Design, Synthesis, and Tripeptidyl Peptidase II Inhibitory Activity of a Novel Series of (*S*)-2,3-Dihydro-2-(4-alkyl-1*H*-imidazol-2-yl)-1*H*-indoles

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Received July 1, 2002

Butabindide, **1**, was previously reported as a potent inhibitor ($IC_{50} = 7$ nM) of the serine protease enzyme tripeptidyl peptidase II (TPPII), an endogenous protease that degrades cholecystokinin-8 (CCK-8). We found that **1** has some inherent chemical instability, yielding diketopiperazine **2** fairly readily under mimicked physiological conditions. We therefore prepared imidazoles **3**, which are void of **1's** inherent instability, and have found that our novel analogues maintained comparable TPPII inhibitory activity (e.g.,for **3c**, $IC_{50} = 4$ nM) as **1**.

Introduction

In 1998, 18% of the U.S. population could be classified as obese compared to 12% in 1991.[1] This is viewed as a concern within our medical community because obesity has been correlated with a number of diseases and disorders, including diabetes, hypertension, gall bladder disease, and osteoarthritis.[2] More critically significant is the determination that obesity is also attributable for 280000 deaths per year in the U.S.[3] New therapeutic antiobesity agents could help offset the morbidity and economic drain caused by obesity-related diseases, while concurrently yielding yearly revenues in the billion of dollars for the marketers of such products.[4]

There are a number of new strategies currently being explored to help attack the obesity problem, as described recently in a comprehensive perspective of past and present approaches.[4] Among the many approaches being explored is evaluation of various satiety-enhancing agents. One reported endogenous satiety agent is the octapeptide cholecystokinin-8 (CCK8), which has demonstrated significant in vivo inhibitory feeding effects. Some researchers have attempted to exploit this desired effect of CCK8 by preparing non-peptidyl agonists of CCK8, and these compounds are currently at various stages of development. In lieu of CCK8 mimics, an alternative approach recently reported involved efforts toward enhancing CCK8's natural response by inhibiting CCK8's normal degradation.[5−8] CCK8 is degraded biochemically into two inactive metabolites, a pentapeptide and a tripeptide, via a serine protease enzyme identified as tripeptidyl peptidase II (TPPII, EC 3.4.14.10).[5] Butabindide (**1**) has been reported as a potent inhibitor (7 nM) of TPPII and thus has been viewed as a useful agent to increase endogenous levels of CCK8. The biochemical inhibition of TPPII by **1** has reportedly translated to the desired hypothesized in vivo effects, i.e., mice dosed with **1** ate 50% less than saline-control-dosed animals over a 1 h period.[5]

From a chemistry perspective, we questioned the inherent stability of **1**. We were concerned that **1** might spontaneously cyclize to generate the six-membered diketopiperazine **2** with concurrent elimination of *n*-butylamine (Scheme 1). We therefore ran a simple stability study under mimicked physiological conditions, i.e., 3 mg of **1** was dissolved in 1 mL of pH 7.0 phosphate-buffered aqueous solution and warmed to 38 °C. Under these conditions, we found 28% of **1** had degraded to diketopiperazine **2** after 8 h. After 22 h, 57% of **1** had been converted to **2**.[9] As a follow-up to these findings, we confirmed the reported 7 nM inhibitory IC_{50} activity for **1** against the TPPII enzyme. Conversely, we found a purified sample of **2** void of any TPPII enzymatic activity ($IC_{50} > 10$ μM).

On the basis of these results, we embarked to design some bioisostere analogues of **1**. We envisioned that our analogues of **1** should possess greater inherent chemical stability than **1**. Concurrently we wanted our analogues to maintain very similar chemical characteristics as **1**, since the biological activity of **1** had been honed via combinatorial chemical variations. Following these guide-

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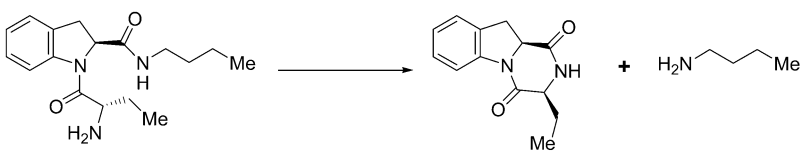
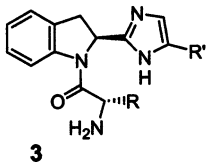
Scheme 1



10.1021/jm0202831 CCC: $22.00
Published on Web 10/26/2002

lines, we identified compounds of general structure **3** as potential preliminary targets.



3

3a R=Pr, R'=H (racemic)
3b R=Pr, R'=Me
3c R=Pr, R'=Et
3d R=Et, R'=Me

We visualized that the imidazole of **3** would mimic the amide of **1**, which was supported by previous reports of imidazole bioisostere equivalence for the amide moiety.[10] Consistent with our defined objectives, imidazoles **3** are devoid of **1**'s instability liability but maintain potentially desirable chemical features of **1**, even possessing both the H bonding donor and acceptor character of **1**'s amide group. These assumed chemical characteristic similarities between structures **1** and **3** were further validated empirically through some molecular modeling work. We began our molecular modeling work by constructing a homology model for the active site of TPPII, using the crystal structure of subtilisin as a prototypical protease template.[11] After the TPPII receptor was designed, **1** was docked into its designated active site. This docking revealed important insights with respect to the key molecular interactions between **1** and TPPII (Figures 1 and 2). Subsequent overlapping of **3** with **1** in the active site of the TPPII model confirmed that consistent binding interactions were maintained for both molecules relative to key TPPII protein residues (Figure 3). These molecular modeling results supported our initial, intuitive notion that minimal disruption to the character and conformation of **1** would occur by introduction of the imidazole moiety of analogues **3**.

On the basis of all the results, rationales, and ensuing conclusions discussed above, synthetic efforts were initiated toward imidazoles **3**. Following are described our initial experimental results related to the preparation and activities of **3**.

