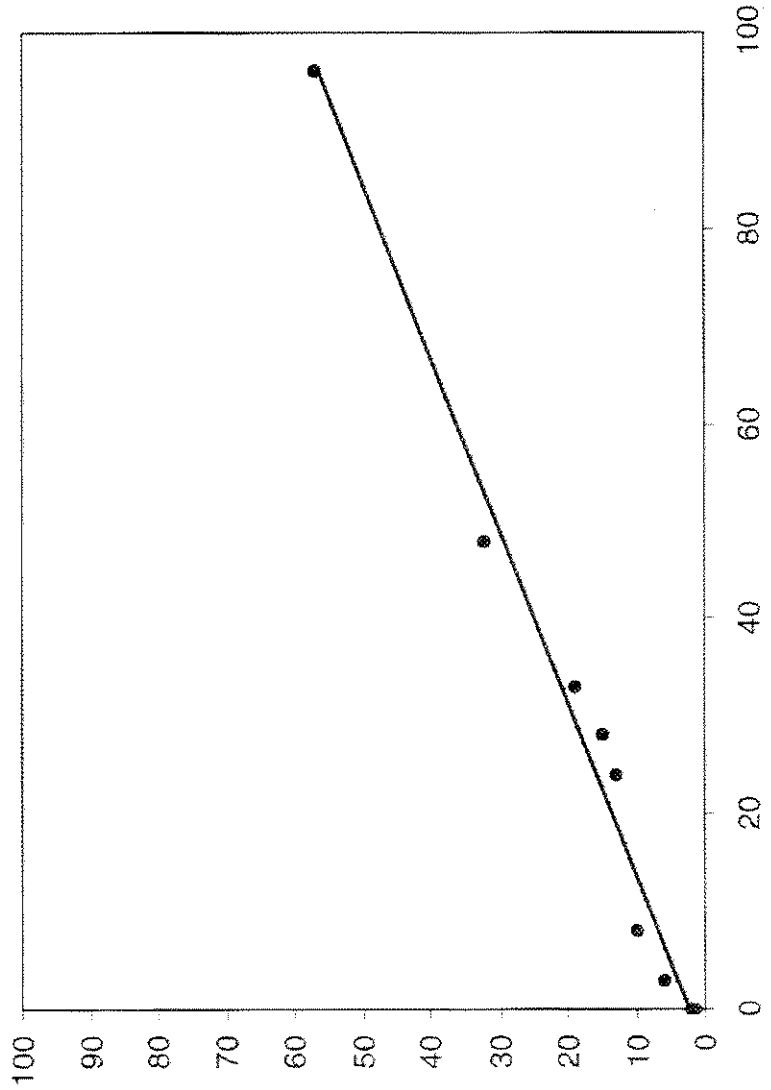


FIG. 1

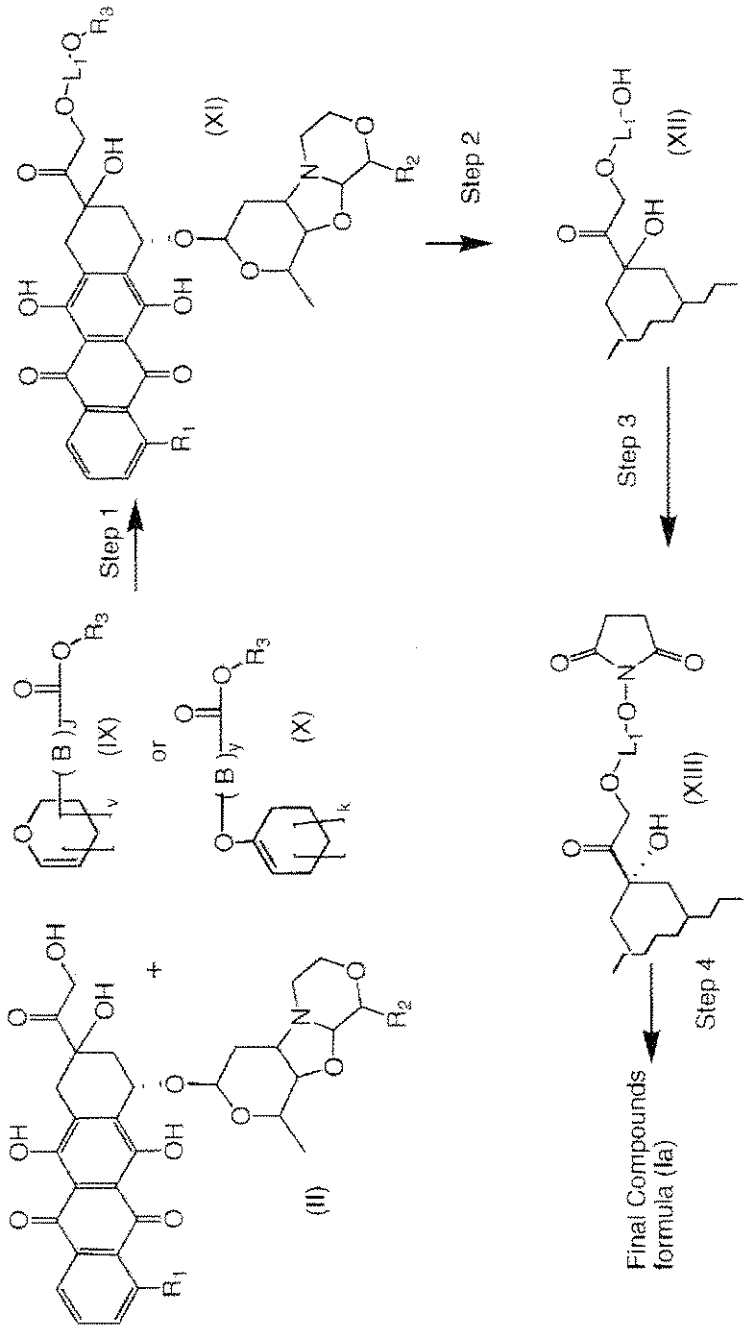

Step 1
Step 2
Step 3
Step 4
(II)
(IX)
(X)
(XI)
(XII)
(XIII)
or
Final Compounds formula (Ia)

