Tetrahedron Vol. 49, No. 17, pp. 3433-3448, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00

Conformationally Constrained Nonpeptide β - Turn Mimetics of Enkephalin

Benjamin Gardner•, Hiroshi Nakanishi* and Michael Kahn +*

+Department of Pathobiology, University of Washington, Seattle, Washington 98195

*Molecumetics Institute, 2023 120th Avenue N.E., Bellevue, Washington 98005

•Department of Chemistry, University of Illinois at Chicago, Chicago, Illinois 60680

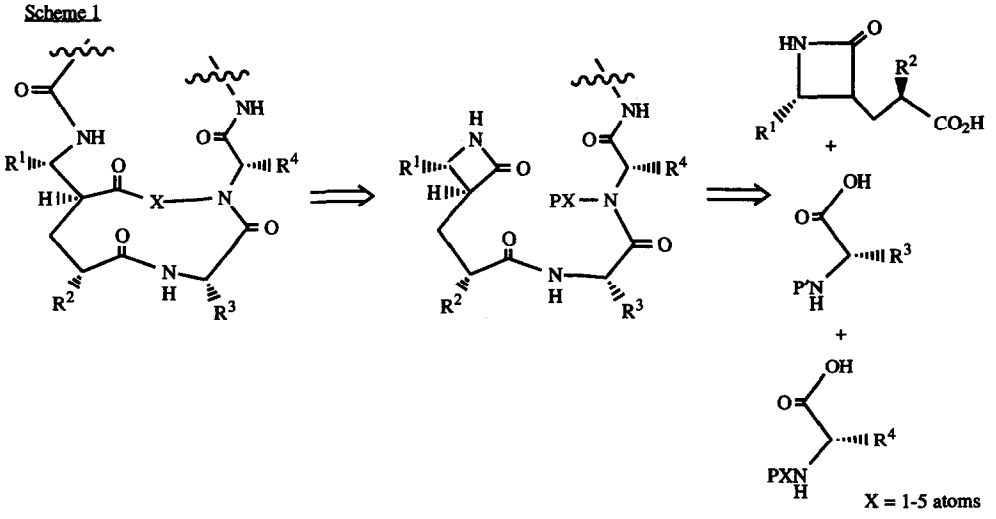
(*Received in USA* 1 *March* 1993)

Abstract: The design, synthesis and *in vitro* biological analysis of a family of conformationally constrained nonpeptide mimetics incorporating a 4→1 β-turn prosthetic unit to examine the proposed biological significance of this conformer is described.

Introduction

The isolation and identification in 1975 of the endogenous opioid pentapeptides, methionine and leucine enkephalin[1], initiated an intensive effort to delineate the relationship between enkephalin and morphine[2,3,4]. The inherent mobility of the enkephalin framework, its rapid degradation *in vivo*[5] and the existence of multiple receptor subtypes[6,7] have hampered the assessment of its bioactive conformations. Conformationally constrained peptides[8] or peptidomimetics[9,10,11] should facilitate this task. Several turn conformations have been proposed based upon computational models[12,13,14,15], X-ray crystallography[16,17] and spectroscopic studies[18,19,20]. We have recently developed a third generation reverse turn mimetic system[23,24]. The retrosynthetic analysis is outlined in Scheme 1. This strategy,

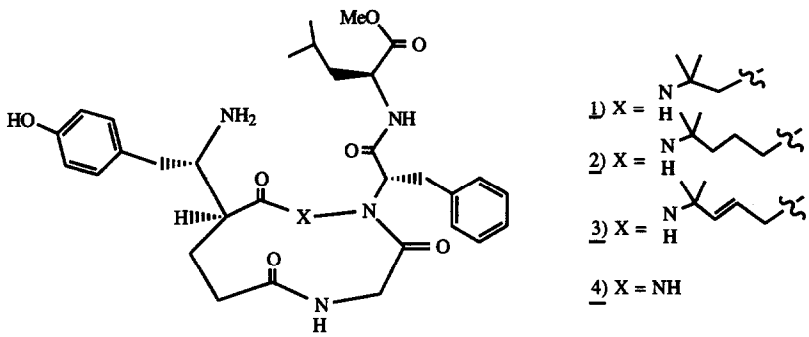
Scheme 1



through variation of the X group linker, provides access to a family of conformationally constrained reverse turn mimetics with reliable control and variation of side-chain orientations, backbone distances,

ϕ and ψ dihedral angles and flexibility/rigidity. This is a critical feature for assessing the importance of induced fit mechanisms, potentially addressing questions of agonism versus antagonism as well as the importance of mobility in antigenicity. The system allows for the introduction of natural or unnatural amino acid side chain functionality using a modular component synthesis which is readily amenable to solid phase peptide synthesis (SPPS). We have utilized our third generation mimetic system to synthesize small molecule conformationally contrained peptidomimetics of members of the immunoglobulin supergene family[23,24]. Previously, we have reported the design and synthesis of a conformationally constrained enkephalin analog which incorporated an 11-membered ring 5→2 β-turn mimetic[21,22]. We now wish to report the design, synthesis and evaluation in an *in vitro* binding assay, of a family of leucine-enkephalin analogs 1-4 (Fig 1) incorporating a family of 4→1 β-turn mimetics, to evaluate the significance of the proposed[32,33,35,36,37,38,39] bioactive relevance of this conformation.

Figure 1



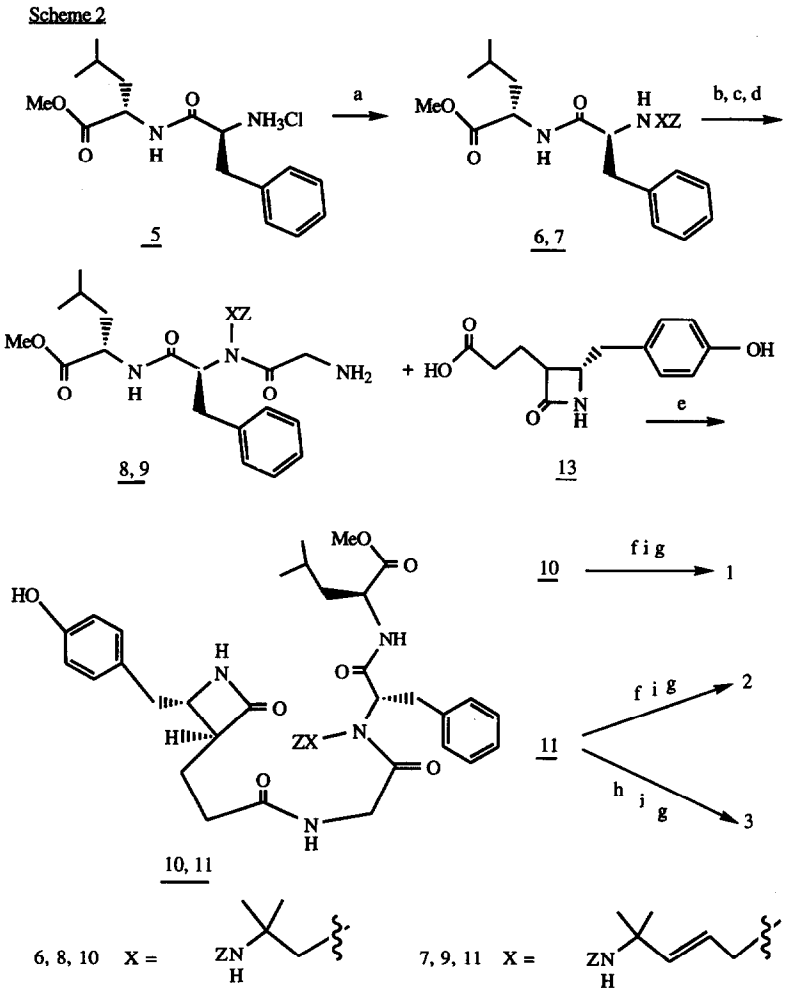
Synthesis

The syntheses of compounds 1-3 are outlined in Scheme 2. Reductive amination of the leu-phe dipeptide 5 with the requisite aldehyde (14 or 15) proceeded smoothly to afford 6 or 7[25]. Acylation of the secondary amine was effected with the acid chloride of Fmoc glycine in high yield using the silver cyanide protocol[26]. Deprotection with diethylamine in acetonitrile provided 8 or 9. Aqueous diimide coupling with azetidinone 13 afforded the nascent β-turn mimetics 10 or 11 in 84% yield. Cyclization was effected either through catalytic hydrogenolysis to provide macrocycles 1 or 2 or under phase transfer conditions to afford 3 with near quantitative conversion.

The synthesis of 4 is detailed in Scheme 3. Boc-phenylalanyl hydrazide methyl ester 16 was prepared according to the procedure of Hoffman[27]. Saponification and aqueous carbodiimide coupling with methyl leucinate proceeded smoothly to afford 17 in 88% yield. Adduct 18 was prepared via the mixed anhydride coupling of benzyl glycinate to azetidinone carboxylic acid 12. The coupling of 17 and 18 was most effectively performed again utilizing the Rich procedure[26] to provide the penultimate species 19. Deprotection and concomitant cyclization affords 4 in high yield. Syntheses of the synthetic

components 12, 13, 14 and 15 are depicted in Schemes 4 and 5 and described in the experimental section.