Chemistry

To initiate our studies discussed above, we required compounds **1** and **2** to evaluate their biological activities. **1** can be prepared as previously described[7] (Scheme 2). We altered from the reported preparation slightly, which proved to be a bit more expedient for larger scale preparation (Scheme 3). Ester **6**[12] was prepared from

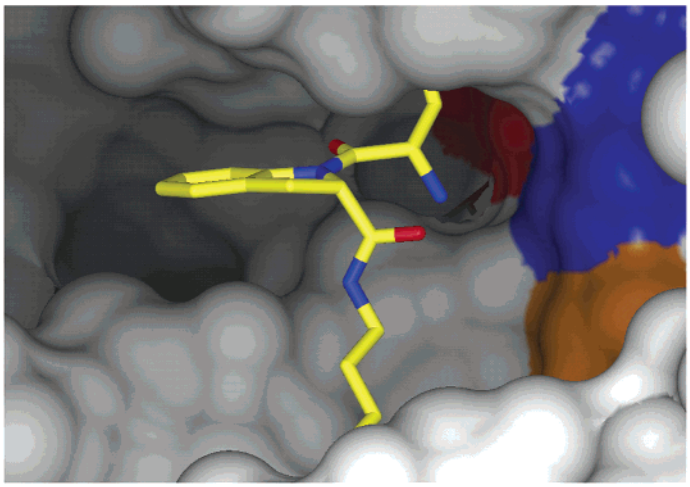


Figure 1. Putative binding mode of butabindide into a model of the active site of TPPII. The surface of the catalytic triad residues is color-coded according to the underlying residue: Asp (red), His (blue), and Ser (orange).

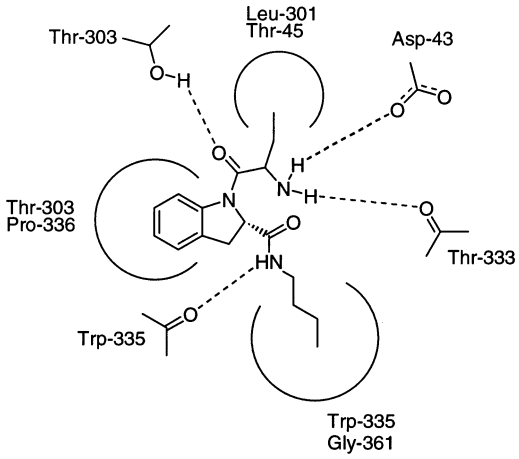


Figure 2. Analysis of the putative binding interactions between butabindide and TPPII based on the docking of the inhibitor into a model of the protein active site.

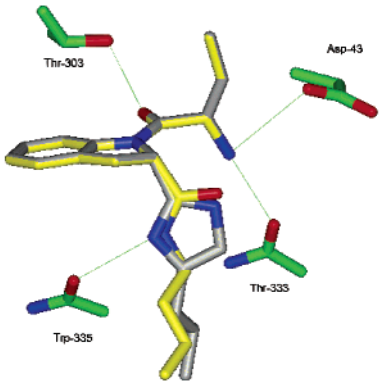
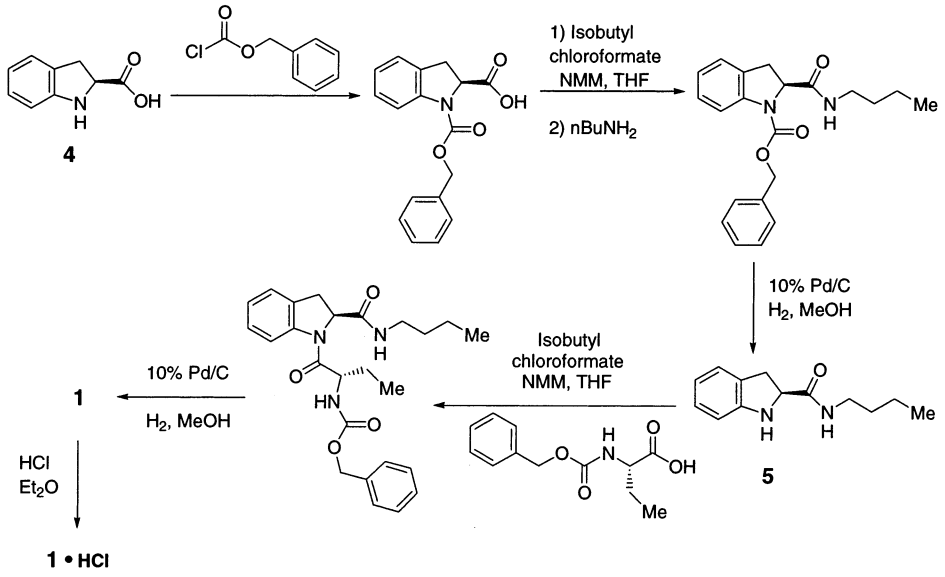


Figure 3. Comparison of the docked structure of **1** (yellow carbon atoms) with the imidazole mimic **3** (gray carbon atoms). A number of important residues are shown. Putative hydrogen bonds are indicated by thin green lines.

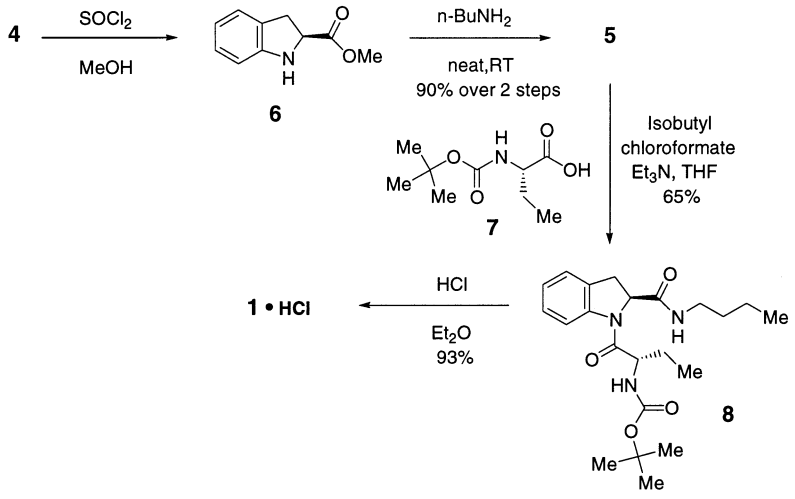
the commercially available *S*-indoline carboxylic acid **4**, which was then reacted neat with butylamine (*n*-$BuNH_2$) to yield amide **5** in good yield. Then **5** was reacted with the activated ester of *tert*-butoxycarbonyl (BOC) protected amino acid **7** to give **8**, which was subsequently deprotected with ethereal HCl. Pure **1** precipitated directly from this reaction mixture as its HCl salt. Simply warming **1**'s HCl salt neat at 165 °C for 30 min yielded a pure sample of **2**.

