Experimental.

Synthesis of *tert*-butyloxycarbonyl-L-methionine-L-leucine-L-phenylalanine methyl ester (Boc-Met-Leu-Phe-OMe or Boc-MLF-OMe). Peptides were assembled via standard EDC [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride] mediated couplings [with HOBT (1-hydroxybenzotriazole) to suppress racemization]. *tert*-Butyloxycarbonyl (t-Boc) was used as an amino protecting group, and was removed with TFA prior to coupling. Boc-Leu-OH (2.0g) was dissolved in 70 mL of dichloromethane and 2 mL of DMF. The resulting solution was cooled to 0 °C on an ice bath. EDC (1.69 g, 1.1 eq) and HOBt (1.19 g, 1.1 eq) were added and the reaction mixture was kept at 0 °C for 15 min. Phenylalanine methyl ester hydrochloride (HCl-NH_3-Phe-OMe, 1.73 g) was dissolved in 40 mL dichloromethane and treated with triethylamine (1.12 mL, 1 eq) to liberate the free amine. This solution was added dropwise to the leucine solution. The mixture was allowed to stir overnight. The reaction was quenched with the addition of an equal volume of 1 N sodium bicarbonate. The organic layer was separated and washed with brine. The solution was dried with sodium sulfate and the solvent removed *in vacuo*. The dimer recrystallized from dichloromethane and hexane to give 2.81 g (89 %) as a white solid. Boc-Met-OH (0.90 g) was dissolved in 25 mL of dichloromethane. The resulting solution was cooled to 0 °C on an ice bath. EDC (0.69 g, 1.0 eq) and HOBt (0.49 g, 1.0 eq) were added and the reaction mixture was kept at 0 °C for 15 min. Boc-Leu-Phe-OMe (1.4 g) was dissolved in a solution of 25 mL of trifluoroacetic acid and 12.5 mL dichloromethane. After stirring for 45 min, the solvent was removed *in vacuo*. The yellow oil that remained was dissolved in 40 mL dichloromethane and the solvent was evaporated *in vacuo* to remove excess TFA. The process was repeated twice yielding the trifluoroacetate salt, which was used without further purification. The above trifluoroacetate salt was dissolved in 20 mL dichloromethane. One equivalent of triethylamine (0.5 mL) was added to liberate the free amine. The amine was added dropwise to a stirring solution of the Boc protected methionine. The mixture was allowed to stir overnight. The reaction mixture was transferred to separatory funnel and washed with an equal volume of 1M bicarbonate, 1 M citric acid, brine, and water. The organic layer was dried

with sodium sulfate and the solvent was removed *in vacuo*. The tripeptide was isolated as a white solid (1.7 g, 90 %) was used without further purification.

Reductive Amination of Met-Leu-Phe Ester. Boc-MLF-OMe (1.0 g) was dissolved in a solution of 10 mL of trifluoroacetic acid and 20 mL dichloromethane. After stirring for 45 min, the solvent was removed *in vacuo*. The yellow oil that remained was dissolved in 40 mL dichloromethane and the solvent was removed *in vacuo* to remove excess TFA. This process was repeated twice yielding the trifluoroacetate salt, which was used without further purification. The TFA salt was dissolved in 10 mL dichloromethane. One equivalent of triethylamine (0.89 mL) was added to liberate the free amine. The amine was added dropwise to a stirring solution of the aromatic aldehyde (1.2 eq) in dichloromethane. After 5 min, sodium triacetoxyborohydride (neat, 3 eq) was added. The flask was covered with foil and allowed to stir for 3-5 d, until TLC indicated the absence of starting material. The reaction was quenched by addition (with vigorous stirring) of saturated sodium bicarbonate solution. The organic layer was separated and washed with bicarbonate, then brine. The organic layer was dried with sodium sulfate and the solvent was removed *in vacuo*. The material was purified via flash chromatography using dichloromethane/ethyl acetate as eluant.

N-(6-Nitro-3,4-dimethoxy)benzyl L-methionine-L-leucine-L-phenylalanine methyl ester (Nv-MLF-OMe, 4). The above procedure was followed, giving 0.50 g (43 %) of the title compound as a yellow syrup, R_f 0.34, 90:10 dichloromethane:ethyl acetate. ^{1}H-NMR ($CDCl_3$): δ 7.65 (d, J = 8.8, 1H), 7.20 (m, 6H), 7.01 (d, J = 7.6, 1H), 4.69 (dd, J = 7.2, 6.0, 1H), 4.55 (m, 1H), 3.91 (s, 3H), 3.85 (s, 3H), 3.81 (d, J = 12.4, 1H), 3.62 (s, 3H), 3.54 (d, J = 12.4, 1H), 3.19 (dd, J = 2.8, 5.0, 1H), 3.06 (dd, J = 5.6, 10.5, 1H), 2.95 (dd, J = 7.0, 10.5, 1H), 2.50 (m, 2H), 1.99 (s, 3H), 1.97 (m, 2H), 1.48 (m, 2H), 1.73 (m, 1H), 0.85 (d, J = 6.4, 3 H), 0.83 (d, J = 6.4, 3 H). ^{13}C NMR ($CDCl_3$): δ 173.4, 171.8, 171.6, 153.42, 147.85, 140.90, 135.79, 129.22, 128.96, 128.31, 126.82, 114.23, 107.76, 61.56, 56.61, 56.10, 53.38, 52.05, 50.76, 50.40, 41.33, 37.71, 32.88, 60.46, 24.63, 22.69, 21.77, 15.17. HRMS: MH^+ $C_{30}H_{43}O_8N_4S$ calc 619.2802; found

