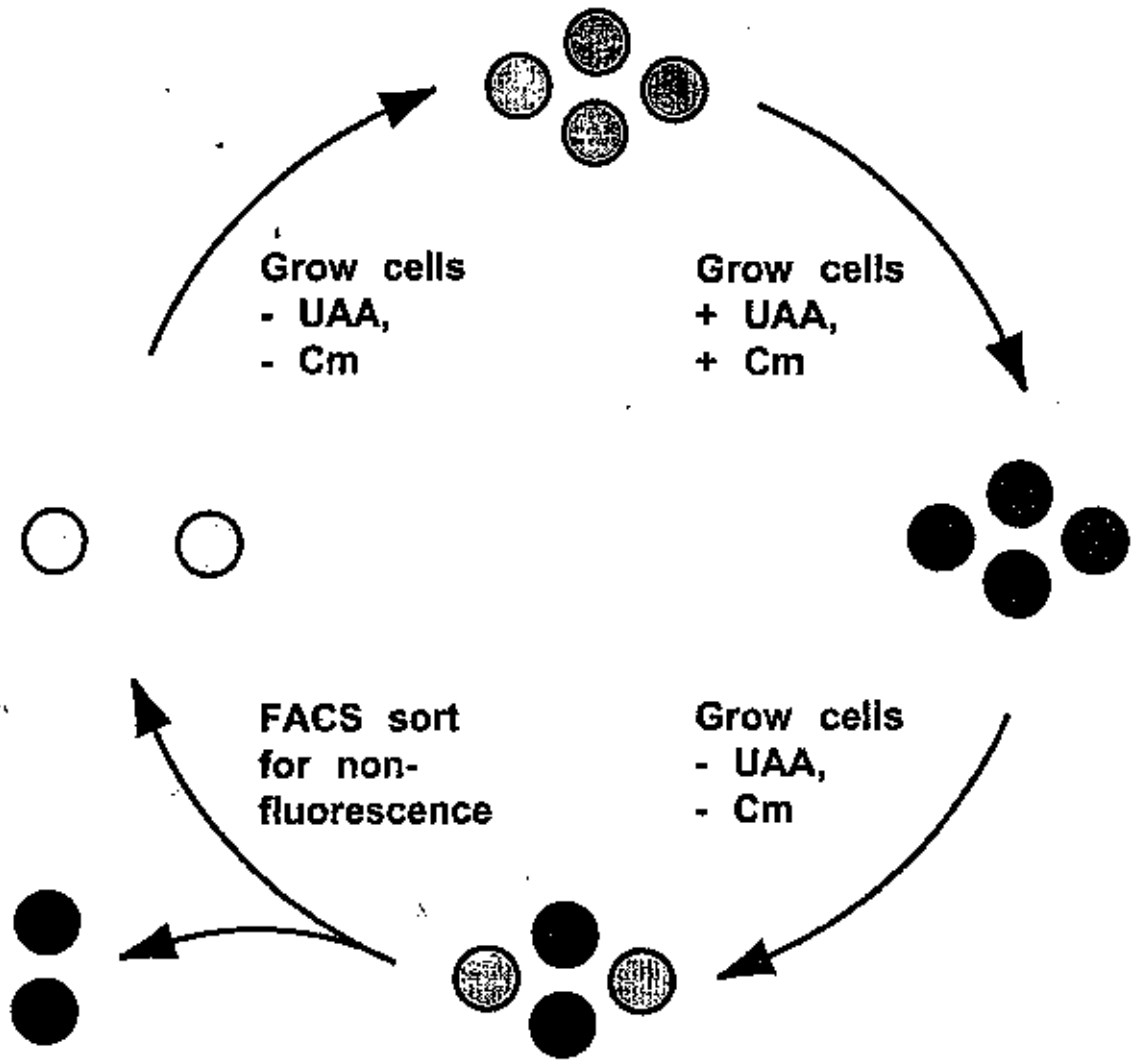

Grow cells
- UAA,
- Cm
Grow cells
+ UAA,
+ Cm
FACS sort
for non-
fluorescence
Grow cells
- UAA,
- Cm

Figure 7C

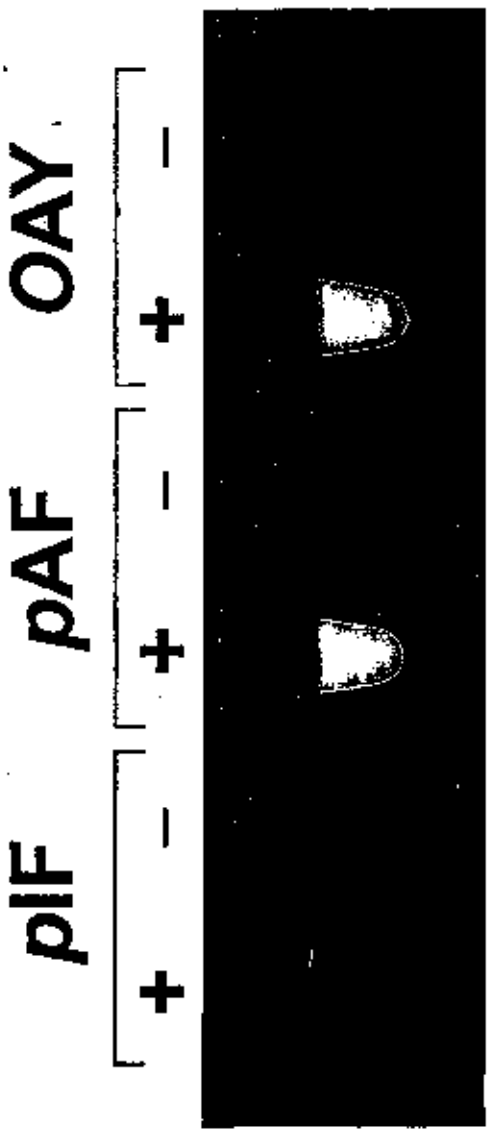


Figure 8A

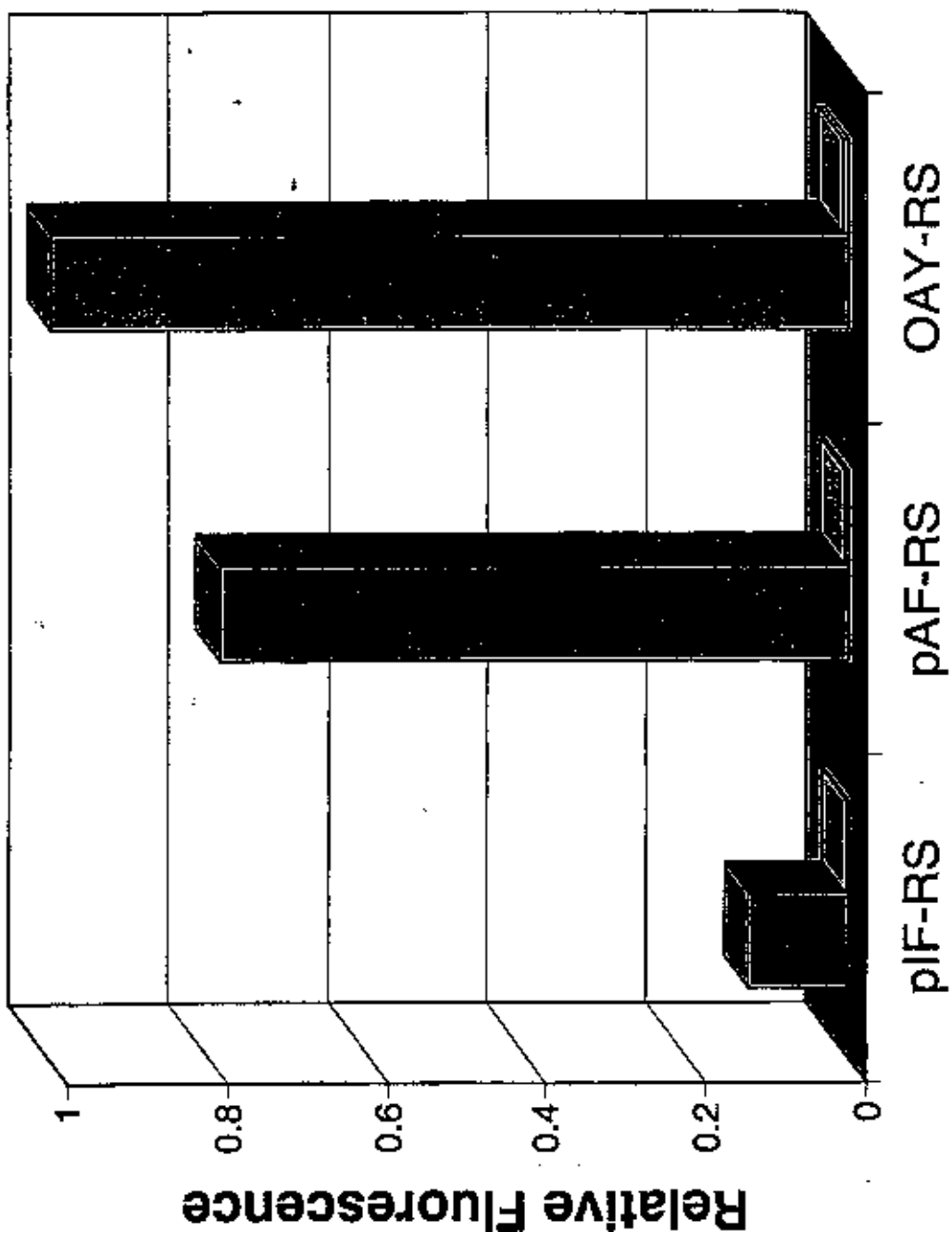


Figure 8B

19 OCT 2005

Cm IC_{50} (μg/mL)	pIF-RS	pAF-RS	OAY-RS(1)
– Unnatural	< 5	< 5	< 5
+ Unnatural	**75**	**100**	**120**