Synthesis of compounds **3** (Scheme 4) also began from commercially available **4**. Similar to the scheme employed for **1**, the initial reaction was conversion of **4** to **6**. Ester **6** was then treated with methanolic ammonia at room temperature to yield amide **9**[13] in respectable yield as previously reported. **9** was acylated with acetyl chloride (AcCl) in the presence of triethylamine (Et_3N) to give **10**. Nitrile **11** was efficiently generated following a literature dehydration procedure,[14] simply by adding trichloroacetyl chloride (Cl_3CCOCl) to a 0 °C mixture of primary amide **10** and Et_3N in dichloromethane (CH_2Cl_2). This procedure proved to be quite amenable to larger scale preparations. To this point in the synthetic scheme, no racemization had been observed, as determined by chiral high-pressure liquid chromatography (HPLC) analysis of crystallized **11**.[15] Imidate **12** was generated by the classical treatment of a nitrile with HCl and alcohol to yield the crystalline product. **12** proved to be hygroscopic and was either quickly used after filtering or maintained in an anhydrous environment. Generally **12** was immediately reacted with an aminoketone **13** (two steps)[16] to yield indoline-imidazoles **14**. Judging by chiral HPLC, it was determined that both crude **14a** and crude **14b** were 85:15 mixtures of enantiomers, implying that some isomerization had occurred in one of the two prior steps. Fortunately, trituration of crude **14a** with acetonitrile yielded optically pure material. Compound **14b** proved to be slightly less amenable to such purification but still yielded a reasonable quantity of enantiomerically pure material. The racemic material isolated from the synthesis of **14b** proved to be useful in pursuing product **3e**, as will be described below. **14** was deacetylated in refluxing 6 N HCl to give **15**. Generally, noramine **15** was used fairly quickly after generation because air oxidation to the indole proved to be somewhat problematic, as determined by HPLC/MS. Coupling of the appropriate noramine **15** with preformed BOC-amino acid fluoride **16b**[17] generated compounds **17b**, **17d**, and **17e**. Com-

Scheme 2



Scheme 3



pound **17c** was prepared by coupling noramine **15a** with **16c**. Compounds **17b**−**d** could be carried on in the sequence without further purification because they were prepared relatively cleanly from enantiomerically pure samples of **15a** or **15b** (≥99% ee). Compound **17e** required additional purification because it was prepared from racemic predecessors **14b** and **15b**. Using the racemic **15b** as a starting material in the amide coupling reaction with **16b** yielded a mixture of diastereomers **17d** and **17e** as coproducts. Fortunately preparative HPLC easily separated compound **17e** as a single compound from the previously identified **17d**, which had initially been generated from optically pure **15b**. All compounds **17** were subsequently deprotected easily with trifluoroacetic acid (TFA) to yield related desired targets **3b**−**e**.

Racemic compound **3a** was prepared in a similar manner as described above, utilizing racemic indoline **15a** as the starting material.

Results and Discussion

Experimental details related to TPPII enzyme isolation and subsequent determinations of TPPII inhibitory activities are described in the Experimental Section. The compiled biological results of TPPII inhibitory activities are outlined in Table 1.

For our initial biological experiment, we reproduced the reported TPPII inhibitory activity for **1** (7 nM). As mentioned in the Introduction, for our initial chemical experiments, we confirmed **1**'s inherent instability, then prepared a pure sample of **1**'s major degradation product, **2**, for follow-up biological evaluation. Our plan was to pursue one of two possible paths, dependent on the relative activity of **2**. If **2** had surprisingly proven to be an extremely potent inhibitor of TPPII, we would have prepared an array of diketopiperazines to explore the related structure−activity relationship (SAR) of **2**, concluding that **1**'s assumed activity was due to a small percentage of **2** generated in situ during testing. How-

Scheme 4

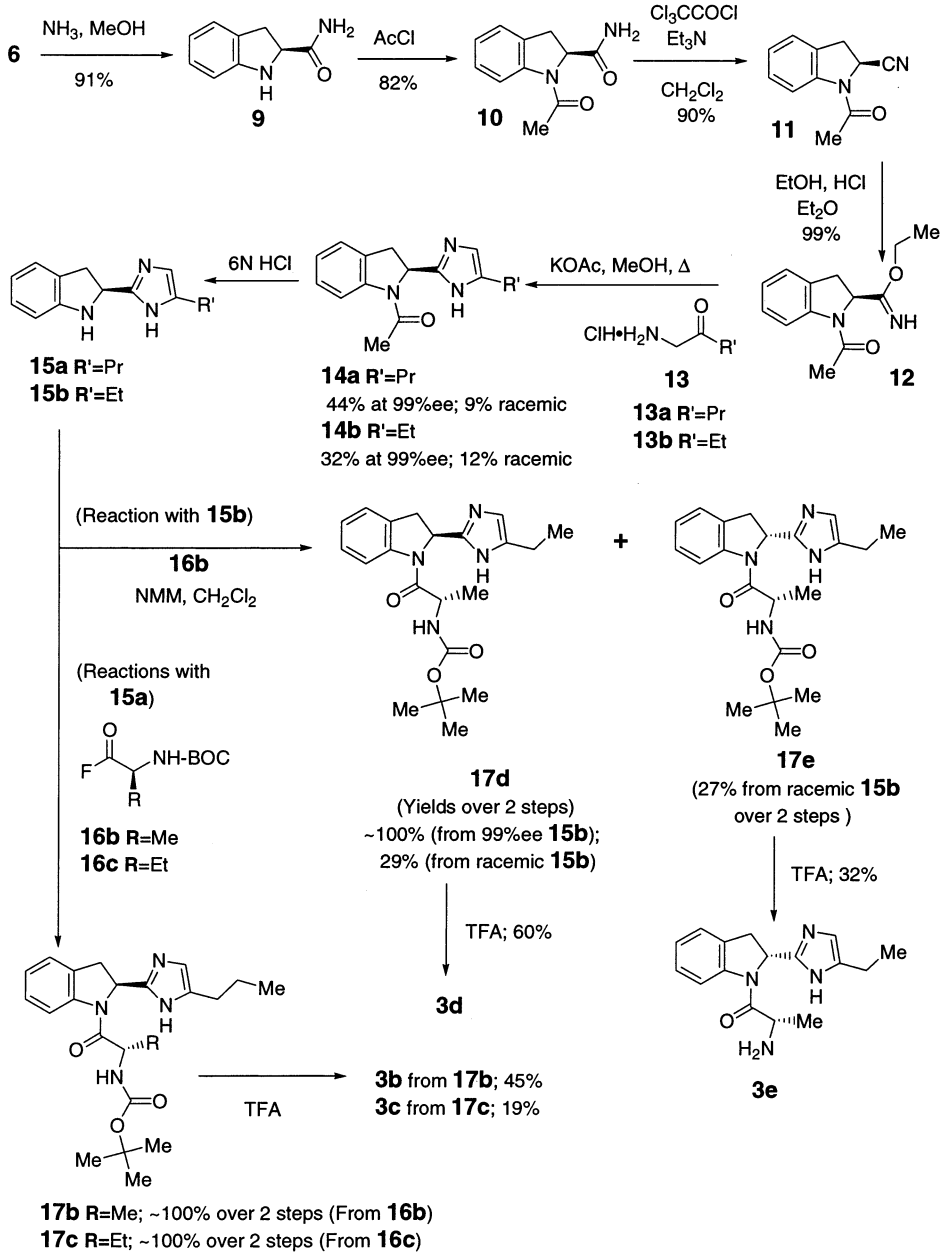


Table 1. Inhibition of TPPII Enzyme

compd	IC_{50} [a] (nM)	n [b]
1	7 ± 1	3
2	>10000	2
3a	36 ± 4	3
3b	6 ± 1	2
3c	4 ± 1	2
3d	23 ± 3	2
3e	>10000	1
14a	>10000	1