619.2800. IR 3291.7 (br), 1745.2(s), 1650.0 (s), 1643.4 (s), 1518.7 (s), 1329.4 (w), 1273.9 (m), 1217.3(w) cm^{-1}.

N-(6-Nitro-3,4-methylenedioxy)benzyl L-methionine-L-leucine-L-phenylalanine methyl ester (Pip-MLF-OMe). The above procedure was followed, giving 0.35 g (61 %) of the title compound as a yellow syrup, R_f 0.21, 90:10 dichloromethane:ethyl acetate. ^{1}H-NMR ($CDCl_3$): δ 7.51 (s, 1H), 7.49 (d, J = 7.5 hz, 1H), 7.2 (m, 6H), 6.97 (s, 1H), 6.68 (d J = 8.1, 1H), 6.10 (s, 2H), 4.85 (dd J = 10.1 and 6.15, 1H), 4.46 (m, 1H), 3.76 (s, 2H), 3.71 (s, 3H), 3.21 (dd, J = 6.4 and 4.9), 3.15 (dd, J = 5.6 and 13.8, 1H), 3.06 (dd, J = 6.6 and 13.8, 1H), 2.53 (dd, J = 8.0 and 8.0), 2.07 (s, 3H), 2.03 (m, 2H), 1.82 (m, 1H), 1.61 (m, 2H), 0.93 (d, J = 6.8, 3H), 0.90 (d, J = 6.8, 3H). ^{13}C NMR ($CDCl_3$): δ 173.40, 171.51, 151.85, 147.14, 142.68, 135.66, 131.47, 129.09, 128.36, 126.88, 111.14, 105.64, 12.90, 61.26, 53.28, 52.29, 51.29, 50.41, 41.02, 37.95, 32.83, 30.65, 24.86, 21.91, 15.41. HRMS: MH^+ $C_{29}H_{38}O_8N_4S$ calc 603.2489; found 603.2471. IR 3278.5 (br), 2954.2 (br), 2913.55 (w), 1744.3 (s, br), 1638.4 (s), 1504.0 (s), 1482.3 (m), 1256 (s), 1035.0 (w), 700.5 (w) cm^{-1}.

Peptide N-formylation. Formic acid (2.2 eq, 96 % ACS reagent grade) was added to dichloromethane (approximately 30 mL per mmol) at 0 °C. EDC (1.1 eq) was added and the reaction mixture was allowed to stir for 10 min. The amine was dissolved in dichloromethane and added dropwise to the solution. The mixture was stirred overnight, allowing the ice to melt. The mixture was quenched with the addition of a solution of saturated sodium bicarbonate. The organic layer was washed with brine, separated, dried ($NaSO_4$), and the solvent removed *in vacuo*. The product was observed as a mixture of conformers (TLC indicated a single spot). The material was used without further purification.

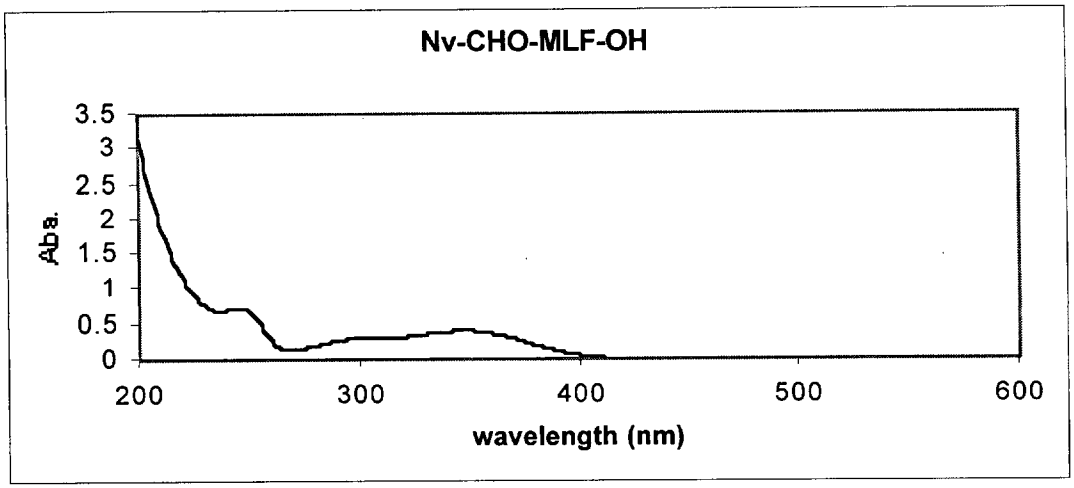
N-Formyl-N-(6-nitro-3,4-dimethoxy)benzyl L-methionine-L-leucine-L-phenylalanine methyl ester (Nv-CHO-MLF-OMe, 5). The above procedure was followed, giving 0.37 g (94 %) of the title compound as a yellow syrup, R_f 0.65, 90:10 dichloromethane:ethyl acetate. ^{1}H-NMR ($CDCl_3$): δ 8.55 and 8.25 (s, 1H), 7.69 and 7.571 (s, 1H), 7.27 (m, 3H), 7.08 (m, 2H), 7.02 and 6.98 (s, 1H), 6.77 and 6.53 (d, J = 8.1 and 7.8, 1H),

