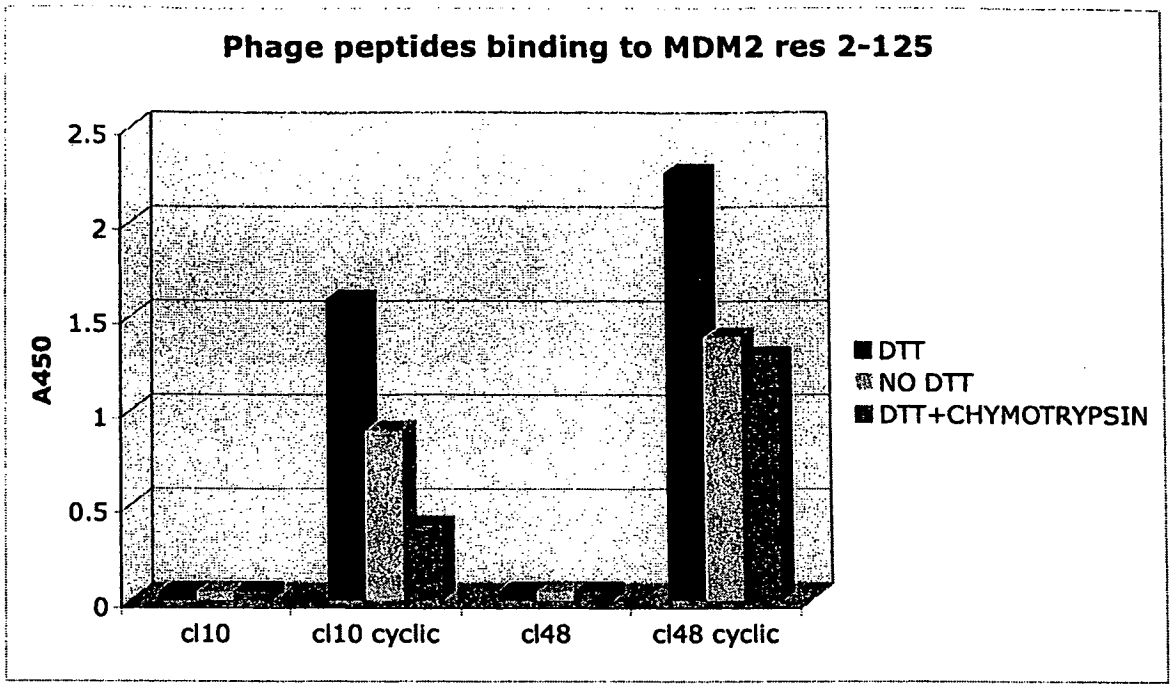

Phage peptides binding to MDM2 res 2-125
A450
2.5
2
1.5
1
0.5
0
cl10
cl10 cyclic
cl48
cl48 cyclic
DTT
NO DTT
DTT+CHYMOTRYPSIN