[a] IC_{50}'s from Sigma plot *C*/*R* curves. [b] Number of total Sigma plot *C*/*R* curves.

ever, finding **2** devoid of any desirable activity, we pursued the alternative path of designing and preparing bioisosteres of **1**, with the requirement that the bioisosteres be inherently more stable to the cyclization liability noted for **1**. Pleasingly we found that our imidazole bioisosteres **3** maintain good TPPII inhibitory activities. More specifically, compound **3c** (4 nM), the direct analogue of **1**, retains activity comparable to that of **1** (7 nM).

Our related SAR of other initial imidazoles prepared (**14a** and **3a**,**b**,**d**,**e**) is also reflective of the SAR previously described for **1**.[5] We found **14a** to be devoid of any desirable activity, supporting the conclusion of a need for the related basic center. Our data also support the SAR that some lipophilicity on the amino acid side chain R correlates with improved activity. This is demonstrated by comparing the single variable altered molecules **3a**, **3b**, and **3c** [**3c** (R = Et; 4 nM) ≥ **3b** (R = Me; 6 nM) > **3a** (R = H; racemic, 36 nM]. A similar conclusion that some lipophilicity is desirable at the R′ region of **3** for improved activity is supported by comparing molecule **3b** (R′ = Pr; 6 nM) with singly altered analogue **3d** (R′ = Et; 23 nM). Finally, comparison of compound **3d** (*S*,*S* stereochemistry, 23 nM) with its respective epimer **3e** (*R*,*S* stereochemistry, >10000 nM) clearly demonstrates the enzymatic stereospecificity requirement for good inhibitors.

Besides finding compounds at least as potent as **1**, our other initial criterion was that our compounds should prove to be inherently more stable than **1**. Since imidazoles **3** are devoid of the cyclization potential noted for **1**, it was not surprising to find that under the same mimicked physiological conditions where **1** degrades 57% over 22 h, compound **3c** remains 100% pure after the same time frame.

In conclusion, we have identified novel imidazoles **3** as extremely potent inhibitors of TPPII, and they possess greater inherent stability than the pharmacophore after which they were modeled, i.e., **1**. We are continuing further chemical and biological endeavors around TPPII inhibitors, and these results will be reported in subsequent publications.

Experimental Section

Compounds listed in Table 1 (**1**, **2**, **3a**−**e**, and **14a**) were all characterized by 360 MHz ^{1}H NMR (Bruker AM 360 WB), mass spectrometry (Finnegan 3300), and HPLC (>98% purity at 214 and 254 nm; Hewlett-Packard series 1050 HPLC with a 3 μm, 3.3 mm × 50 mm Supelco AZB+ C18 column, using a gradient mobile phase of 4:96:0.1 acetonitrile/water/TFA to 100:0:0.1 acetonitrile/water/TFA over 8.5 min at a flow rate of 1.20 mL/min and a total run time of 9.5 min). ^{1}H NMR of key intermediates was also run on the 360 MHz NMR spectrometer. The related ^{1}H NMR results for both intermediates and final products are included in the Experimental Section. Also, final products **3** and **2** were evaluated by high-resolution mass spectrometry (HRMS) analyses (Autospec E high-resolution magnetic sector mass spectrometer). These HRMS measurements were carried out internally by our spectrometry department in Spring House, PA. For an initial key intermediate (**14a**) and for a representative final product (**3d**), C, H, and N elemental analyses were carried out by our internal analytical research department in Beerse, Belgium, and were within the 0.4% limit values of the calculated percent values for C, H, and N. All final products were also assayed for homogeneity by thin-layer chromatography on Whatman MK6F (1 in. × 3 in. × 250 μm) silica gel plates. Melting points were determined on a Thomas-Hoover Unimelt capillary melting point apparatus and are uncorrected. All reagents were commercially available unless otherwise specified, and all reactions were run under an inert atmosphere of Ar or N_2 unless otherwise specified. Where required, preparative purifications were performed on a Gilson semipreparative HPLC unit (column, YMC ODS-A 30 mm × 100 mm, 5 μm; temperature, ambient; flow rate, 35 mL/min; mobile phase consisting of (A) 10:90 acetonitrile/ water with 0.1% trifluoroacetic acid or (B) 90:10 acetonitrile/ water with 0.1% TFA; gradient, linear gradient from mobile phase A to mobile phase B over 9 min; wavelength, 254 nm).

Molecular Modeling. A homology model of the active site of TPPII was calculated using Modeller, version 4e (Accelrys Inc., San Diego, CA), using the crystal structure of subtilisin as the template.[11] Docking of **1** was performed using the program Gold, version 1.1, with the standard default settings.[18] Visualization was done using the InsightII software of Accelrys Inc. (San Diego, CA).

[*S*-(*R,*R**)]-1-(2-Amino-1-oxobutyl]-*N*-butyl-2,3-dihydro-1*H*-indole-2-carboxamide Hydrochloride (1:1) (Butabindide, 1).** Ester **6**[12] (21.62 g, 0.122 mol) was added neat to *n*-$BuNH_2$ (18 g, 0.245 mol) at 10 °C, and then the neat reaction mixture was slowly warmed to room temperature. After 4 h, an additional portion of *n*-$BuNH_2$ (9 g, 0.122 mol) was added to the reaction mixture. After another 2 h, the reaction mixture was treated with hexane and the resulting solid was filtered, rinsed liberally with hexane, and then dried under reduced pressure to yield 24.13 g (90%) of **5** as a white solid.