FIG. 2

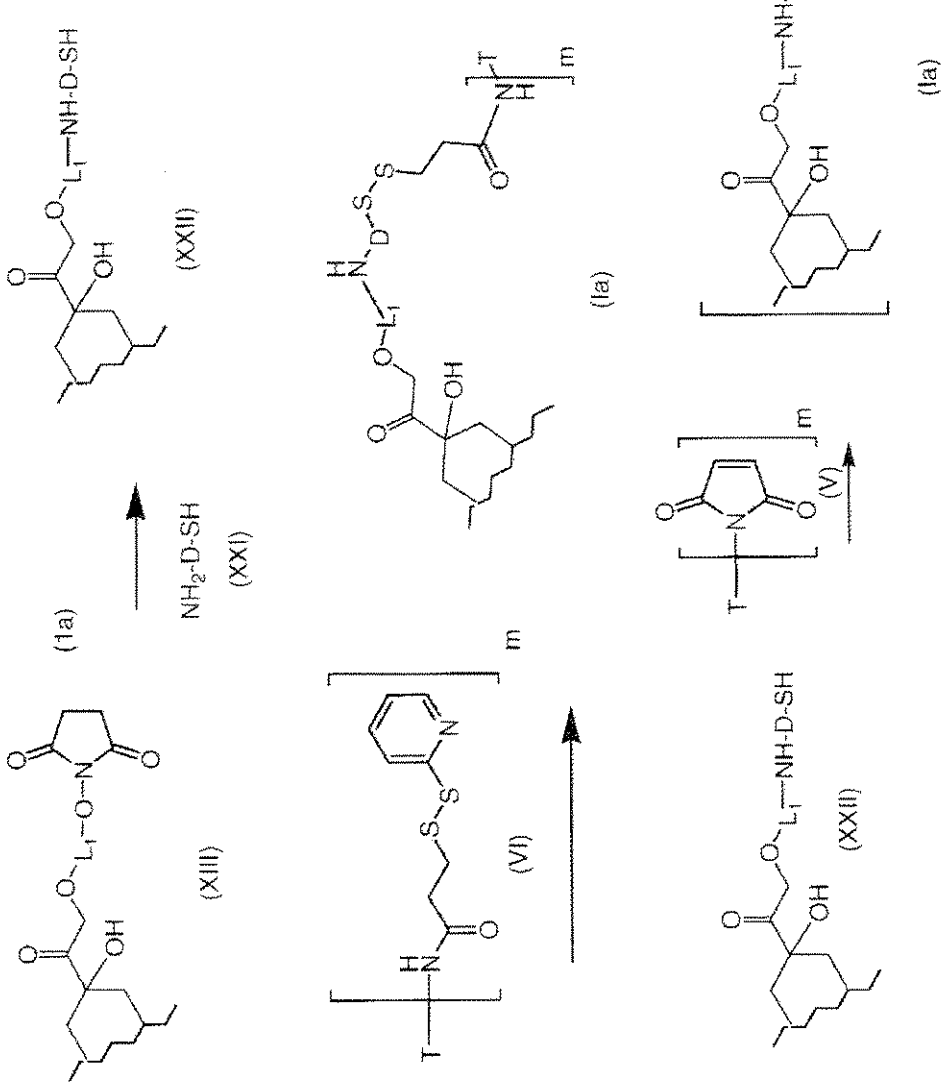


FIG._3a

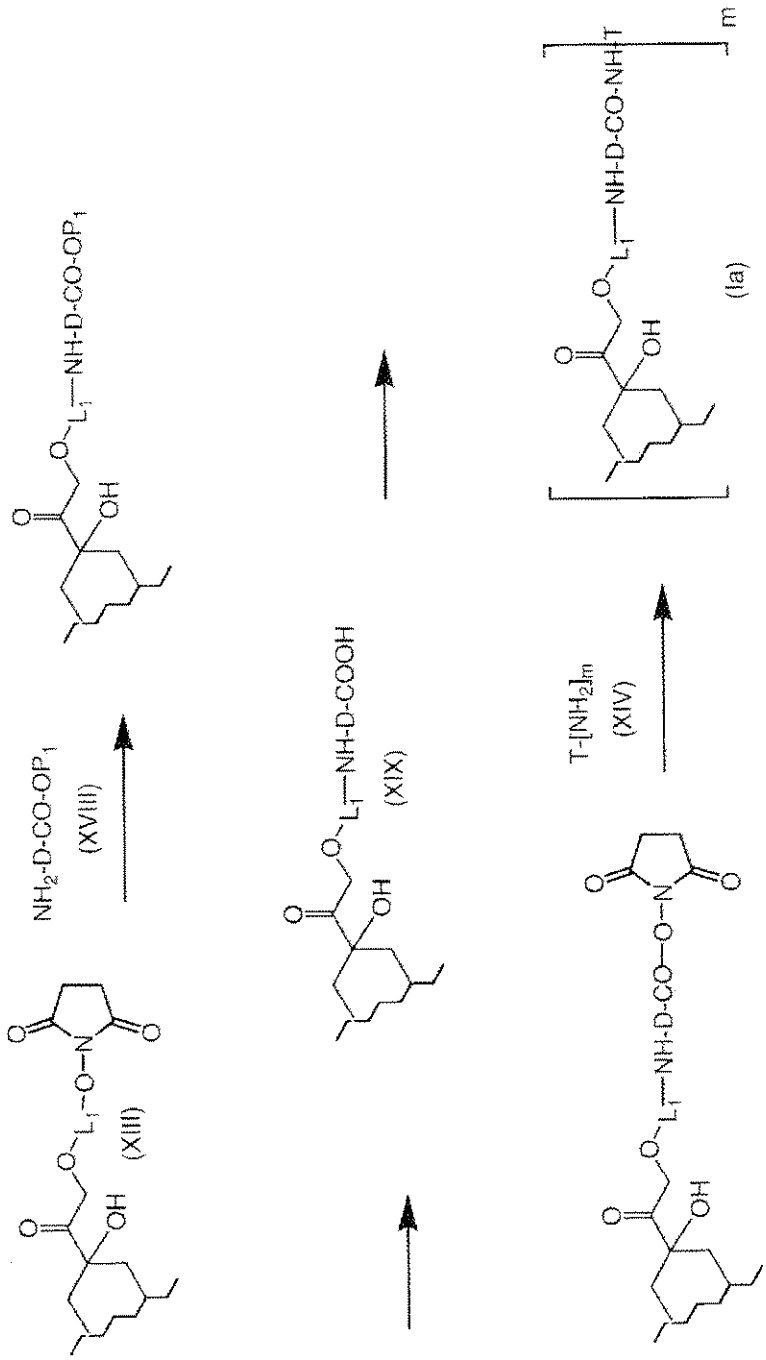


FIG._3b

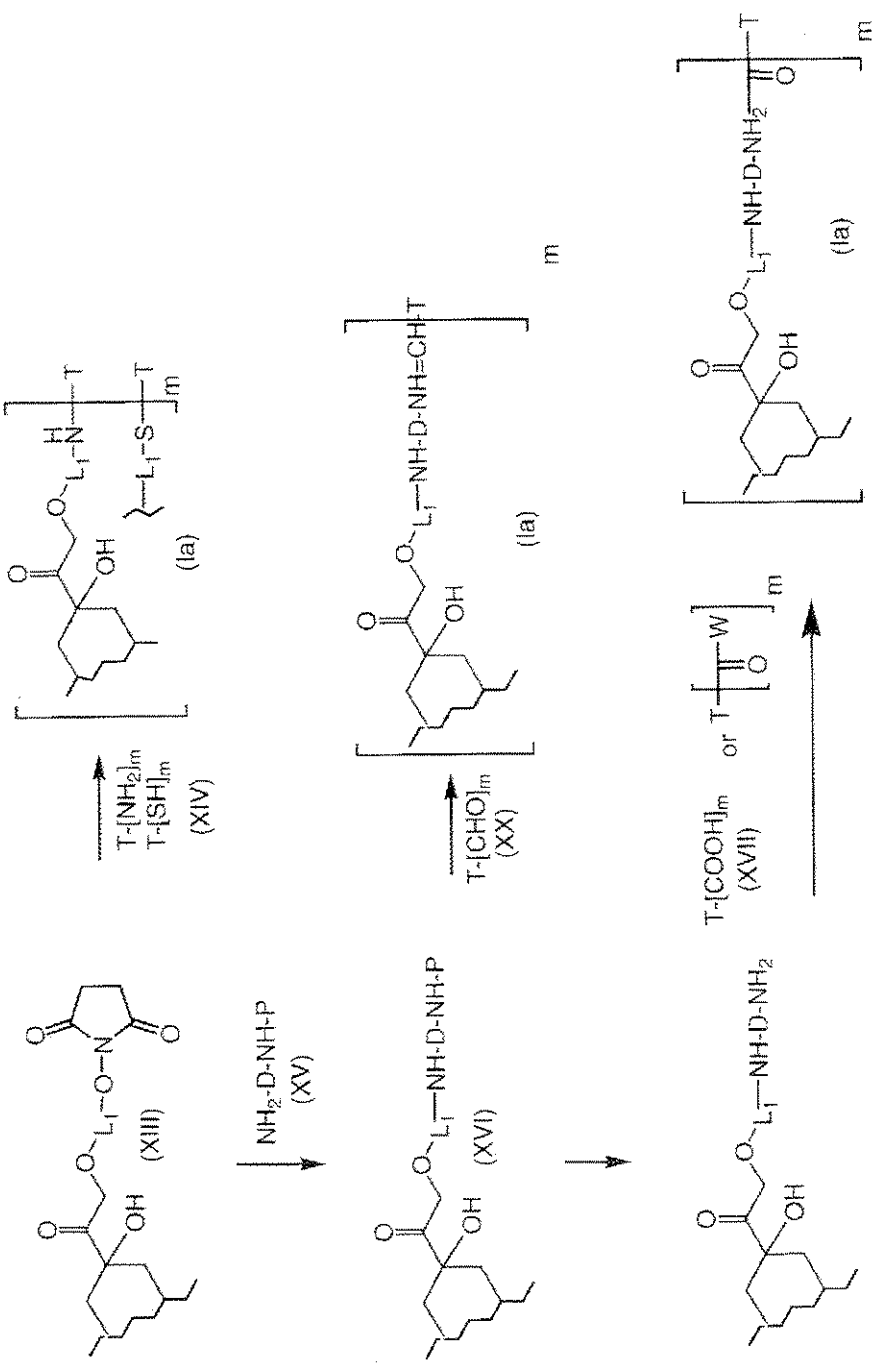

T-[NH2]m
T-[SH]m
(XIV)
(Ia)
T-[CHO]m
(XX)
(Ia)
m
T-[COOH]m
(XVII)
or
(Ia)
NH2-D-NH-P
(XV)
(XIII)
(XVI)

FIG. 3c

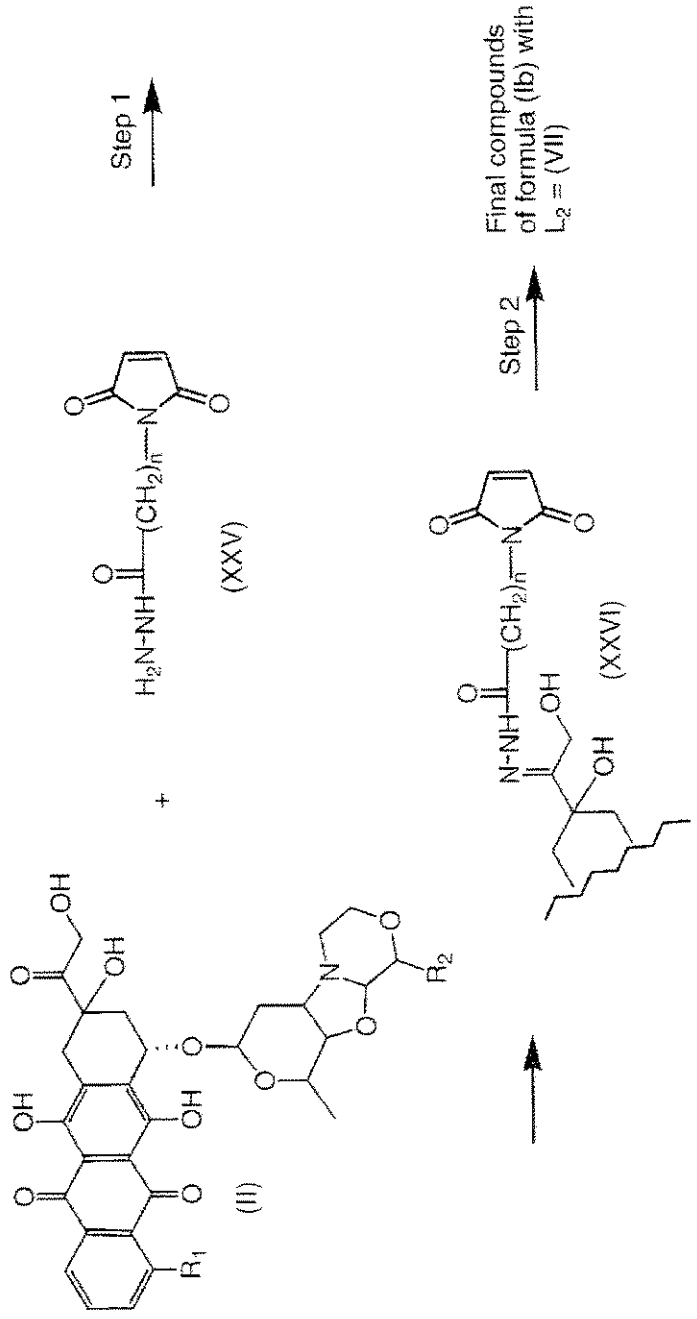

Step 1
Step 2
Final compounds of formula (Ib) with L_2 = (VII)
(II)
(XXV)
(XXVI)