Figure 1

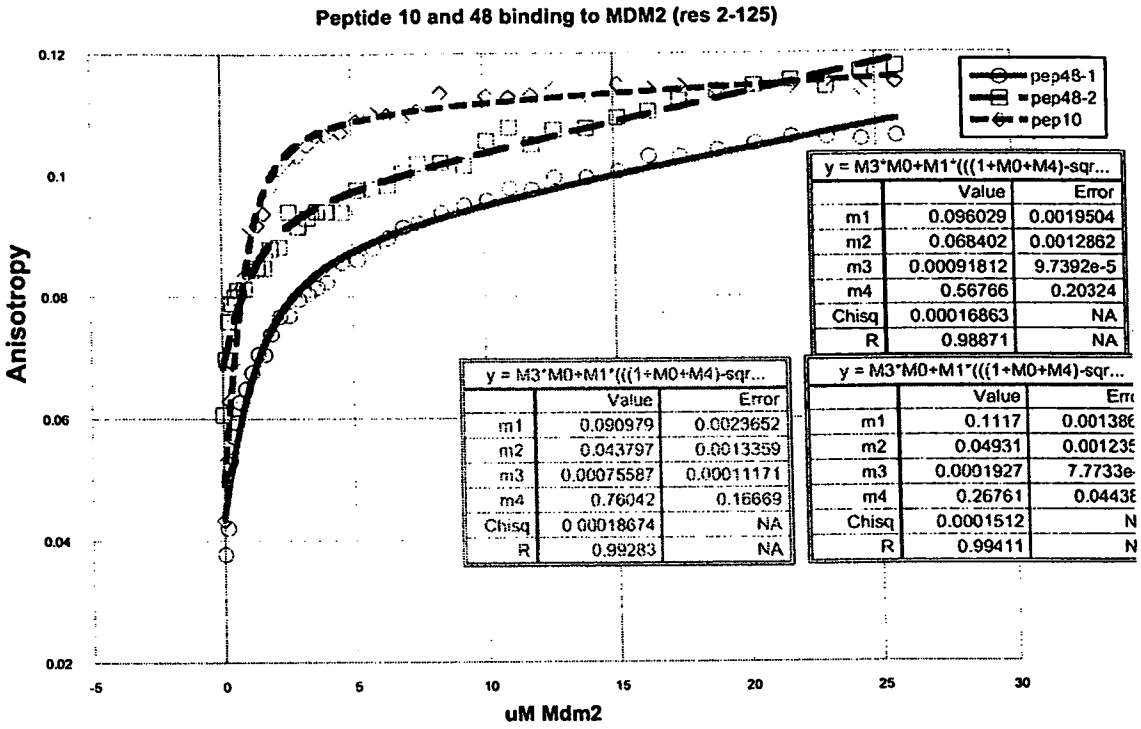

Peptide 10 and 48 binding to MDM2 (res 2-125)
Anisotropy
uM Mdm2
pep48-1
pep48-2
pep10
y = M3*M0+M1*(((1+M0+M4)-sqr...
Value Error
m1 0.096029 0.0019504
m2 0.068402 0.0012862
m3 0.00091812 9.7392e-5
m4 0.56766 0.20324
Chisq 0.00016863 NA
R 0.98871 NA
y = M3*M0+M1*(((1+M0+M4)-sqr...
Value Error
m1 0.090979 0.0023652
m2 0.043797 0.0013359
m3 0.00075587 0.00011171
m4 0.76042 0.16669
Chisq 0.00018674 NA
R 0.99283 NA
y = M3*M0+M1*(((1+M0+M4)-sqr...
Value Err
m1 0.1117 0.00138
m2 0.04931 0.00123
m3 0.0001927 7.7733e-
m4 0.26761 0.0443
Chisq 0.0001512 N
R 0.99411 N

Figure 2

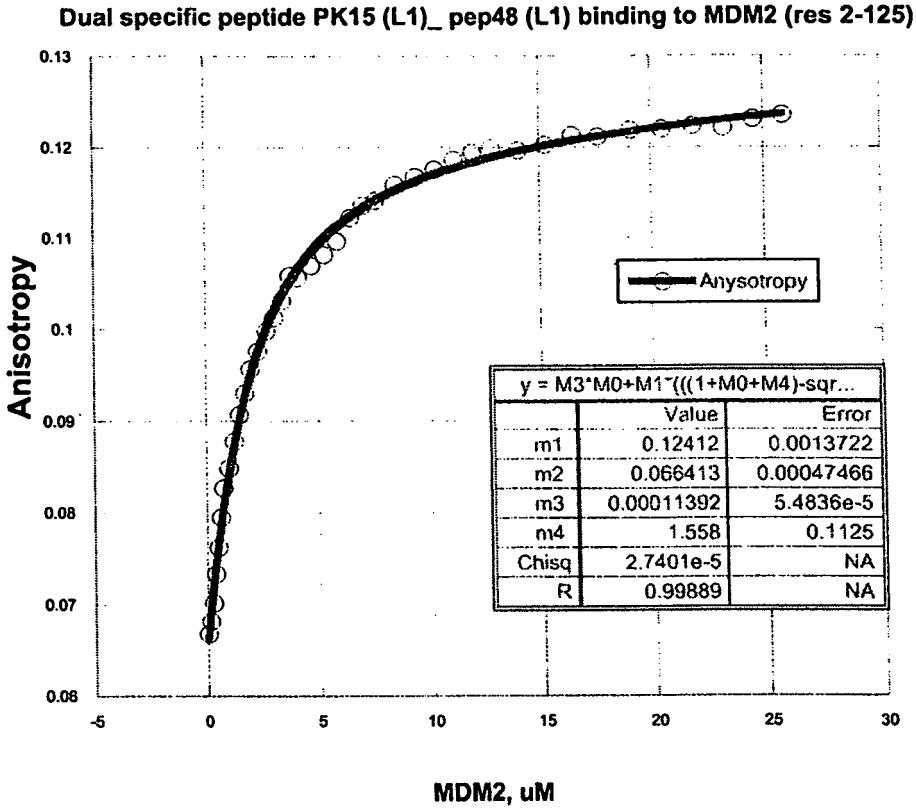

Dual specific peptide PK15 (L1)_ pep48 (L1) binding to MDM2 (res 2-125)
Anisotropy
MDM2, uM
Anysotropy
y = M3*M0+M1*(((1+M0+M4)-sqr...
Value
Error
m1 0.12412 0.0013722
m2 0.066413 0.00047466
m3 0.00011392 5.4836e-5
m4 1.558 0.1125
Chisq 2.7401e-5 NA
R 0.99889 NA

Figure 3

Fluorescence anisotropy titration curves of Fmoc-Lys-Mca, Lys-Mca, Mca-OH, Fmoc-Gly-OH, Fmoc-Phe-OH with BSA