Scheme 2



(a) (CBZ)NHC(CH3)2CHO (14) or (CBZ)NHC(CH3)2 CH=CHCHO(15), NaBH(OAc)3 (2eq), THF, rt.; (b) Fmoc Gly, $SOCl_2$, CH_2Cl_2, DMAP (cat); (c) AgCN, benzene, 50° C; (d) Et_2NH, CH_3CN, rt.; (e) EDC (1.5 eq), HOBT (1.0 eq), THF/H_2O (1:1), rt.; (f) H_2 (1 atm.), 5% Pd/C (cat), MeOH, rt; (g) HCl/EtOAc; (h) cyclohexene: MeOH (1:4), palladium black (cat.), 60° C.

Conformational Analysis

Monte Carlo conformational searches were carried out for the mimetic ring structures using BATCHMIN 3.5x with MM2/MMOD force field parameters. Hydration energies were included in the calculation using a generalized Born solvation model[28]. All conformations within 4.18 kJ/mol from the

global minimum are listed in Table 1 for each mimetic ring system. The numbers listed for the population (pop) were calculated from the Boltzmann distribution assuming thermal equilibrium with low energy barriers between conformers[29].

To the best of our knowledge, no structural data on conformationally constrained biologically active 4→1 β-turn enkephalin analogues is available[30], therefore, we have compared mimetics 1–4 with idealized type I, I′, II and II′ β-turns. The idealized turns were generated by energy minimization of a tetrapeptide sequence consisting of either all alanines or alanines with one or two glycines depending on the steric requirements of the particular turn types with torsional constraints at the defined angles[31]. The actual comparisons were made using six-position root-mean-square (rms) values at the four alpha carbons in the turn, the carbonyl carbon at the *i*, and the amide nitrogen at the *i+3* positions. Two other parameters are also listed in the table which characterize β-turns, *i.e.*, the distance between (d) the C_α^i and C_α^{i+3} carbons, and torsional angle (ε) between the $C_\alpha^i - C^i$ and $N^{i+3} - C_\alpha^{i+3}$ bonds, of the equivalent bonds in the mimetics.

The presence of glycine residues at the second and third position enables enkephalin to theoretically form any type of β-turn, although only a type I′ 4→1 β-turn was observed by x-ray crystallagraphy[32,33]. The lowest energy conformer in the 10 membered ring system (4) is an excellent mimic for an idealized type I′ β-turn with a 6-atom rms deviation of 0.22 Å. The 12 membered ring mimetic (1) can also adopt a type I′ β-turn; however the C_α^1 to C_α^4 distance is quite large (7Å) (Table 1).

Table 1

	ΔE kJ/mol	pop %	d Å	ε deg	rms deviation (Å) I	I′	II	II′
1	0.000	23.2	7.02	-26	0.876	1.009	0.795	0.920
	1.363	13.5	6.47	117	0.883	1.410	1.088	1.052
	1.964	10.6	5.21	58	0.799	1.004	0.756	0.761
	3.089	6.7	6.68	-70	1.038	0.639	0.756	0.914
	3.133	6.6	6.83	25	0.839	1.135	0.912	0.933
	3.596	5.5	6.22	-31	0.749	0.736	0.599	0.820
	4.049	4.6	6.42	-9	0.979	0.776	0.799	0.883
2	0.000	37.1	7.99	-9	1.463	1.303	1.307	1.285
	2.510	13.6	8.63	165	1.675	1.560	1.583	1.535
	2.820	12.0	7.13	132	1.164	1.570	1.298	1.229
	3.990	7.5	8.82	-141	1.984	1.480	1.738	1.712
3	0.000	36.5	6.95	104	1.174	1.566	1.295	1.205
	3.640	8.5	6.67	-137	1.392	1.064	1.099	1.182
	8.680	8.3	6.22	162	0.892	1.269	0.999	0.937
	3.720	8.2	7.02	-29	0.900	1.144	0.887	0.964
4	0.000	21.9	5.01	-83	1.133	0.220	0.695	0.869
	0.555	17.6	5.08	26	0.359	1.171	0.729	0.768
	0.742	16.3	4.99	12	0.336	0.966	0.578	0.670
	1.437	12.3	6.06	-127	1.251	0.472	0.931	0.985
	1.818	10.6	6.09	-147	1.427	0.583	1.081	1.136
	2.061	9.6	5.31	9	0.430	0.746	0.339	0.524

Pharmacologic Evaluation

The 4→1 β-turn enkephalin mimetics (1-4) were evaluated for their ability to bind at the μ and δ opioid receptors. Briefly, binding at μ and δ receptors was measured in a twice-washed P2 membrane fraction obtained from whole rat brain (minus cerebellum) using a 50 mM Tris-HCl buffer (pH 7.4 at 37°C). Assay tubes contained 0.8 ml of membrane homogenate (0.5 mg of protein), 0.1 ml of ^{3}H-labeled ligand (1.0 nM DTLET or 1.0 nM DSLET for δ, 2.0 nM DAMGO for μ), and 0.1 ml of the test compound in replicates of three. After 60 min. incubation at 37 °C, reactions were terminated by rapid filtration on Whatcom GF/B glass-fiber filters and a subsequent 10 ml wash with ice-cold buffer. Filters were prepared for liquid scintillation counting Specific binding was calculated as the difference in radioactivity bound in the absence and presence of 10 μl of levorphanol[34]. The results of these assays are depicted in table 2.

Table 2

Mimetic	δ		μ	
	concentration(μM)	% inhibition	concentration(μM)	% inhibition
1	0.1	0%	1000	9%
2	0.1	0%	1000	0%
3	›10	50%	8	50%
4	›10	50%	›10	50%

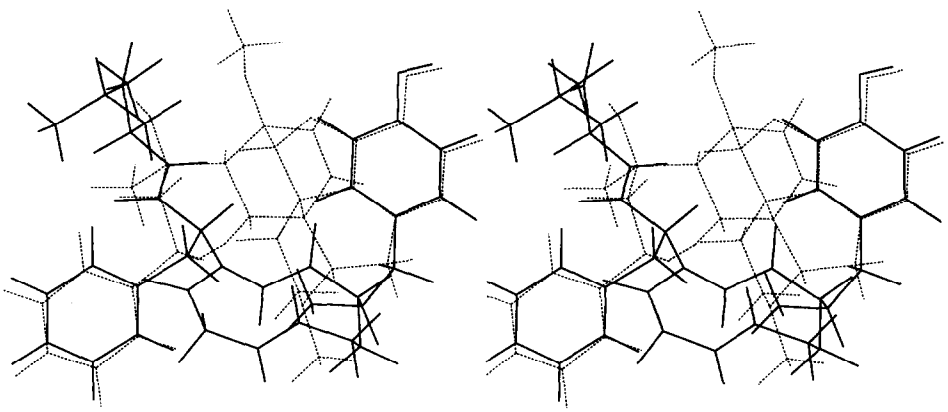


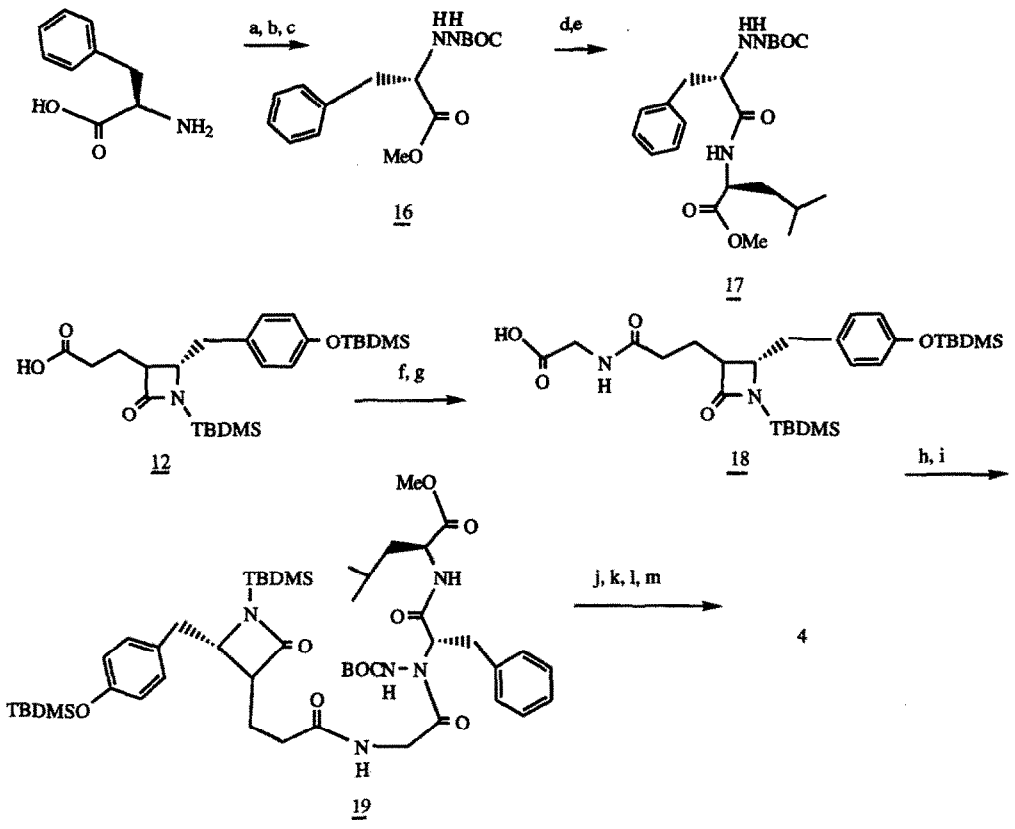
Figure 2 The tyramide and phenyl ring moieties of enkephalin mimetic 4 (solid line) can be perfectly aligned with those of the morphine analog PET, 7-[1-phenyl-3-hydroxybutyl-3-] endoethenotetrahydrothebaine (dashed line) by a flexible fitting procedure without any steric conflict.