6.46 and 6.29 (d, J = 8.1 and 7.8, 1H), 5.10 (d, J = 16.1, 1H), 4.83 (m, 1H), 4.81 (m, 1H), 4.76 and 4.46 (m and dd, J = 7.4, 7.4, 1H), 4.26 (m, 1H), 3.97 and 3.94 (s, 3H), 3.92 (s, 3H), 3.71 and 3.69 (s, 3H), 3.07 (m, 2H), 2.48 (m, 2H), 2.18 (m, 3H), 2.05 and 2.04 (s, 3H), 1.41 (m, 3H), 0.89 and 0.85 (d, J = 6.1, 3H), 0.82 and 0.80 (d, J = 6.1, 3H). ^{13}C NMR ($CDCl_3$): δ (171.51 and 171.30), (170.87 and 170.75), (169.43 and 169.13), (164.71 and 163.82), 153.34, (148.63 and 147.87), 140.71, (135.67 and 135.35), 129.08, (128.50 and 128.38), (127.09 and 126.93), 125.51, (113.19 and 111.66), (108.74 and 107.78), 60.94, (57.00 and 56.70), (56.42 and 56.30), (53.24 and 53.14), (52.28 and 52.18), 49.20, 44.70, (41.26 and 40.68), 37.77, (30.62 and 30.30), 27.60, (24.78 and 24.68), (22.86 and 22.59), 22.03 and 21.81), 15.35. HRMS: MH^+ $C_{31}H_{43}O_9N_4S$ calc. 647.2750; found 647.2753. IR 3298.5 (br), 1744.5 (s), 1649.99 (s), 1519.889 (s), 1331.0 (w), 1274.5 (s), 1218.2 (w), 1061.89 (w) cm^{-1}.

N-Formyl-N-(6-nitro-3,4-methylenedioxy)benzyl L-methionine-L-leucine-L-phenylalanine methyl ester (Pip-CHO-MLF-OMe, 6). The above procedure was followed, giving 0.12 g (90 %) of the title compound as a yellow syrup, R_f 0.41, 90:10 dichloromethane:ethyl acetate. ^{1}H-NMR ($CDCl_3$): δ (8.49 and 8.15, s 1H), (7.57 and 7.51, s 1H), 7.24 (m, 4H), 7.08 (m, 3H), (6.91 and 6.84, s, 1H), 6.63 (d J = 8.1), 6.08 (m, 2H), 4.93 (d, J = 16.9), 4.86 (d, J = 16.9), 4.80 (m, 1H), (4.66 and 4.34, m 1H), 4.28 (m, 1H), (3.70 and 3.68, s, 3H), 3.07 (m, 2H), 2.44 (m, 2H), 2.19 (m, 3H), 2.04 (s, 3H), 1.52 (m, 3H), (0.88 and 0.85 d, J = 6.6, 6.0), (0.85 and 0.83 d, J = 6.0, 6.3). ^{13}C NMR ($CDCl_3$): δ (171.41 and 171.27), 171.01 and 170.90), 168.93, (164.67 and 163.95), (152.14 and 152.04), (147.67 and 146.83), (142.23 and 141.98), (135.61 and 135.38), 128.79, (128.37 and 128.29), (126.95 and 126.84), (109.23 and 108.02), (106.17 and 105.50), (103.21 and 102.88), 59.72, 56.12, (53.24 and 53.14), (52.31 and 52.21), (52.14 and 51.84), 48.15, 44.31, (41.06 and 40.61), 37.68, (30.36 and 30.12), (30.12 and 29.88), 27.73, 24.66, (22.72 and 22.56), (22.07 and 21.88), 15.25. HRMS: MH^+ $C_{30}H_{39}O_9N_4S$ calc 631.2438; found 631.2442. IR: 3289.6 (br), 2916.7 (w), 2869.8 (w), 1714.9 (s), 1780.3 (s, br), 1522.4 (m), 1484.1 (w), 1436.4 (w), 1331.2 (w), 1259.0 (m), 1034.7 (w) cm^{-1}.