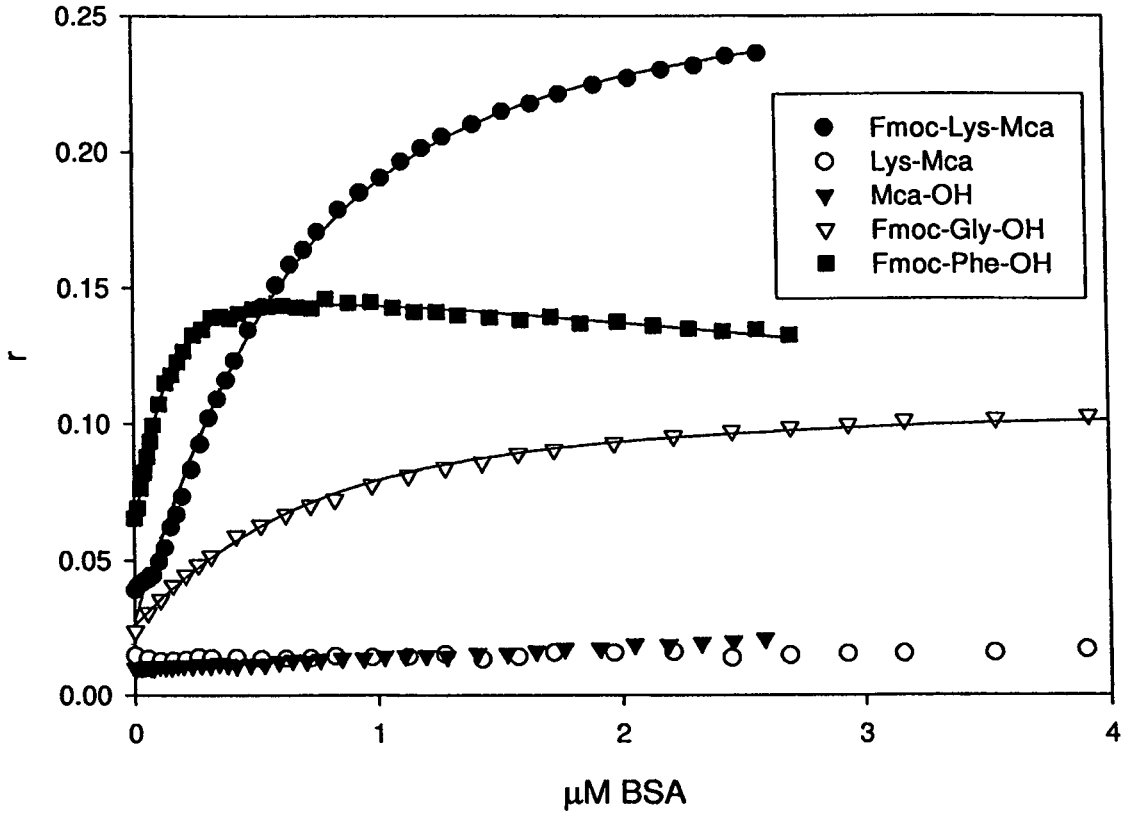


Figure 4

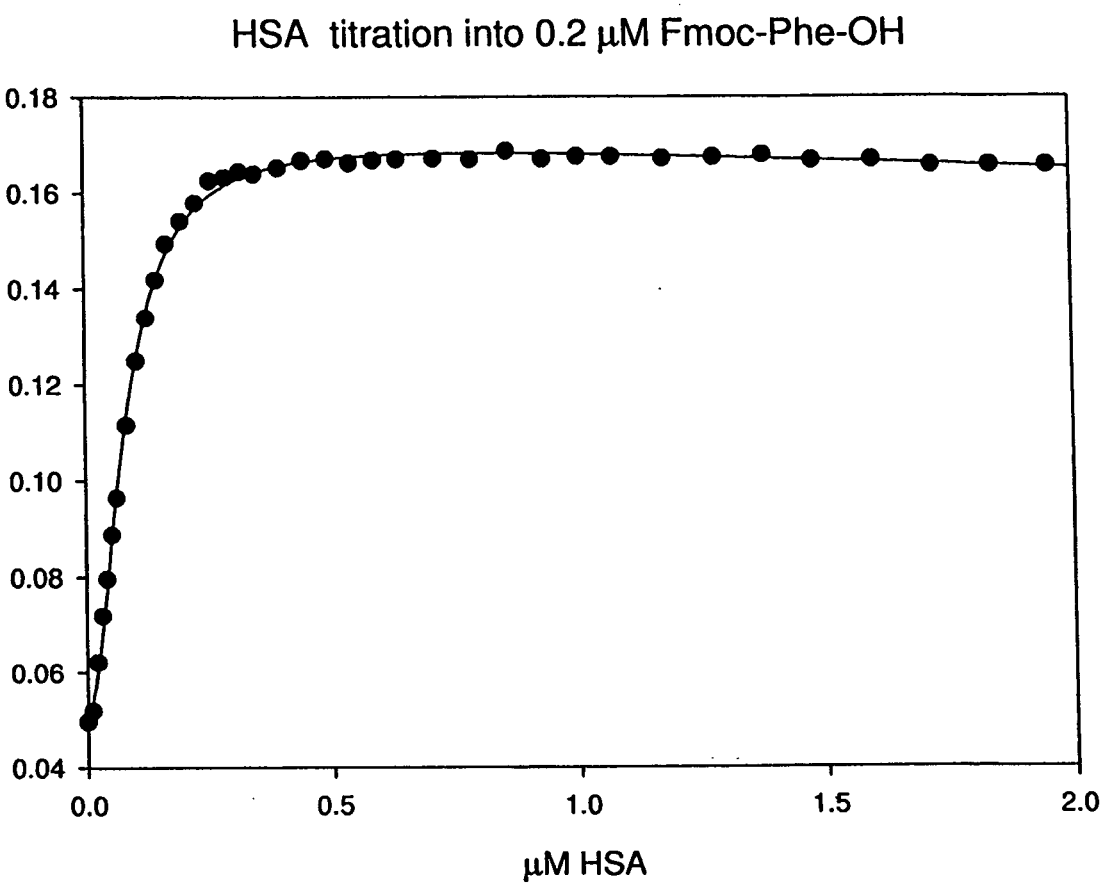

HSA titration into 0.2 µM Fmoc-Phe-OH
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.0
0.5
1.0
1.5
2.0
µM HSA