Discussion

The conformation(s) of enkephalin have been studied extensively over the past 15 years. Despite this effort, knowledge of the bioactive conformation of enkephalin at either the μ or δ receptor remains shrouded. Additionally, the fact that enkephalin and rigid opiates bind to the same receptor(s) has tempted and intrigued chemists to decipher this structural relationship. In 1976, Bradbury et al proposed a β-bend model stabilized by an intramolecular hydrogen bond between the N-H of Phe^4 and the C=O of Tyr^1 which produces a spatial disposition between the Phe^4 aromatic ring and the tyramine segment of Tyr^1, analogous to that existing between the corresponding moieties in the potent morphine analogue PEO[35,36]. Further support for the relevance of this confirmation was provided by energy calculations on the potent [$DAla^2$, Met^5] enkephalin analogue, the lowest energy conformer of which contained a folded structure with a turn centered on residues 2 and 3.[37,38,39]. However, evidence contrary to the biological significance of a 4→1 β-turn has been presented by Freidinger[40], and additionally by Schiller in the analysis of the conformations of 13- and 14-membered rigid cyclic analogues[41].

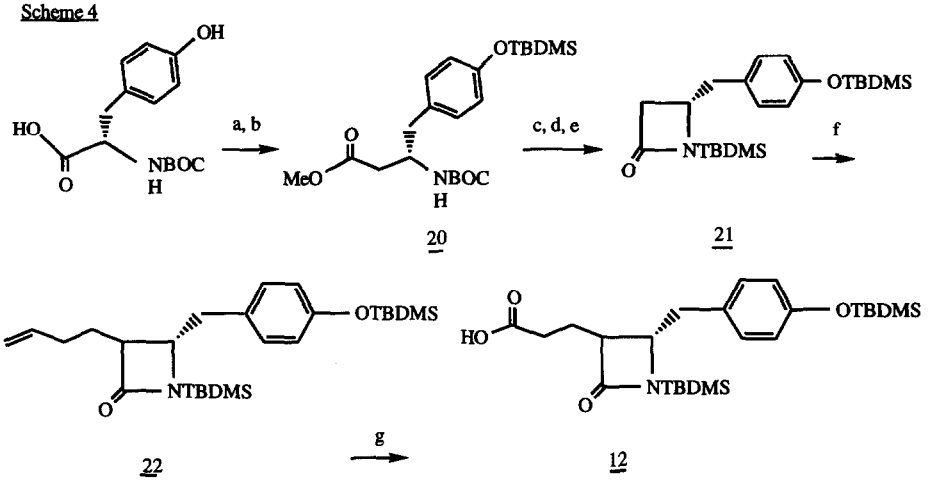
To further examine this hypothetical bioactive confirmation, we have synthesized a family of 4→1 β-turn mimetics (1-4). The lowest energy conformer of the 10 membered ring system 4 is an excellent mimic of an idealized type I′ β-turn (6 atom rms deviation 0.22Å, table 1) and displays excellent overlap with the critical Phe^4 aromatic ring and tyramine moieties of PET (Fig. 2) , yet it is essentially devoid of biological activity (table 2). Only the 14-membered ring analog 3, which has a rather expanded loop structure demonstrates any, albeit minimal, binding activity at the μ receptor. The results of this investigation can be interpreted as casting signficant doubt on the biological relevance of a 4→1 β-turn conformation for enkephalin. A similar series of experiments involving conformationally constrained analogues of 5→2 enkephalin β-turn mimetics is underway. It is hoped that this type of a systematic approach to the synthesis of constrained reverse turn analogs in conjunction with multiple peptide synthetic strategies will clarify the situation as to the biological significance of these and other proposed receptor bound reverse turn conformations. These experiments and further details concerning the conformational properties of analogs 1-4 will be reported in due course.

Scheme 3



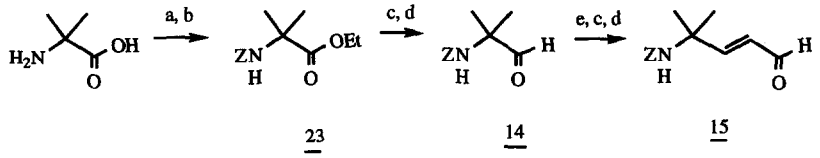
(a) $NaNO_2$, 1N H_2SO_4 (aq); (b) CH_2N_2, (c) Tf_2O, 2,6-lutidine, $H_2NNHBOC$, -78° → 0° C; (d) aq. NaOH, MeOH; (e) EDC, HOBT, methyl leucinate; (f), isobutyl chloroformate, NMM, benzyl glycinate, THF; (g) 10% Pd/C, EtOH, H_2 (1 atm), (h) $(COCl)_2$, CH_2Cl_2, 0° C; (i) compound 17, AgCN, benzene; (j) TFA, CH_2Cl_2, 0° C; (k) Et_3N; (1) TBAF, THF; (m) HCl, EtOAc.

Scheme 4



(a) TBDMSCl (1.3 eq), Et_3N (2.35 eq), DMAP (cat), CH_2Cl_2, r.t.; (b) 1. i-BuOCOCl (1.1 eq), NMM (1.1 eq), THF, 0° C; 2. CH_2N_2 in Et_2O (3.0 eq); 3. AgO_2CPh/Et_3N (cat), MeOH, rt.; (c) HCl in EtOAc, 0° C; (d) TBDMSCl (2.5 eq), Et_3N (4.0 eq), DMAP (cat); (e) t-BuMgCl in Et_2O (1.05 eq), 0° C → rt.; (f) 1. LDA (1.3 eq), THF, -78° C, 30 min; 2. 1-butenyl bromide (1.45 eq), -78° C → rt., 6 hrs; (g) $NaIO_4$ (4.1 eq), $RuCl_3$ (cat), 1:1:2 CCl_4:CH_3CN:H_2O, rt.

Scheme 5



(a) TsOH, EtOH, benzene, Δ; (b) Benzyl chloroformate (1.1 eq), Et_3N (2.5 eq), THF, 0° C; (c) Dibal, Toluene, -78° → 0° C, (d) PDC, CH_2Cl_2, rt., 4 Å mol sieves; (e) $\phi_3P{=}CHCO_2Me$, benzene, reflux.

Experimental Section

General. ^1H and ^{13}C spectra were recorded at 300 and 400 MHz (^1H) and at 75.4 and 100.6 MHz (^{13}C). The FAB mass spectra were recorded at the NIH Mass Spectral Facility, Department of Biochemistry, Michigan State University, East Lansing, MI. IR spectra were recorded on an FTIR spectrophotometer in $CDCl_3$ solution using NaCl solution cells. Unless stated otherwise, all reactions were run under an atmosphere of argon in oven-dried glassware. Ether and tetrahydrofuran were distilled from sodium-benzophenone ketyl Methanol was distilled from sodium methoxide. Benzene and dichloromethane were distilled from calcium hydride. Column chromatography was performed using E. Merck silica gel (230-400 mesh). Analytical TLC was performed with precoated silica gel 60F $_{254}$ (E. Merck) plates. Chromatographic eluent composition is reported "volume:volume" without exception.

Preparation of 12-membered ring 4→1 β-turn mimetic (1). – 3 mg of glassine solid (10) was dissolved in 1.5 ml freshly distilled MeOH. A catalytic amount of 5% Pd/C was added and the reaction was stirred under 1 atm. H_2 (g) for 12 hours. The reaction was filtered through a celite pad and the volatiles were removed under reduced pressure to yield 2.5 mg (95%) of glassine solid (1). ^1H NMR (300MHz, $CDCl_3$) δ 7.55 (br d, J=8.4 Hz, 1 H), 7.37-7.14 (m, 5H), 6.82 (d, J=8.4 Hz, 2H), 6.66 (d, J=8.4

Hz, 2H), 5.72 (br s, 1 H),, 4.71 - 4.62 (m, 1 H), 4.62 - 4.52 (m, lH), 3.74 (s, 3H), 3.71- 3.38 (m, 3H), 3.23 - 2.97 (m, 4H), 2.76 - 2.62 (m, lH), 2.58-2.43 (m, 2H), 2.39 - 2.18 (m, 2H), 2.15 - 1.98 (m, lH), 1.94 - 1.82 (m, lH), 1.68 - 1.48 (m, 3H), 1.26 (s, 3H), 1.20 (s, 3H), 0.87 - 0.82 (m, 6H); ^{13}C NMR (75.429 MHz, $CDCl_3$) δ 173.09, 172.10, 171.17, 169.04, 164.48, 137.17, 137.13, 130.48, 128.98, 128.94, 127.32, 127.25, 115.95, 77.19, 63.07, 59.56, 57.87, 54.87, 52.40, 51.01, 42.52, 40.86, 37.79, 34.07, 29.61, 29.26, 29.00, 24.89, 22.84, 21.57, 19.78; IR ($CDCl_3$) 3405, 3302, 2962, 2930, 1740, 1655, 1605, 1520, 1270, 1160, 1090; MS (FAB) m/z 638 (of corresponding carboxylic acid). The hydrochloride salt was generated by dissolving 2.5 mg of the above glassine solid in 1.0 ml 50/50 EtOAc/CH_2Cl_2 (v/v), cooling to 0°C under argon, and adding 5 drops of EtOAc saturated with HCl The reaction was stirred for 5 minutes at 0°C. The volatiles were removed under reduced pressure to yield 2.5 mg of a water soluble white hydrochloride salt. ^1H NMR (300 MHz, D_2O) δ 7.36-7.17 (m, 5H), 6.96 (d, J=9.5 Hz, 2H), 6.72 (d, J=9.5 Hz, 2H), 4.83 - 4.74 (m, lH), 4.38 - 4.29 (m, lH), 3.84-3.78 (m, 3H), 3.77-3.27 (m, 2H), 3.62 (s, 3H), 3.26-2.98 (m, 2H), 2.82 - 2.77 (m, lH), 2.60 - 2.46 (m, 2H), 2.38 - 2.13 (m, 2H), 2.03 - 1.76 (m, 2H), 1.60 - 1.37 (m, 3H), 1.19 (s, 3H), 1.16 (s, 3H), 1.16 (S, 3H), 0.80 - 0.72 (m, 6H).