Compound **7** (Sigma, 20.32 g, 0.1 mol) was dissolved in CH_2Cl_2 (500 mL), then cooled to 0 °C. Then Et_3N (14 mL, 0.1 mol) was added neat, followed by neat isobutyl chloroformate (12.9 mL, 0.1 mol). This mixture was stirred for 1.5 h at 0 °C, and then **5** was added neat. After 6 h, the reaction mixture was extracted sequentially with H_2O (200 mL), saturated aqueous $NaHCO_3$ (100 mL), 2 N citric acid (100 mL), saturated aqueous $NaHCO_3$ once again (100 mL), and then brine (100 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to yield 42.87 g of whitish solid. This material was triturated in ice-cold Et_2O, filtered, and rinsed with ice-cold Et_2O (150 mL). After it was air-dried, there remained 26.35 g (65%) of **8** as a white solid (TLC, 40% EtOAc/hexane, R_f = 0.35, trace impurity at R_f = 0.4; HPLC, 98.1%, one impurity).

Compound **8** was suspended in a 0 °C 1 N ethereal HCl solution (500 mL), and then gaseous HCl was bubbled in for 20 min while stirring the mixture. The original heterogeneous mix became homogeneous during the HCl addition. After the HCl addition, the reaction mixture was warmed to room temperature. Almost immediately a precipitate began to fall out of solution. After the mixture was stirred for an additional 3 h, the reaction mixture was recooled to 0 °C and then filtered and rinsed with ice-cold Et_2O (200 mL). The resulting solid was dried under high vacuum for 16 h to yield 19.51 g (93%) of desired **1** as a white solid hydrochloride (1:1): mp 140−145 °C (TLC, 80:20:5 $CHCl_3$/CH_3OH/HCOOH, R_f = 0.5, homogeneous; HPLC, 99%, one impurity); ^{1}H NMR (DMSO-d_6) δ 0.8−0.9 (t, 3H), 0.95−1.05 (t, 3H), 1.2−1.35 (m, 2H), 1.35−1.5 (m, 2H), 1.75−2.05 (m, 2H), 3.05−3.2 (bd, 3H), 3.4−3.7 (m, 3H), 5.1−5.2 (d, 1H), 7.05−7.15 (t, 1H), 7.2−7.4 (m, 2H), 8.05−8.15 (d, 1H), 8.5−8.65 (bs, 3H), 8.75−8.85 (t, 1H).

(3*S*,10a*S*)-Ethyl-2,3,10,10*a*-tetrahydropyrazino[1,2-*a*]-indole-1,4-dione (2). Compound **1**·HCl (0.076 g, 0.000 22 mol) was placed neat in an oil bath prewarmed to 140 °C and was stirred as the temperature of the bath was elevated slowly. After 15 min, the temperature of the oil bath had risen to 152 °C and bubbling of the starting material was noted. After an additional 15 min, the oil bath had reached a temperature of 168 °C and the reaction had become a homogeneous, stirring oil. After the mixture was stirred for an additional 15 min at 168 °C, heating was discontinued. After the mixture was cooled, the brown residue was triturated with Et_2O (10 mL) and filtered through Dicalite. The filtrate was treated with hexane (15 mL) and cooled to 0 °C. The resulting solid was filtered and rinsed three times with hexane (1 mL each). After it was air-dried, there remained 12 mg (23%) of **2** as a white solid: mp 169−170 °C (TLC, 80:20:5 $CHCl_3$/CH_3OH/HCOOH, $R_f = 0.75$; HPLC, 100%); ^{1}H NMR ($CDCl_3$) δ 1.05−1.15 (t, 3H), 1.90−2.05 (m, 1H), 2.10−2.20 (m, 1H), 3.35−3.45 (dd, 1H), 3.6−3.7 (dd, 1H), 4.1−4.2 (t, 1H), 4.75−4.85 (t, 1H), 5.8−5.9 (bs, 1H), 7.05−7.15 (t, 1H), 7.25−7.3 (m, 2H), 8.1−8.15 (d, 1H). HRMS calcd for $C_{13}H_{14}N_2O_2$: 230.1056. Found: 230.1056.

(*S*)-1-Acetyl-2,3-dihydro-1*H*-indole-2-carboxamide (10). (*S*)-2,3-Dihydro-1*H*-indole-2-carboxamide[13] (**9**) (4.86 g, 0.030 mol) was suspended in trichloromethane ($CHCl_3$) (400 mL). The mixture was cooled to 0 °C. Et_3N (6.26 mL, 0.045 mol) was added neat followed by neat AcCl (3.21 mL, 0.045 mol), which was added dropwise over 2 min. After 30 min, TLC (80:20:5 $CHCl_3$/MeOH/HCOOH) showed that the reaction was incomplete. Maintaining the reaction mixture at 0 °C, more Et_3N (6.26 mL) was added, followed 15 min later with dropwise addition of AcCl (3.21 mL). TLC showed the reaction was still incomplete (∼80%). Therefore, third portions of Et_3N (6.26 mL) and AcCl (3.21 mL) were added. After an additional 15 min, ice-cold water (200 mL) was added. The mixture was stirred for 10 min, and the solid was filtered and rinsed with water (3 × 100 mL) and $CHCl_3$ (2 × 75 mL). The sample was allowed to dry overnight, yielding 4.71 g (77%) of **10** as a white solid: mp >260 °C (TLC, 80:20:5 $CHCl_3$/CH_3OH/HCOOH, R_f = 0.60); ^{1}H NMR (DMSO-d_6) δ 2.05 (s, 3H), 2.95−3.05 (d, 1H), 3.55−3.65 (dd, 1H), 4.85−4.95 (dd, 1H), 6.9−7.0 (t, 1H), 7.1−7.25 (m, 2H), 7.25−7.35 (bs, 1H), 7.7−7.8 (bs, 1H), 8.0−8.1 (d, 1H).

(*S*)-1-Acetyl-2,3-dihydro-1*H*-indole-2-carbonitrile (11). **10** (4.13 g, 0.020 mol) was suspended in CH_2Cl_2 (175 mL). The mixture was cooled to 0 °C, and Et_3N (8.44 mL, 0.061 mol) was added neat. Cl_3CCOCl (3.39 mL, 0.030 mol) in CH_2Cl_2 (20 mL) was added dropwise over 20 min. After 2 h, ice/water (200 mL) was added, the phases were separated, and the organic phase was reextracted with 3 N HCl and then with a saturated aqueous $NaHCO_3$ solution. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to yield 4.61 g of brown solid. The solid was triturated with ice-cold Et_2O (30 mL) and filtered and the resulting solid was rinsed with ice-cold Et_2O (twice), yielding 3.12 g (83%) of **11** as a tan solid: mp 134−135 °C (TLC, 80:20:5 $CHCl_3$/CH_3OH/HCOOH, R_f = 0.85); ^{1}H NMR ($CDCl_3$) δ 2.35−2.5 (bs, 3H), 3.35−3.75 (bm, 2H), 5.05−5.2 (bd, 0.5H), 5.35−5.5 (bd, 0.5H), 7.05−7.15 (t, 1H), 7.15−7.35 (m, 2.5H), 8.1−8.2 (bd, 0.5H).