FIG. 4

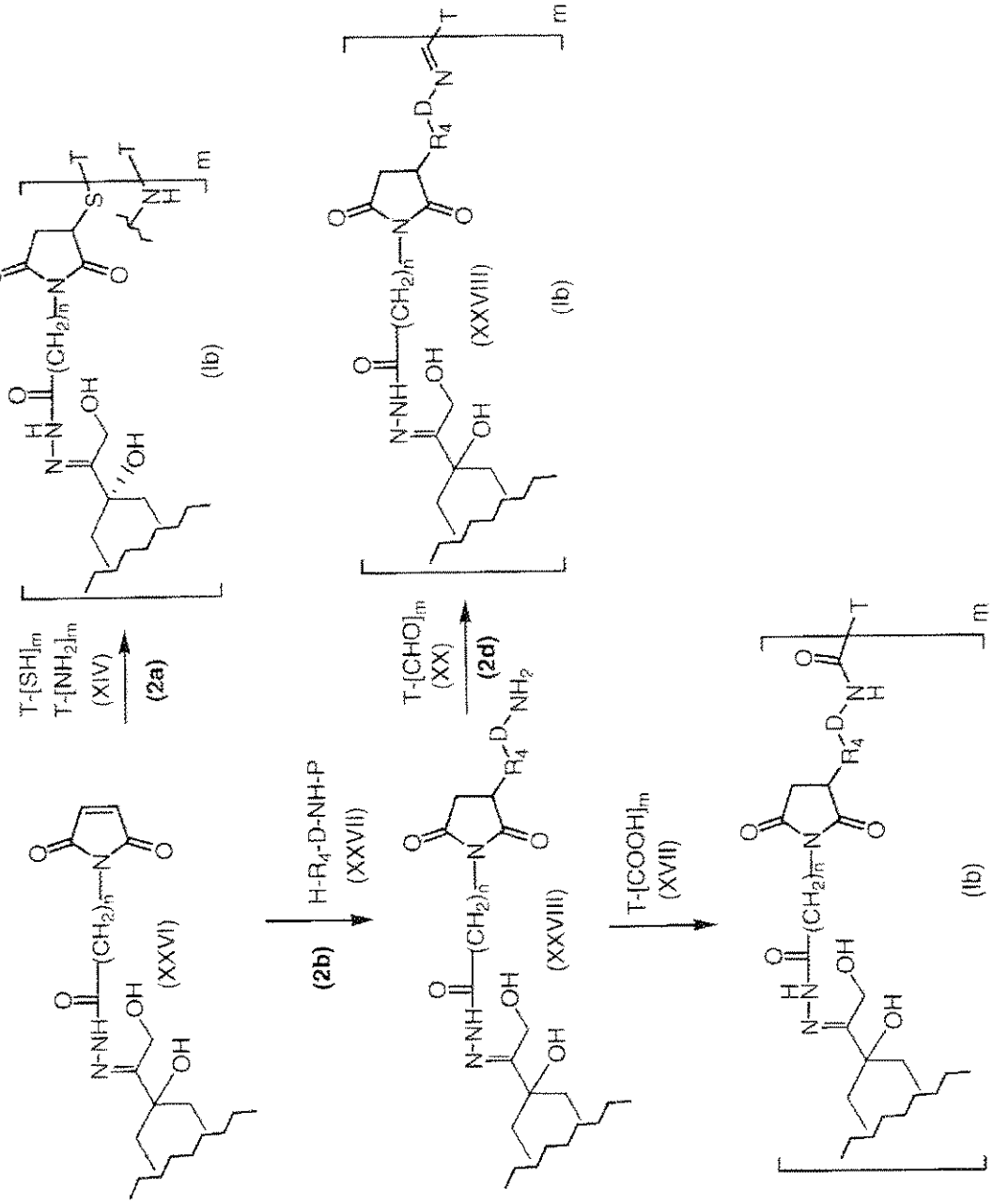


FIG. 5a

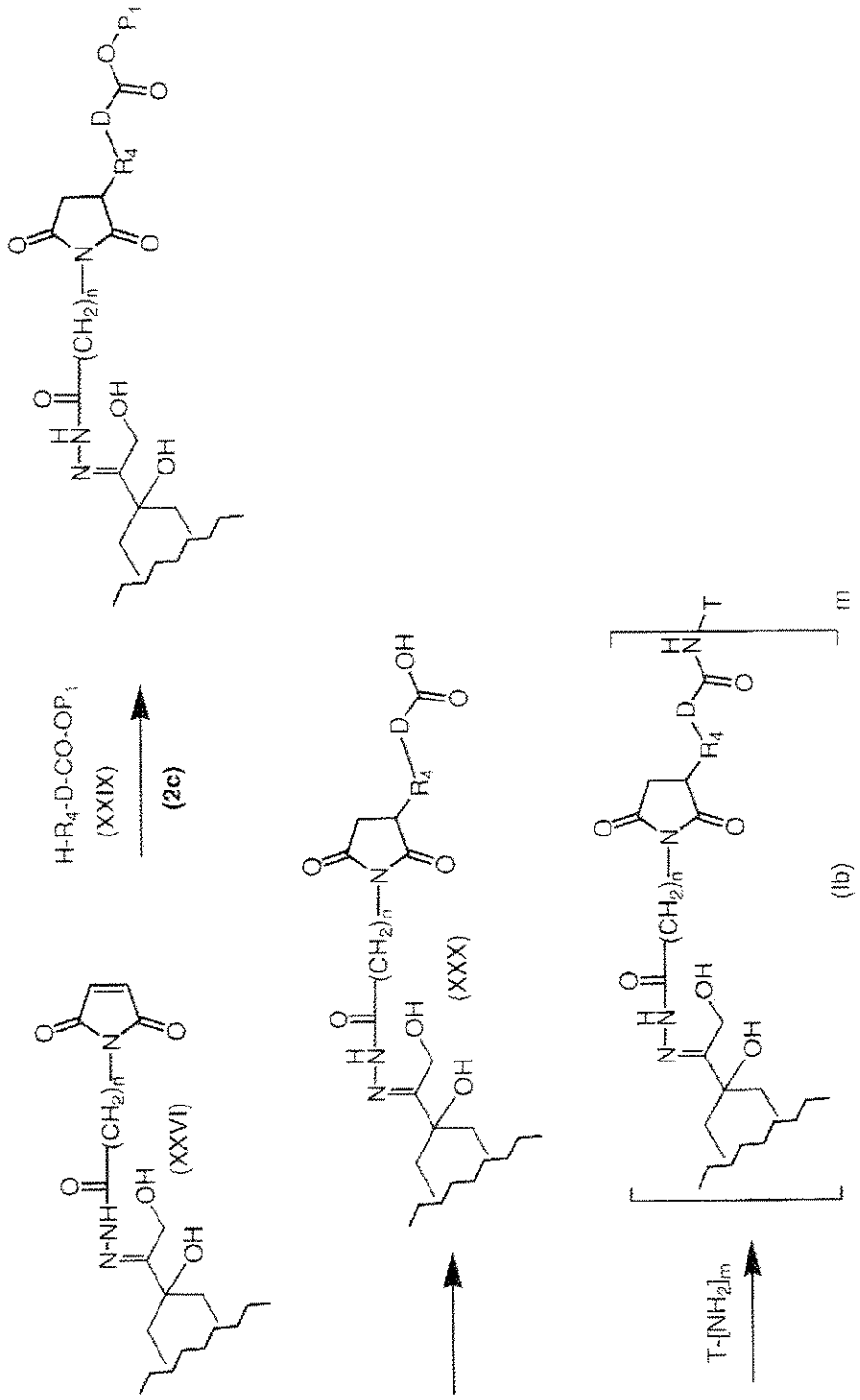


FIG. 5b

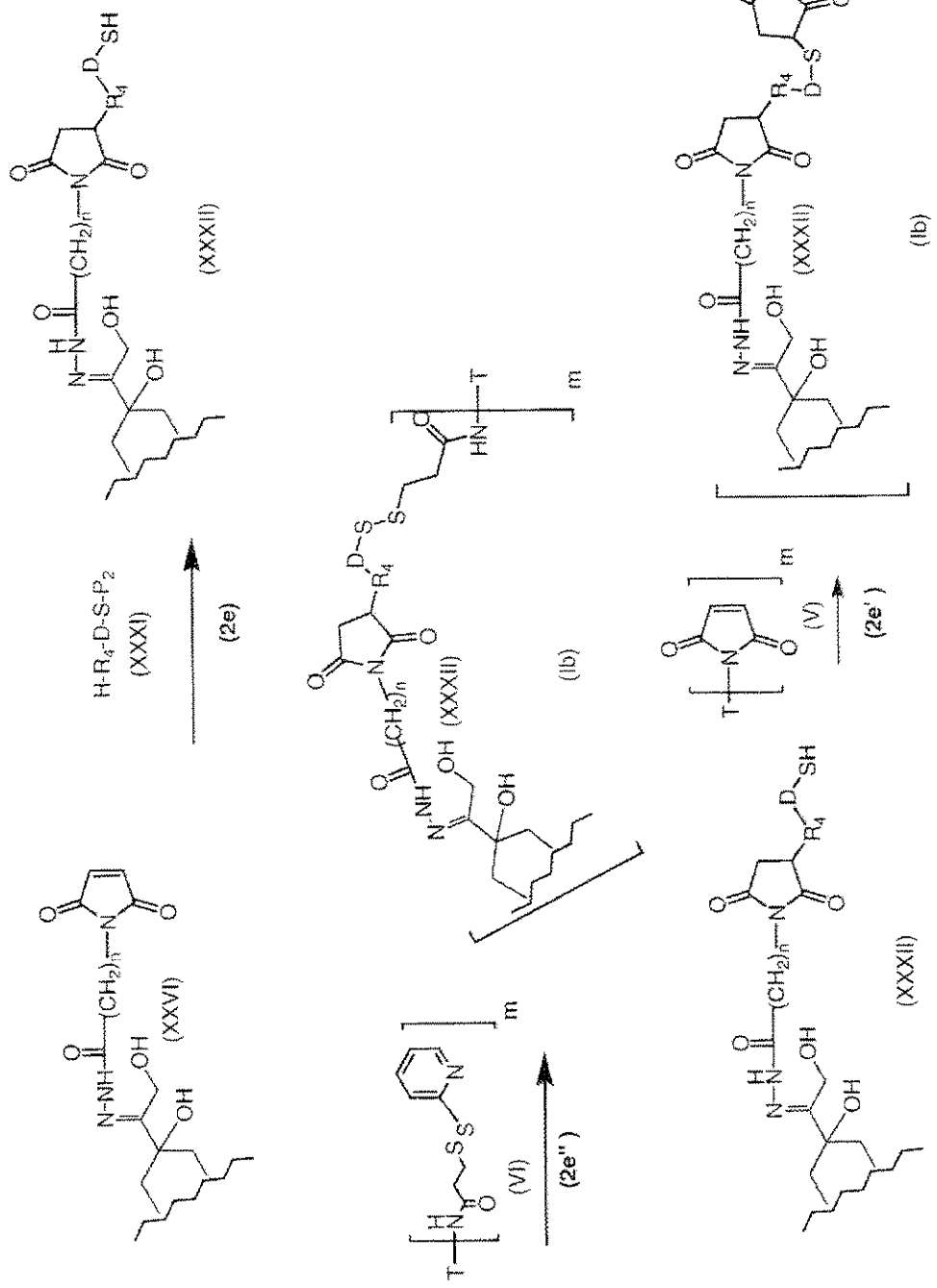


FIG._5c

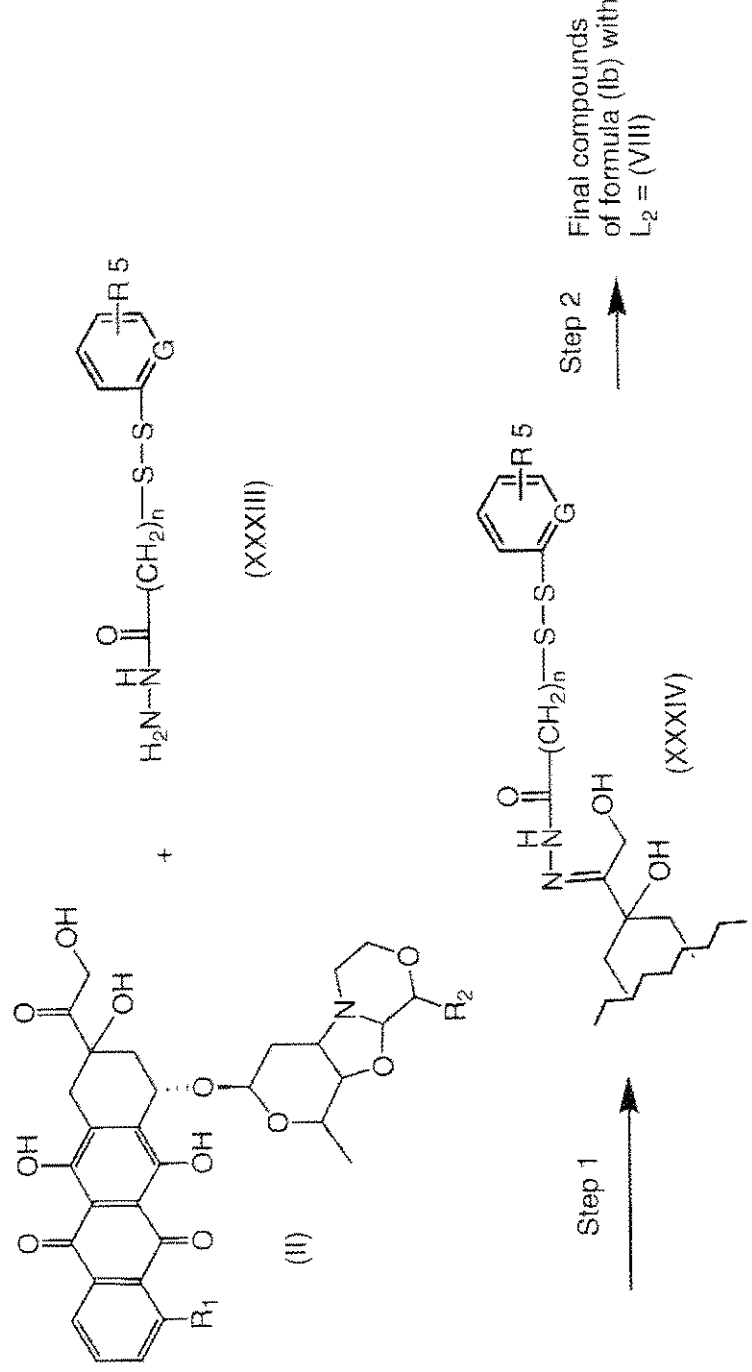

Step 1
Step 2
Final compounds of formula (Ib) with L_2 = (VIII)
(II)
(XXXIII)
(XXXIV)

FIG. 6

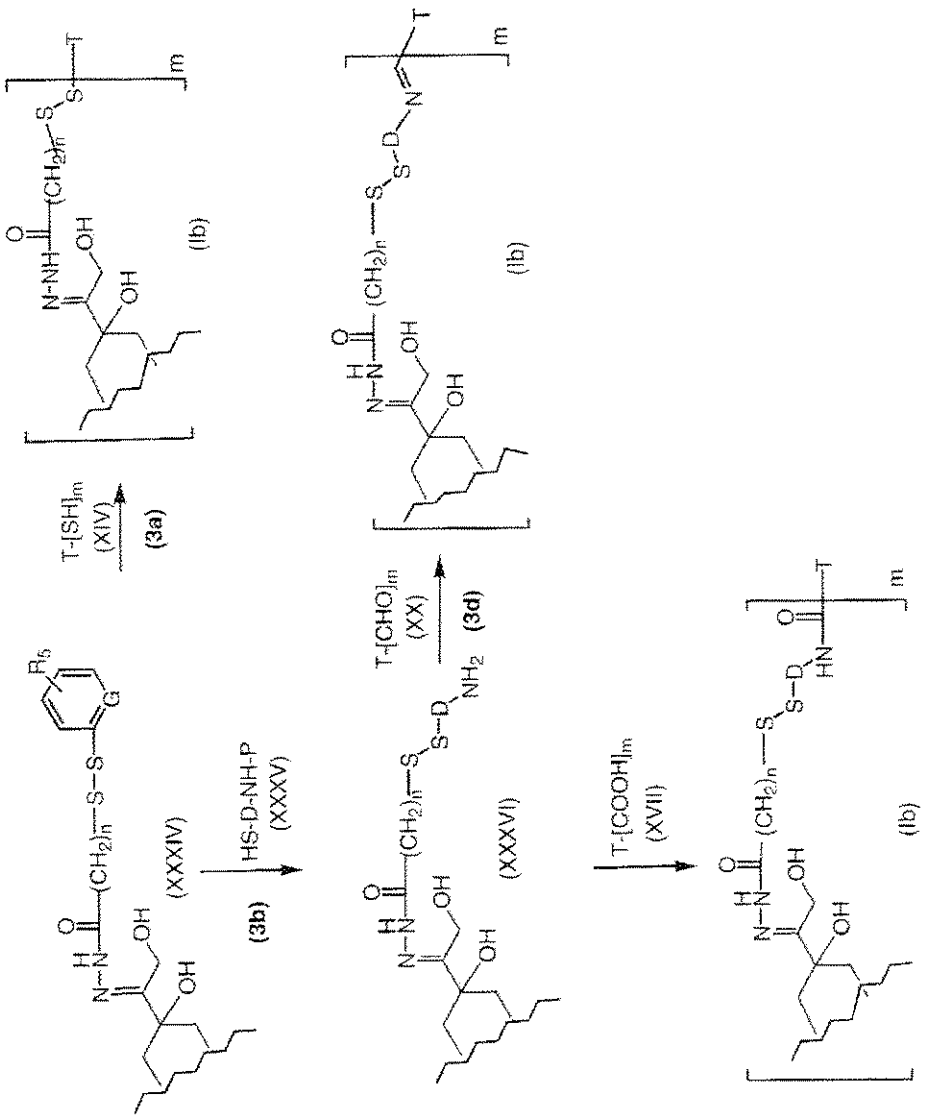

T-[SH]m
(XIV)
(3a)
(Ib)
(XXXIV)
(3b)
HS-D-NH-P
(XXXV)
(XXXVI)
T-[CHO]m
(XX)
(3d)
(IIb)
T-[COOH]m
(XVII)
(Ib)