Preparation of 14-membered ring 4→1 β-turn mimetic (2). – 19 mg of glassine solid (11) was dissolved in 2.0 ml of freshly distilled MeOH. A catalytic amount of 5% Pd/C was added and the reaction was stirred under 1 atm. H_2 (g) for 12 hours. The reaction was filtered through a celite pad and the volatiles were removed under reduced pressure to yield 16 mg (99%) of a glassine solid (2). R_f 0.25 in 7:93 NH_3-MeOH:CH_2Cl_2; ^1H NMR (400 MHz, $CDCl_3$) δ 7.38 - 7.11 (m, 5H), 6.97 (d, J=8.4 Hz, 2H), 6.77 (d, J=9.0 Hz, lH), 6.68 (d, J=8.4 Hz, 2H), 6.58 (br s, lH), 6.24 (br s, lH), 4.58 - 4.52 (m, lH), 4.13-4.02 (m, lH), 4.37 -4.28 (m, lH), 3.73 (s, 3H), 3.78-3.59 (m, 2H), 3.48-3.37 (m, lH), 3.32-3.18 (m, 2H), 3.11-2.98 (m, lH), 2.93 - 2.77 (m, 2H), 2.75 - 2.02 (m, 6H), 1.72 - 1.51 (m, 3H), 1.51 - 1.20 (m, 2H), 1.12 (s, 3H), 1.08 (s, 3H), 0.98 - 0.92 (m, 6H), 0.87 (m, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 171.39, 170.23, 169.92, 169.72, 155.89, 137.10, 130.48, 129.29, 128.91, 128.76, 127.11, 127.00, 115.50, 54.94, 52.33, 51.11, 50.10, 42.75, 41.69, 41.12, 39.85, 39.19, 34.49, 30.10, 30.09, 29.92, 25.10, 24.88, 23.94, 23.92, 22.79, 21.89, 18.88; MS (FAB) m/z 680. The hydrochloride was generated as previously described. ^1H NMR (300 MHz, D_2O) δ 7.26-7.07 (m, 5H), 7.02 (d, J=8.4 Hz, 2H), 6.96 (d, J=8.6 Hz, lH), 6.72 (d, J=8.4 Hz, 2H), 4.28-4.20 (m, lH), 3.92-3.69 (m, 2H), 3.54 (s, 3H), 3.22-2.93 (m, 3H), 2.89 - 2.70 (m, lH), 2.69-2.48 (m, 2H), 2.42-2.16 (m, 2H), 2.07-1.73 (m, 2H), 1.59 - 1.26 (m, 3H), 1.25-1.17 (m, 2H), 1.12 (s, 6H), 0.79-0.65 (m, 8H).

Preparation of unsaturated 14-membered ring 4→1 β-turn mimetic (3). – 2 mg glassine solid (11) was dissolved in 1.5 ml 20:80 cyclohexene: MeOH. A catalytic amount of palladium black was added and the reaction was stirred for 10 minutes at 60° C. The palladium was removed by vacuum filtration and volatiles were removed under reduced pressure to yield 1.7 mg clear solid which was chromatographed using 6:94 NH_3-MeOH:CH_2Cl_2 to yield 1.4 mg (84%) of a clear glassine solid (3). The hydrochloride was generated as previously described. ^1H NMR (300 MHz, D_2O) δ 7.27 - 7.06 (m, 5H), 7.00 (d, J=8.9 Hz, 2H), 6.73 (d, J=8.9 Hz, 2H), 5.63 (d, J = 16.6 Hz, lH), 5.58 - 5.42 (m, lH), 4.88 - 4.80 (m, lH), 4.24 - 4.17 (m, lH), 4.06 - 3.64 (m, 4H), 3.53 (s, 3H), 3.21 - 2.96 (m, 2H), 2.88 - 2.77 (m, lH), 2.67-2.45 (m, 2H), 2.38 - 2.18 (m, 2H), 2.07 - 1.72 (m, 2H), 1.58 - 0.96 (m, 3H), 1.24 (s, 6H), 0.78-0.62 (m, 6H).

Preparation of 10-membered ring 4→1 β-turn mimetic (4). – 10 mg of a clear oil (19) was dissolved in 2.5 ml freshly distilled CH_2Cl_2 and cooled to -10°C. 500 µl of anhydrous TFA was added and the reaction was stirred at -10° C for 3 hours. The volatiles were removed under reduced pressure, and the residue was dried under high vacuum for 30 minutes, and subsequently dissolved in 2.5 ml of CH_2Cl_2. The solution was cooled to 0°C and 200 µl of Et_3N was added. The reaction was stirred at 0°C for 1 hour. Volatiles were removed under reduced pressure and the residue was dissolved in 2 ml freshly distilled THF. The solution was cooled to 0°C and 50 mg of TBAF was added. The reaction was stirred to room temperature over the course of 2 hours. The volatiles were removed under reduced pressure and the residue was chromatographed using 6:94 MeOH:CH_2Cl_2 as eluent to yield 8 mg(94%) glassine solid (4). ^1H NMR (300 MHz, $CDCl_3$) δ 7.36-7.16 (m, 5H), 6.98 (d, J = 8.4 Hz, 2H), 6.84 (d, J = 9.2 Hz, lH), 6.74 (d, J = 8.4 Hz, 2H), 6.42-6.38 (m, lH), 6.30 (s, lH), 5.42-5.36 (m, lH), 4.55-4.43 (m, lH), 4.31 - 3.98 (m, 2H), 3.72 (s, 3H), 3.48-3.41 (m, lH), 3.37-3.04 (m, 2H), 2.88 - 2.74 (m, 3H), 2.13-2.03 (m, 2H), 2.02-1.83 (m, 2H), 1.66-1.46 (m, 3H), 0.94-0.83 (m, 6H); ^{13}C NMR (75.429 MHz, $CDCl_3$) δ 173.12, 172.43, 172.21, 170.64, 170.19, 155.30, 136.01, 129.48, 129.00, 128.83, 128.51,

127.31, 115.89, 58.29, 58.24, 56.43, 56.27, 55.10, 52.55, 50.94, 41.70, 40.73, 40.29, 24.90, 24.00, 22.71, 21.86; MS (FAB) m/z 596. The hydrochloride salt was generated as previously described.

Preparation of dipeptide fragment (5). – 499 mg (1.51 mmol) of methyl leucinate, 400 mg (1.51 mmol) of N-t-Boc-phenylalanine, and 204 mg (1.51 mmol) HOBT were dissolved in 7 ml of freshly distilled CH_2Cl_2 and cooled to 0°C. 435 mg (2.27 mmol) of EDC was added and the reaction was stirred to room temperature over the course of 2 hours. The reaction was diluted to 200 ml with CH_2Cl_2, washed with 40 ml sat. aq. NH_4Cl, 40 ml sat. aq. $NaHCO_3$, 40 ml brine, and dried over Na_2SO_4. The volatiles were removed under reduced pressure and the residue chromatographed using 3:97 MeOH:CH_2Cl_2 to yield 540 mg of a white glassine solid. Rf 0.4 in 3:97 MeOH:CH_2Cl_2; ^1H NMR (300 MHz, $CDCl_3$) δ 7.35 - 7.18 (m, 5H), 6.24 (d, J = 10.0 Hz, 1H), 4.95 (br s, 1H), 4.65-4.52 (m, 1H), 4.40-4.29 (m, 1H), 3.71 (s, 3H), 3.17-3.02 (m, 2H), 1.65-1.45 (m, 3H), 1.42 (s, 9H), 0.93-0.87 (m, 6H). 530 mg of the solid was dissolved in 10 ml EtOAc and cooled to 0°C. 3 ml EtOAc saturated in HCl was added and the reaction was stirred to room temperature over 12 hours. The disappearance of starting material was monitored by thin layer chromatography. The volatiles were removed under reduced pressure to yield 390 mg (83%) of a white glassine solid (5).