Figure 8C

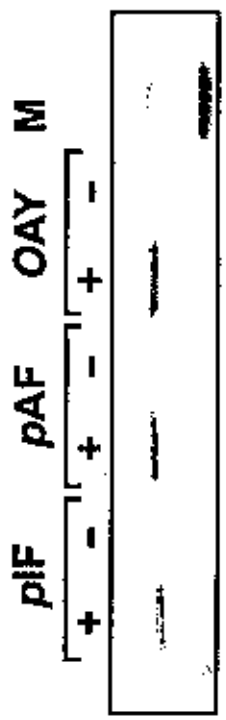


Figure 8D

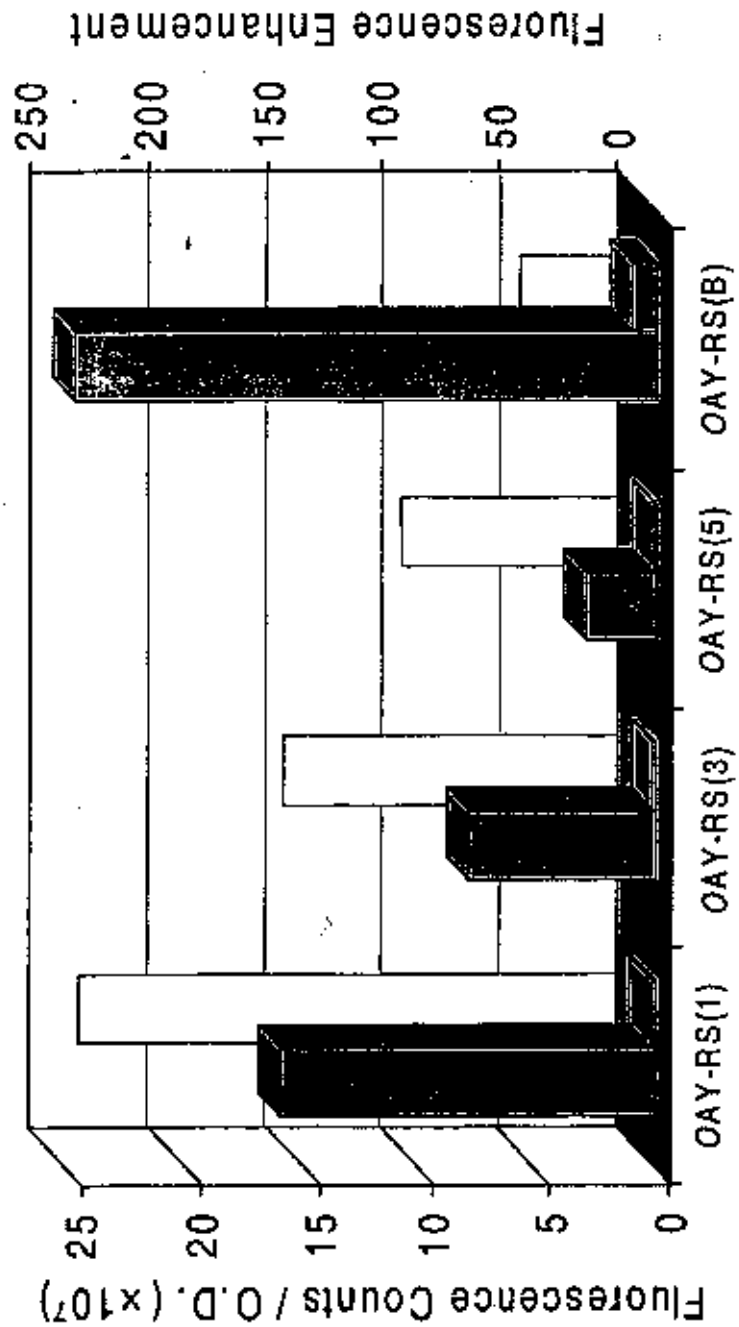

Fluorescence Enhancement
250
200
150
100
50
0
OAY-RS(B)
OAY-RS(5)
OAY-RS(3)
OAY-RS(1)
Fluorescence Counts / O.D. (x10⁷)
25
20
15
10
5
0

Figure 9

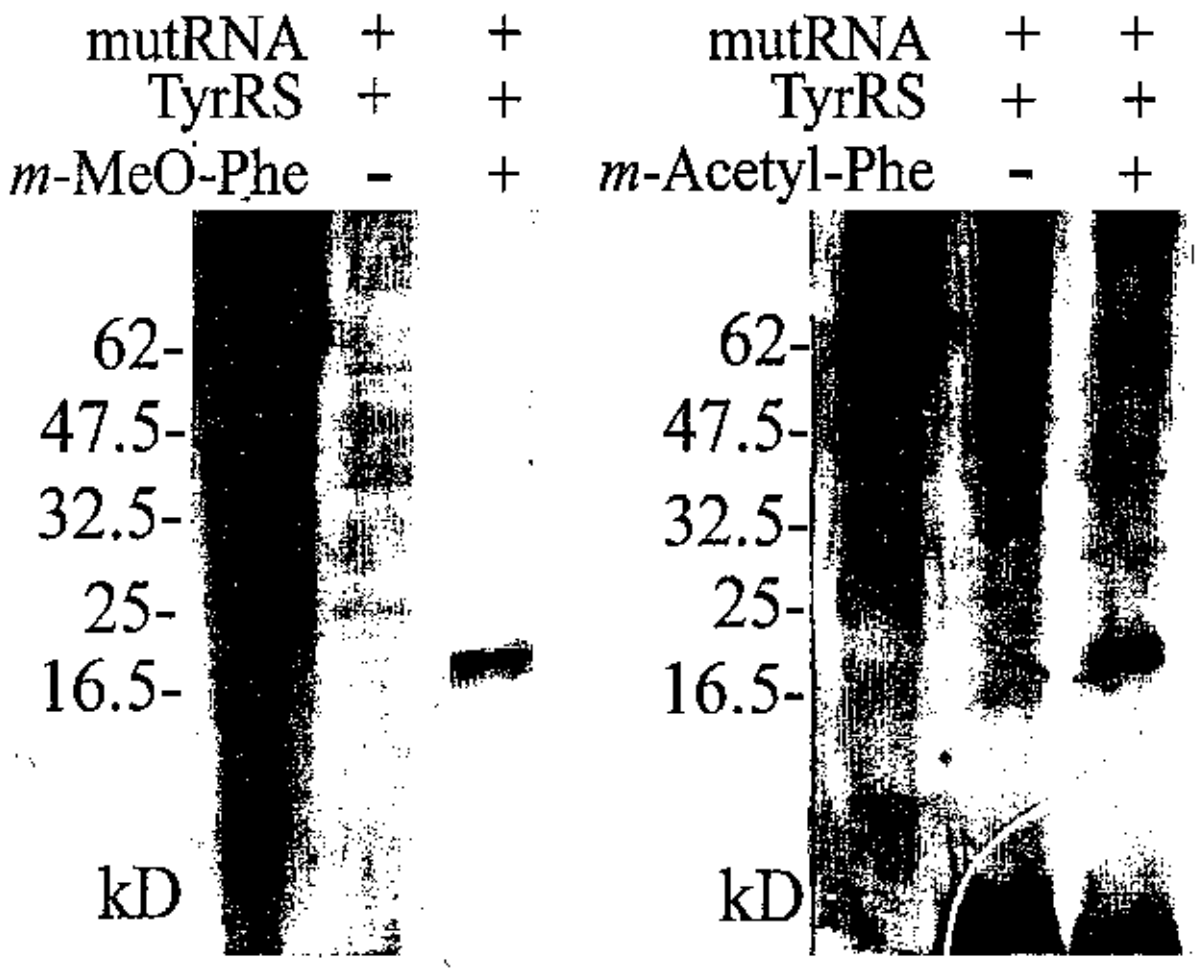

mutRNA + +
TyrRS + +
m-MeO-Phe - +
62-
47.5-
32.5-
25-
16.5-
kD
mutRNA + +
TyrRS + +
m-Acetyl-Phe - +
62-
47.5-
32.5-
25-
16.5-
kD