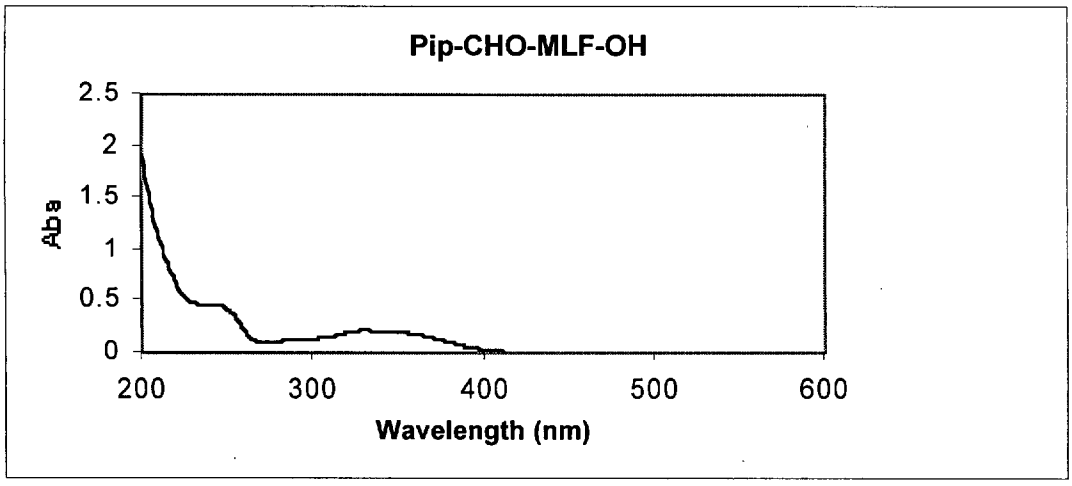
Ester Hydrolysis. The methyl ester (0.57 mmol) was dissolved in a mixture of tetrahydrofuran (9 mL), methanol (2.9 mL), and water (0.9 mL). The resulting solution was cooled to 0 °C with an ice bath and lithium hydroxide (119 mg, 5 eq) was added (as a solid). The reaction was kept at 0 °C and was monitored via TLC. After 3-4 h, TLC indicated that no methyl ester remained. The reaction was quenched with an equal volume of 1N $KHSO_4$ and 25 mL of dichloromethane was added. The organic layer was separated, washed with saturated brine, and dried with sodium sulfate. The solvent was removed *in vacuo.*

N-Formyl-N-(6-nitro-3,4-dimethoxy)benzyl L-methionine-L-leucine-L-phenylalanine (Nv-CHO-MLF-OH). The above procedure was followed, giving 0.26 g (73 %) of the title compound as a white solid, which was used without further purification. ^{1}H-NMR ($CDCl_3$): δ (8.21 and 8.17, s 1H), (7.68 and 7.56 s, 1H), 7.48 (d, J = 8.4), 7.20 (m, 6H), (7.02 and 6.91, s, 1H), 5.02 (d J = 16.2), 4.79 (m, 2H), 4.52 (d, J = 7.5), (4.35 and 4.15, m, 1H), (3.95 and 3.93, s, 3H), (3.90 and 3.89, s, 3H), 3.16 (m, 1H), (3.02 and 2.97 dd, J = 14.4, 6.6 and 14.4, 6.6), 2.40 (m, 2H), 2.17 (m, 1H), 2.02 (s, 3H), 1.92 (m, 1H), 1.42 (m, 3H), 0.84 and 0.79 (d J = 5.7, 6.0), (0.79 and 0.77, d, J = 6.0, 6.0). ^{13}C NMR ($CDCl_3$): δ 173.16, 171.93, 169.50, 164.71, 153.48, (148.80 and 147.99), 140.74, (135.92 and 135.61), (129.32 and 129.22), 128.42, 126.94, 125.07, (113.42 and 111.69), (108.79 and 107.83), 60.30, 56.78, (56.52 and 56.35), 53.64, (52.60 and 52.0), 49.76, 44.18, (41.06 and 40.51), 36.96, 30.63, (30.30 and 30.11), 24.82, (22.83 and 22.58), (22.04 and 21.77), 15.40. HRMS: MH^+ $C_{30}H_{41}O_9N_4S$ calc 633.2594; found 633.2599. IR 3303.2 (br) 2958.0 (br), 1729.9 (s), 1713.3(s), 1680.8(s), 1659.34(s), 1650.16(s), 1643.63(s),



1555.6 (m), 1536.7, 1519.0, 1503.9, 1453.8 (m), 1332.8 (m), 1219.1, (m) 1062.29 (w), 986.32(w), 872.95(w), 797.1(w), 735.68v, 701.32(w) cm^{-1}. The UV spectrum is shown.