(*S*)-Ethyl 1-acetyl-2,3-dihydro-1*H*-indole-2-carboximidate Monohydrochloride (12). Compound **11** (2.81 g, 0.0151 mol) was suspended in Et_2O (200 mL). Ethanol (0.97 g, 0.0214 mol) was added, and the mixture was cooled to 0 °C. HCl (gas) was bubbled in for 45 min. The mixture was removed from the ice bath and stirred. After 20 min, a residue began to collect on the sides of the flask. The residue was scratched with a spatula, and a white solid precipitated out. After 1 h, the sample was filtered, rinsed with Et_2O, and air-dried quickly, yielding 3.99 g (99%) of **12**. Compound **12** was immediately placed under Ar and was generally used for subsequent reactions within the same working day (TLC, 80:20:5 $CHCl_3$/CH_3OH/HCOOH, R_f = 0.80).

(*S*)-1-Acetyl-2,3-dihydro-2-(4-propyl-1*H*-imidazol-2-yl)-1*H*-indole (14a). Intermediate **12** (0.047 mol) in methanol (MeOH) (200 mL) was treated with potassium acetate (KOAc) (18.45 g, 0.188 mol). The mixture was heated to reflux under argon. To this was slowly added a solution of **13a**[16] (12.93 g, 0.094 mol) in MeOH (95 mL) over 45 min. After the addition was complete, the mixture was allowed to stir overnight at reflux, then concentrated. The concentrate was taken up in CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The aqueous layer was extracted with a second portion of CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated to an oily residue. The residue was triturated with Et_2O, and the resulting solid was filtered. This filtered solid (1.13 g, 9%) proved to be a racemic mixture of desired **14a**, as determined by chiral column analysis.[15] The resulting filtrate from this Et_2O trituration was concentrated under reduced pressure, and the resulting residue was triturated with ice-cold CH_3CN. The resulting solid was filtered to yield 4.97 g of product as a white solid. This material was further triturated in Et_2O to yield 4.67 g of pure product **14a** as a white solid. The combined filtrates from the latter two triturations were combined, concentrated under reduced pressure, and purified by flash silica gel column chromatography to yield an additional 0.83 of **14a**. The combined return was 5.53 g (44%) of desired **14a** as a white solid: mp 174−175°C (TLC, 10% CH_3OH in CH_2Cl_2, R_f = 0.42, homogeneous); chiral LC analysis,[15] 99% ee; ^{1}H NMR (DMSO-d_6) δ 0.8−0.9 (t, 3H), 1.45−1.55 (m, 2H), 1.95−2.1 (bs, 3H), 2.35−2.45 (t, 3H), 3.05−3.2 (d, 2H), 3.55−3.7 (t, 3H), 5.5−5.6 (d, 2H), 6.55−6.65 (bs, 1H), 6.95−7.05 (t, 1H), 7.1−7.25 (m, 2H), 7.95−8.1 (bs, 1H), 11.6−11.8 (bs, 1H). Anal. Calcd for $C_{16}H_{19}N_3O'$: C, 71.35; H, 7.11; N, 15.60. Found: C, 71.38; H, 6.92; N, 15.61.

(*S*)-1-Acetyl-2,3-dihydro-2-(4-ethyl-1*H*-imidazol-2-yl)-1*H*-indole (14b). 14b was prepared in a similar manner as **14a**, with the exception that **13b** was used in place of **13a**. Workup varied somewhat from that for **14a**. The following details are based on a 0.015 mol scale of **12**. After the original reaction mixture was concentrated, the residue was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. The aqueous phase was washed with a second portion of CH_2Cl_2 and the organic phases were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure, yielding a yellow oil. This material was triturated with Et_2O (40 mL) to yield a solid precipitate. The solid was filtered and rinsed with ice-cold Et_2O to yield 0.46 g of white solid. This filtered solid proved to be the racemic mixture of desired **14b** (12%), as determined by chiral column analysis.[15] The resulting Et_2O filtrates were combined and concentrated under reduced pressure to give 2.66 g of yellow tinted solid. This material was triturated in Et_2O (20 mL) to give an additional portion of solid. This solid was filtered and rinsed with Et_2O twice to yield 0.89 g of desired **14b** as a white solid: mp 136−139 °C (TLC, 80:20:5 $CHCl_3$/CH_3OH/HCOOH, R_f = 0.7, homogeneous; chiral LC analysis,[15] 99% ee). The resulting filtrate was again concentrated to yield 1.56 g of residue, which was purified by preparative HPLC to yield an additional 0.34 g of **14b**. The total return of optically pure **14b** was 1.23 g (32%): ^{1}H NMR (DMSO-d_6) δ 1.05−1.15 (bs, 3H), 1.95−2.1 (bs, 3H), 2.3−2.5 (bs, 2H), 3.1−3.2 (d, 1H), 3.55−3.65 (t, 1H), 5.55−5.65 (d, 1H), 6.4−6.5 (bs, 0.5H), 6.65−6.75 (bs, 0.5H), 6.95−7.05 (t, 1H), 7.15−7.3 (m, 2H), 7.95−8.05 (d, 1H).

(*S*)-2,3-Dihydro-2-(4-propyl-1*H*-imidazol-2-yl)-1*H*-indole (15a). Compound **14a** (0.46 g, 0.001 70 mol) was combined with aqueous HCl (6 N aqueous, 20 mL) and immediately warmed in a 100 °C oil bath under a nitrogen atmosphere. After 3.5 h, the heat was removed and the sample was cooled to 0 °C. Then 3 N NaOH (35 mL) was slowly added. Basification to neutral pH was completed with saturated aqueous $NaHCO_3$. The aqueous solution was then extracted with $CHCl_3$ (2 × 40 mL). The organic extracts were combined, dried over Na_2SO_4, and filtered, and the resulting solution of **15a** was used for subsequent reactions without further purification (TLC, 80:20:5 $CHCl_3$/CH_3OH/HCOOH, R_f = 0.55).

(*S*)-2,3-Dihydro-2-(4-ethyl-1*H*-imidazol-2-yl)-1*H*-indole (15b). Compound **15b** was prepared in a similar manner as **15a** and was also used for subsequent reactions without

further purification (TLC, 80:20:5 $CHCl_3/CH_3OH/HCOOH$, R_f = 0.65).