Figure 10

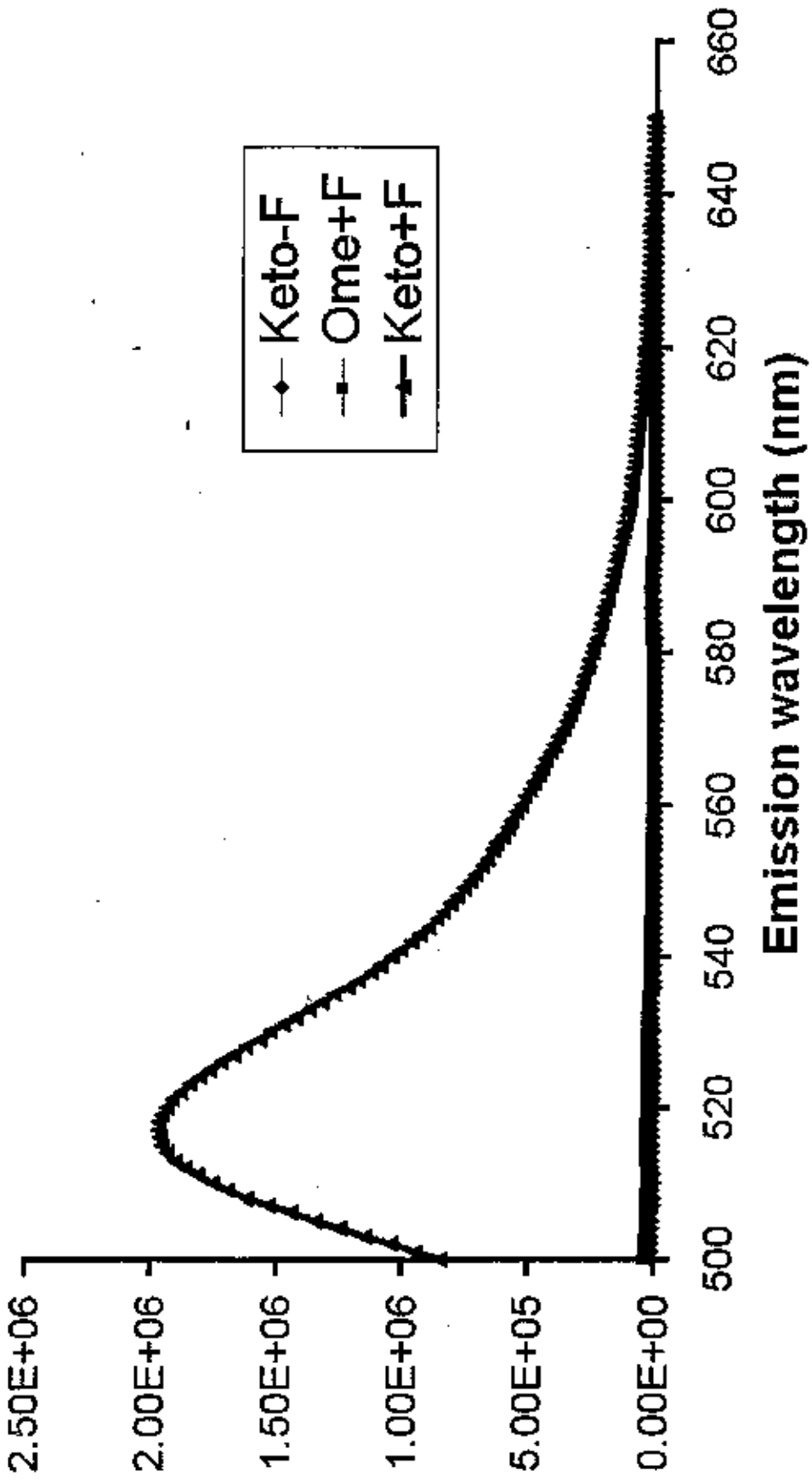

Keto-F
Ome+F
Keto+F
Fluorescence Unit
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
500
520
540
560
580
600
620
640
660
Emission wavelength (nm)