N-Formyl-N-(6-nitro-3,4-methylenedioxy)benzyl L-methionine-L-leucine-L-phenylalanine (Pip-CHO-MLF-OH). The above procedure was followed, giving 0.06 g (71 %) of the title compound as a white solid. The product was purified using preparative TLC (Analtech 20 X 20 cm 1000 microns silicagel GF uniplate) eluted with ethyl acetate containing 1 % acetic acid. The material of interest was scraped off and removed from the silica with ethyl acetate (R_f = 0.4). ^{1}H-NMR ($CDCl_3$): δ 8.17 and 8.10 (s, 1H), 7.57 and 7.49 (s, 1H), 7.23 (m, 5H), 6.93 (s, br), 6.81 (s, 1H), 6.09 and 6.06 (s, 2H), 4.79 (m, 3H), 4.50 (m, 1H), 4.30 and 4.18 (m, 1H), 3.21 (m, 1H), 3.05 (dd, J = 9.0 and 6.0, 1H), 2.35 (m, 2H), 2.10 (m, 2H), 1.97 (s, 3H), 1.40 (m, 3H), 0.86 and 0.83 (d, J = 7.2 and 5.4, 6H). ^{13}C NMR ($CDCl_3$) δ (152.26 and 152.10), 147.94, 147.02, (142.45 and 142.07), 135.86, 129.38, 128.34, 126.93, 109.66, 108.25, (106.32 and 105.57), (103.36 and 103.00), 60.04, 53.72, 52.03, 43.92, (41.05 and 40.63), 37.37, (30.49 and 30.27), 29.42, 27.62, 24.74, (22.81 and 22.64), (22.05 and 21.86), 15.36. HRMS: MH^+ $C_{29}H_{37}O_9N_4S$ calc 617.2281; found 617.2275. IR 3220.94 (br), 2945.6 (w), 2932.7 (w), 1680.95 (s, br), 1666.8 (s), 1660.0 (s), 1593.2 (w), 1530.9 (s), 1509.3 (s), 1486.2 (w), 1333.3 (m), 1263.6 (s), 1200.9 (m), 1135.0 (w) 1036.2 (w) cm^{-1}. The UV spectrum is shown.



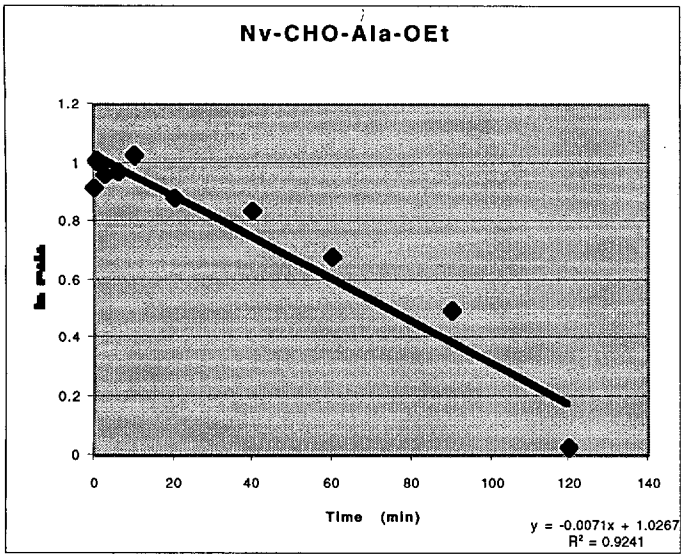
Preparative HPLC. An Alltech Econosil 10 μ C18 10 X 250 mm column was used. Conditions unless otherwise specified: flow rate of 3.5 mL/min, UV detection at 220 nm,

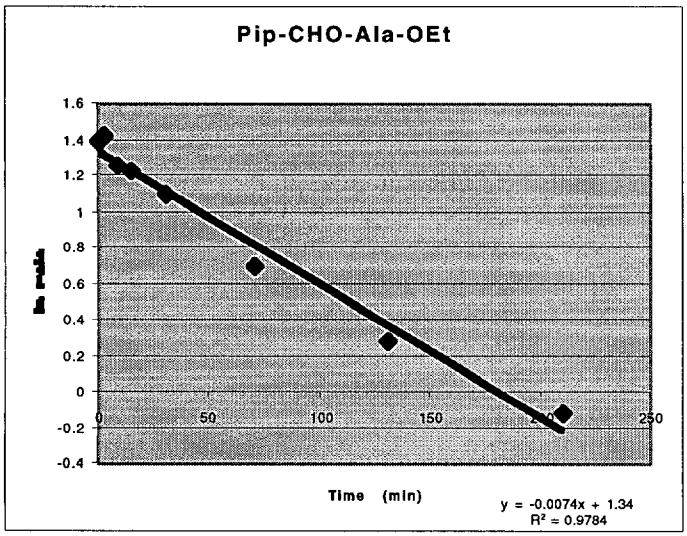
solvents (water and acetonitrile) were filtered prior to use and degassed *via* a helium sparge. Approximately 20mg of crude material was injected for each run. The following gradient was used to separate Pip-CHO-MLF-OH: Solvent A: H_2O with 0.1 % trifluoroacetic acid; Solvent B: CH_3CN with 0.1 % trifluoroacetic acid; 0 min, 40% B; 20 min, 95% B. The material of interest had a retention time of 9.8 min.

Analytical HPLC. An Alltech Econosil 5 μ C18 4.6 X 250 mm column was used. Conditions unless otherwise specified: flow rate of 1 mL/min, UV detection at 220 nm, solvents (water and acetonitrile) were filtered prior to use and degassed via a helium sparge.