(*S*)-1,1-Dimethylethyl [1-(fluorocarbonyl)propyl]carbamate (16c). (*S*)-2-(*tert*-butoxycarbonylamino)butyric acid (2.03 g, 0.010 mol) dissolved in CH_2Cl_2 (25 mL) was placed in a cooling bath at −10 °C. While the reaction mixture was being stirred, pyridine (0.77 g, 0.010 mol) was added neat, followed by 2,4,6-trifluoro-1,3,5-triazine (4.66 g, 0.0345 mol) neat. After the mixture was stirred for 1 h, ice-cold water (75 mL) was added. More CH_2Cl_2 (45 mL) was added, and the mixture was shaken. The organic phase was separated and washed with ice-cold water again (100 mL), and then the organic phase was dried over $MgSO_4$, filtered, and concentrated to yield 2.29 g (total weight (TW) = 2.05 g) of crude **16c**, which was used without further purification.

{2-Oxo-2-[2-(4-propyl-1*H*-imidazol-2-yl)-2,3-dihydroindol-1-yl]ethyl}carbamic Acid *tert*-Butyl Ester (17a). Fluorocarbonylmethylcarbamic acid *tert*-butyl ester[17] (**16a**) was reacted with racemic **15a** in the typical manner as described for intermediate **17b**. The crude **17a** (51% over two steps) was used in the subsequent step without further purification (TLC, 90:9:1 $CHCl_3/CH_3OH/NH_4OH$, R_f = 0.22).

[2*S*-[1(*R),*R**]]-{1-Methyl-2-oxo-2-[2-(4-propyl-1*H*-imidazol-2-yl)-2,3-dihydroindol-1-yl]ethyl}carbamic Acid *tert*-Butyl Ester (17b).** *N*-Methylmorpholine (NMM, 0.17 g, 0.0017 mol) was added to a 0 °C solution of intermediate **15a** (0.39 g, 0.0017 mol) in $CHCl_3$ (75 mL). Then intermediate **16b**[17] (0.33 g, 0.0017 mol) was added neat. After 0.5 h, the reaction mixture was washed with water (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and then brine (50 mL), dried over Na_2SO_4, filtered, and concentrated, yielding 0.77 g (TW = 0.68 g over two steps) of **17b** as an oil, which was used without further purification (TLC, 80:20:5 $CHCl_3$/MeOH/HCOOH, R_f = 0.75).

[2*S*-[1(*R),*R**]]-{1-[2-(4-Propyl-1*H*-imidazol-2-yl)-2,3-dihydroindole-1-carbonyl]propyl}carbamic Acid *tert*-Butyl Ester (17c).** Starting from intermediate **16c**, compound **17c** was prepared in a similar manner as **17b** and was also used for the subsequent reaction without further purification, with the crude yield proving to be ∼100% over two steps (TLC, 80:20:5 $CHCl_3$:MeOH:HCOOH, R_f = 0.8).

[2*S*-[1(*R),*R**]]-{1-Methyl-2-oxo-2-[2-(4-ethyl-1*H*-imidazol-2-yl)-2,3-dihydroindol-1-yl]ethyl}carbamic Acid *tert*-Butyl Ester (17d).** Starting from intermediate **15b**, compound **17d** was prepared in a similar manner as **17b** and was also used for the subsequent reaction without further purification, with the crude yield proving to be ∼100% over two steps (TLC, 80:20:5 $CHCl_3$/MeOH/HCOOH, R_f = 0.7).

[2*R*-[1(*S),*R**]]-{1-Methyl-2-oxo-2-[2-(4-ethyl-1*H*-imidazol-2-yl)-2,3-dihydroindol-1-yl]ethyl}carbamic Acid *tert*-Butyl Ester (17e).** Compound **17e** was prepared in a similar manner as **17b**, except the starting material **15b** used for this reaction was racemic; i.e., it contained 50% of the *R* enantiomer. Also, after the typical workup, the crude product was purified by preparative HPLC to separate **17e** from **17d**. Compound **17e** (27%) eluted in the front run, with an additional sample of **17d** (29%) eluting in the latter fractions. The resulting **17e**, after lyophylization, was used for the subsequent reaction without further purification (TLC for **17e** same as for **17d**, 80:20:5 $CHCl_3$/MeOH/HCOOH, R_f = 0.7).

2-Amino-1-[2-(4-propyl-1*H*-imidazol-2-yl)-2,3-dihydroindol-1-yl]ethanone (3a). TFA (10 mL) was cooled to 0°C and added to **17a** (0.40 g, 0.0010 mol) with stirring. After 15 min, the excess TFA was removed under reduced pressure. The residue was partitioned between aqueous $NaHCO_3$ and $CHCl_3$. The $CHCl_3$ organic phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was triturated in CH_3CN to yield a white solid, which was filtered and rinsed with a small amount of ice-cold CH_3CN to yield 0.137 g (48%) of **3a** as a white solid: mp 168−170 °C (TLC, 90:9:1 $CHCl_3/CH_3OH/NH_4OH$, R_f = 0.37, homogeneous; HPLC, 98.6%, one impurity); ^{1}H NMR (DMSO-d_6) δ 0.85−0.9 (t, 3H), 1.45−1.6 (m, 2H), 2.35−2.45 (t, 2H), 3.1−3.2 (bd, 2H), 3.5−3.7 (m, 2H), 5.55−5.65 (d, 1H), 6.55−6.65 (bs, 1H), 7.0−7.1 (t, 1H), 7.15−7.3 (m, 2H), 8.0−8.2 (bs, 1H). HRMS calcd for $C_{16}H_{20}N_4O$ (MH^+): 285.1715. Found: 285.1722.

[2*S*-[1(*R),*R**]]-α-Methyl-2,3-dihydro-*β*-oxo-2-(4-propyl-1*H*-imidazol-2-yl)-1*H*-indole-1-ethanamine (3b).** TFA (4 mL) was cooled to 0 °C and added to **17b** (0.68 g, 0.0017 mol) with stirring. After 15 min, the excess TFA was removed under reduced pressure. The residue was dissolved in water (12 mL) and extracted with Et_2O (2 × 15 mL). The aqueous phase was basified with saturated aqueous $NaHCO_3$ and then reextracted with Et_2O (5 mL), then with $CHCl_3$ (2 × 40 mL). The $CHCl_3$ organic phases were combined, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to leave 0.51 g of clear oil. Hexane (35 mL) was added, and the mixture was held overnight. The material was then scratched, and the resulting solid was filtered and rinsed (ice-cold hexane, 3 × 2 mL). After it was air-dried, there remained 0.237 g (45%) of **3b** as a white solid: mp 68−69 °C (TLC, 80:20:5 $CHCl_3/CH_3OH/HCOOH$, R_f = 0.3, homogeneous; HPLC, 100%); ^{1}H NMR ($CDCl_3$) δ 0.85−0.9 (t, 3H), 1.15−1.2 (d, 3H), 1.45−1.55 (m, 2H), 2.35−2.45 (t, 2H), 3.3−3.4 (m, 3H; 2H exchangeable on treatment with D_2O; post D_2O treatment, d, 1H), 3.55−3.7 (m, 2H), 5.65−5.75 (d, 1H), 6.55−6.65 (bs, 1H), 6.95−7.05 (t, 1H), 7.1−7.2 (t, 1H), 7.2−7.3 (d, 1H), 8.05−8.2 (bs, 1H). HRMS calcd for $C_{17}H_{22}N_4O$ (MH^+): 299.1872. Found: 299.1881.