Figure 11

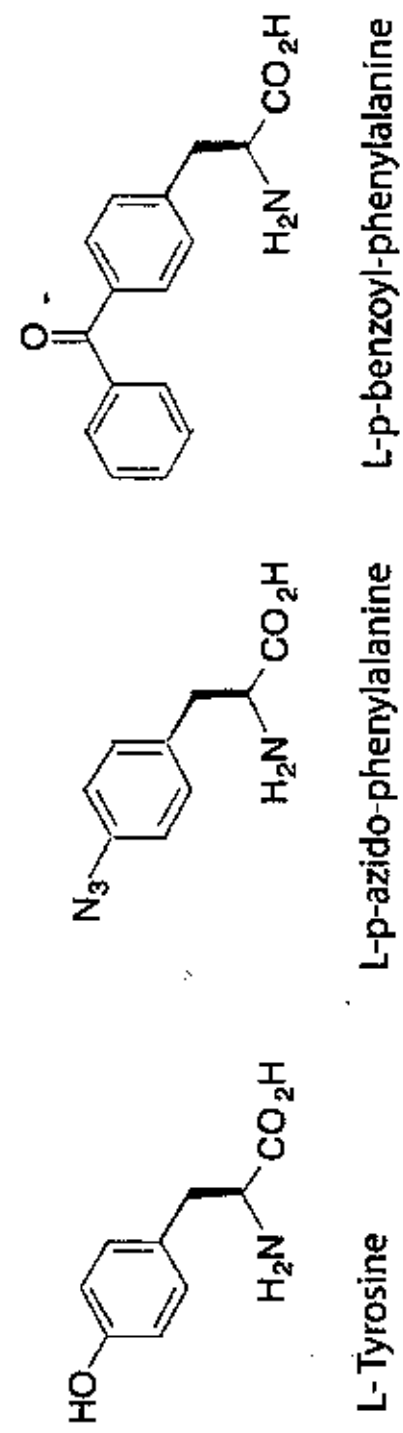

L-Tyrosine
L-p-azido-phenylalanine
L-p-benzoyl-phenylalanine

Figure 12

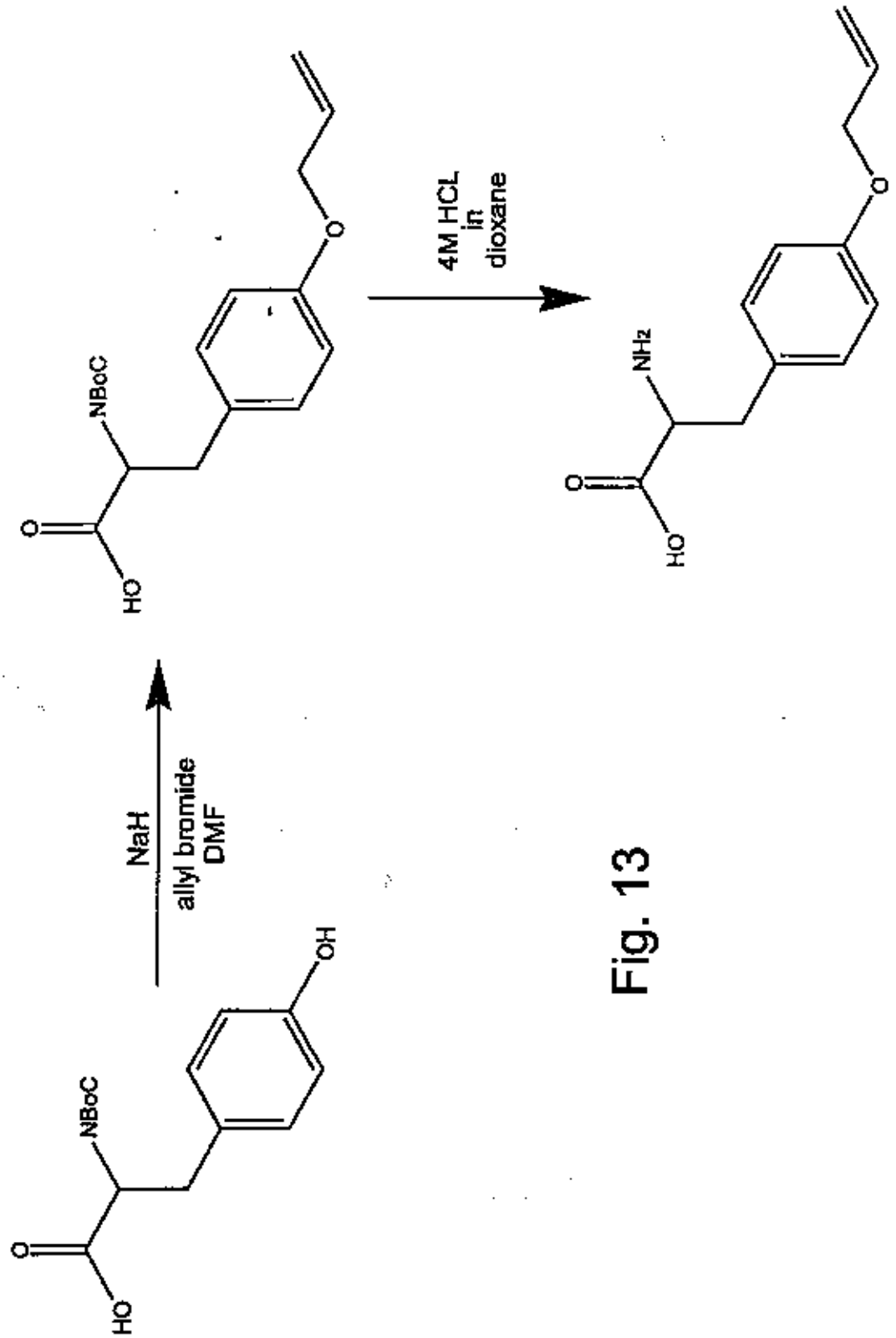


Fig. 13

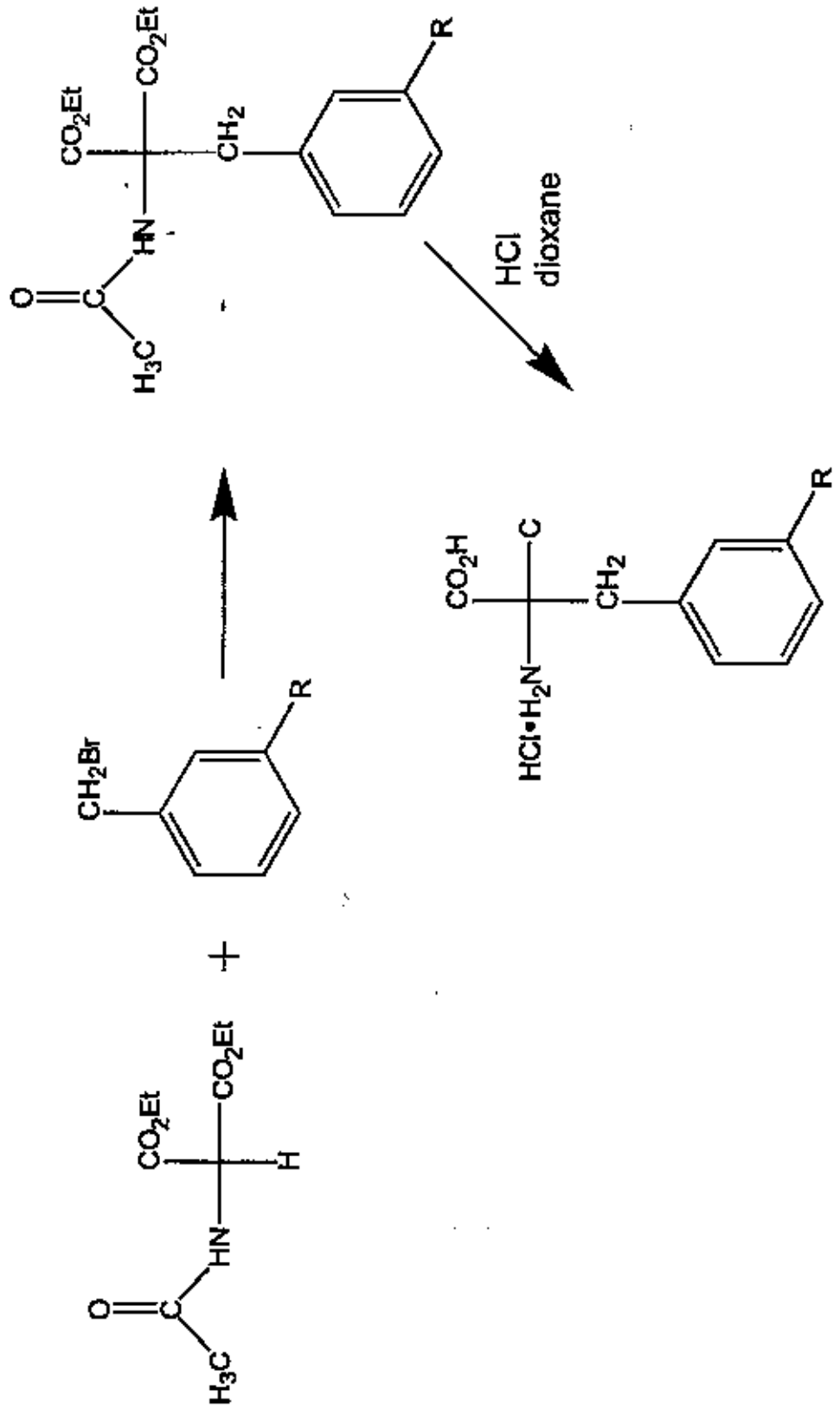

CO2Et
CO2Et
CH2
R
HN
O
C
H3C
HCl
dioxane
CO2H
C
CH2
R
HCl•H2N
CH2Br
R
+
CO2Et
CO2Et
H
HN
O
C
H3C