Irradiation of Nitrobenzylformylalanines. A 0.01 M solution of each alanine derivative (Pip-CHO-Ala-OEt and Nv-CHO-Ala-OEt) was prepared in acetonitrile. The solution was irradiated (with rapid magnetic stirring) in a Pyrex round bottom flask in a Rayonet reactor fitted with 350 nm UV lamps. Reaction progress was followed by diluting a 25 μL aliquot of the solution with an additional 75 μL of solvent, loading 10 μL of this diluted solution into a 20 μL loop, and injecting it into the HPLC operating at a flow rate of 1.2 mL/min. An isocratic elution system was used for Nv-CHO-Ala-OEt: 85 % A/15 %B. The retention times are 3.7 min (Nv-CHO-Ala-OEt) and 3.0 min (CHO-Ala-OEt). The following elution gradient was utilized for Pip-CHO-Ala-OEt: 0 min, 0% B; 10 min, 10% B. The retention times are 10.7 min (Pip-CHO-Ala-OEt) and 5.3 min (CHO-Ala-OEt). The area of the resulting peaks was used to determine amount of starting material remaining and was used in graphical data. Plots of time vs. moles for each photolysis show first order decay. Plots of time vs. ln moles are linear with a 0.42 s^{-1} rate at the same flux.

Irradiation of Caged Chemotactic Peptides. The derivatized peptides (Nv-CHO-MLF-OH and Pip-CHO-MLF-OH) were irradiated in a manner similar to that described above. A 5 mM solution in acetonitrile was irradiated (with rapid magnetic stirring) in a Pyrex round bottom flask in a Rayonet reactor fitted with 350 nm UV lamps. The progress of the reaction was followed by taking 15 μL aliquots of the reaction mixture at the following time points: 0, 3, 8, 15, 30, 90, 120, 150 min, which were injected via a 20 μL loop. The following gradient was