FIG._7a

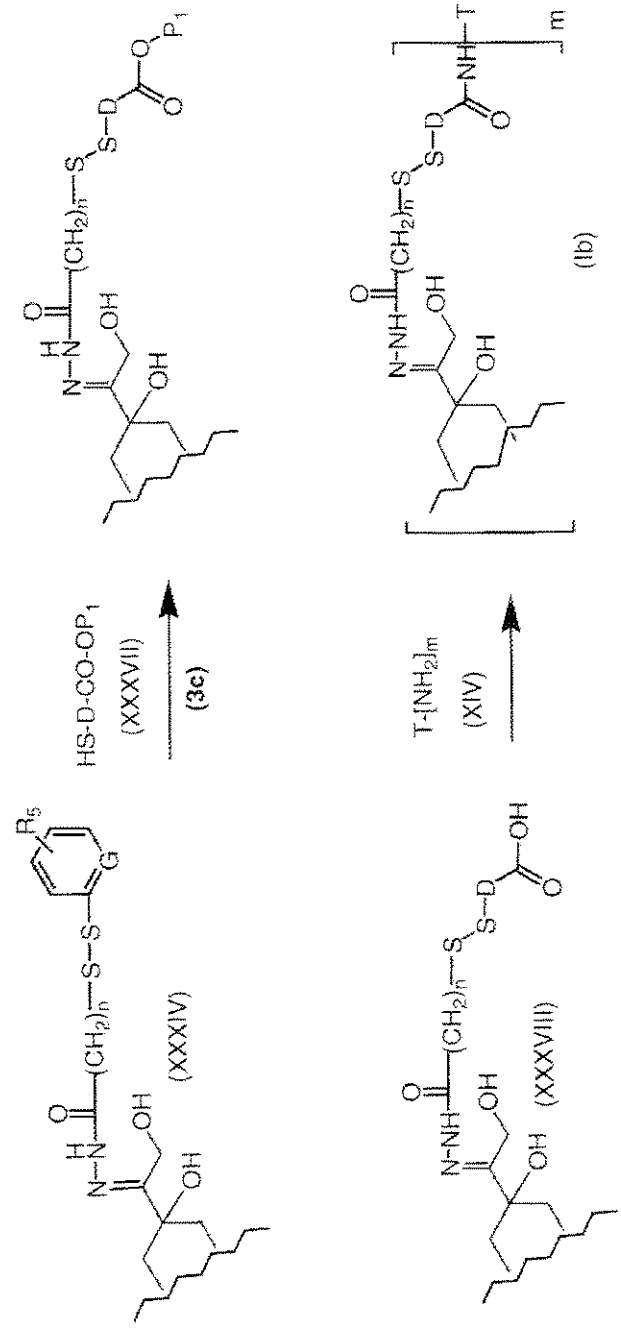


FIG._7b

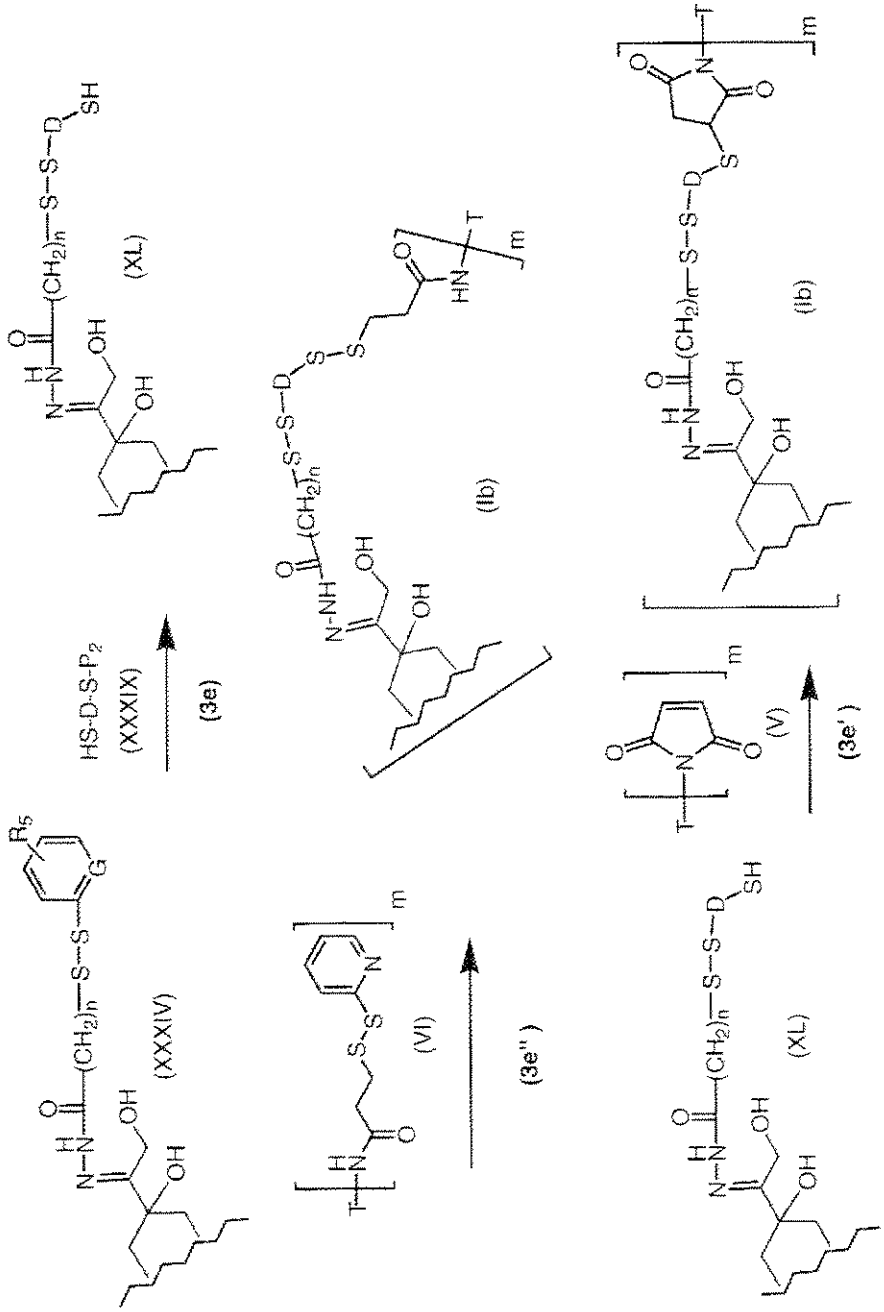


FIG._7c

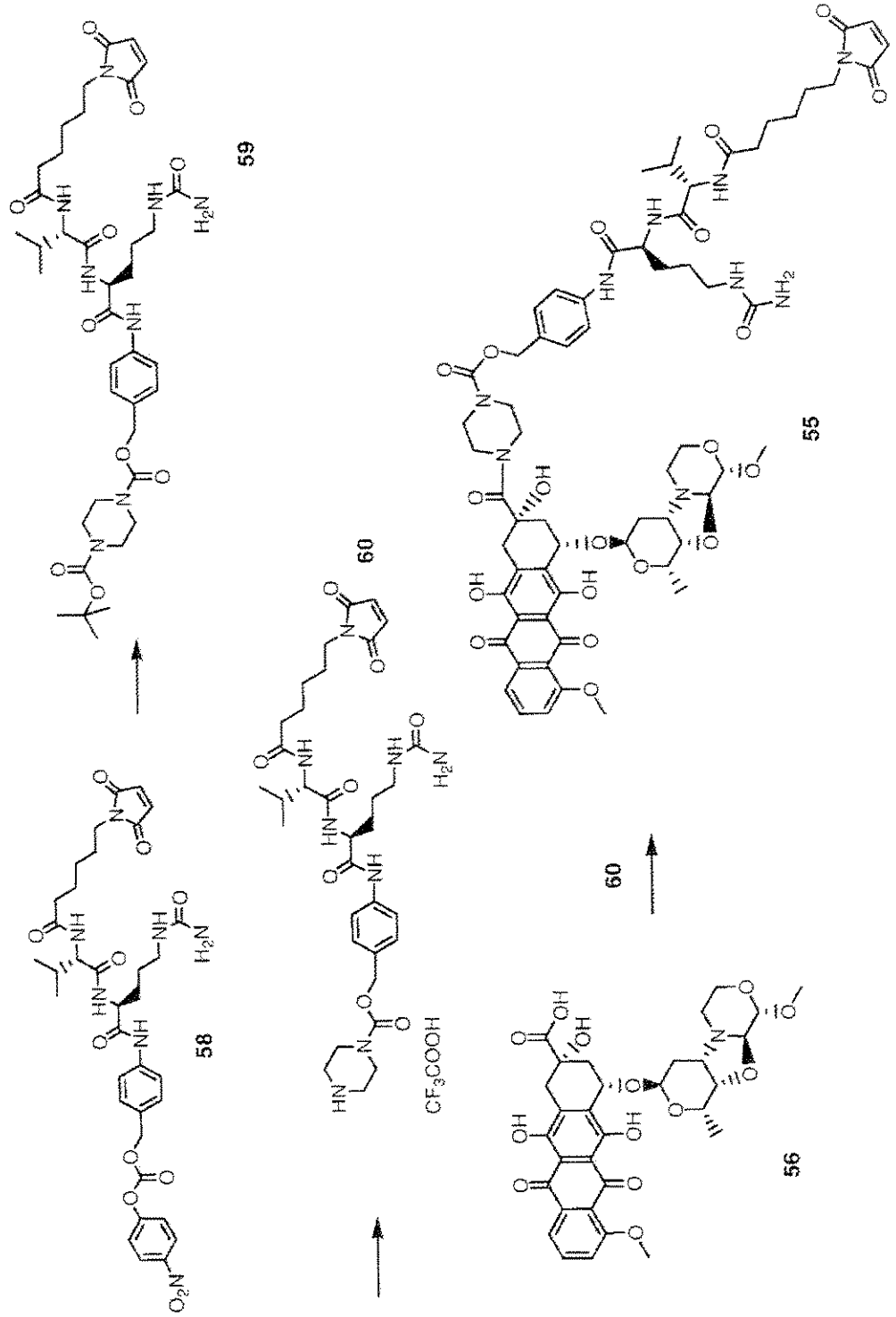


FIG.7d

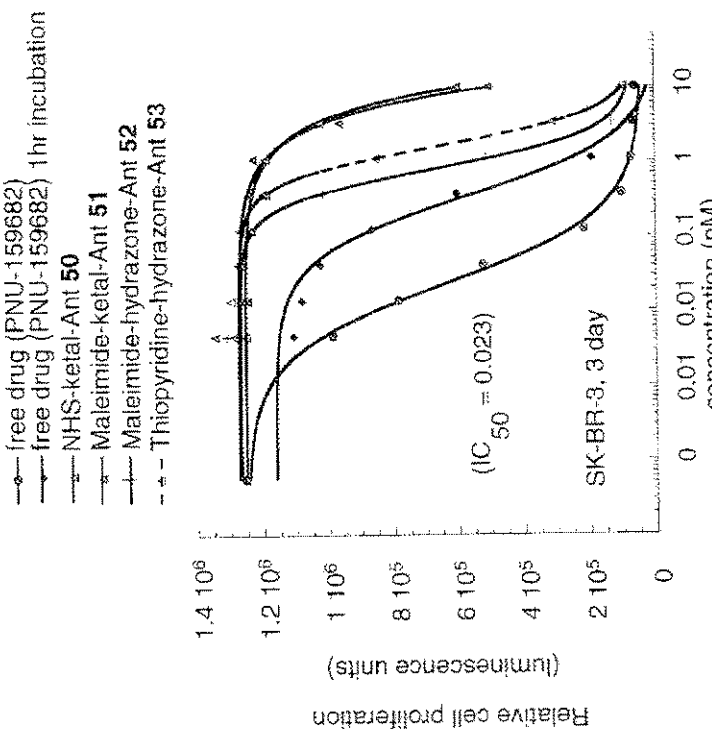

free drug (PNU-159682)
free drug (PNU-159682) 1hr incubation
NHS-ketal-Ant 50
Maleimide-ketal-Ant 51
Maleimide-hydrazone-Ant 52
Thiopyridine-hydrazone-Ant 53
Relative cell proliferation
(luminescence units)
1.4 10^6
1.2 10^6
1 10^6
8 10^5
6 10^5
4 10^5
2 10^5
0
0 0.01 0.01 0.1 1 10
concentration (nM)
SK-BR-3, 3 day
(IC50 = 0.023)

FIG._8

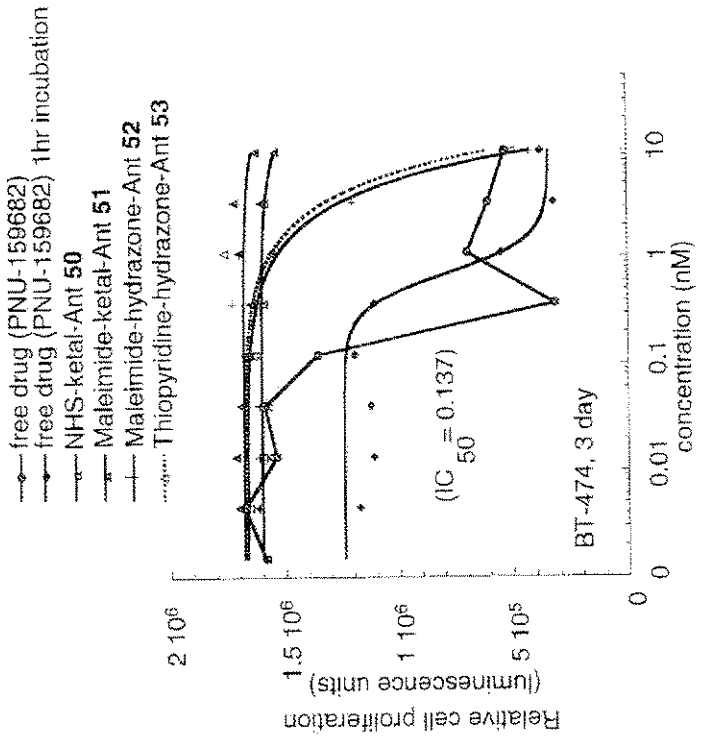

free drug (PNU-159682)
free drug (PNU-159682) 1hr incubation
NHS-ketal-Ant 50
Maleimide-ketal-Ant 51
Maleimide-hydrazone-Ant 52
Thiopyridine-hydrazone-Ant 53
Relative cell proliferation
(luminescence units)
2 10^6
1.5 10^6
1 10^6
5 10^5
0
0 0.01 0.1 1 10
concentration (nM)
BT-474, 3 day
(IC50 = 0.137)

FIG._9

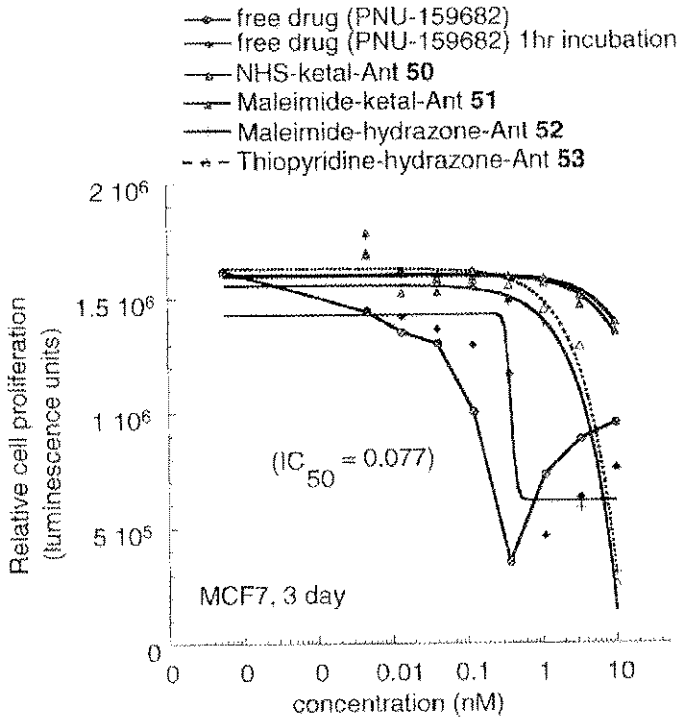

free drug (PNU-159682)
free drug (PNU-159682) 1hr incubation
NHS-ketal-Ant 50
Maleimide-ketal-Ant 51
Maleimide-hydrazone-Ant 52
Thiopyridine-hydrazone-Ant 53
Relative cell proliferation
(luminescence units)
2 10^6
1.5 10^6
1 10^6
5 10^5
0
(IC50 = 0.077)
MCF7, 3 day
0 0 0 0.01 0.1 1 10
concentration (nM)