Figure 5

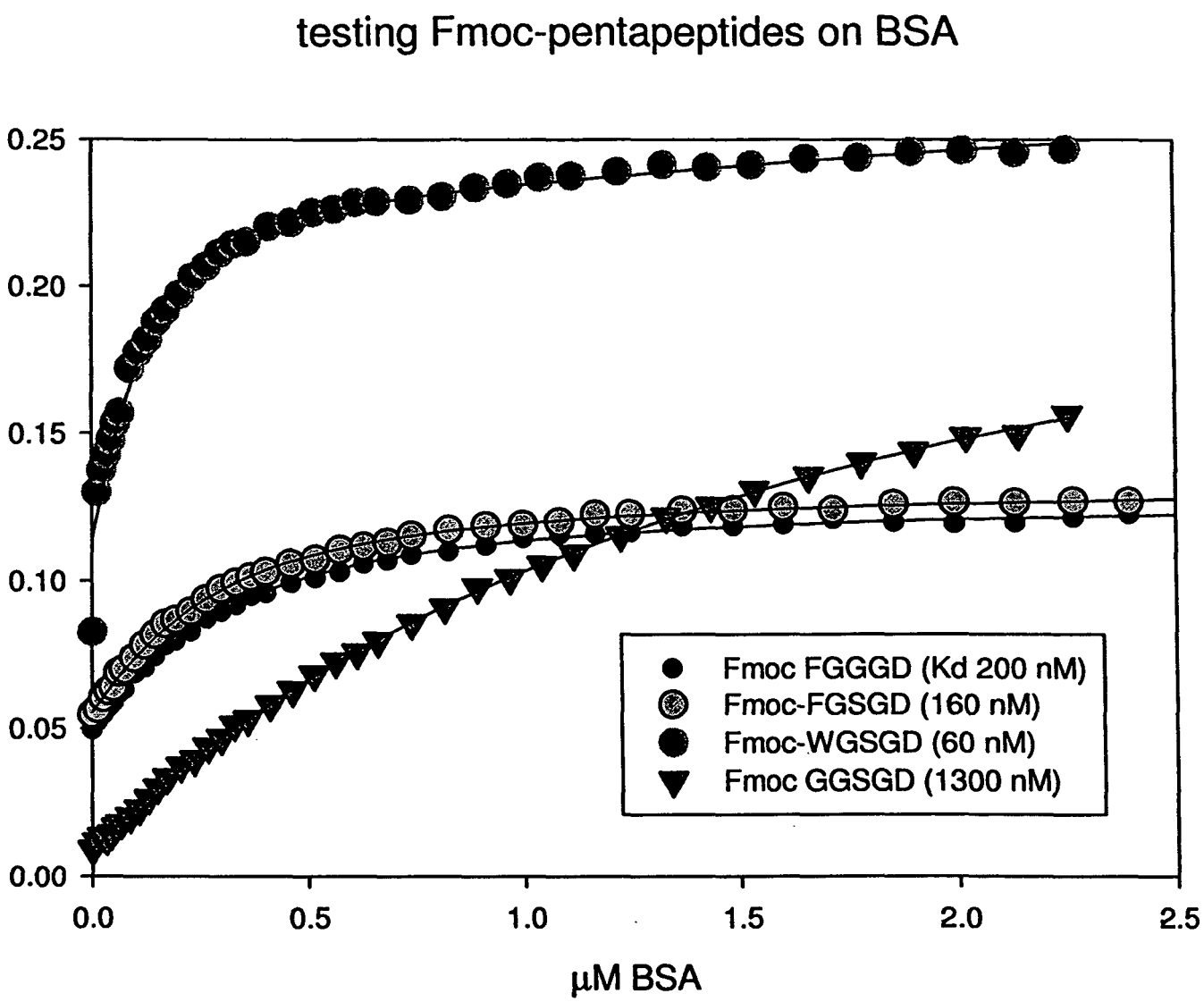
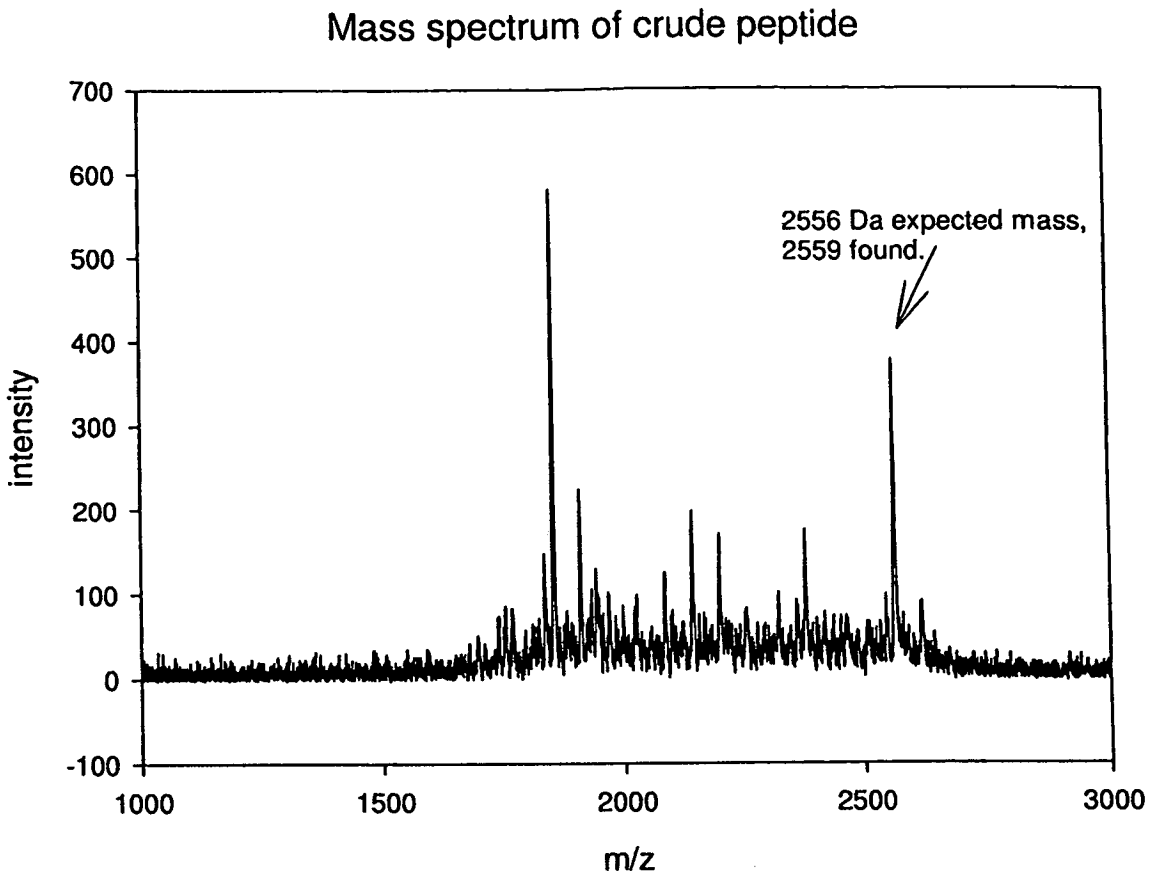

testing Fmoc-pentapeptides on BSA
0.25
0.20
0.15
0.10
0.05
0.00
0.0
0.5
1.0
1.5
2.0
2.5
µM BSA
Fmoc FGGGD (Kd 200 nM)
Fmoc-FGSGD (160 nM)
Fmoc-WGSGD (60 nM)
Fmoc GGSGD (1300 nM)

Figure 6

A


Mass spectrum of crude peptide
2556 Da expected mass,
2559 found.
intensity
700
600
500
400
300
200
100
0
-100
1000
1500
2000
2500
3000
m/z