Fig. 14



Fig. 15A

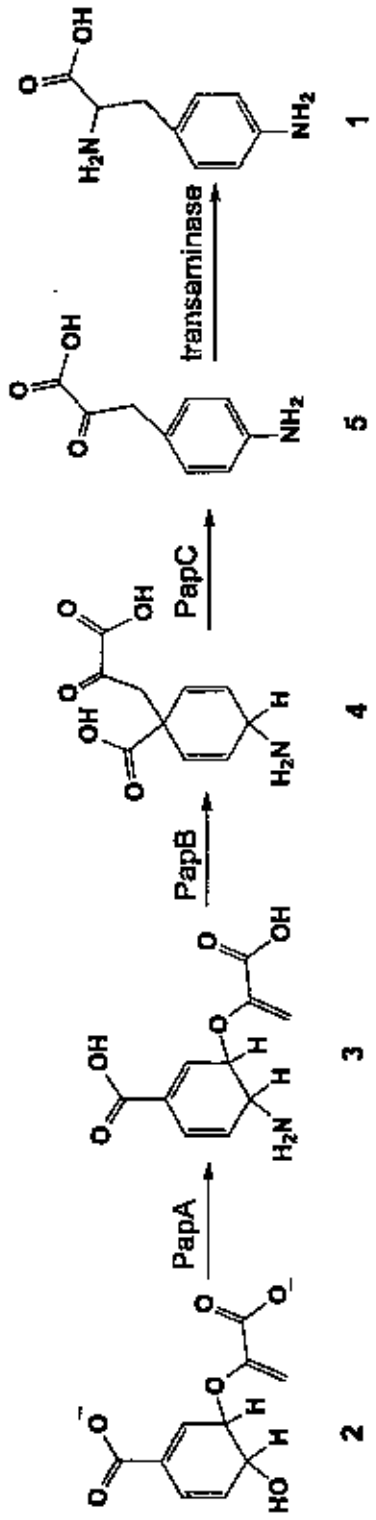


Fig. 15B

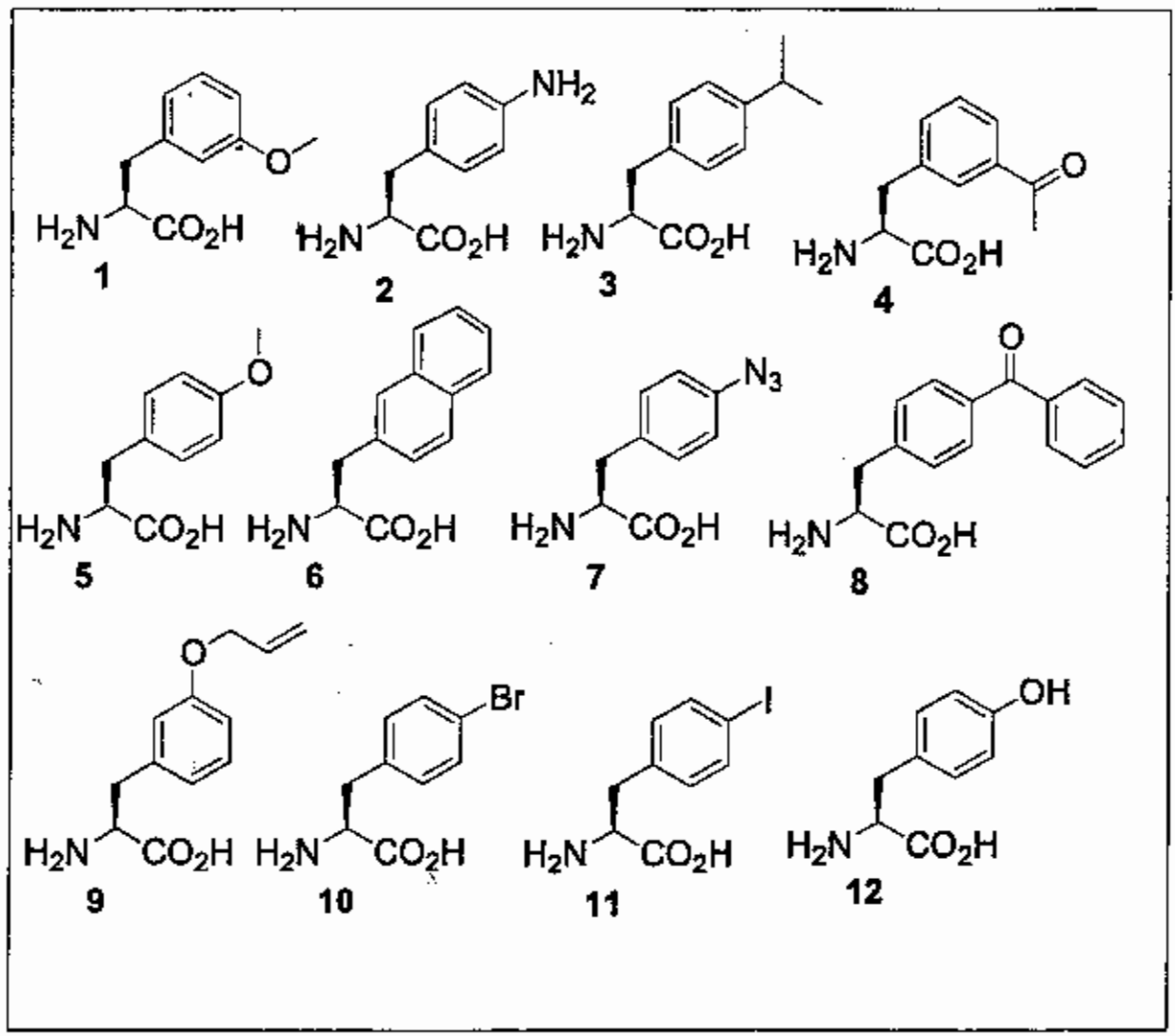


Fig. 16

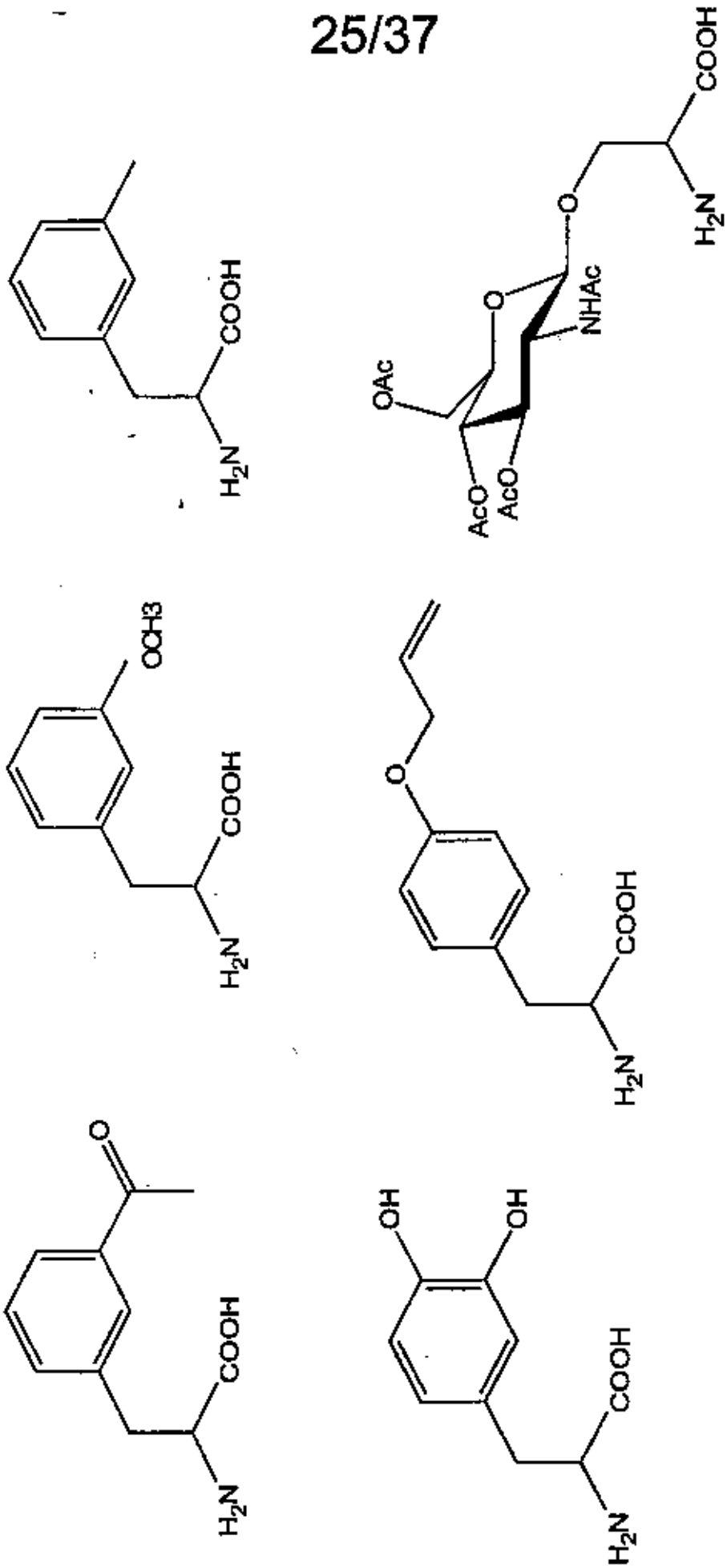


Fig. 17

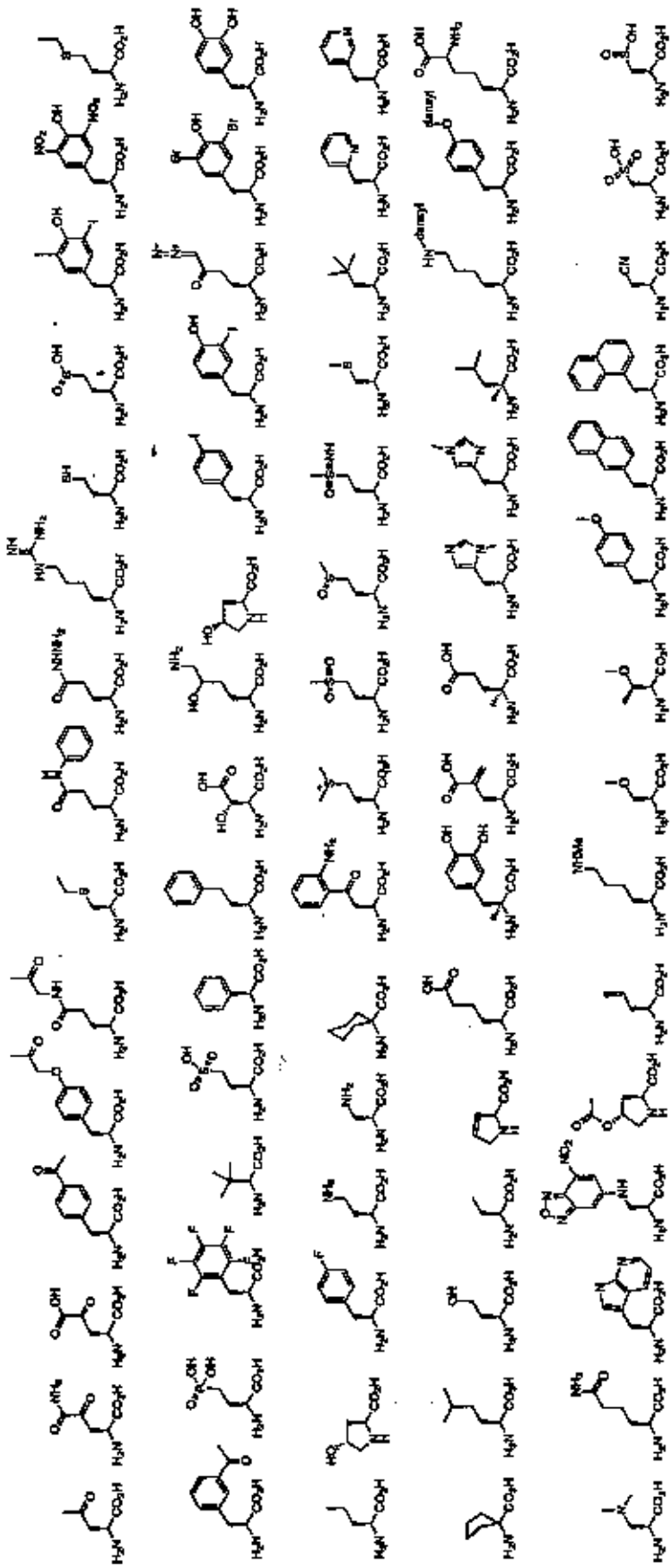


Fig. 18

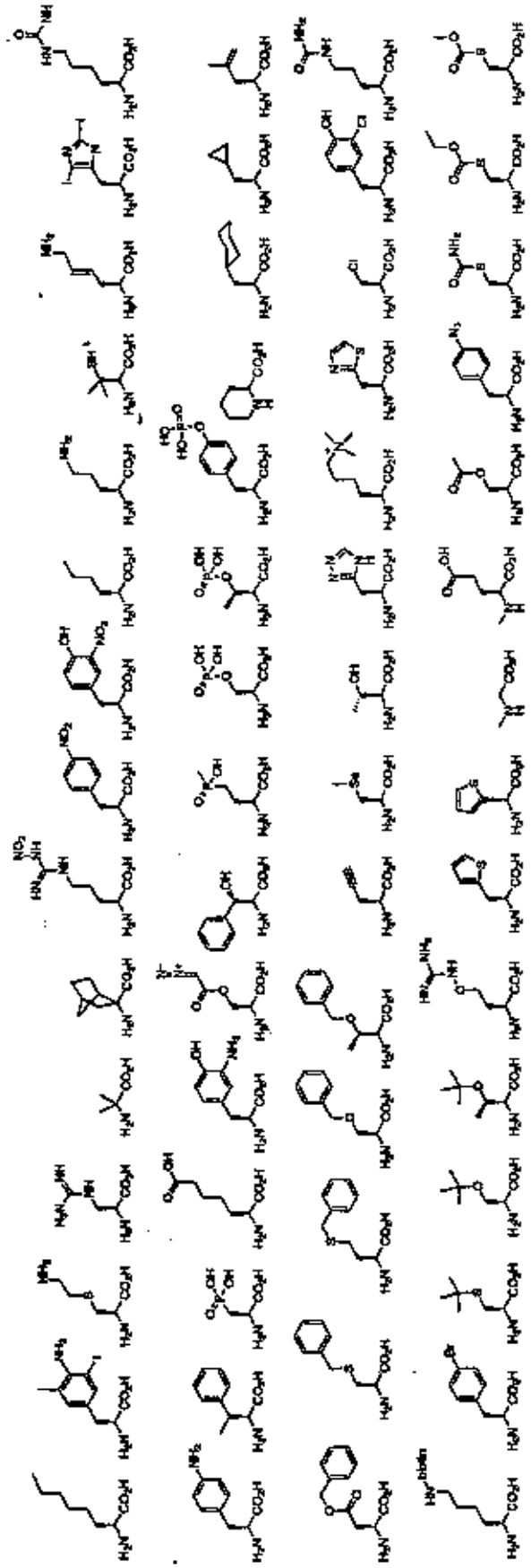


Fig. 19

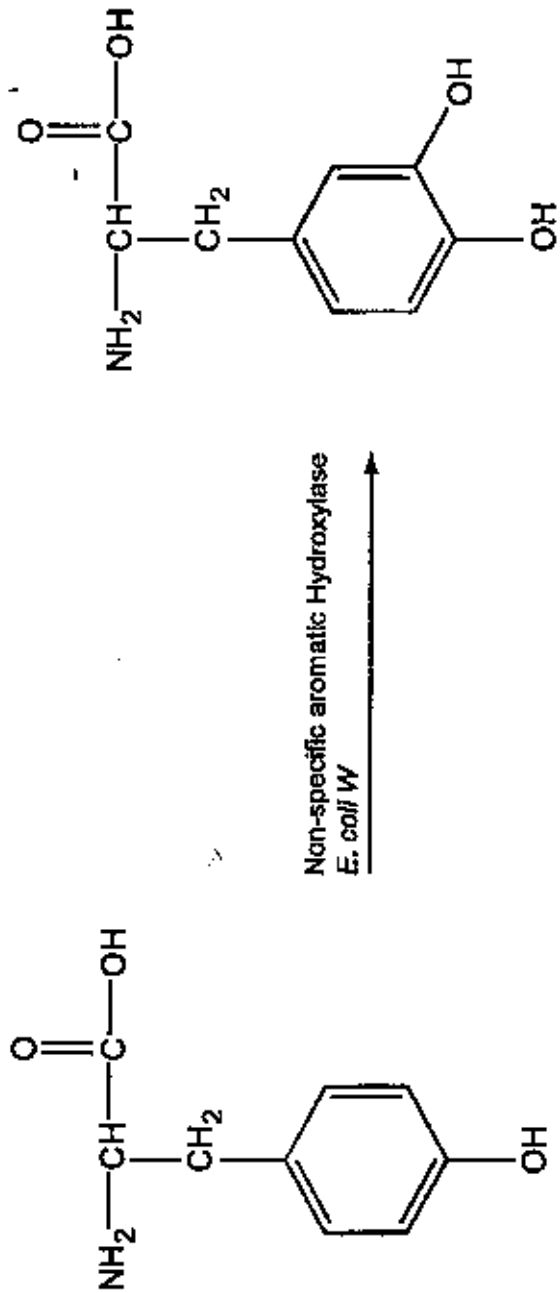


Fig. 20

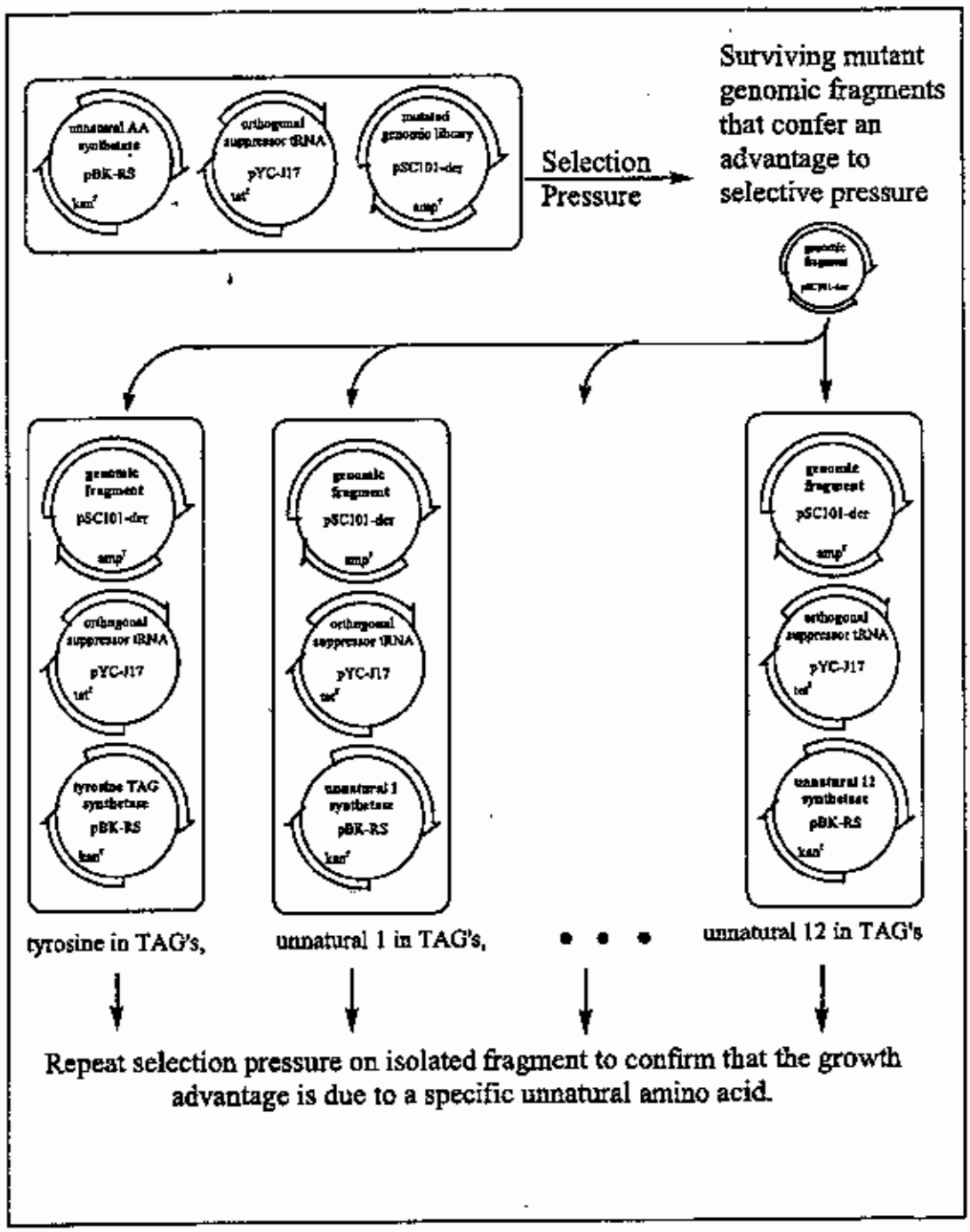

unnatural AA synthetase
pBK-RS
kan^r
orthogonal suppressor tRNA
pYC-J17
tet^r
mutated genomic library
pSC101-der
amp^r
Selection Pressure
Surviving mutant genomic fragments that confer an advantage to selective pressure
genomic fragment
pSC101-der
amp^r
orthogonal suppressor tRNA
pYC-J17
tet^r
tyrosine TAG synthetase
pBK-RS
kan^r
unnatural 1 synthetase
pBK-RS
kan^r
unnatural 12 synthetase
pBK-RS
kan^r
tyrosine in TAG's,
unnatural 1 in TAG's,
unnatural 12 in TAG's
Repeat selection pressure on isolated fragment to confirm that the growth advantage is due to a specific unnatural amino acid.