FIG._10

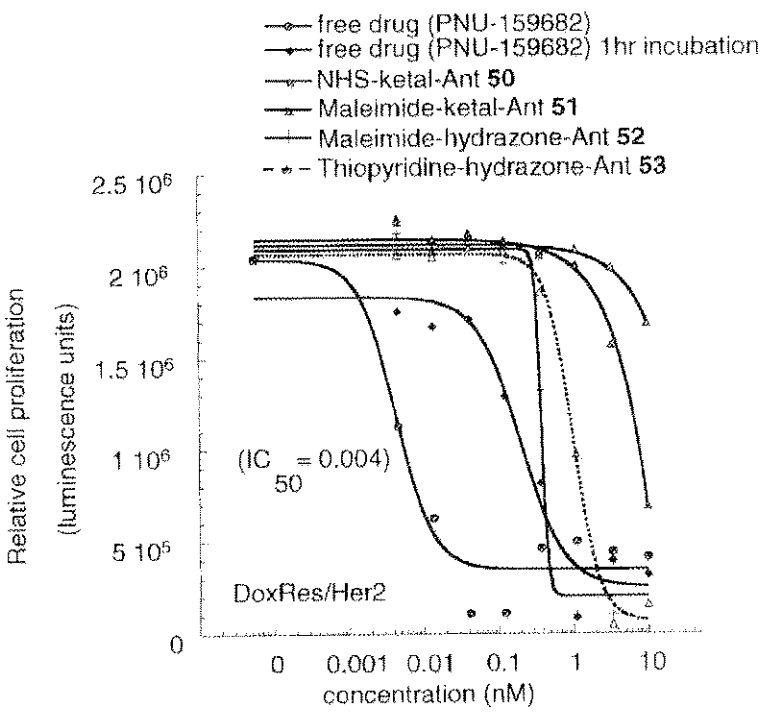

free drug (PNU-159682)
free drug (PNU-159682) 1hr incubation
NHS-ketal-Ant 50
Maleimide-ketal-Ant 51
Maleimide-hydrazone-Ant 52
Thiopyridine-hydrazone-Ant 53
Relative cell proliferation
(luminescence units)
2.5 10^6
2 10^6
1.5 10^6
1 10^6
5 10^5
0
(IC50 = 0.004)
DoxRes/Her2
0 0.001 0.01 0.1 1 10
concentration (nM)

FIG._11

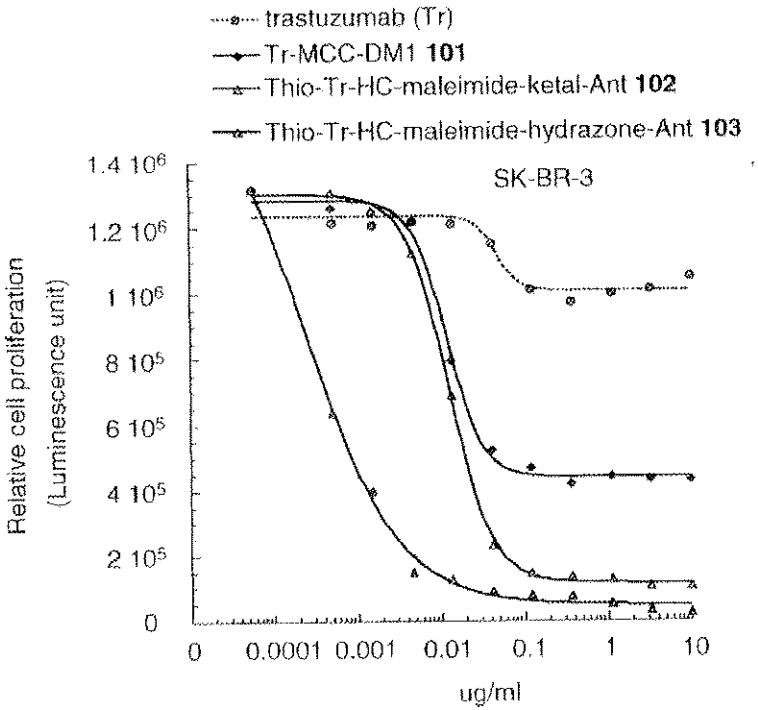

trastuzumab (Tr)
Tr-MCC-DM1 101
Thio-Tr-HC-maleimide-ketal-Ant 102
Thio-Tr-HC-maleimide-hydrazone-Ant 103
SK-BR-3
Relative cell proliferation
(Luminescence unit)
1.4 10^6
1.2 10^6
1 10^6
8 10^5
6 10^5
4 10^5
2 10^5
0
0 0.0001 0.001 0.01 0.1 1 10
ug/ml

FIG._12

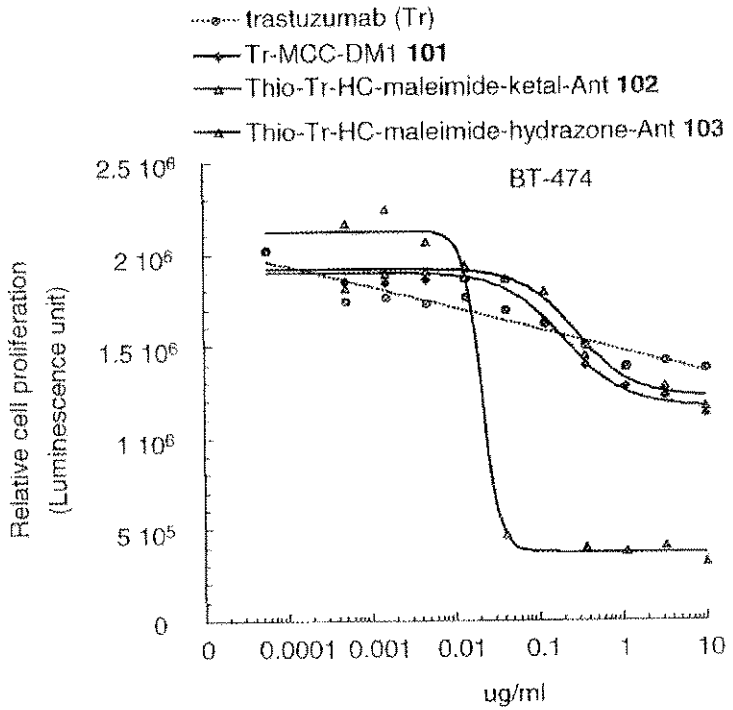

trastuzumab (Tr)
Tr-MCC-DM1 101
Thio-Tr-HC-maleimide-ketal-Ant 102
Thio-Tr-HC-maleimide-hydrazone-Ant 103
BT-474
Relative cell proliferation
(Luminescence unit)
2.5 10^6
2 10^6
1.5 10^6
1 10^6
5 10^5
0
0 0.0001 0.001 0.01 0.1 1 10
ug/ml

FIG._13

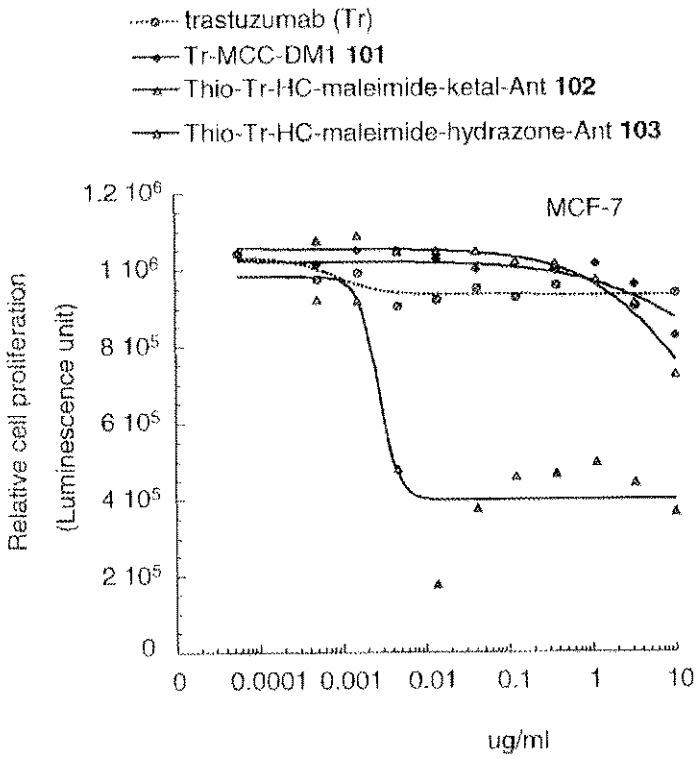

trastuzumab (Tr)
Tr-MCC-DM1 101
Thio-Tr-HC-maleimide-ketal-Ant 102
Thio-Tr-HC-maleimide-hydrazone-Ant 103
MCF-7
Relative cell proliferation
(Luminescence unit)
1.2 10^6
1 10^6
8 10^5
6 10^5
4 10^5
2 10^5
0
0 0.0001 0.001 0.01 0.1 1 10
ug/ml

FIG._14

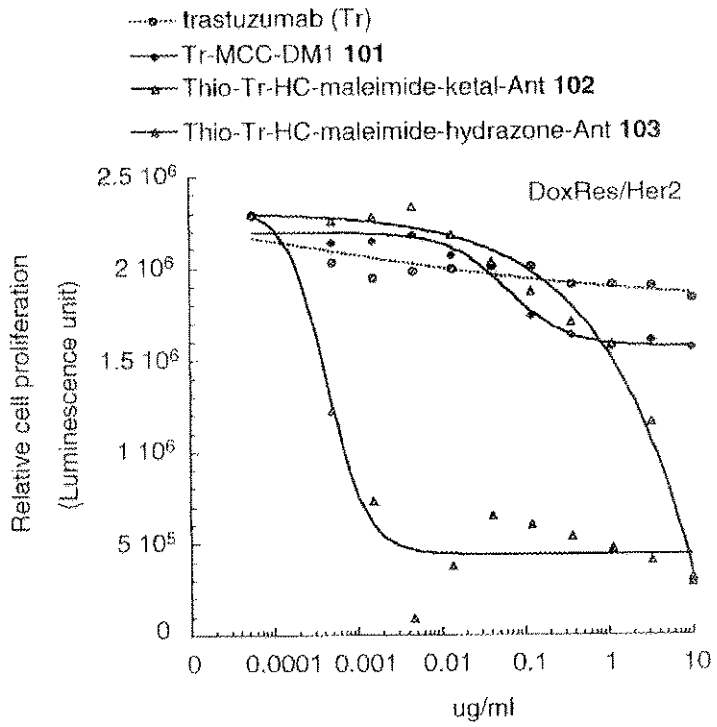

trastuzumab (Tr)
Tr-MCC-DM1 101
Thio-Tr-HC-maleimide-ketal-Ant 102
Thio-Tr-HC-maleimide-hydrazone-Ant 103
DoxRes/Her2
Relative cell proliferation
(Luminescence unit)
2.5 10^6
2 10^6
1.5 10^6
1 10^6
5 10^5
0
0 0.0001 0.001 0.01 0.1 1 10
ug/ml

FIG._15

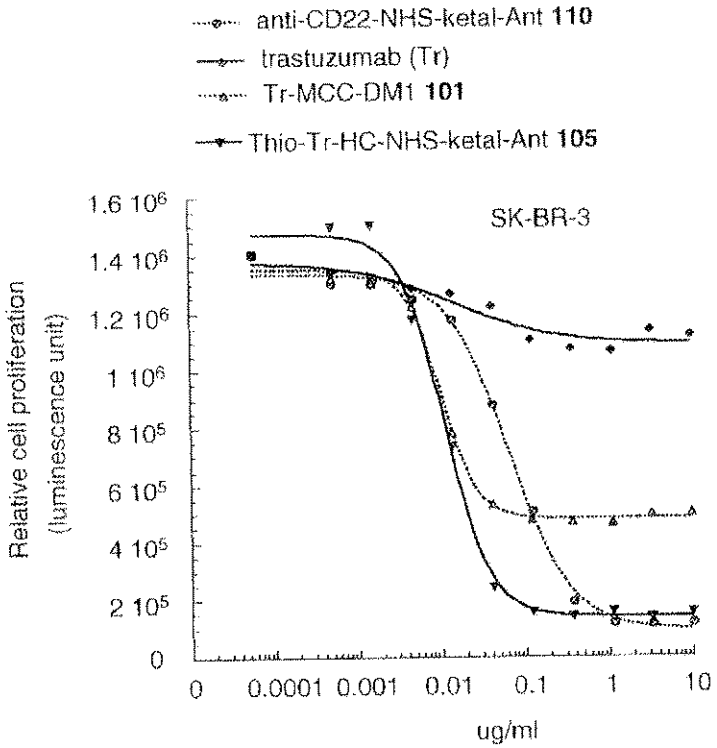

anti-CD22-NHS-ketal-Ant 110
trastuzumab (Tr)
Tr-MCC-DM1 101
Thio-Tr-HC-NHS-ketal-Ant 105
SK-BR-3
Relative cell proliferation
(luminescence unit)
1.6 10^6
1.4 10^6
1.2 10^6
1 10^6
8 10^5
6 10^5
4 10^5
2 10^5
0
0 0.0001 0.001 0.01 0.1 1 10
ug/ml