B

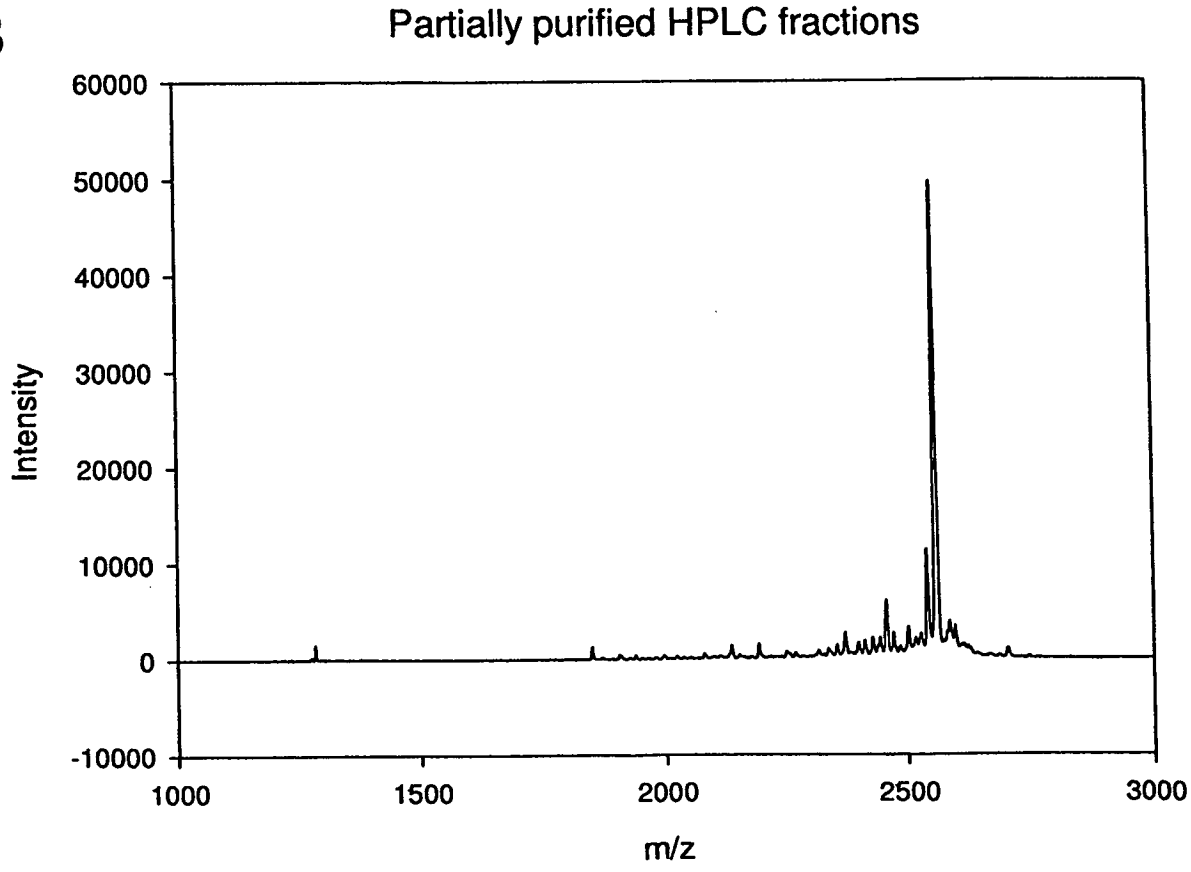
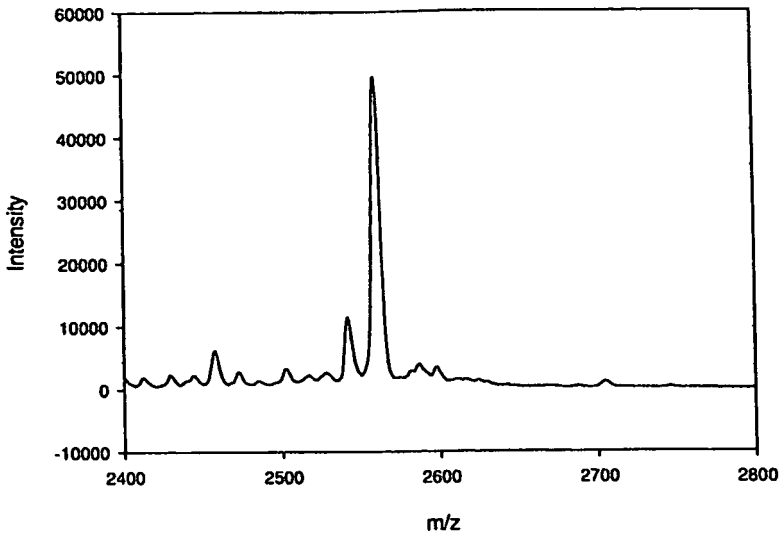

Partially purified HPLC fractions
Intensity
60000
50000
40000
30000
20000
10000
0
-10000
1000
1500
2000
2500
3000
m/z