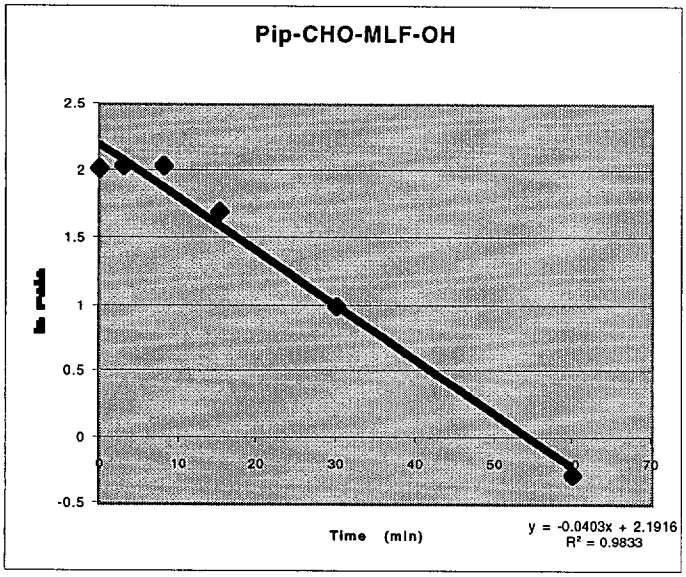


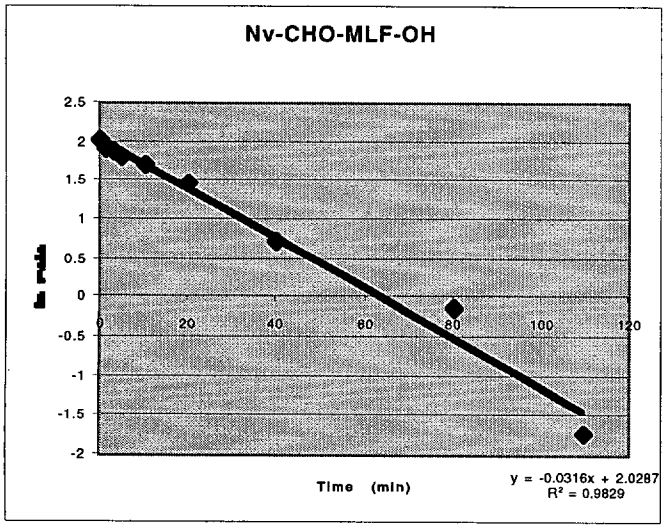


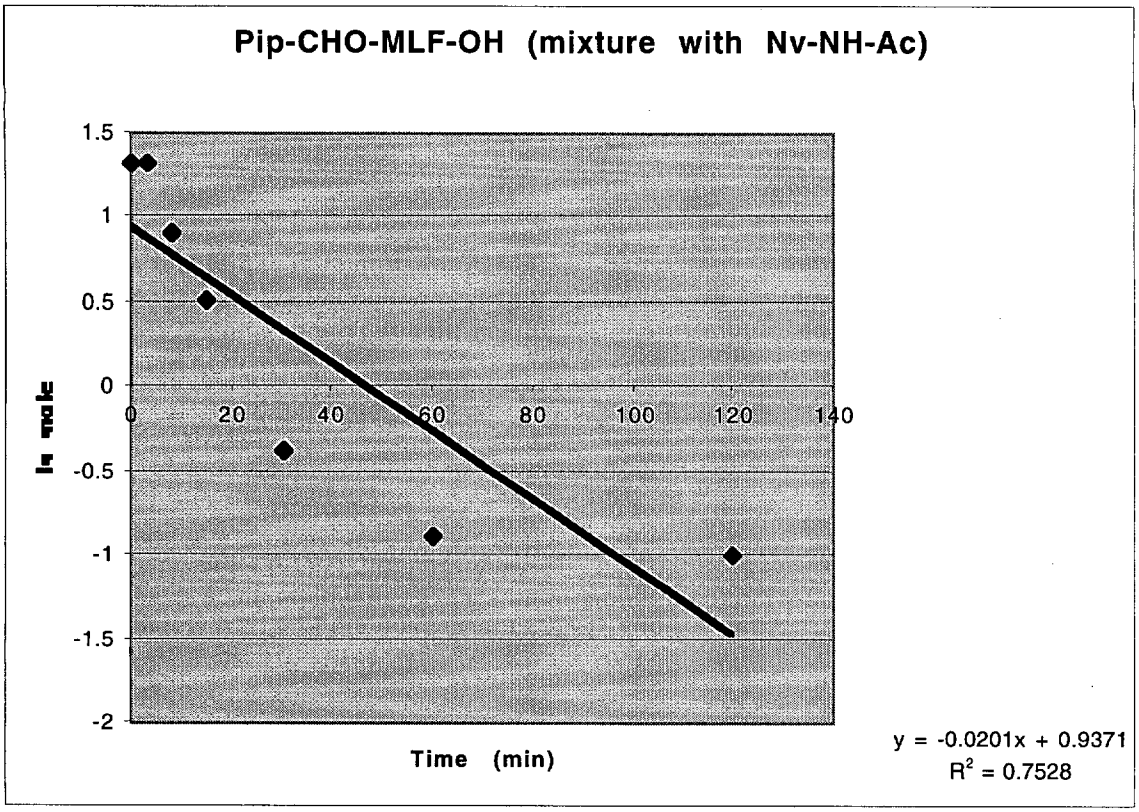
utilized: 0 min, 20% B; 20min, 90% B; 30min, 95% B. Compounds were identified according to their characteristic retention times as compared with known standards as follows: CHO-M(O)LF-OH, 6.3 min; CHO-MLF-OH 7.9 min; NV-NH-Ac10.8 min; Nv-CHO-MLF-OH, 11.1 min; Pip-CHO-MLF-OH 11.6 min. A plot of time vs. moles for each photolysis shows a first order decay. A plot of time vs. ln moles is linear. The rate for Nv-CHO-MLF-OH is 1.89 s^{-1}, while that for Pip-CHO-MLF-OH is 2.41 s^{-1}.

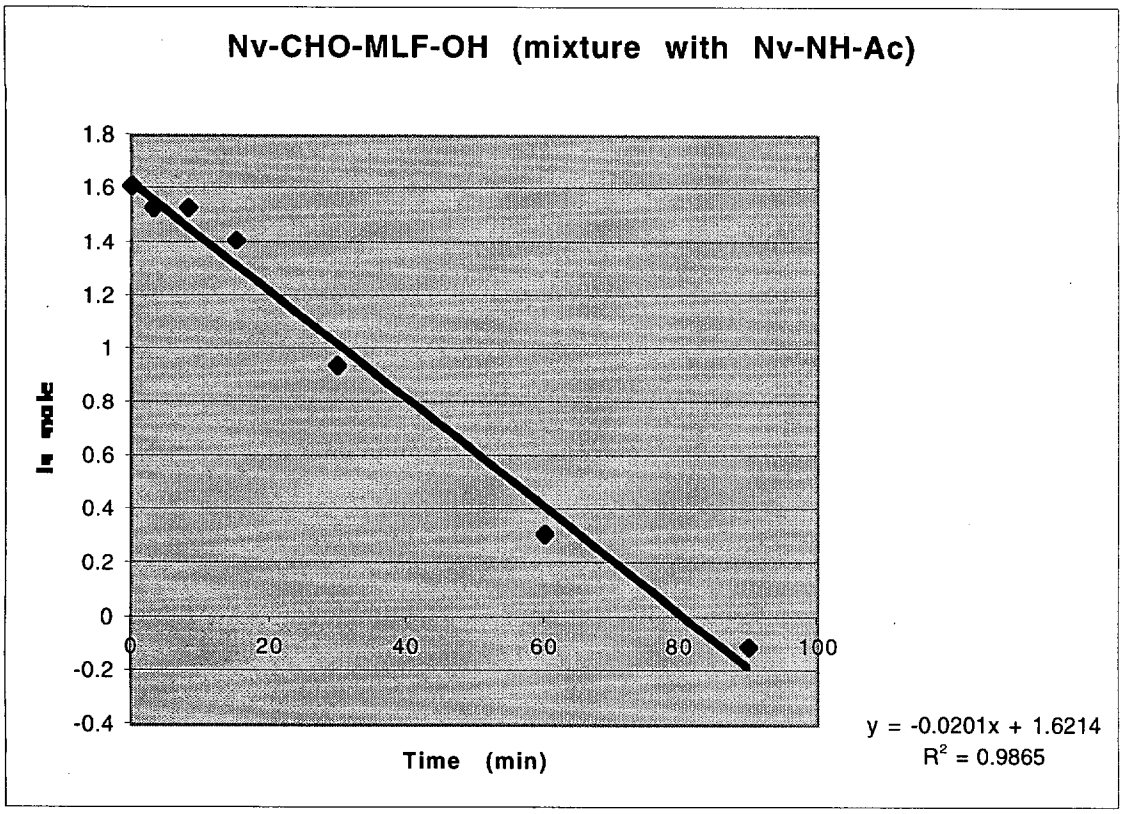
Quantum yield determination. Both derivatives were irradiated alone (data shown above) and with an equimolar concentration of Nv-NH-Ac (in the same flask) to determine quantum yield. Two separate experiments were conducted with Nv-CHO-MLF-OH. They are shown below: A

quantum yield of 0.024 is obtained. The photolysis of Pip-CHO-MLF-OH was carried out in the same manner as that of Nv-CHO-MLF-OH. Analysis of the data give a quantum yield of 0.041.