FIG._16

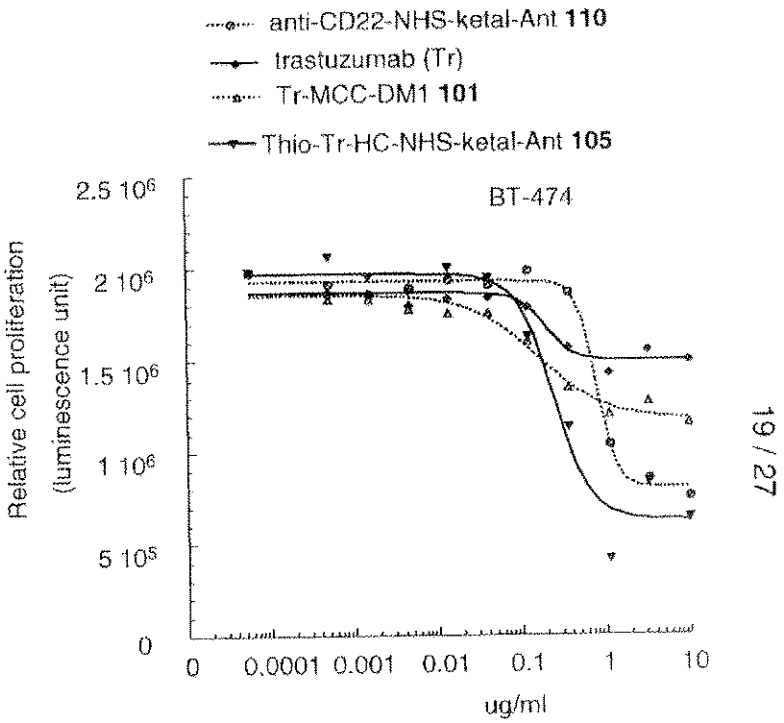

anti-CD22-NHS-ketal-Ant 110
trastuzumab (Tr)
Tr-MCC-DM1 101
Thio-Tr-HC-NHS-ketal-Ant 105
BT-474
Relative cell proliferation
(luminescence unit)
2.5 10^6
2 10^6
1.5 10^6
1 10^6
5 10^5
0
0 0.0001 0.001 0.01 0.1 1 10
ug/ml

FIG._17

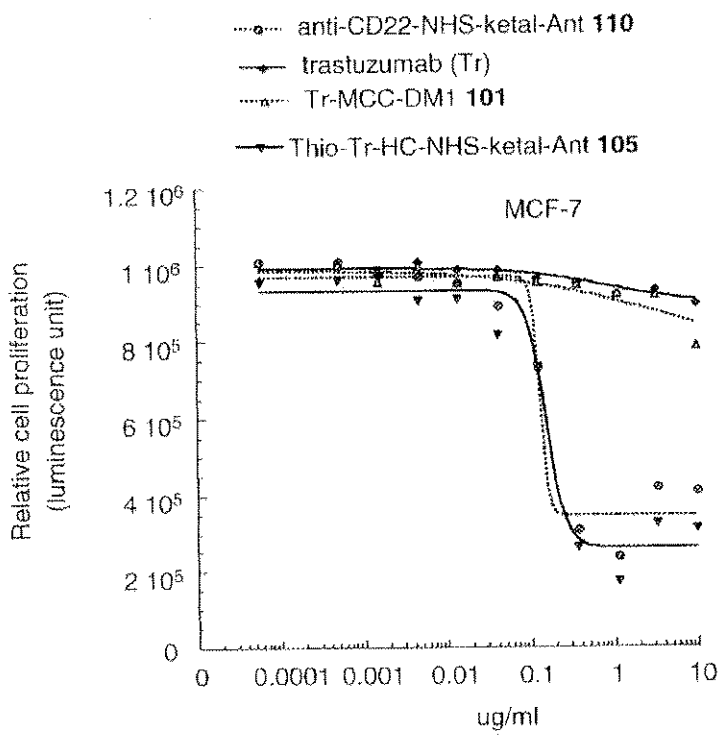

anti-CD22-NHS-ketal-Ant 110
trastuzumab (Tr)
Tr-MCC-DM1 101
Thio-Tr-HC-NHS-ketal-Ant 105
MCF-7
Relative cell proliferation (luminescence unit)
1.2 10^6
1 10^6
8 10^5
6 10^5
4 10^5
2 10^5
0
0 0.0001 0.001 0.01 0.1 1 10
ug/ml

FIG._18

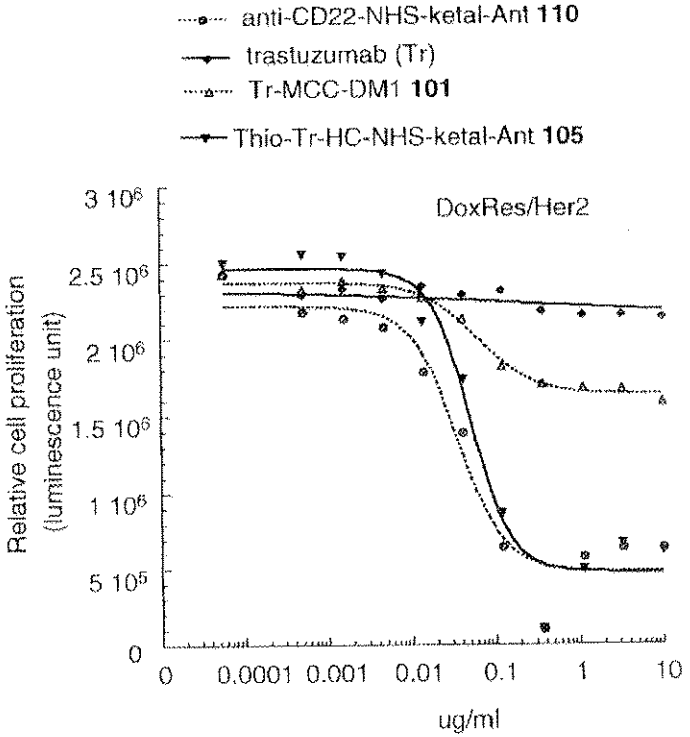

anti-CD22-NHS-ketal-Ant 110
trastuzumab (Tr)
Tr-MCC-DM1 101
Thio-Tr-HC-NHS-ketal-Ant 105
DoxRes/Her2
Relative cell proliferation (luminescence unit)
3 10^6
2.5 10^6
2 10^6
1.5 10^6
1 10^6
5 10^5
0
0 0.0001 0.001 0.01 0.1 1 10
ug/ml

FIG._19

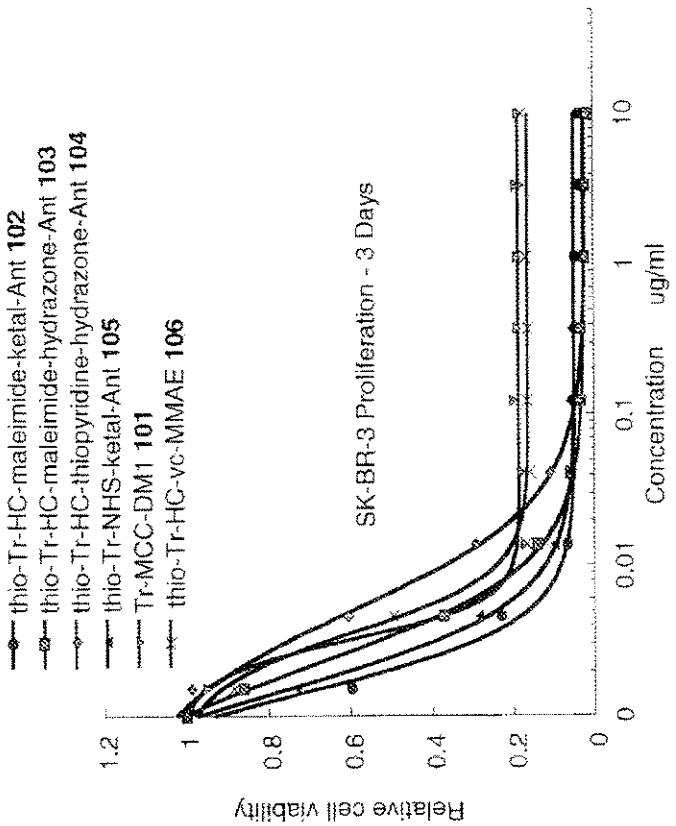

thio-Tr-HC-maleimide-ketal-Ant 102
thio-Tr-HC-maleimide-hydrazone-Ant 103
thio-Tr-HC-thiopyridine-hydrazone-Ant 104
thio-Tr-NHS-ketal-Ant 105
Tr-MCC-DM1 101
thio-Tr-HC-vc-MMAE 106
SK-BR-3 Proliferation - 3 Days
Relative cell viability
Concentration ug/ml
0
0.2
0.4
0.6
0.8
1
1.2
0.01
0.1
1
10

FIG._20

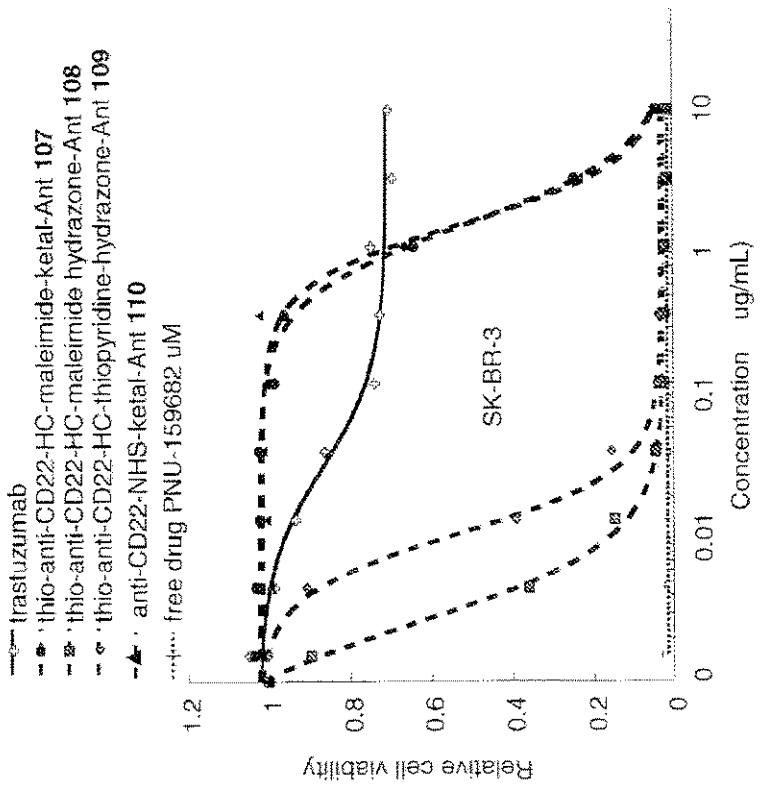

trastuzumab
thio-anti-CD22-HC-maleimide-ketal-Ant 107
thio-anti-CD22-HC-maleimide hydrazone-Ant 108
thio-anti-CD22-HC-thiopyridine-hydrazone-Ant 109
anti-CD22-NHS-ketal-Ant 110
free drug PNU-159682 uM
SK-BR-3
Relative cell viability
Concentration ug/mL)
0
0.2
0.4
0.6
0.8
1
1.2
0.01
0.1
1
10

FIG._21

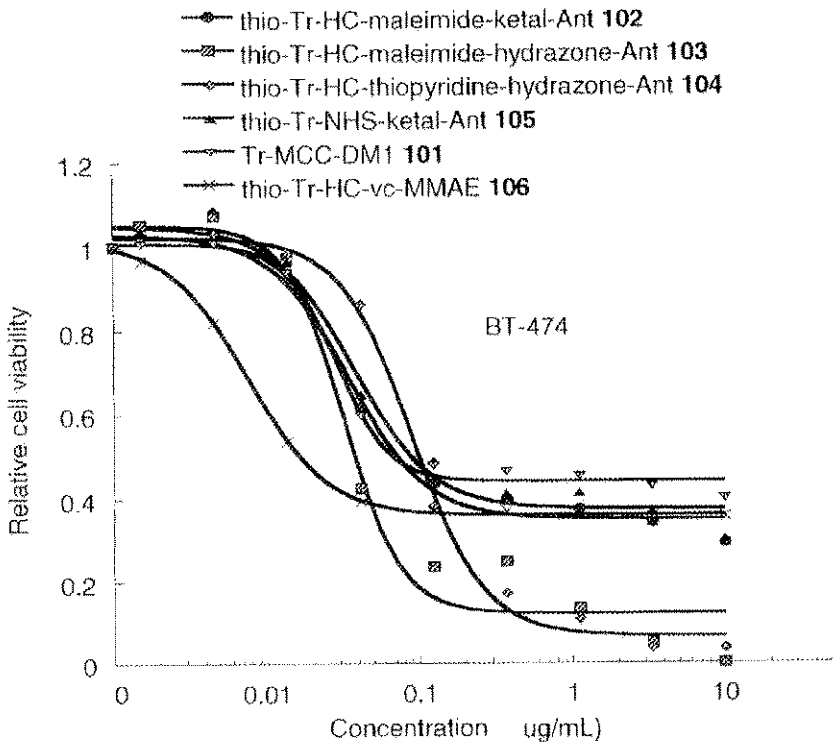

thio-Tr-HC-maleimide-ketal-Ant 102
thio-Tr-HC-maleimide-hydrazone-Ant 103
thio-Tr-HC-thiopyridine-hydrazone-Ant 104
thio-Tr-NHS-ketal-Ant 105
Tr-MCC-DM1 101
thio-Tr-HC-vc-MMAE 106
BT-474
Relative cell viability
Concentration ug/mL)
0
0.01
0.1
1
10
0.2
0.4
0.6
0.8
1
1.2