Figure 7

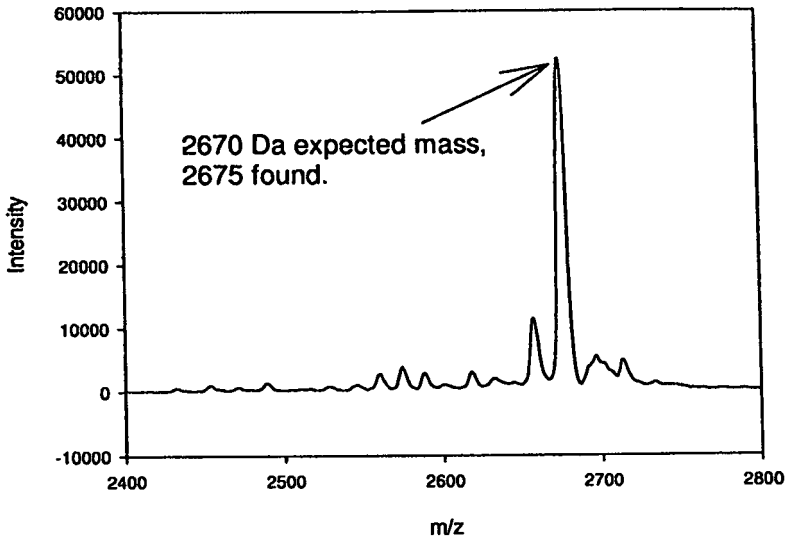
C

Starting material prior to reaction with TBMB



D

Post TBMB reaction (after 3 minutes)