[2*S*-[1(*R),*R**]]-α-Ethyl-2,3-dihydro-*β*-oxo-2-(4-propyl-1*H*-imidazol-2-yl)-1*H*-indole-1-ethanamine Hydrochloride (3c).** Compound **17c** (1.24 g, 0.003 mol) and TFA (4 mL), both precooled in an ice bath, were combined. After 10 min, the mixture was concentrated. The concentrate was dissolved in water and extracted twice with Et_2O. The aqueous layer was separated and basified with saturated $NaHCO_3$. The resulting basic aqueous solution was then extracted twice with $CHCl_3$. The combined $CHCl_3$ organic extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was flash-chromatographed on a silica gel column, eluting with 25:1 $CHCl_3$/MeOH. The desired fractions were combined and concentrated under reduced pressure to yield 0.33 g of clear oil. This material was dissolved in Et_2O (10 mL) and treated with 3 mL of 1 M HCl in Et_2O. The precipitate was filtered, rinsed with Et_2O, and dried under vacuum to yield 0.24 g (19%) of desired product **3c** as a white solid: mp >200°C (TLC, 80:20:5 $CHCl_3/CH_3OH/HCOOH$, R_f = 0.5, homogeneous; HPLC, 99.4%, one impurity). NMR of **3c**'s free base, as the **3c** salt itself, yielded a noninterpretable spectra containing multiple rotamers with poor resolution. ^{1}H NMR of free base **3c** (DMSO-d_6): δ 0.85−0.95 (m, 6H), 1.4−1.55 (m, 3H), 1.65−1.75 (m, 1H), 2.35−2.45 (t, 2H), 3.35−3.45 (m, 3H; 2H exchangeable on treatment with D_2O; post D_2O treatment, d, 1H), 3.45−3.55 (bt, 1H), 3.55−3.7 (m, 1H), 5.65−5.75 (d, 1H), 6.5−6.6 (bs, 1H), 7−7.1 (t, 1H), 7.15−7.25 (t, 1H), 7.25−7.3 (d, 1H), 8.1−8.2 (bd, 1H). HRMS calcd for $C_{18}H_{24}N_4O$ (MH^+): 313.2028. Found: 313.2014.

[2*S*-[1(*R),*R**]]-α-Methyl-2,3-dihydro-*β*-oxo-2-(4-ethyl-1*H*-imidazol-2-yl)-1*H*-indole-1-ethanamine (3d).** Starting from **17d**, **3d** was prepared and worked up in a similar manner as was **3b** to yield 60% of **3d** as a white solid: mp 116−118 °C (TLC, 80:20:5 $CHCl_3/CH_3OH.HCOOH$, R_f = 0.25, homogeneous; HPLC, 99.3%, one impurity); ^{1}H NMR (DMSO-d_6) δ 1.1−1.15 (t, 3H), 1.2−1.25 (d, 3H), 1.45−1.55 (m, 2H), 2.4−2.5 (t, 2H), 3.35−3.45 (m, 3H; 2H exchangeable on treatment with D_2O; post D_2O treatment, d, 1H), 3.6−3.75 (m, 2H), 5.7−5.75 (d, 1H), 6.55−6.65 (bs, 1H), 7.0−7.1 (t, 1H), 7.2−7.25 (t, 1H), 7.25−7.3 (d, 1H), 8.1−8.25 (bs, 1H). Anal. Calcd for $C_{16}H_{20}N_4O)'$: C, 67.58; H, 7.09; N, 19.70. Found: C, 67.31; H, 6.90; N, 19.54.

[2*R*-[1(*S),*R**]]-α-Methyl-2,3-dihydro-*β*-oxo-2-(4-ethyl-1*H*-imidazol-2-yl)-1*H*-indole-1-ethanamine (3e).** Starting from **17e**, **3e** was prepared and worked up in a similar manner as was **3b** to yield 32% of **3e** as a white solid: mp 73−74 °C (TLC, 80:20:5 $CHCl_3/CH_3OH/HCOOH$, R_f = 0.3, homogeneous; HPLC, 99.8%, one impurity); ^{1}H NMR (DMSO-d_6) δ 0.7−0.85 (bs, 3H), 1.05−1.15 (t, 3H), 1.85−2.0 (bs, 2H, both exchangeable on treatment with D_2O), 2.35−2.45 (m, 1H), 2.45−2.55 (m, 1H), 3.05−3.2 (d, 1H), 3.5−3.7 (m, 2H), 5.8−5.9 (bd, 1H),

6.5 (s, 0.5H), 6.7 (s, 0.5H), 7.0−7.1 (t, 1H), 7.2−7.3 (m, 2H), 8.05−8.2 (bs, 1H), 11.65−11.9 (bd, 1H, exchangeable on treatment with D_2O). HRMS calcd for $C_{16}H_{20}N_4O$ (MH^+): 285.1715. Found: 285.1723.

Tripeptidyl Peptidase II (TPPII) Enzyme Isolation and Related Determination of Inhibitory Activity. TPPII enzyme was purified from a rat liver postlysosomal fraction using DEAE ion exchange chromatography followed by an S-200 size exlusion column and hydroxyl apatite chromatography.[19] To the protein that eluted at 50 mM potassium phosphate, glycerol was added to a final concentration of 30% and the protein was stored at −70 °C.

TPPII activity was evaluated at room temperature using 100 μM H-Ala-Ala-Phe-AMC as the substrate in an assay buffer of pH 7.5 consisting of 50 mM KH_2PO_4, 1 mM EGTA, 1 mM DTT, 100 μM Bestatin, and 100 nM Thiorphan. Inhibitors were added at a final DMSO concentration of 1%. Assay incubations were measured in the fluoroscan (Labsystems) at 405 nm (excitation at 355 nm) in the linear part of the reaction (less than 15% of hydrolysis).[5] IC_{50} values were determined using Sigma plot *C/R* curves.

Acknowledgment. We appreciate the assistance of William Jones for obtaining high-resolution mass spectral data.

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JM0202831