FIG._22

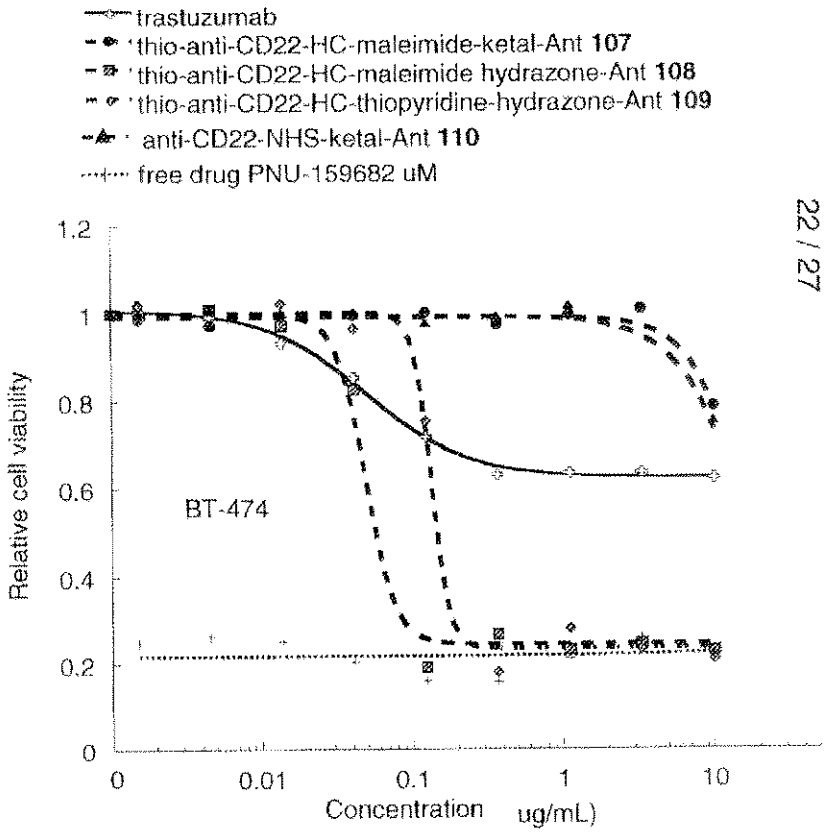

trastuzumab
thio-anti-CD22-HC-maleimide-ketal-Ant 107
thio-anti-CD22-HC-maleimide hydrazone-Ant 108
thio-anti-CD22-HC-thiopyridine-hydrazone-Ant 109
anti-CD22-NHS-ketal-Ant 110
free drug PNU-159682 uM
BT-474
Relative cell viability
Concentration ug/mL)
0
0.01
0.1
1
10
0.2
0.4
0.6
0.8
1
1.2

FIG._23

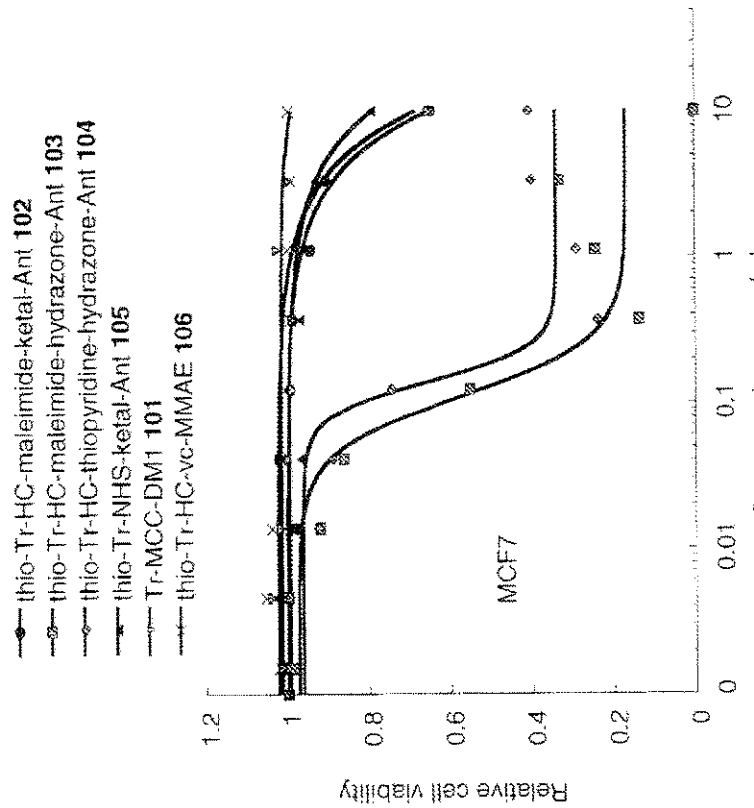

thio-Tr-HC-maleimide-ketal-Ant 102
thio-Tr-HC-maleimide-hydrazone-Ant 103
thio-Tr-HC-thiopyridine-hydrazone-Ant 104
thio-Tr-NHS-ketal-Ant 105
Tr-MCC-DM1 101
thio-Tr-HC-vc-MMAE 106
MCF7
Relative cell viability
Concentration ug/mL
0
0.01
0.1
1
10
0.2
0.4
0.6
0.8
1
1.2

FIG. 24

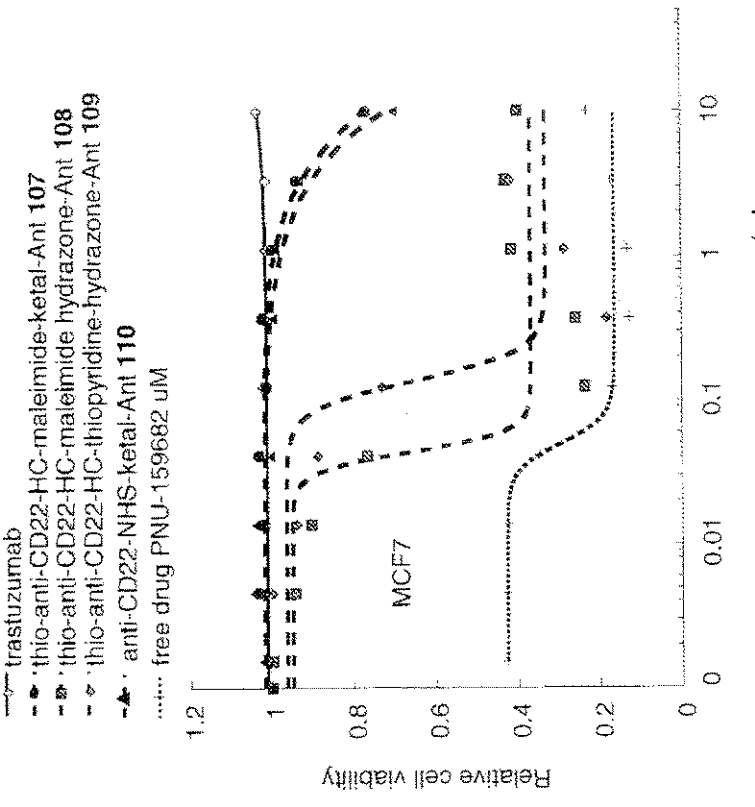

trastuzumab
thio-anti-CD22-HC-maleimide-ketal-Ant 107
thio-anti-CD22-HC-maleimide hydrazone-Ant 108
thio-anti-CD22-HC-thiopyridine-hydrazone-Ant 109
anti-CD22-NHS-ketal-Ant 110
free drug PNU-159682 uM
MCF7
Relative cell viability
Concentration ug/mL
0
0.01
0.1
1
10
0.2
0.4
0.6
0.8
1
1.2

FIG. 25

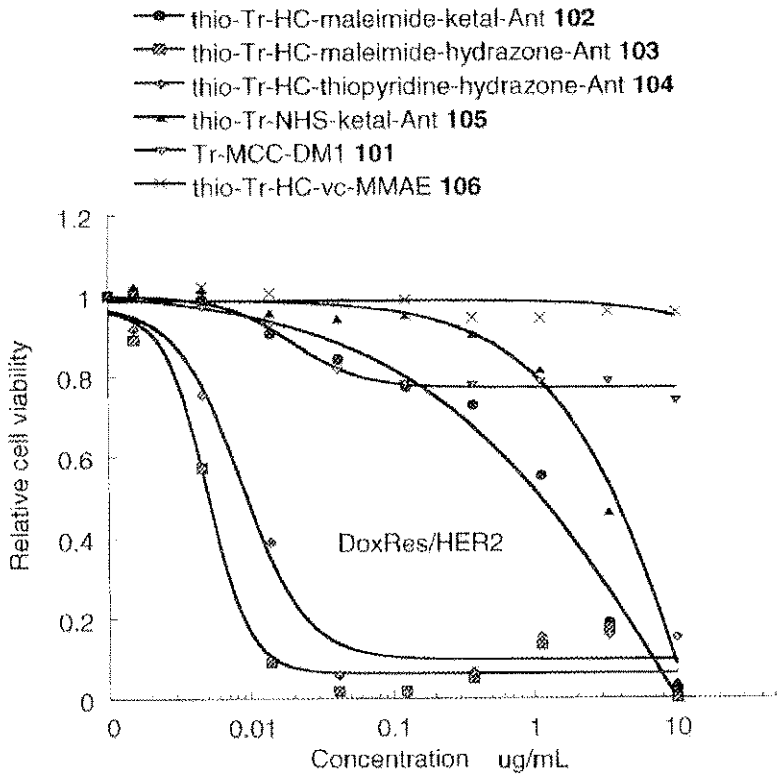

thio-Tr-HC-maleimide-ketal-Ant 102
thio-Tr-HC-maleimide-hydrazone-Ant 103
thio-Tr-HC-thiopyridine-hydrazone-Ant 104
thio-Tr-NHS-ketal-Ant 105
Tr-MCC-DM1 101
thio-Tr-HC-vc-MMAE 106
Relative cell viability
1.2
1
0.8
0.6
0.4
0.2
0
DoxRes/HER2
0
0.01
0.1
1
10
Concentration ug/mL

FIG._26

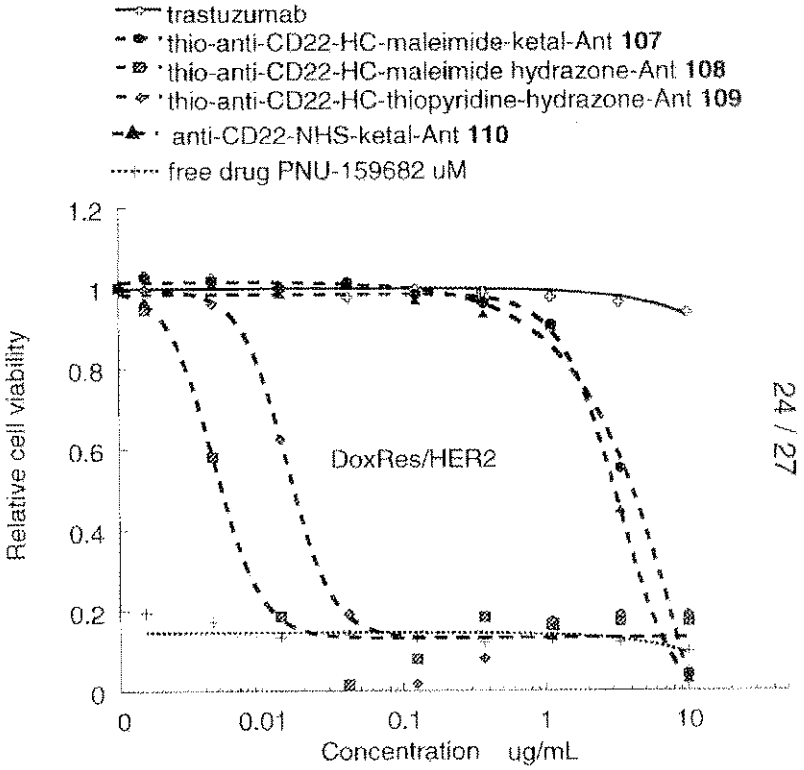

trastuzumab
thio-anti-CD22-HC-maleimide-ketal-Ant 107
thio-anti-CD22-HC-maleimide hydrazone-Ant 108
thio-anti-CD22-HC-thiopyridine-hydrazone-Ant 109
anti-CD22-NHS-ketal-Ant 110
free drug PNU-159682 uM
Relative cell viability
1.2
1
0.8
0.6
0.4
0.2
0
DoxRes/HER2
0
0.01
0.1
1
10
Concentration ug/mL