E

Purified bicyclic Fmoc-W-GGGA-Pep48-PK15

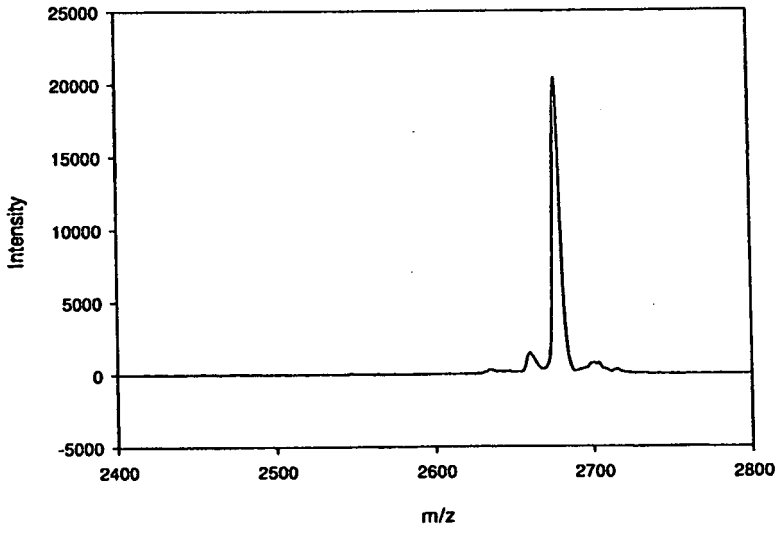


Figure 9

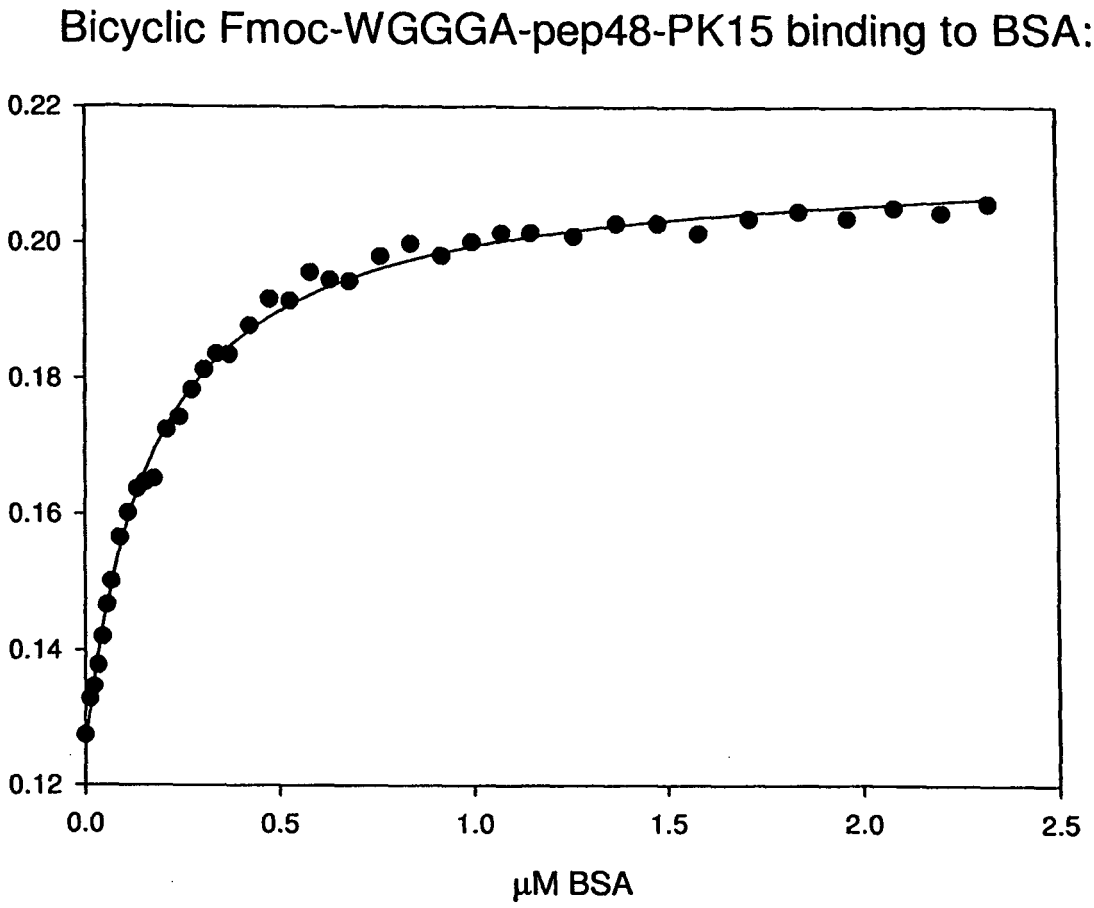

Bicyclic Fmoc-WGGGA-pep48-PK15 binding to BSA:
0.22
0.20
0.18
0.16
0.14
0.12
0.0