Fig. 21

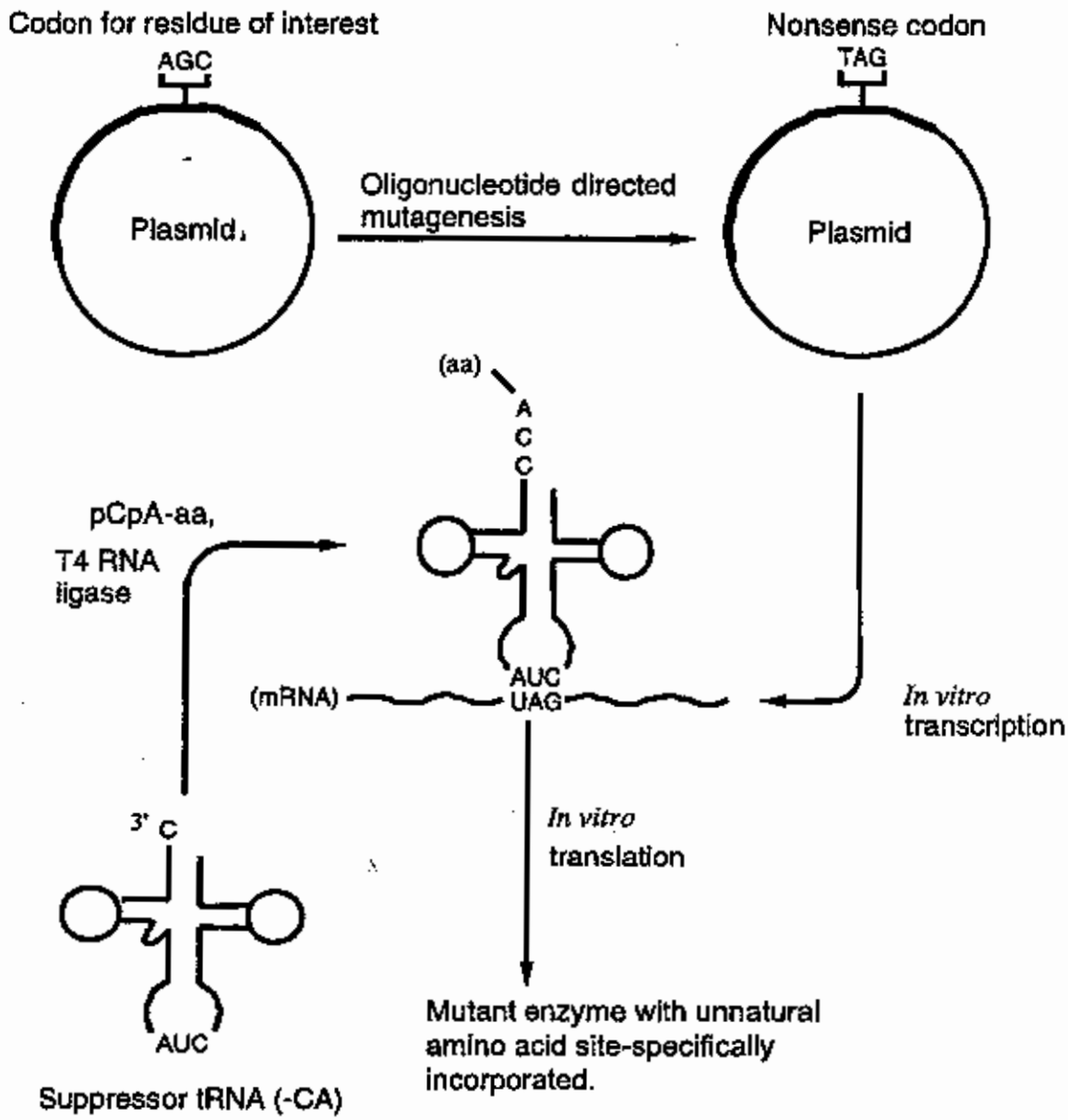
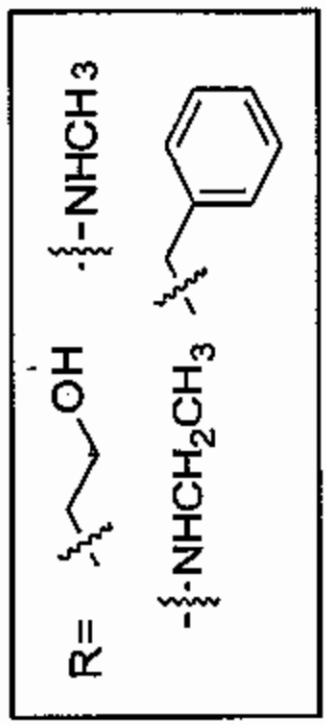

Codon for residue of interest
AGC
Nonsense codon
TAG
Plasmid
Oligonucleotide directed mutagenesis
Plasmid
(aa)
A
C
C
pCpA-aa,
T4 RNA ligase
AUC
UAG
(mRNA)
In vitro transcription
3' C
In vitro translation
AUC
Mutant enzyme with unnatural amino acid site-specifically incorporated.
Suppressor tRNA (-CA)

Fig. 22


R=
OH
NHCH3
NHCH2CH3

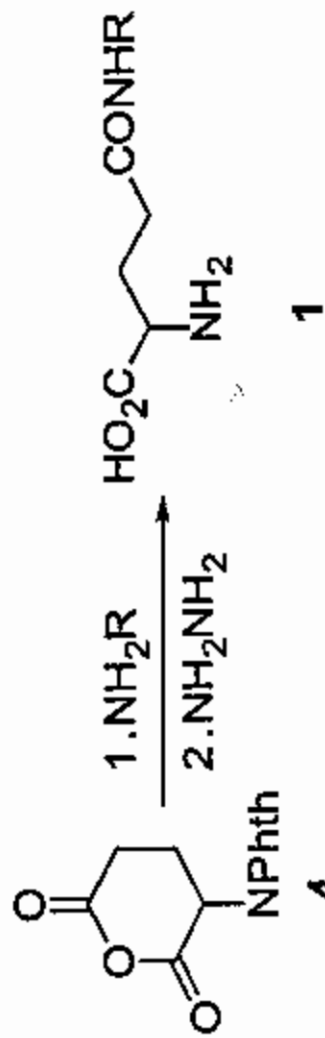
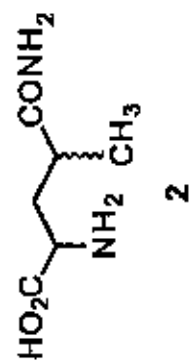
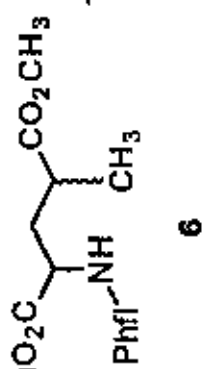
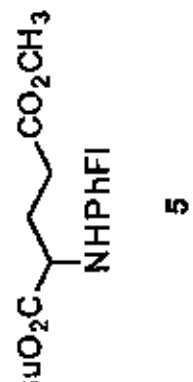

1.NH2R
2.NH2NH2
HO2C
CONHR
NH2
1
O
O
O
NPhth
4

Fig. 23







1. NH_4OH
2. TFA

1. $KN(Si(CH_3)_3)_2$, THF, -78°C
2. MeI

Fig. 24

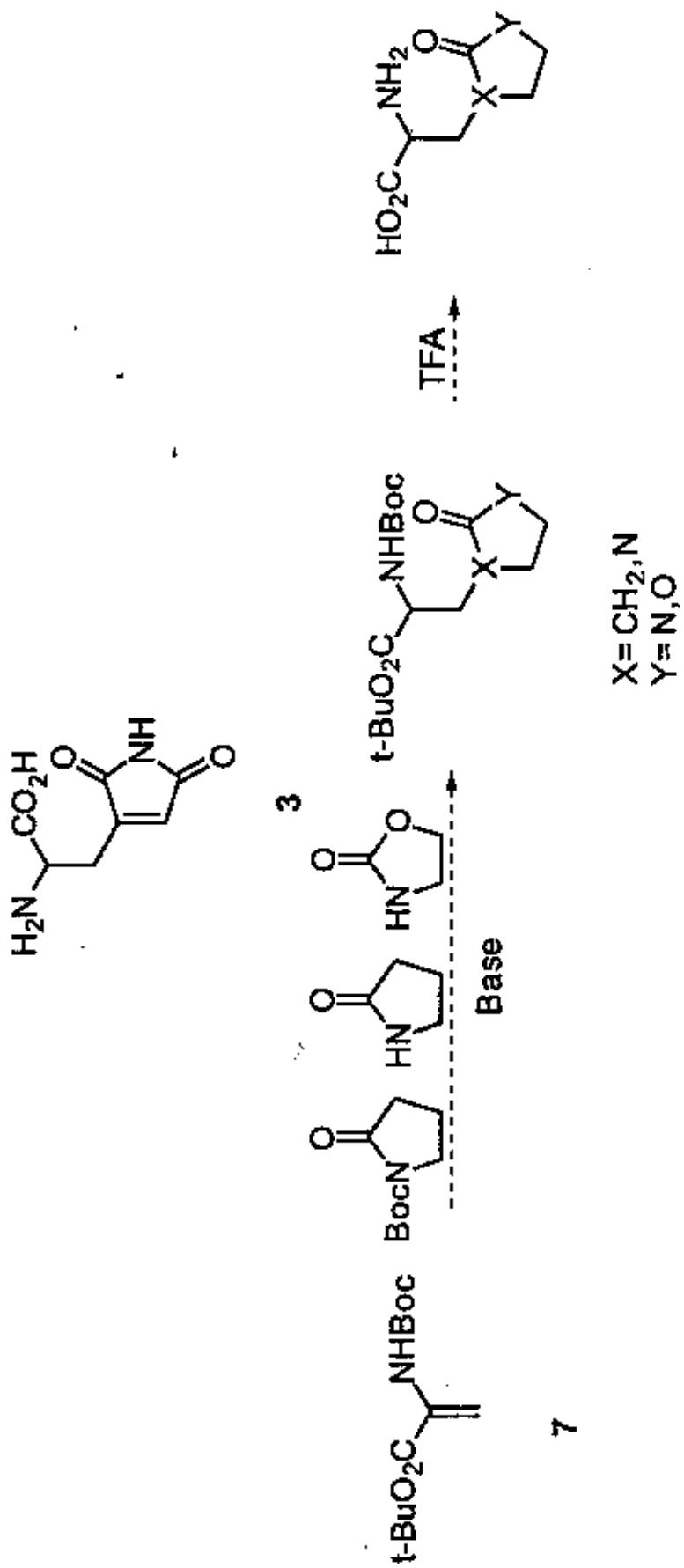


Fig. 25

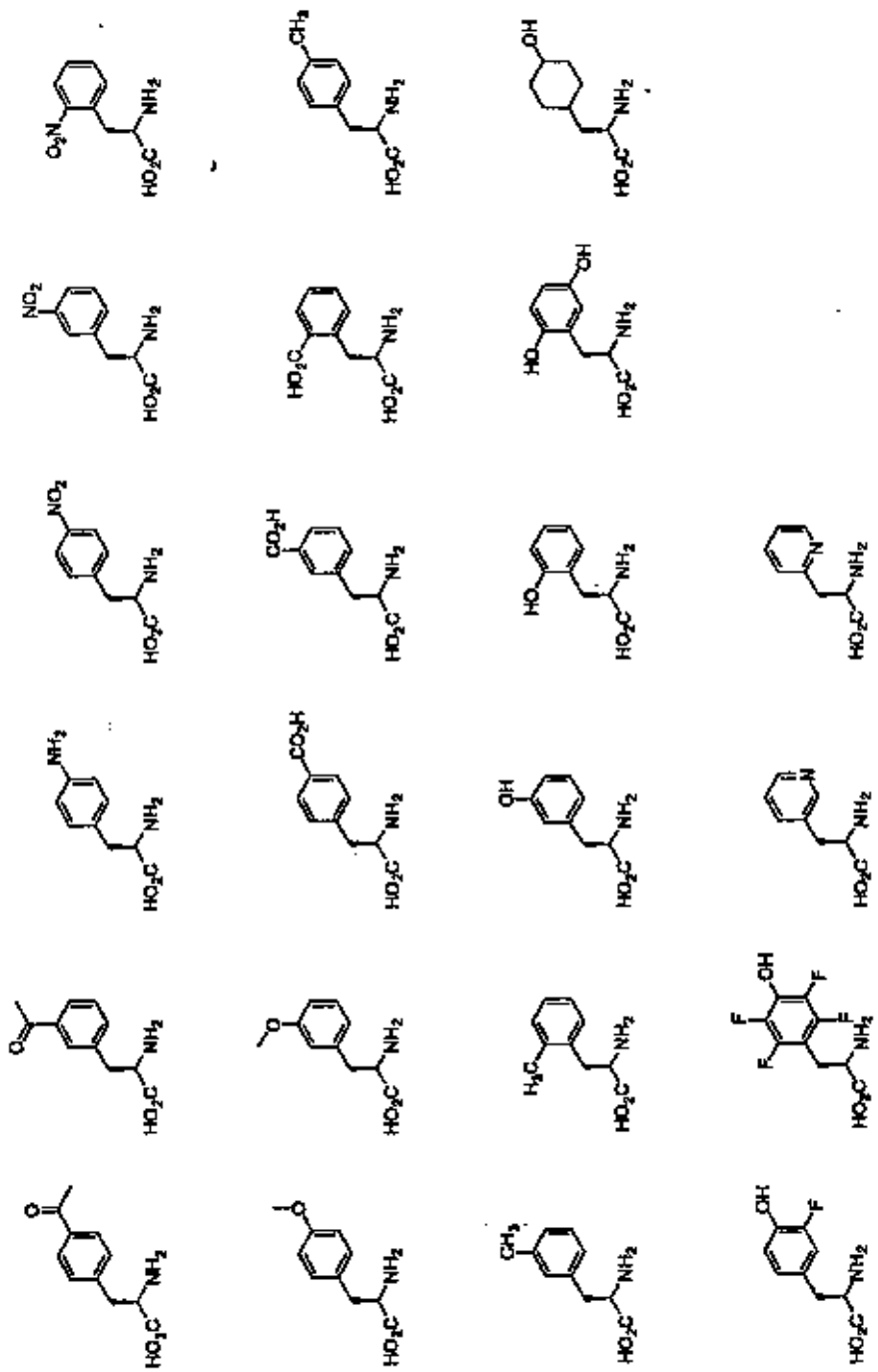
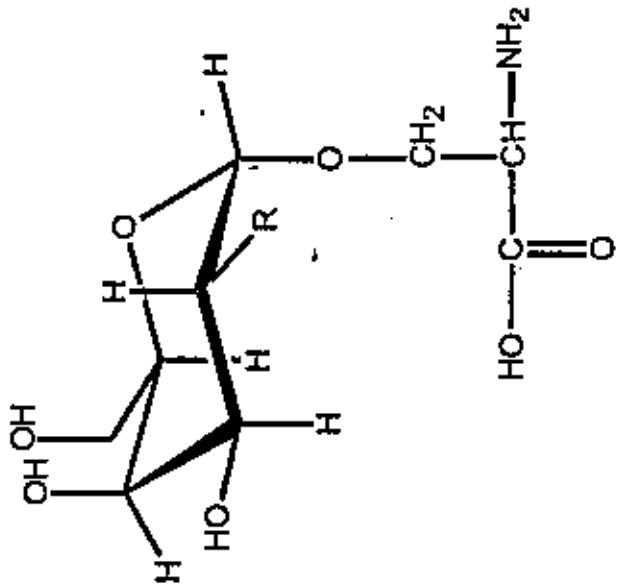


Fig. 26

EtO2C
CO2Et
NHAc
1.NaOEt,EtOH
2.
Br
X
EtO2C
CO2Et
AcHN
12 M HCl
reflux
HO2C
NH2
X
X=OOCH3,CO2Me,CH3,OMe,NO2,I,Br

Fig. 27



Enzyme
in vivo

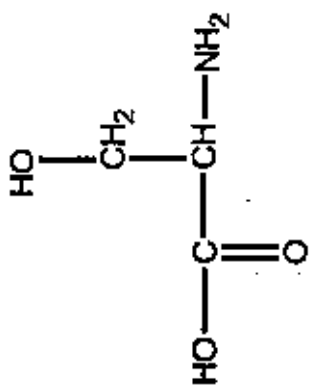


Fig. 28

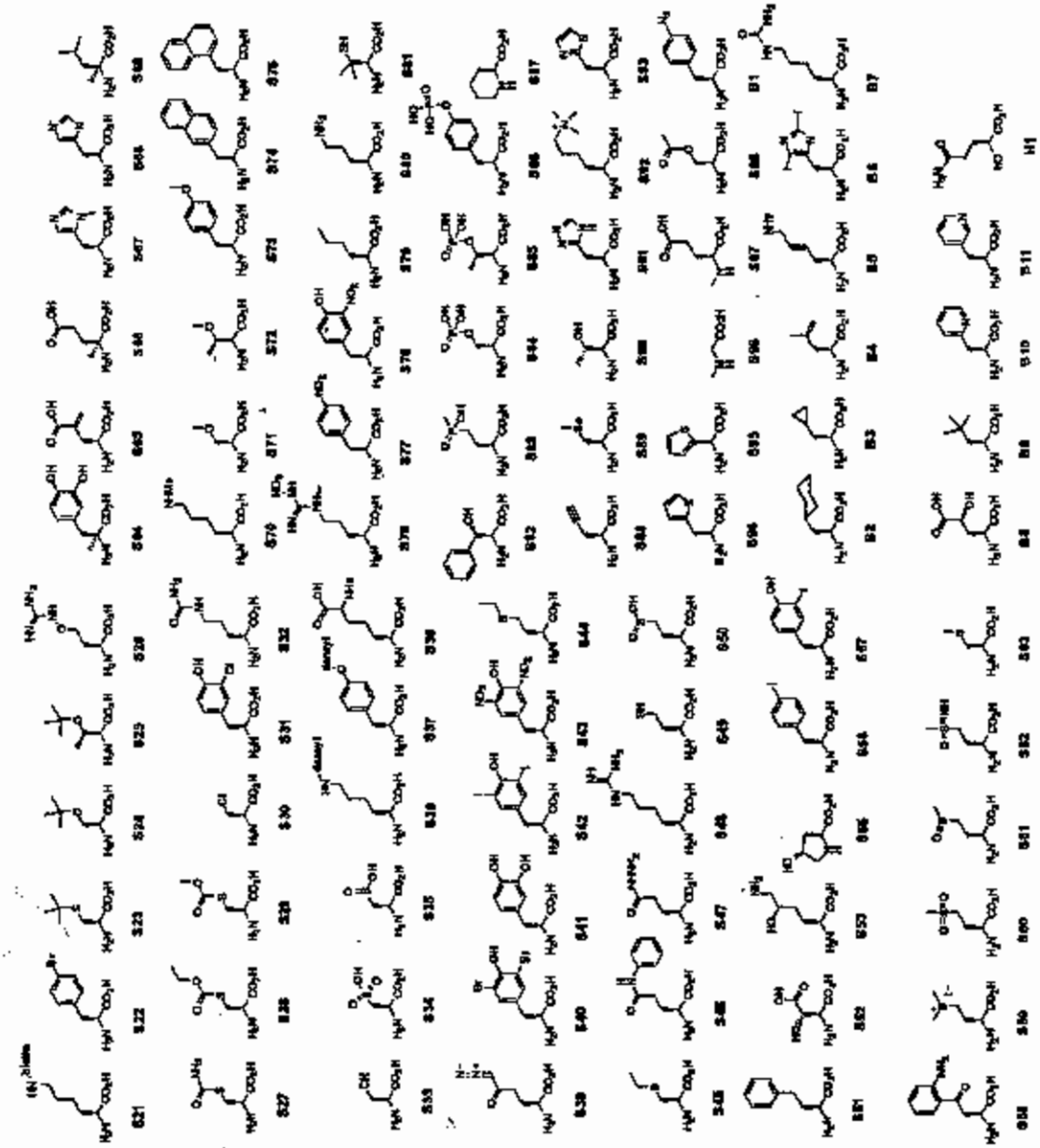


Fig. 29