FIG._27

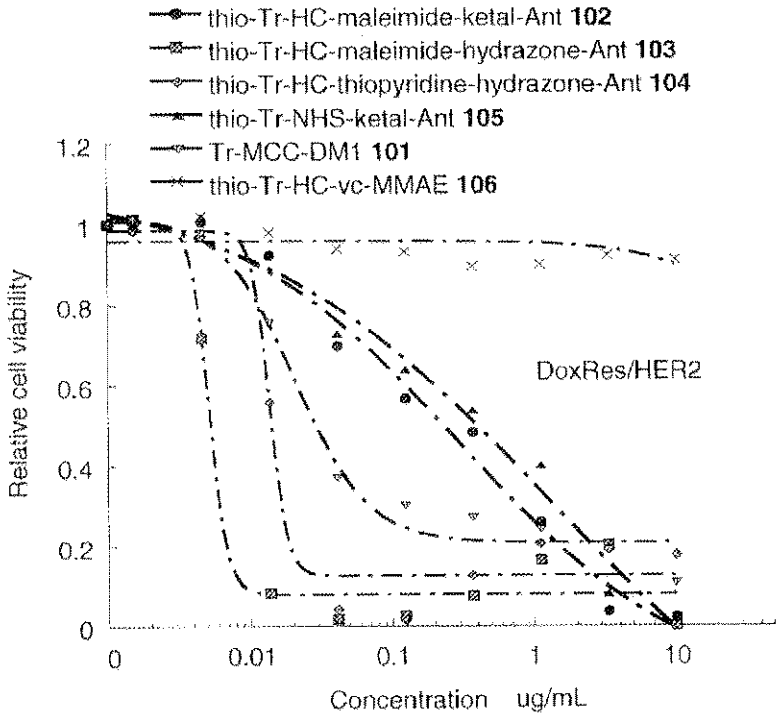

thio-Tr-HC-maleimide-ketal-Ant 102
thio-Tr-HC-maleimide-hydrazone-Ant 103
thio-Tr-HC-thiopyridine-hydrazone-Ant 104
thio-Tr-NHS-ketal-Ant 105
Tr-MCC-DM1 101
thio-Tr-HC-vc-MMAE 106
Relative cell viability
1.2
1
0.8
0.6
0.4
0.2
0
DoxRes/HER2
0
0.01
0.1
1
10
Concentration ug/mL

FIG._28

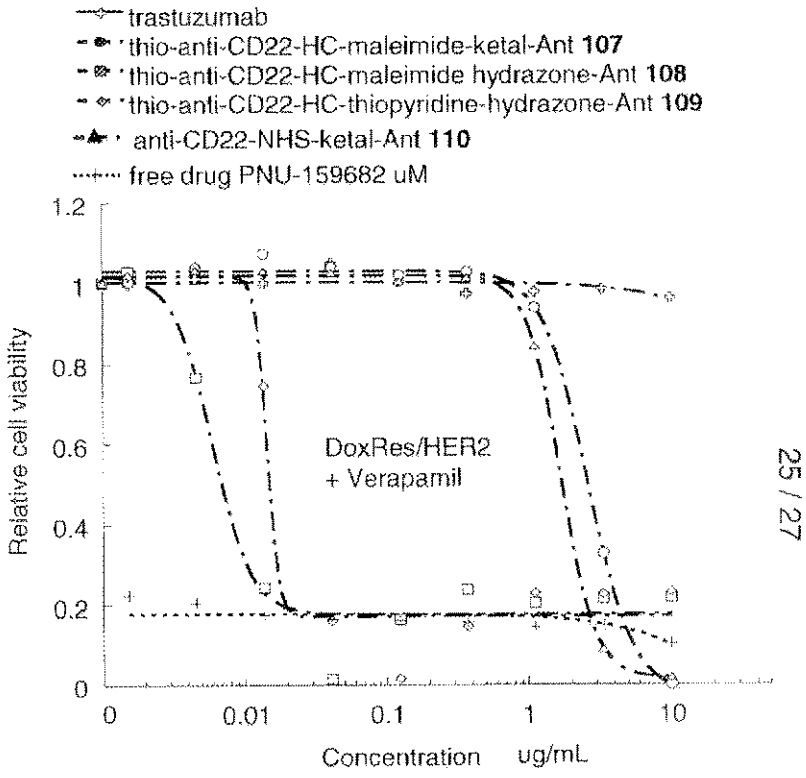

trastuzumab
thio-anti-CD22-HC-maleimide-ketal-Ant 107
thio-anti-CD22-HC-maleimide hydrazone-Ant 108
thio-anti-CD22-HC-thiopyridine-hydrazone-Ant 109
anti-CD22-NHS-ketal-Ant 110
free drug PNU-159682 uM
Relative cell viability
1.2
1
0.8
0.6
0.4
0.2
0
DoxRes/HER2
+ Verapamil
0
0.01
0.1
1
10
Concentration ug/mL

FIG._29

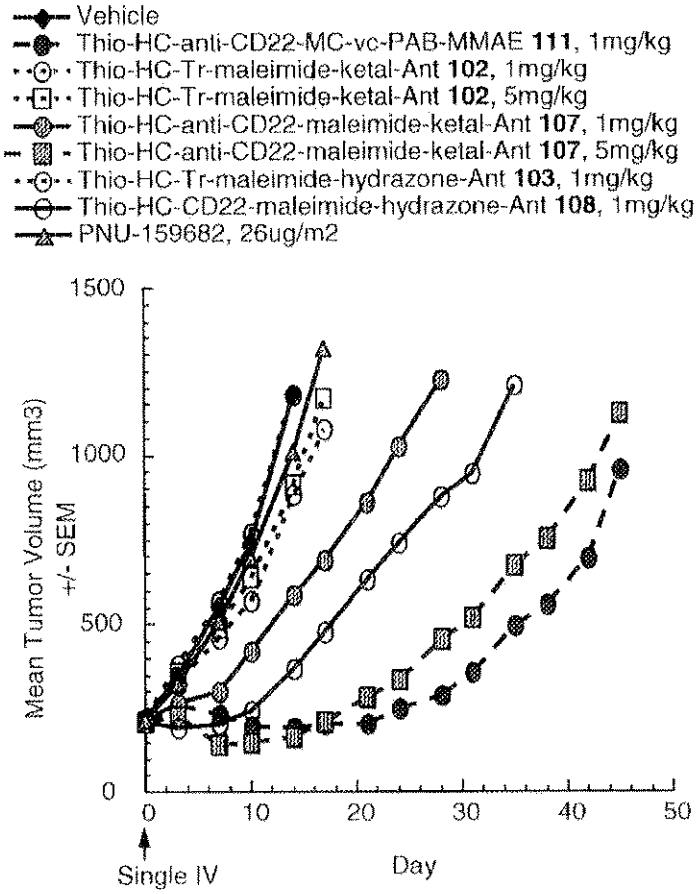

Vehicle
Thio-HC-anti-CD22-MC-vc-PAB-MMAE 111, 1mg/kg
Thio-HC-Tr-maleimide-ketal-Ant 102, 1mg/kg
Thio-HC-Tr-maleimide-ketal-Ant 102, 5mg/kg
Thio-HC-anti-CD22-maleimide-ketal-Ant 107, 1mg/kg
Thio-HC-anti-CD22-maleimide-ketal-Ant 107, 5mg/kg
Thio-HC-Tr-maleimide-hydrazone-Ant 103, 1mg/kg
Thio-HC-CD22-maleimide-hydrazone-Ant 108, 1mg/kg
PNU-159682, 26ug/m2
Mean Tumor Volume (mm3)
+/- SEM
1500
1000
500
0
0
10
20
30
40
50
Single IV
Day

FIG._30

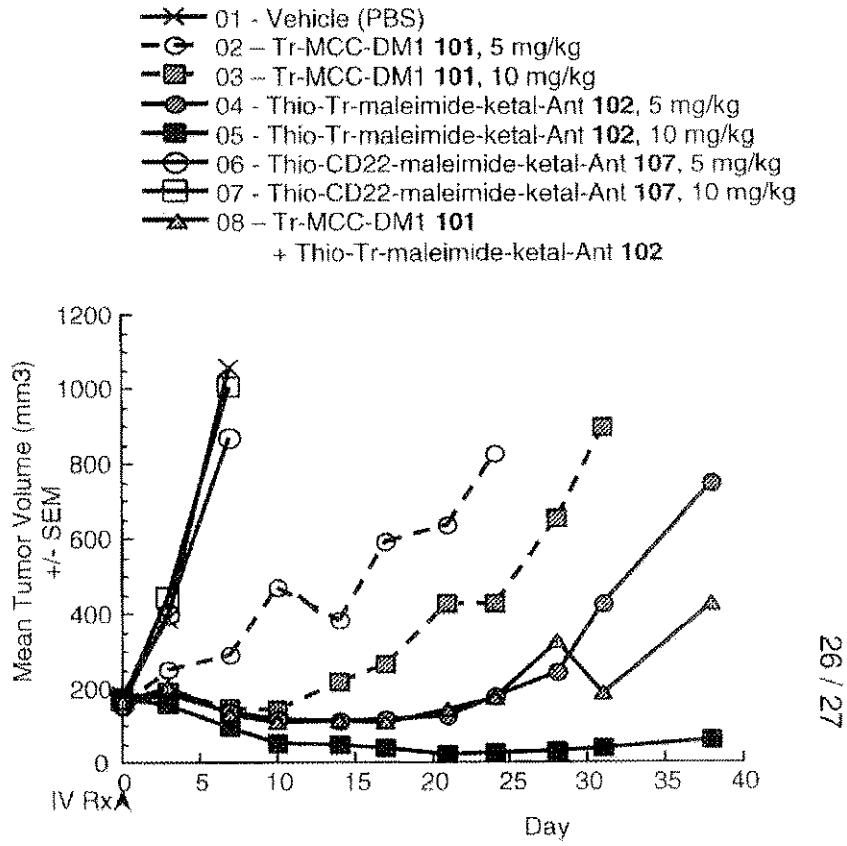

01 - Vehicle (PBS)
02 – Tr-MCC-DM1 101, 5 mg/kg
03 – Tr-MCC-DM1 101, 10 mg/kg
04 - Thio-Tr-maleimide-ketal-Ant 102, 5 mg/kg
05 - Thio-Tr-maleimide-ketal-Ant 102, 10 mg/kg
06 - Thio-CD22-maleimide-ketal-Ant 107, 5 mg/kg
07 - Thio-CD22-maleimide-ketal-Ant 107, 10 mg/kg
08 – Tr-MCC-DM1 101
+ Thio-Tr-maleimide-ketal-Ant 102
Mean Tumor Volume (mm3)
+/- SEM
1200
1000
800
600
400
200
0
0
5
10
15
20
25
30
35
40
IV Rx
Day

FIG._31

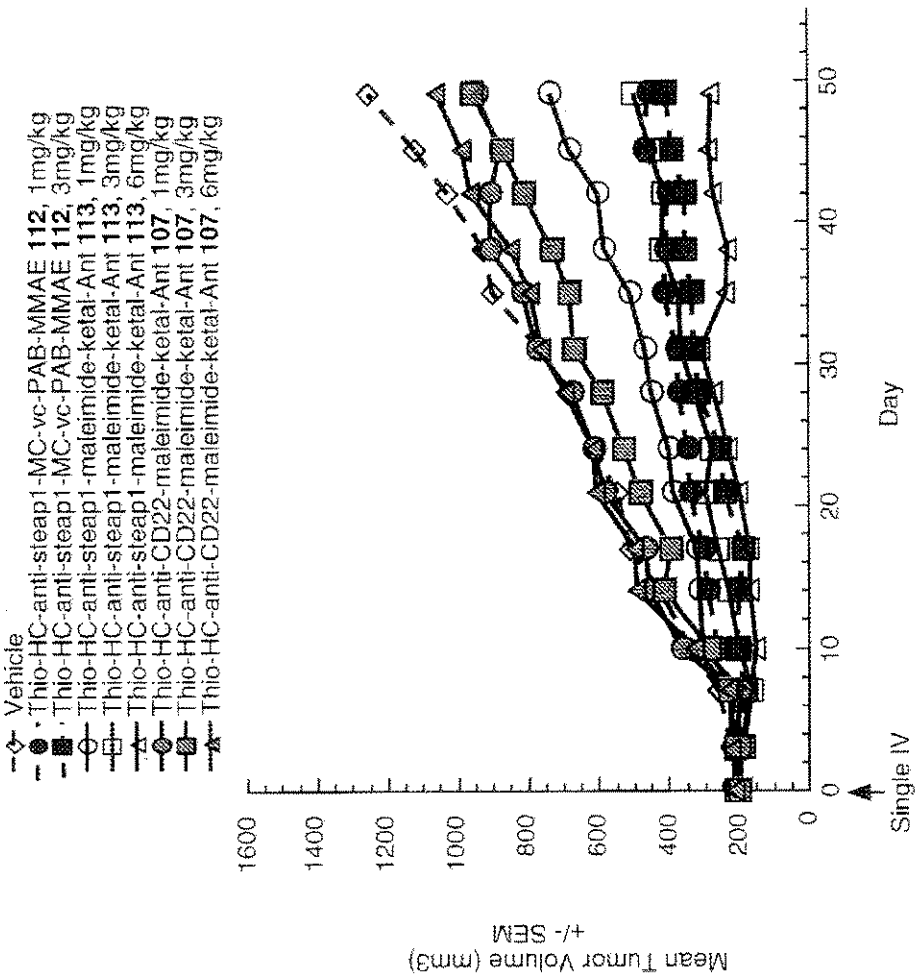

Vehicle
Thio-HC-anti-steap1-MC-vc-PAB-MMAE 112, 1mg/kg
Thio-HC-anti-steap1-MC-vc-PAB-MMAE 112, 3mg/kg
Thio-HC-anti-steap1-maleimide-ketal-Ant 113, 1mg/kg
Thio-HC-anti-steap1-maleimide-ketal-Ant 113, 3mg/kg
Thio-HC-anti-steap1-maleimide-ketal-Ant 113, 6mg/kg
Thio-HC-anti-CD22-maleimide-ketal-Ant 107, 1mg/kg
Thio-HC-anti-CD22-maleimide-ketal-Ant 107, 3mg/kg
Thio-HC-anti-CD22-maleimide-ketal-Ant 107, 6mg/kg
Mean Tumor Volume (mm3)
+/- SEM
1600
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
Day
Single IV

FIG. 32