0.5
1.0
1.5
2.0
2.5
µM BSA

Figure 8

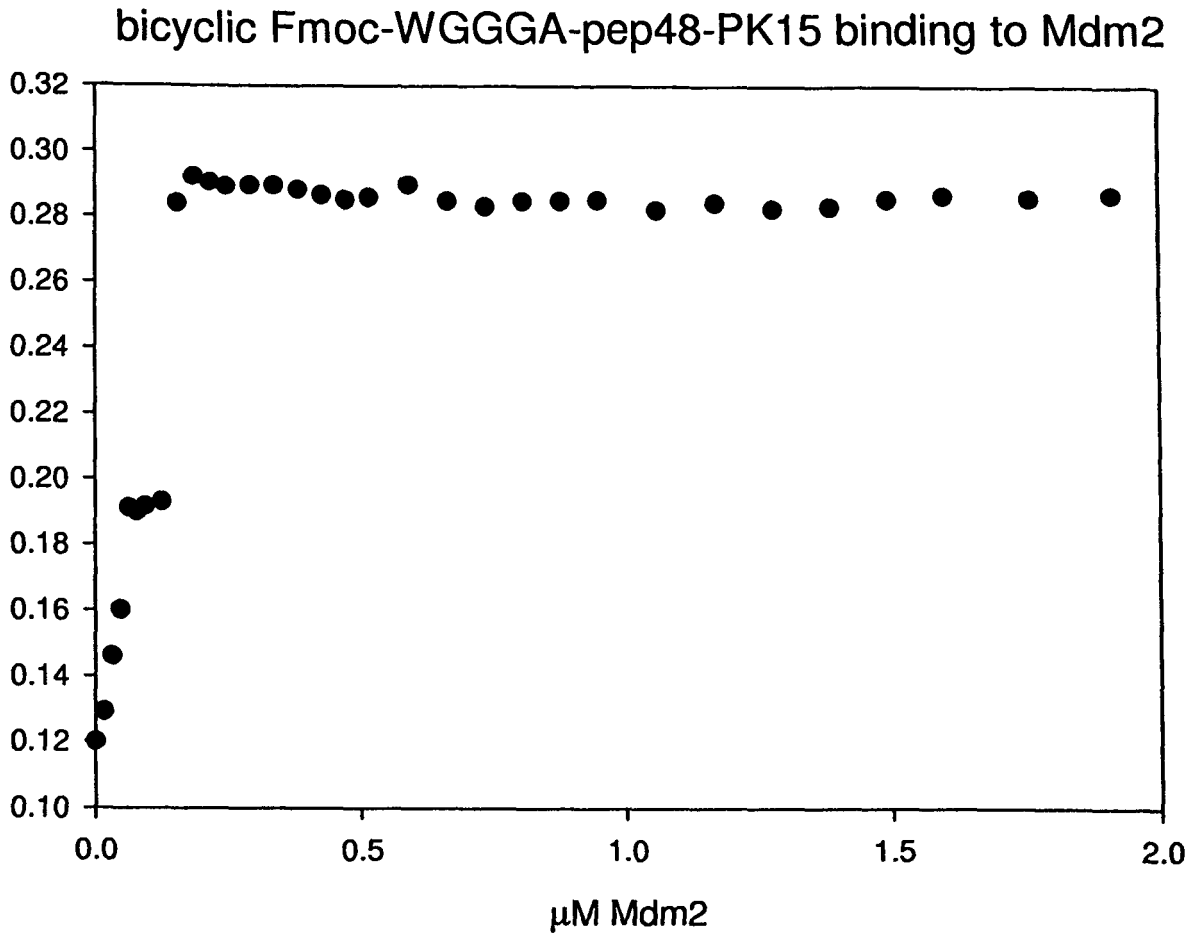

bicyclic Fmoc-WGGGA-pep48-PK15 binding to Mdm2
0.32
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.0
0.5
1.0
1.5
2.0
µM Mdm2

Figure 9