Preparation of secondary amine (6). – 40 mg (0.128 mmol) of dipeptide fragment (5) and 29 mg (.128 mmol) of aldehyde (14) were dissolved in 4 ml of THF under argon. 82 mg (.384 mmol) of sodium triacetoxyborohydride was added and the reaction was stirred at room temperature overnight. The reaction was diluted to 100 ml with EtOAc, washed with 30 ml of sat. aq. $NaHCO_3$, 30 ml brine, and dried over Na_2SO_4. The residue was chromatographed using 3:97 MeOH:CH_2Cl_2 as eluent to yield 51 mg(81%) of a clear oil (6). Rf 0.4 in 3:97 MeOH: CH_2Cl_2; ^1H NMR (300 MHz, $CDCl_3$) δ 7.59 (d, J = 10.0 Hz, 1H), 7.39-7.17 (m, 10H), 5.05-4.88 (m, 3H), 4.68-4.59 (m, 1H), 3.66 (s, 3H), 3.33-3.14 (m, 2H), 2.78-2.50 (m, 3H), 1.66-1.40 (m, 3H), 1.28 (s, 3H), 1.24 (s, 3H), 0.95-0.89 (m, 6H).

Preparation of secondary amine (7). – The procedure used in the preparation of (6) was repeated using 45 mg (0.144 mmol) of dipeptide fragment (5), 36 mg (0.144 mmol) of aldehyde (15), 92 mg (0.432 mmol) of sodium triacetoxyborohydride, and 30:70 EtOAc:Hex as mobile phase during chromatographic purification to yield 60 mg (80%) of a clear oil (7). Rf 0.3 in 40:60 EtOAc:Hex; ^1H NMR (300 MHz, $CDCl_3$) δ 7.62 (d, J=9.6 Hz, 1H), 7.38-7.19 (m, 10H), 5.59 (d, J=16.7 Hz, 1H), 5.44-5.35 (m, 1H), 5.03 (s, 2H), 4.82 (br s, 1H), 4.68-4.60 (m, 1H), 3.72 (s, 3H), 3.43-3.35 (m, 1H), 3.28-3.03 (m, 3H), 2.75-2.63 (m, 1H), 1.68-1.50 (m, 3H), 1.39 (s, 3H), 1.35 (s, 3H), 0.98-0.87 (m, 6H).

Preparation of tertiary amide (8). – 26 mg (0.086 mmol) of Fmoc-glycine was suspended in 3 ml of freshly distilled CH_2Cl_2 under argon. 63 µl (0.860 mmol) of $SOCl_2$ and 5µl of DMF were added and the reaction was stirred for 1 hour at room temperature. Volatiles were removed under reduced pressure and the residue was dried for 30 min. under high vacuum. 43 mg (0.086 mmol) of secondary amine (6) was azeotroped with 3x4 ml of freshly distilled benzene and dissolved in 3 ml of freshly distilled benzene. This solution was added to the acid chloride of Fmoc-glycine along with 23 mg (0.172 mmol) AgCN. The reaction was stirred at room temperature for 12 hours, diluted to 7 ml with EtOAc and filtered through a pad of celite. The volatiles were removed under reduced pressure and the residue was chromatographed using 1:3 EtOAc: Toluene as eluent to yield 40 mg of a clear oil Rf 0.5 in 30:70 EtOAc:Toluene; ^1H NMR (300 MHz, $CDCl_3$) δ 7.79 (d, J = 8.0 Hz, 2H), 7.63 (d, J = 7.5 Hz, 2H), 7.50-7.18 (m, 13H, 7.09 (d, J = 7.5Hz, 2H), 5.7 (br s, 1H), 5.59-5.54 (m, 1H), 5.03 (d, J = 3.2 Hz, 2H), 4.63-4.53 (m, 1H), 4.44-4.37 (m, 2H), 4.27-4.21 (m, 1H), 4.11-3.96 (m, 2H), 3.62-3.57 (m, 2H), 3.52 (s,3H), 3.26-3.18 (m, 1H), 3.13-2.92 (m, 2H), 1.82-1.48 (m, 3H), 1.28 (s, 3H), 1.22 (s, 3H), 0.89-0.92 (m, 6H). 20mg of the above oil was dissolved in 2 ml of CH_3CN. 100µl Et_2NH was added and the reaction was stirred at room temperature for 1 hour. The volatiles were removed under reduced pressure and the residue was chromatographed using 7:93 NH_3-MeOH:CH_2Cl_2 as eluent to yield 12 mg (51%) of a clear oil (8). Rf 0.2 in 5:95 NH_3 - MeOH:CH_2Cl_2; ^1H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, J=8.9 Hz, 1H), 7.42-7.06 (m, 10H), 5.75 (br s, 1H), 5.10-4.98 (m, 2H), 4.62-4.53 (m, 1H), 4.02-3.94 (m, 1H), 3.68-3.56 (m, 2H), 3.52 (s, 3H), 3.49-3.39 (m, 1H), 3.24-3.14 (m, 1H), 3.12-2.93 (m, 2H), 1.82-1.48 (m, 3H), 1.26 (s, 3H), 1.20 (s, 3H), 0.99-0.92 (m, 6H).

Preparation of tertiary amide (9). – Compound (9) was prepared according to the procedure used to prepare (8) to afford 18 mg (64%) of clear oil (9). Rf 0.25 in 5:95 NH_3-MeOH:CH_2Cl_2; ^1H NMR (300 MHz, $CDCl_3$) δ 7.38-7.17 (m, 10H), 6.84 (d, J=7.6 Hz, 1H), 5.72 (d, J=15.5 Hz, 1H), 5.32-5.22 (m, 1H), 5.01 (s, 2H), 4.90-4.80 (m, 2H), 4.56-4.46 (m, 1H), 3.86 - 3.55 (m, 2H), 3.65 (s, 3H), 3.40 (br s, 2H), 3.36-3.17 (m, 2H), 1.78-1.49 (m, 3H), 1.33 (s, 6H), 0.96-0.84 (m, 6H).

Preparation of mimetic precursor (10). – 4.5 mg (0.018 mmol) of azetidinone (13), 10 mg (0.018 mmol) of primary amine (8), and 3mg (0.020 mmol) of HOBT were dissolved in 1 ml of 50:50 THF:H_2O (v/v) and cooled to 0°C. 7 mg (0.032 mmol) of EDC was added and the reaction was stirred to room temperature over the course of 12 hours. The reaction was diluted to 50 ml with CH_2Cl_2, washed with 15 ml sat. aq. $NaHCO_3$, 15 ml sat. aq. NH_4Cl, 15 ml brine, and dried over Na_2SO_4. The volatiles were removed under reduced pressure, and the residue was chromatographed using 5:95 NH_3:MeOH:CH_2Cl_2 as eluent to provide 12 mg (84%) glassine solid (10). R_f 0.3 in 7:93 NH_3-MeOH:CH_2Cl_2; ^1H NMR (300 MHz, $CDCl_3$) δ 7.41 - 7.18 (m, 9H), 7.06 (d, J = 7.5 Hz, 2H), 7.02 (d, J = 8.4 Hz, 2H), 6.76 (d, J = 8.4 Hz, 2H), 6.56-6.59 (m, lH), 5.69 (br s, lH), 5.63 (br s, lH), 5.04 (s, 2H), 4.62-4.53 (m, lH), 4.17-3.96 (m, 2H), 3.90-3.78 (m, lH), 3.77-3.58 (m, 2H), 3.57 (s, 3H), 3.27-3.18 (m, lH), 3.17 -2.87 (m 2H),2.86-2.78 (m, 2H), 2.71-2.56 (m, 2H), 2.50 - 2.32 (m, 2H), 2.25 - 1.98 (m, 2H), 1.72-1.40 (m, 3H), 1.26 (s, 3H), 1.23 (s, 3H), 0.94-0.86 (m, 6H).

Preparation of mimetic precursor (11). – Compound (11) was prepared according to the procedure used to prepare 10 to yield 36 mg (85%) of glassine solid (11) after chromatography. R_f 0.3 in 5.95 NH_3-MeOH:CH_2Cl_2; ^1H NMR (300 MHz, $CDCl_3$) δ 7.38-7.16 (m, 10H), 6.98 (d, J = 8.4 Hz, 2H), 6.73 (d, J=3.4 Hz, 2H), 6.15 (br s, lH), 5.74 (d, J =16.6 Hz, 1H), 5.38 - 5.24 (m, lH), 5.08 - 4.88 (m, 3H), 4.56-4.42 (m, lH), 4.03-3.63 (m, 4H), 3.63 (s, 3H), 5.08-4.88 (m, 3H), 4.56-4.42 (m, lH), 4.03-3.63 (m, 4H), 3.63 (s, 3H), 3.39-3.11 (m, 2H), 2.79-2.70 (m, 2H), 2.61-2.49 (m, 2H), 2.40-2.23 (m, lH), 2.20-2.02 (m, lH), 2.01-1.89 (m, lH), 1.84-1.66 (m, 2H), 1.63-1.43 (m, 3H), 1.36 (s, 3H), 1.32 (s, 3H), 0.90-0.81 (m, 6H).

Preparation of β-lactam acid (12). – 625 mg (1.36 mmol) of (22) was dissolved in 2.5 ml CCl_4, 2.5 ml McCN, and 5 ml H_2O. 1.19 g (5.6 mmol, 4.1 eq) of $NaIO_4$ and 50 mg of $RuCl_3$ were added to the solution and the reaction was stirred at room temperature overnight. The aqueous layer was saturated with NaCl and the reaction was diluted with 25 ml EtOAc and 10 ml brine. The layers were separated and the aqueous layer was re-extracted with 3x25 ml EtOAc. The combined organic layers were washed with 30 ml brine, dried over Na_2SO_4, and the solvent removed in vacuo to yield 630 mg (97%) of (12) as a tan oil ^1H NMR (300 MHz, $CDCl_3$) δ7.01 (d, J = 8.4 Hz, 2H), 6.78 (d, J = 8.4 Hz, 2H), 3.35 - 3.30 (m, 1H), 3.20-3.13 (m, 1H), 2.92 - 2.83 (m, 1H), 2.53 - 2.42 (m, 1H), 2.07 - 1.82 (m, 3H), 1.73 - 1.58 (m, 1H), 0.99 (s, 9H), 0.96 (s, 9H), 0.32 (s, 3H), 0.27 (s, 3H), 0.18 (s, 6H).

Preparation of azetidinone (13). – 10 mg (0.021 mmol) of azetidinone (12) was dissolved in 3ml acetonitrile and cooled to 0°C. 200 µl of 49% aqueous HF was added and the reaction was stirred at 0°C for 1 hour. The volatiles were removed under reduced pressure to yield 8 mg (90%) of glassine solid (13). ^1H NMR (300 MHz, CD_3OD) δ 6.91 (d, J = 8.6 Hz, 2H), 6.61 (d, J = 8.6 Hz, 2H), 3.82 - 3.74 (m, lH), 3.39 - 3.32 (m, lH), 2.80 - 2.56 (m, 2H), 2.41 - 2.29 (m, 2H), 2.06 - 1.84 (m, 2H).

Preparation of N-benzyloxycarbonyl - aminoisobutyraldehyde (14) – 2.32 g (8.75 mmol) of ethyl ester (23) was dissolved in 50 ml of freshly distilled toluene and cooled to -78° C under argon. 20.4 ml (30.6 mmol, 3.5 eq) of 1.5 M Dibal in toluene was added to the reaction, and warmed to 0° C over the course of 2 hours. The reaction was quenched with a saturated solution of Rochelle's salt and extracted with 3x100 ml of Et_2O. The combined ethereal layers were dried over Na_2SO_4 and the volatiles were removed under reduced pressure. The resulting amorphous white solid was dissolved in 30 ml of freshly distilled CH_2Cl_2. 8.23 g (21.8 mmol, 2.5 eq) of PDC and 2.0g of 4A powdered molecular sieves were added to the reaction and the resulting suspension was stirred under argon overnight. The reaction was filtered through a pad of celite and the volatiles were removed under reduced pressure. The residue was chromatographed using 20:80 EtOAc:Hex to yield 1.80 g of (14) (93%) as a clear oil R_f 0.25 in 20:80 EtOAc:Hex; ^1H NMR (300 MHz, $CDCl_3$) δ9.42 (s, 1 H), 7.37 - 7.29 (m, 5H), 5.36 (br s, 1 H), 5.12 (s, 2H), 1.38 (s, 6H).

Preparation of (15). – 402 mg (1.82 mmol) of (14) and 730 mg (2.18 mmol, 1.2 eq), of carbomethoxy triphenylphosphorane were dissolved in 30 ml of freshly distilled benzene and refluxed overnight. The volatiles were removed under reduced pressure and the residue chromatographed using 20:30 EtOAc:Hex to yield 420 mg(83%) of a clear oil ^1H NMR (300 MHz, $CDCl_3$) δ 7.43 - 7.27 (m, 5H), 7.03 (d, J = 15.1 Hz, 1H), 5.86 (d, J = 15.1 Hz, 1H), 5.05 (s, 2H), 4.86 (br s, 1H), 3.76 (s, 3H), 1.48 (s, 6H). 106 mg (.383 mmol) of the α,β-unsaturated ester was dissolved in 4 ml of freshly distilled toluene and cooled to -78° C under argon. 892 µl (1.34 mmol, 3.5 eq) of 1.5 M Dibal in toluene was added to the reaction. The solution was stirred 30 minutes at -78° C and an additional 30 minutes at 0° C. The reaction was quenched with aqueous Rochelle's salt, diluted to 100 ml with Et_2O, washed with 25

ml H_2O, 25 ml brine, and dried over Na_2SO_4. The volatiles were removed *in vacuo* to afford the crude allylic alcohol as an oily residue. Without purification the alcohol was dissolved in 5 ml of freshly distilled CH_2Cl_2 under argon. 360 mg (0.958 mmol) of PDC and 1g of crushed 4Å molecular sieves were added and the reaction was stirred for 2 hours. The solution was diluted to 100 ml with Et_2O, filtered through a pad of celite, and the volatiles were removed in vacuo. The oily residue was chromatographed using 6:1 Hexane: EtOAc to afford 82 mg (86%) of (15) as a clear oil ^1H NMR ($CDCl_3$, 300 MHz) δ 9.18 (d, J = 4.1 Hz, 1H), 7.38 - 7.02 (m. 5H), 6.76 (d, J = 15.6 Hz, 1H), 5.96 (m, 1H), 5.01 (s, 2H), 4.89 (br s, 1H), 1.71 (s, 6H).

Preparation of Boc-phenylalanyl hydrazide methyl ester (16). – 2.0 g (12.1 mmol) of (D)-phenylalanine was dissolved in 13 ml of 1N H_2SO_4 (aq) To this solution 919 mg (13.3 mmol) of $NaNO_2$ was gradually added over the course of 1 hour. The reaction was stirred at room temperature overnight and vigorously extracted with 4x25 ml EtOAc. The combined organic layers were washed with 25 ml brine and dried over Na_2SO_4. Volatiles were removed under reduced pressure and the residue was dissolved in 7 ml EtOAc and treated with excess ethereal CH_2N_2. The volatiles were again removed under reduced pressure and the residue was chromatographed using 60:40 EtOAc:Hex as eluent to afford (73%) of clear oily hydroxy acid. ^1H NMR (300 MHz, $CDCl_3$) δ 7.19 - 6.84 (m, 5H), 3.62 (s, 3H), 3.41-3.32 (m, lH), 3.19 - 2.83 (m, 2H). 1.59 g (8.83 mmol) of the hydroxy acid was dissolved in 5 ml of CH_2Cl_2 and cooled to -78°C under argon. 1.63 ml (9.71 mmol) of triflic anhydride was added, followed by 1.23 ml (10.59 mmol) of 2,6-lutidine. The reaction was stirred for 5 minutes at -78°C. A solution of 2.33 g (17.7 mmol) of t-butyl carbazate in 3 ml CH_2Cl_2 was added dropwise. The reaction was stirred to room temperature over the course of three hours, diluted to 100 ml with CH_2Cl_2, washed with 25 ml sat. aq. $NaHCO_3$, 25 ml brine, and dried over Na_2SO_4. The volatiles were removed under reduced pressure and the residue was chromatographed using 25:75 EtOAc:Hex as eluent to yield 2.10 g (81%) of glassine solid (16). R_f 0.3 in 25:75 EtOAc:Hex; ^{1} H NMR (300 MHz, $CDCl_3$) δ 7.21 - 6.85 (m, 5H), 5.20 (br s, lH), 3.76-3.66 (m, lH), 3.59 (s, 3H), 3.20-2.83 (m, 2H), 1.41 (s, 9H).

Preparation of dipeptide hydrazide (17). – 140 mg (0.476 mmol) of phenylalanyl hydrazide methyl ester (16) was dissolved in 2 ml of MeOH. 520 µl of 1N aqueous NaOH was added and the reaction was stirred for 2 hours at room temperature. The volatiles were removed under reduced pressure and the residue was dissolved in 10 ml of distilled water. The pH was adjusted to 3 with 1N aqueous HCl and the resulting suspension was extracted with 3x30 ml EtOAc. The combined organic layers were washed with 20 ml of brine and dried over Na_2SO_4. The volatiles were removed to yield 130 mg of a clear glassine solid. ^1H NMR (300 MHz, CD_3OD) δ 7.17-7.08 (m, 5H), 3.73-3.67 (m, 1H), 2.96-2.79 (m, 2H), 1.35 (s, 9H). 130 mg (0.464 mmol) of the above acid, 63 mg (0.464 mmol) of HOBT, 84 mg (0.464 mmol) of methyl leucinate hydrochloride, and 65 µl (0.464 mmol) of Et_3N were dissolved in 5 ml of freshly distilled THF and cooled to 0°C. 133 mg (0.696 mmol) EDC was added and the reaction was stirred to room temperature over the course of two hours. The reaction was diluted to 100 ml with CH_2Cl_2, washed with 30 ml sat. aq. NH_4Cl, 30 ml sat. aq. $NaHCO_3$, 30 ml brine, and dried over Na_2SO_4. The volatiles were removed under reduced pressure and the resulting crude oil was chromatographed using 35:65 EtOAc:Hex as eluent to yield 171 mg (88%) of a white glassine solid (17). Rf 0.4 in 40:60 EtOAc:Hex; ^1H NMR (300 MHz, $CDCl_3$) δ 7.62 (br s, 1H), 7.38-7.23 (m, 5H), 6.21 (br s, 1H), 4.62-4.53 (m, 1H), 3.79-3.76 (m, 1H), 3.65 (s, 3H), 3.28 - 2.76 (m, 2H), 1.68-1.56 (m, 3H), 1.41 (s, 9H), 0.98 - 0.89 (m, 6H).

Preparation of azetidinone adduct (18). – 202 mg (0.423 mmol) of azetidinone (12) was dissolved in 3 ml of freshly distilled THF under argon and cooled to 0°C. 50µl (0.444 mmol) of NMM and 58 µl (0.444 mmol) of isobutyl chloroformate were added sequentially. The reaction was stirred for 15 minutes at 0°C. To the resulting solution was added 90 mg (0.444 mmol) of benzyl glycinate hydrochloride and an additional 50µl of NMM. The reaction was warmed to room temperature over the course of 3 hours. The reaction was diluted to 100 ml with EtOAc, washed with 20 ml sat. aq. NH_4Cl, 20 ml sat. aq. NH_4Cl, 20 ml brine, and dried over Na_2SO_4. The volatiles were removed under reduced pressure, and the residue was chromotographed using 40:60 EtOAc:Hex as eluent to provide 250 mg of a clear oil The residue was dissolved in 3 ml of EtOH and stirred with a catalytic amount of 10% Pd/C for 12 hours under 1 atm. of H_2(g). The solids were removed by vacuum filtration and the volatiles were removed under reduced pressure to yield 170 mg (91%) of a white glassine solid (18). ^1H NMR (300 MHz, $CDCl_3$) δ 7.01 (d, J = 8.7 Hz, 2H), 6.77 (d, J = 8.7 Hz, 2H), 4.03-3.98 (m, 2H), 3.49-3.41 (m, 1H),

3.19-3.11 (m, 1H), 2.98 - 2.93 (m, 1H), 2.58-2.49 (m, 1H), 2.25-2.19 (m, 2H), 1.91-1.66 (m, 2H), 0.97 (s, 18H), 0.28 (s, 3H), 0.23 (s, 3H), 0.18 (s, 6H).

Preparation of mimetic precursor (19). – 62 mg (0.116 mmol) of azetidinone (18) was azeotroped with 3x4 ml of benzene, dissolved in 1 ml of 20:80 THF:Et_2O, and cooled to 0°C under argon. 11 µl (0.127 mmol) of oxalyl chloride was added followed by 3 µl of DMF. The reaction was stirred for 2 hours at 0°C and 45 minutes at room temperature. Volatiles were removed under reduced pressure and the residue was dissolved in 1 ml of freshly distilled benzene. 61 mg (0.151 mmol) of hydrazide (17) was added to the solution, followed by 31 mg (0.232 mmol) of AgCN. The reaction was stirred at 50° for 12 hours, diluted to 10 ml with EtOAc and filtered through a pad of celite. The volatiles were removed under reduced pressure and the residue was chromatographed using 50:50 EtOAc: Hex as eluent to yield 52 mg (49%) of a white glassine solid (19). R_f 0.4 in 60:40 EtOAc:Hex; ^1H NMR (300 MHz, $CDCl_3$) δ 7.38 - 7.18 (m, 5H), 7.01 (d, J = 8.7 Hz, 2H), 6.77 (d, J = 8.7 Hz, 2H), 6.37 (br s, 1H), 6.23 (br s, 1H), 4.59 - 4.32 (m, 2H), 4.21 - 3.99 (m, 2H), 3.69 (s, 3H), 3.39-3.10 (m, 3H), 3.02-2.93 (m, 1 H), 2.58-2.44 (m, 2H), 2.07-1.46 (m, 7H), 1.42 (m, 9H), 0.98 (s, 9H), 0.95 (s, 9H), 0.90 - 0.82 (m, 6H), 0.30 (s, 3H), 0.25 (s, 3H), 0.18 (s, 6H).

Preparation of N-t-butyloxy carbonyl-O-t-butyl dimethylsilyl-homo-L-tyrosine methyl ester (20). – 2.00g (7.11 mmol) N-t-Boc-(L)-tyrosine was dried for 3 hours under high vacuum at 35°C in a 200 ml round-bottom flask. To this was added 1.39 g (9.24 mmol, 1.3 eq.) of TBDMSCl and 50 mg of DMAP, followed by 40 ml of CH_2Cl_2. 2.33 ml (16.7 mmol, 2.35 eq) of Et_3N was added slowly to the reaction. The resulting solution was stirred at room temperature overnight under argon. The reaction was diluted to 200 ml with CH_2Cl_2 and washed with 2x40 ml sat. aq. NH_4Cl, 40 ml H_2O, and 40 ml brine. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield 3.19 g of a light pink oil ^1H NMR (300 MHz, $CDCl_3$) δ 7.01 (d, J = 9.4 Hz, 2H), 6.75 (d, J = 9.4 Hz, 2H), 4.92 (br d, J = 10.1 Hz, 1H), 4.54 - 4.45 (m, 1H), 3.13 - 2.93 (m, 2H), 1.42 (s, 9H), 0.95 (s, 9H), 0.18 (s, 6H). The oil was dissolved in 25 ml of freshly distilled THF and cooled to 0° C. 860 µl (7.82 mmol, 1.1 eq) of NMM was added, followed by 1.01 ml (7.82 mmol, 1.1 eq.) of isobutyl chloroformate. The reaction was stirred at 0° C for 15 minutes at which time 75 ml of cold ethereal CH_2N_2 in (3.5 eq) was added. The reaction was allowed to warm to room temperature with stirring overnight. The volatiles were removed under reduced pressure and the solids removed by vacuum filtration through a pad of celite to yield 3.4 g of a light yellow oil, which was dissolved in 35 ml of freshly distilled methanol To this was added 2.0 ml of a methanolic solution containing 50 mg silver benzoate and 50 µl of Et_3N. The reaction was stirred for 1 hour at room temperature and filtered through celite. The volatiles were removed under reduced pressure and the residue redissolved in CH_2Cl_2. The organic layer was washed with 1N HCl, sat. aq. $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and the solvent removed in vacuo to provide 3.0 g of a clear oil, which was chromatographed using 20:80 EtOAc:Hex to provide 2.21 g (73%) of a clear oil (20). R_f 0.5 in 30:70 EtOAc:Hex; ^1H NMR (300 MHz, $CDCl_3$) δ7.03 (d, J = 8.8 Hz, 2H), 6.73 (d, J = 8.8 Hz, 2H), 5.02 (br d, J = 12.4 Hz, 1H), 3,62 (s, 3H), 4.10 (m, 1H), 2.78 (m, 2H), 2.48 (m, 2H), 1.40 (s, 9H), 0.96 (s, 9H), 0.18 (s, 6H).

Preparation of β-lactam (21). – 430 mg (1.02 mmol) of (20) was dissolved in 25 ml of EtOAc and cooled to O°C. 5.0 ml of EtOAc saturated with HCl was added and the reaction was stirred to room temperature overnight. The volatiles were removed under reduced pressure and the residue was dried for 2 hours under high vacuum and then placed under an argon atmosphere. 384 mg (2.55 mmol, 2.5 eq) of TBDMSCl and 50 mg of DMAP were added to the residue. 22 ml of freshly distilled CH_2Cl_2 was added followed by 569 µl (4.08 mmol, 4.0 eq) of Et_3N dropwise. The reaction was stirred overnight at room temperature, and diluted to 200 ml with CH_2Cl_2. The organic layer was washed with 2x40 ml sat. aq. NH_4Cl, 40 ml sat. aq. $NaHCO_3$, 40 ml brine, and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was azeotroped 3x30 ml with freshly distilled benzene. The residue was dissolved in 60 ml of freshly distilled Et_2O under argon and cooled to O° C. 536 µl (1.05 eq) of 2.0M t-BuMgCl was added slowly to the reaction. The solution was allowed to warm to room temperature and quenched with sat. aq. NH_4Cl The reaction was partitioned between 30 ml H_2O and 150 ml Et_2O. The aqueous layer was extracted with two additional 30 ml portions of ether, and the combined ethereal layers were washed with brine and dried over Na_2SO_4. The volatiles were removed under reduced pressure and the residue was chromatographed on silica gel using 15:85 EtOAc:Hex as eluent to provide 320 mg (83%) of (21) as a clear oil R_f 0.5 in 20:30 EtOAc:Hex; ^1H NMR (300 MHz,

$CDCl_3$) δ 7.02 (d, J = 8.8 Hz, 2H), 6.26 (d, J = 8.8 Hz, 2H), 3.76-3.63 (m, 1H), 3.22-2.95 (m, 2H), 2.72-2.42 (m, 2H), 1.00 (s, 9H), 0.97 (s, 9H), 0.31 (s, 3H), 0.26 (s,3H), 0.19 (s, 6H).

Preparation of β-lactam (22). – 760 mg (2.01 mmol) of (21) was azeotroped with 3x10 ml of benzene and dissolved in 3 ml of THF. This solution was added to a solution of LDA (2.01 mmol, 1.3 eq) in THF and stirred at -78° C for 30 minutes. 296 µl (2.91 mmol, 1.45 eq) of 4-bromo-1-butene was added slowly to the resulting enolate. The reaction was stirred for an additional 1 hour at -78° C and then allowed to warm to room temperature over the course of 5 hours. The reaction was cooled to 0° C and quenched with sat. aq. NH_4Cl The reaction was diluted to 100 ml with Et_2O, washed with 30 ml sat. aq. NH_4Cl, 30 ml H_2O, 30 ml brine, and dried over Na_2SO_4. The volatiles were removed under reduced pressure and the residue was chromatographed using 10:90 EtOAc:Hex to yield 780 mg (84%) of (22) as a clear oil R_f 0.5 in 15:85 EtOAc:Hex; ^1H NMR (300 MHz, $CDCl_3$) δ 7.02 (d, J = 8.8 Hz, 2H), 6.77 (d, J = 8.8 Hz, 2H), 5.66 - 5.51 (m, 1H), 4.88-4.78 (m, 2H), 3.38-3.31 (m, 1H), 3.20-3.12 (m, IH), 2.84-2.78 (m, IH), 2.53 - 2.42 (m, IH), 1.76 - 1.31 (m, 4H), 0.99 (s, 9H), 0.95 (s, 9H), 0.31 (s, 3H), 0.27 (s, 3H), 0.17 (s, 6H).

Preparation of ethyl-N-benzyloxycarbonyl - aminoisobutyrate (23). – 2.0 g (19.4 mmol) of benzyloxycarbonyl aminoisobutyric acid and 4.06 g (21.3 m mol, 1.1 eq) of toluenesulfonic acid were placed in a 200 ml round bottom flask and dissolved in 60 ml of benzene and 5 ml of ethanol The reaction was refluxed with a Dean Stark trap for 6 hours. The volatiles were removed and the resulting white solid was washed with 2x30 ml Et_2O and dried for 3 hours under high vacuum. The white solid was dissolved in 175 ml of THF and cooled to O°C. 6.75 ml (48.5 mmol, 2.5 eq) of Et_3N was added, followed by 3.03 ml (21.3 mmol, 1.1 eq) of benzyl chloroformate. The reaction was warmed to room temperature over 2 hours, filtered through a pad of celite, and the solvent was removed under reduced pressure. The residue was chromatographed using 20:80 EtOAc:Hex as eluent to afford 3.80 g (74%) of (23) as a clear oil R_f 0.4 in 40:60 EtOAc:Hex; ^1H NMR (300 MHz, $CDCl_3$) δ 7.38 - 7.29 (m, 5H), 5.42 (br s, 1H), 5.09 (s, 2H), 4.19 (q, J =9.3Hz, 2H), 1.57 (s, 6H), 1.23 (t, J = 7.2Hz, 3H).

Acknowledgements

We thank Dr. Donald Hansen, Searle, for his assistance in obtaining the binding data. We also gratefully acknowledge the generous financial support of the Camille and Henry Dreyfus Foundation, the NIH GM38260 and the Molecumetics Institute, and the assistance of Kathy Hjelmeland in the preparation of this manuscript. M.K. is an Established Investigator of the American Heart Association.

References

1. Hughes, J.; Smith, T.W.; Kostelitz, H. W.; Fothergill, L. A.; Morgan, B. A.; Morris, H. R. *Nature* **1975**, *258*, 577.

2. Hansen, P. E.; Morgan, B. A. *The Peptides: Analysis, Synthesis, Biology* Udenfried, S.; Meienhofer, J., Eds.; Academic Press, Orlando, FL, **1990**, *6*, 269-321.

3. Schiller, P. W. *The Peptides: Analysis, Synthesis, Biology* Udenfired, S. & Meienhofer, J., Eds. Academic Press, Orlando, Fl, **1990**, *6*, 219-268.

4. Portoghese, P. S.; Sultana, M.; Nagase, H.; Takemori, A. E. *J. Med. Chem.* **1988**, *31*, 281.

5. Roques, B. P.; Garbary-Jaureguiberry, C.; Oberlin, R.; Anteunis, M.; Lala, A. K. *Nature* **1976**, *262*, 778.

6. *Opioid Peptides: Medicinal Chemistry* Rapaka, R. S.; Barnett, G.; Hawks, R. L. Eds., NIDA Research Monograph 69, Rockville, MD, **1986**.

7. Mansour, A.; Khachturian, H.; Lewis, M. E.; Akil, H.; Watson, S. J. *Trends in Neuroscience* **1988**, *11*, 308.

8. Hruby, V. J.; Al-Oeidi, F.; Kazmierski, W. *Biochem J.* **1990**, *268*, 249-262.

9. Bélanger, P. C.; Dufresne, C.; Scheigetz, J.; Young, R. N.; Springer, J. P.; Dmitrienko, G. I. *Can. J. Chem.* **1982**, *60*, 1019.

10. Krstenansky, J. L.,; Baranowsky, R. L.,; Currie, B.L., *Biochem. Biophys. Res. Commun. 1982, 109,* 1368.

11. Su., T.; Nakanishi, H.; Xue, L.; Chen, B.; Tuladhar, S.; Johnson, M. E.; Kahn, M. *Biorg. and Med. Chem. Let. in press.*

12. Hassan, M.; Goodman, M. *Biochemistry* **1986**, *25*, 7596, and references therein.

13. Chew, C.; Villar, H. O.; Loew, G. H. *Molec. Pharmacol.* **1991**, *39*, 502.

14. Pettitt, B. M.; Matsunaga, T.; Al-Obeidi, F.; Gehrig, C.; Hruby, V. J.; Karplus, M. *Biophys. J.* **1991**, *60*, 1540.

15. Smith, P. E.; Dana, L. X.; Pettitt, B. M. *J. Am. Chem. Soc* **1991**, *113*, 67.

16. Aubry, A.; Birlirakis, N.; Sakarellos-Daitsiotis, M.; Sakarellos, C.; Marroud, M. *Biopolymers* **1989**, *28*, 27.

17. Griffin, J. F.; Smith, G. D. *Opioid Peptides: An Update,* Rapaka, R. S. & Dhawan, B. N. Eds. NIDA Research Monograph 87; Washington, D. C., **1988**, 41.

18. Hruby, J. V.; Kao, L. F.; Pettitt, B. M.; Karplus, M. *J. Am. Chem. Soc.* **1988**, *110*, 3351.

19. Picone, D.; D'Ursi, A.; Motta, A.; Tacredi, T.; Temussi, P. A. *Eur. J. Biochem.* **1990**, *192*, 433.

20. Mosberg, H. I.; Sobczyk-Kojiro, K.; Subramanian, P.; Crippen, G. M.; Ramalingam, K.; Woodard, R. W. *J. Am. Chem. Soc.* **1990**, *112*, 822.

21. Kahn, M.; Wilke, S.; Chen, B.; Fujita, K. *J. Am. Chem. Soc.* **1988**, *110*, 1638.

22. Kahn, M.; Wilke, S.; Chen, B.; Fujita, K.; Lee, J.; Johnson, M. E. *J. Mol. Recogn* **1988**, *1*, 75.

23. Saragovi, H. U.; Fitzpatrick, D.; Raktabutr, A.; Nakanishi, H.; Kahn, M.; Greene, M. I. *Science* **1991**, *253*, 792.

24. Chen, S.; Chrusciel, R. A.; Nakanishi, H.; Raktabutr, A.; Johnson, M. E.; Sato, A.; Weiner, D.; Hoxie, J.; Saragovi, H. U.; Greene, M. I.; Kahn, M. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 5872.

25. Borch, R. F., *Org.Syn.,* **1972**, 52, 124-7.

26. Rich, D. H.; Tung, R. D.; Dhaon, M K., *J. Org. Chem.*, **1986**, 51, 3350.

27. Hoffman, R. V.; Kim, H, *Tetrahedron Lett.* **1990**, 31, 2953.

28. Still, W. C., Tempczyk, A., Hawley, R., and Hendrickson, T., *J. Am. Chem. Soc.* **1990**, *112*; 6127-6129

29. **NOTE:** In reality, in most of cases studied here, this assumption is not correct. Thus the listed populations should be regarded as merely theoretical. A more detailed study of the solution of

conformation of these enkephalin mimetics incorporating 2D NMR data is beyond the scope of this manuscript and will be reported separately (M. Lee, H. Nakanishi, M. Kahn manuscript in preparation).

30. Siemion, I.Z., Szewczuk, Z., Herman, Z.S. and Stachura, Z. *Mol. Cell. Biochem.* **1981,** *34*, 23-29.

31. Wilmot, C.M. and Thornton, J.M. *Protein Engineering* **1990,** *3*, 479-493

32. Smith, G.D., and Griffin, J.F. *Science* **1978,** *199*, 1214-1216

33. Ishida, T., Kenmotsu, M., Mino, Y., Inoue, M., Fujiwara, T., Tomita, K., Kimura, T. and Sakakibara, S. *Biochem. J.* **1984,** *218*, 677-689

34. Chandrakumar, N. S., Stapelfeld, A., Beardsley, P., Lopez, O. T., Drury, B., Anthony, E., Savage, M. A., Williamson, L. N. and Reichman, M., *J. Med. Chem.* **1992,** *35*, 2928 2938

35. Bradbury, A. F., Smyth, D. G. and Snell, C. R. *Nature* **1976,** *260*, 165-6.

36. Lowe, G. H. and Bart, S. K. *Proc. Natl. Acad. Sci. USA* **1978,** *75*, 7-11.

37. Humblet, C. and DeCoen, J. L. In "Peptides: Proceedings of the Fifth American Peptide Symposium (M. Goodrum and J. Meienhofer eds.) **1977,** pp. 88-91, Wiley, New York.

38. Balodis, Y.Y. Nikiforovich, G.V., Grinsteine, I.V., Vegner, R.E. and Chipens, G.I. *FEBS Lett.* **1978,** *86*, 239-242.

39. Manavalan, P. and Momany, F.A. *I nt. J. Pept. Protein Res.* **1981,** *18*, 256-275.

40. Freidinger, R.M. In "Peptides, Synthesis, Structure, Function" (D.H. Rich and E. Gross, eds.) **1981,** pp. 673-683. Pierce Chemical Co., Rockford, Illinois.

41. Schiller, P.W. and Dimaio, J. In "Peptides: Structure and Function" (V.J. Hruby and D.H. Rich eds.) **1983,** pp. 269-278. Pierce Chemical Co., Rockford, Illinois.