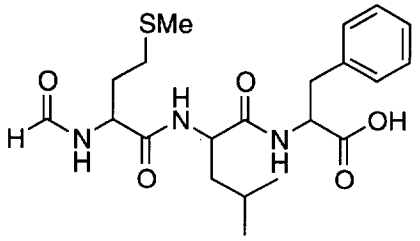




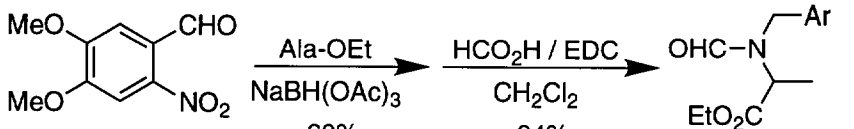

Pip-CHO-MLF-OH (mixture with Nv-NH-Ac)
1.5
1
0.5
0
-0.5
-1
-1.5
-2
0
20
40
60
80
100
120
140
ln rate
Time (min)
y = -0.0201x + 0.9371
R2 = 0.7528


Nv-CHO-MLF-OH (mixture with Nv-NH-Ac)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
0
20
40
60
80
100
Time (min)
y = -0.0201x + 1.6214
R2 = 0.9865

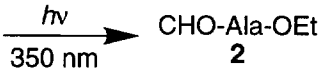
Cell culture and secretion assay. A rat basophilic leukemia RBL-2H3) cell line stably expressing fMLF receptors was used for this study. Cells were cultured in 96-well tissue culture plates overnight. Cells were then washed with HEPES-buffered saline containing 0.1%BSA and treated with and without fMLF or NV-fMLF. β-Hexosaminidase release was assessed as previously reported.[21]



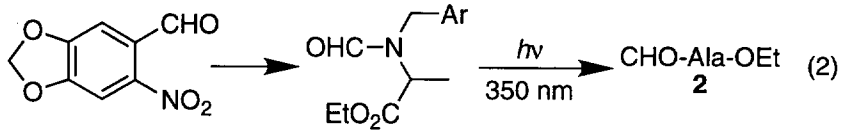
f-Met-Leu-Phe,
a chemotactic peptide



Nv-CHO-Ala-OEt
1

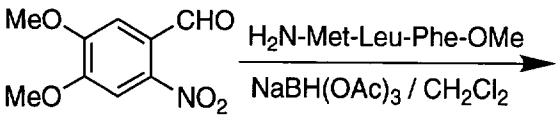


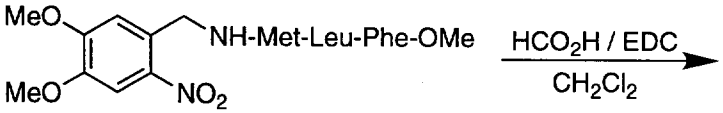
(1)



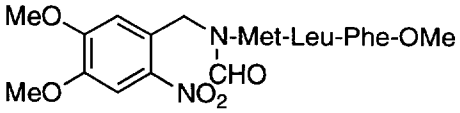
(2)

Pip-CHO-Ala-OEt
3



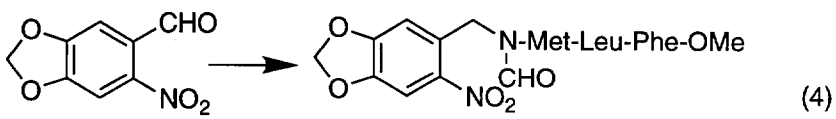


Nv-Met-Leu-Phe-OMe
4



Nv-CHO-Met-Leu-Phe-OMe
5

(3)



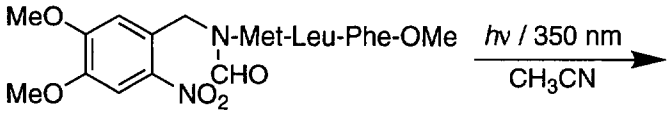
(4)

Pip-CHO-Met-Leu-Phe-OMe
6

Nv-CHO-Met-Leu-Phe-OMe
ED_{50} 10^{-6} M

Pip-CHO-Met-Leu-Phe-OMe
ED_{50} 10^{-6} M

CHO-NH-Met-Leu-Phe-OMe
ED_{50} 10^{-9} M



Nv-CHO-Met-Leu-Phe-OMe

CHO-NH-Met-Leu-Phe-OMe, *no* 67% isolated

CHO-NH-Met-Leu-Phe-OMe (5) (HPLC)

For graphical abstract: