

2-Nitrophenylcarbamoyl-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-N-benzyl-N-methylamide (SDZ NKT 343), a Potent Human NK₁ Tachykinin Receptor Antagonist with Good Oral Analgesic Activity in Chronic Pain Models

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A lead compound which had sub-micromolar affinity for the rabbit NK₁ receptor but negligible affinity for rat NK₁ receptors, **3a**, was discovered by directed screening. 2-Substitution in the ring of the benzylthiourea substituent in the initial lead was found to be important, and halogens (Cl, Br) in this position were found to improve affinity for the human receptor. The activity of a series of 2-halo-substituted benzylthioureas was then optimized by modification of the proline diphenylmethyl amide, guided by a simple conceptual model based on structural overlay between these early antagonists and NK₁ selective peptides. In this way, aromatic amino acid amides were identified which had improved affinity with respect to the starting diphenylmethyl (DPM) amides. The first sub-nanomolar ligand for the human NK₁ receptor which arose from this series, **4af**, combined a 2-chlorobenzylthiourea unit with a 2-naphthylalanine amide. Contemporaneously it was discovered that the benzylthiourea unit could be simplified to a phenylthiourea providing that an appropriate 2-substituent was also incorporated. Combination of these two series gave 2-NO₂ phenylthiourea analogues which led directly to the analogous urea, **5f** (2-nitrophenylcarbamoyl-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-N-benzyl-N-methylamide, SDZ NKT 343), a highly potent ligand for the human NK₁ receptor ($K_i = 0.16$ nM). In addition to its high in vitro potency, **5f** proved to be a potent orally active analgesic in guinea pig models of chronic inflammatory and neuropathic pain. The nature of the 2-aryl substituent was found to be critical for oral activity in this series. Clinical evaluation of **5f** as a novel analgesic agent is currently underway.

Introduction

The tachykinins are a family of neuropeptides which include substance P, neurokinin A, and neurokinin B (SP, NKA, and NKB). These peptides exert their effects through three G-protein-coupled receptor subtypes, the NK₁, NK₂, and NK₃ receptors.¹ SP is the preferred agonist at NK₁ receptors.²

Several non-peptide antagonists at NK₁ receptors, mostly the products of screening and optimization programs, have been described in recent years^{3–10} and some are currently in clinical development.

The development of such agents as novel analgesics is based on recent evidence that NK₁ tachykinin receptors are instrumental in the development and maintenance of the central component of pain and hyperalgesia. Substance P is an important mediator in nociceptive pathways.¹¹ NK₁ receptor antagonists produce only a weak inhibition of acute nociceptive responses in animal models, suggesting that under normal physiological conditions SP is less important than other excitatory transmitters in this pathway. In models of pathological

pain (particularly those involving inflammatory hyperalgesia), however, NK₁ receptors become increasingly important.¹² The role of the NK₁ receptor in neuropathic pain is less clear, but recent evidence in animal models suggests that NK₁ antagonists might be effective.^{13,14} One published report suggests that NK₁ antagonists may show clinical efficacy in certain pain states.¹⁵

Besides pain, the main indications foreseen for these antagonists are emesis, migraine, inflammation, and chronic bronchitis.^{16–18}

Chemistry

The benzyl- and phenylthioureas and their derivatives were prepared by reaction of the central intermediates **2** with a variety of reagents outlined in Scheme 1 by routes A–G. The intermediates **2** were prepared by reaction of an N-protected amino acid with the amines **1** using a variety of standard coupling agents, e.g., mixed anhydride method or DCCI and then deprotection, e.g., with HCl/dioxan. The amines **1** were either commercially available or were amino acid derivatives which were synthesized by reaction of N-protected amino acids with amines using coupling agents, e.g., mixed anhydride method, by analogy with the preparation of intermediates **2**, deprotection, and then isolation of the free base.

Since the intermediates, **2**, were prepared by standard methods, their preparation is described in the Experi-

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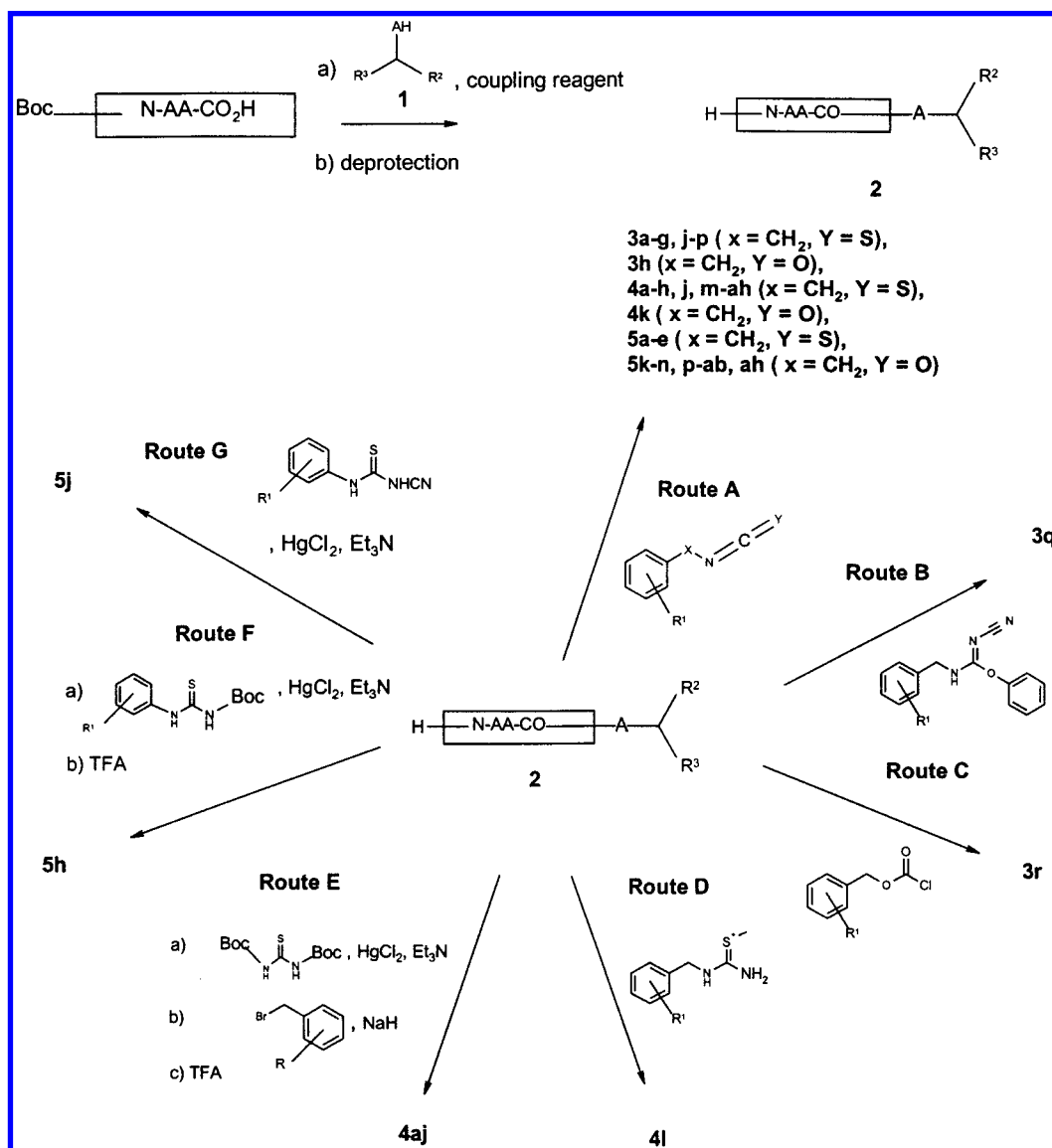
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Scheme 1

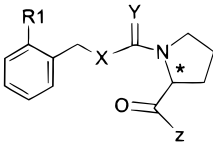


mental Section as exemplified by one example of the class. Full experimental details for the preparation of the individual intermediates is included as Supporting Information. Likewise the repetitive procedures, e.g., the formation of thioureas and ureas from these intermediates, are exemplified by a single example where the synthesis is described in detail and subsequent compounds of the same class have been presented in tabular format with full details included in the Supporting Information.

3g ($\text{R}^1 = \text{NH}_2$, Table 1) was prepared by catalytic reduction of **3f** ($\text{R}^1 = \text{NO}_2$) using H_2/Pd on C. **5ac** ($\text{R} = \text{Ph-CO}_2\text{H}$, Table 4) was prepared by saponification of **5ab** ($\text{R} = \text{Ph-CO}_2\text{CH}_3$) using potassium hydroxide in methanol. The acid **5ac** was converted to the mixed anhydride, under standard conditions, using isobutyl chloroformate for **5ad**, **5ae**, and **5af** ($\text{R} = \text{Ph-2-CO}_2\text{NH}_2$, $\text{Ph-3-CO}_2\text{NH}_2$, $\text{Ph-2-CO}_2\text{NHCH}_3$, Table 4) and then cleavage with ammonia and methylamine as required. The hindered anhydride formed from isopropyl chloroformate was required to suppress opening of the inappropriate anhydride carbonyl when reacted with dimethylamine, furnishing **5ag** ($\text{Ph-2-CO}_2\text{N}(\text{CH}_3)_2$, Table 4). **5aj** and **5ak** ($\text{Ph-2-CH}_2\text{OH}$ and Ph-2-OH , Table 4) were

prepared by route A using the appropriate O-TBDMS-protected isocyanate and subsequent deprotection of the intermediate urea using TBAF in THF. **5al** ($\text{R} = \text{Ph-2-NH}_2$, Table 4) was prepared by catalytic reduction of **5f** ($\text{R}^1 = \text{Ph-2-NO}_2$) using H_2/Pd on C. **5am** ($\text{R} = \text{Ph-2-NHCOCH}_3$, Table 4) was prepared by acetylation of **5al** using acetic anhydride.

The benzyl *N*-cyanoguanidine compound **3q** ($\text{X} = \text{NH}$, $\text{Y} = \text{NCN}$, Table 1) was prepared by reaction of **2** with the appropriately ring-substituted *N*-cyano-*O*-phenylisourea (route B). The benzyl carbamate, **3r** ($\text{X} = \text{O}$, $\text{Y} = \text{O}$, Table 1), and guanidine, **4l** ($\text{Y} = \text{NH}$, Table 2), were prepared by reaction of **2** with the appropriate chloroformate and *N*-benzyl-*S*-methylthiuronium salt (routes C and D respectively). The guanidines **4aj** ($\text{Y} = \text{NH}$, $\text{R}^1 = \text{Cl}$, Table 2) and **5h** ($\text{R}^1 = \text{NO}_2\text{Ph}$, $\text{Y} = \text{NH}$, Table 3) were prepared by a novel route^{19,20} using HgCl_2 coupling of **2** with protected thioureas. In the former case the bis-protected guanidine product of the coupling reaction was deprotonated using NaH and then alkylated with the appropriately ring-substituted benzyl bromide (route E), whereas the latter compound was prepared by direct coupling with a ring-substituted *N*-phenyl-*N*-Boc-thiourea intermediate (route F). The

Table 1. Effect on Rabbit NK₁ Receptor Binding Affinity of Simple Structural Modifications to **3a**


compd no.	R ¹	X	Y	*	Z	rabbit NK ₁ receptor binding <i>K_i</i> , μM, mean ± SEM (<i>n</i> ≥ 3) (IC ₅₀ , μM, <i>n</i> = 2)
3a	OMe	NH	S	<i>S</i>	NHCH(Ph) ₂	0.33 ± 0.11
3b	H	NH	S	<i>S</i>	NHCH(Ph) ₂	(> 5)
3c	Cl	NH	S	<i>S</i>	NHCH(Ph) ₂	0.17 ± 0.06
3d	F	NH	S	<i>S</i>	NHCH(Ph) ₂	1.27 ± 0.53
3e	Br	NH	S	<i>S</i>	NHCH(Ph) ₂	0.17 ± 0.08
3f	NO ₂	NH	S	<i>S</i>	NHCH(Ph) ₂	1.56 ± 0.61
3g	NH ₂	NH	S	<i>S</i>	NHCH(Ph) ₂	(> 50)
3h	OMe	NH	O	<i>S</i>	NHCH(Ph) ₂	(> 50)
3j	OMe	NH	S	<i>R</i>	NHCH(Ph) ₂	(> 5)
3k	OMe	NH	S	<i>S</i>	NHCH(CH ₂ Ph) ₂	(> 50)
3l	OMe	NH	S	<i>S</i>	NHCH ₂ CH(Ph) ₂	(> 5)
3m	OMe	NH	S	<i>S</i>	N(CH ₂ Ph) ₂	(> 50)
3n	OMe	NH	S	<i>S</i>	NHCH(CH ₂ Ph)CON(CH ₃)CH ₂ Ph-(<i>S</i>)	0.26 ± 0.09
3p	OMe	NH	S	<i>S</i>	NHCH(CH ₂ Ph)CON(CH ₃)CH ₂ Ph-(<i>R</i>)	1.28 ± 0.37
3q	OMe	NH	NCN	<i>S</i>	NHCH(Ph) ₂	(> 50)
3r	OMe	O	O	<i>S</i>	NHCH(Ph) ₂	(> 50)

Boc-protected guanidine in each case was then deprotected with TFA. Similar chemistry was employed in the synthesis of the *N*-cyanoguanidine analogue, **5j**, starting from cyanamide (route G).

Biology: Assays in Vitro

The in vitro assessment of the compounds was carried out in binding assays and in the guinea pig ileum organ bath bioassay. Binding assays were carried out using rabbit whole brain membranes, rat whole forebrain membranes, and membranes from Cos-7 cells transiently transfected with the human NK₁ receptor. Guinea pig ileum (GPI) contractions were evoked by the potent NK₁ selective peptide agonist [Sar⁹Met(O₂)¹¹]SP (4.0 nM).

Full experimental details of these assays are presented in the Experimental Section.

Pharmacology: Assays in Vivo

Due to the marked species differences in receptor affinity noted with this class of compounds, a model of carrageenan-induced hyperalgesia in the guinea pig²¹ was used to assess the effects of NK₁ antagonists on inflammatory hyperalgesia in vivo. Carrageenan (1.0%, 100 μL) injected intraplantar into one hind paw induces both a mechanical and thermal hyperalgesia which persists for over 24 h. Subcutaneously administered morphine potently and completely reverses hyperalgesia whereas orally administered aspirin is less potent and only produces a partial reversal of hyperalgesia, as do other NSAIDs.

ED₃₀ was calculated as the dose required to produce a 30% inhibition of hyperalgesia, and the maximum effect achieved was expressed as percentage reversal of the predose hyperalgesia.

Full experimental details of this assay are presented in the Experimental Section.

Results and Discussion

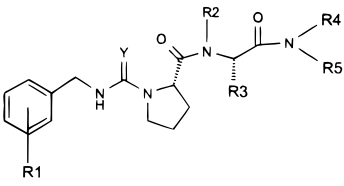
A lead compound, **3a** (Table 1), was discovered by directed screening of a proprietary library of amino acid


amides in a rabbit NK₁ binding assay. **3a** had sub-micromolar affinity for the rabbit NK₁ receptor (*K_i* = 0.33 μM) but negligible affinity for the rat receptor.

SAR of the Lead Structure, 3a. Simple variation of the aromatic ring substitution in this early lead structure established that 2-substitution was important (compare **3a** and **3b**, Table 1) for sub-micromolar affinity at the rabbit NK₁ receptor. 2-Substitution with halogens (Cl and Br but not F) led to a 2-fold increase in affinity. The nitro-substituted compound, **3f**, was 5-fold less potent, and the amino compound, **3g**, was inactive.

In such 2-methoxybenzylthioureas, replacement of the thiourea substructure with urea, **3h**, carbamate, **3r**, or the *N*-cyanoguanidine, **3q** (sometimes considered a thiourea "bioisostere"²²), led to loss of affinity. The chirality of the proline unit appeared to be important, since the D-proline analogue of **3a**, **3j**, was much less active. Several other amino acids (e.g., glycine) were explored as replacements for L-proline but were without activity, suggesting that the conformational constraint afforded by L-proline was critical.

The nature of the hydrophobic prolinamide substituent (diphenylmethyl in **3a**) was then modified. SAR from the initial lead finding established that a simple benzyl substituent on the proline amide was insufficient for significant rabbit NK₁ receptor affinity: two aryl rings were apparently required. The activity of other substituents for the proline amide containing two aryl rings was explored, e.g., dibenzylmethyl, diphenylethyl, and *N*-dibenzyl (**3k–m**), but none were found to be active. It is now known that the majority of non-peptide NK₁ antagonists, whose structures have been published, do not share binding determinants in common with peptide agonists, i.e., they are not peptidomimetics.²³ In the absence of such information, however, a simple conceptual model, in which the diphenylmethyl moiety in **3a** was hypothesized to mimic the Phe-Phe side chains in a panel of NK₁ selective peptide ligands, was used as a basis for design. The proline amide substituent

Table 2. Optimization of a Series of Benzylthiourea Proline Amides for Human NK₁ Receptor Binding Affinity


compd no.	R ¹	Y	R ²	R ³	R ⁴	R ⁵	human NK ₁ receptor binding K _i , nM, mean ± SEM (n ≥ 3) (IC ₅₀ , nM, n = 2)
3n	2-OMe	S	H	CH ₂ Ph	CH ₃	CH ₂ Ph	123.0 ± 34.0
4a	2-Cl	S	H	CH ₂ Ph	CH ₃	CH ₂ Ph	29.9 ± 5.0
4b	2-Br	S	H	CH ₂ Ph	CH ₃	CH ₂ Ph	29.7 ± 15.0
4c	2-F	S	H	CH ₂ Ph	CH ₃	CH ₂ Ph	(290, 480)
4d	2-CF ₃	S	H	CH ₂ Ph	CH ₃	CH ₂ Ph	27.2 ± 10.9
4e	2-NO ₂	S	H	CH ₂ Ph	CH ₃	CH ₂ Ph	77.6 ± 23.2
4f	2-CH ₃	S	H	CH ₂ Ph	CH ₃	CH ₂ Ph	(410, 330)
4g	2-H	S	H	CH ₂ Ph	CH ₃	CH ₂ Ph	1010 ± 47
4h	2,6-diCl	S	H	CH ₂ Ph	CH ₃	CH ₂ Ph	25.9 ± 9.6
4j	3,5-diCF ₃	S	H	CH ₂ Ph	CH ₃	CH ₂ Ph	78.6 ± 32.2
4k	2-Cl	O	H	CH ₂ Ph	CH ₃	CH ₂ Ph	(590, 660)
4l	2-Cl	NH	H	CH ₂ Ph	CH ₃	CH ₂ Ph	18.5 ± 4.5
4m	2-Cl	S	H	CH ₂ Ph	CH ₃	CH ₂ CH ₂ Ph	(2500, 1100)
4n	2-Cl	S	H	CH ₂ Ph	CH ₃	Ph	(100,000, 11700)
4p	2-Cl	S	H	CH ₂ Ph			8000 ± 250
4q	2-Cl	S	H	CH ₂ Ph	CH ₃	CH ₂ -1-naphthyl	17.1 ± 4.1
4r	2-Cl	S	H	CH ₂ Ph	CH ₃	CH ₂ -2-naphthyl	36.0 ± 17.0
4s	2-Cl	S	H	CH ₂ Ph	CH ₃	CH ₂ (3,5-diCF ₃ Ph)	26.9 ± 13.0
4t	2-Cl	S	H	CH ₂ Ph	CH ₃	CH ₂ CH ₂ (3-indolyl)	48 ± 22
4u	2-Cl	S	H	CH ₂ Ph	H	CH ₂ CH ₂ (3-indolyl)	(3700, 6000)
4v	2-Cl	S	H	CH ₂ Ph	CH ₃	CH ₂ (4-pyridyl)	(10000, 2500)
4w	2-Cl	S	CH ₃	CH ₂ Ph	CH ₃	CH ₂ Ph	38 ± 11
4x	2-Cl	S	H	CH ₂ CH ₂ Ph	CH ₃	CH ₂ Ph	(206, 543)
4y	2-Cl	S	H	CH ₂ (4-Ph)Ph	CH ₃	CH ₂ Ph	7.2 ± 1.9
4z	2-Cl	S	H	CH ₂ (3,4-diCl)Ph	CH ₃	CH ₂ Ph	5 ± 1
4aa	2-Cl	S	H	CH ₂ (3-indolyl)	CH ₃	CH ₂ Ph	16.7 ± 5.3
4ab	2-Cl	S	H	CH ₂ (N-Me 3-indolyl)	CH ₃	CH ₂ Ph	4.7 ± 1.1
4ac	2-Cl	S	H	CH ₂ (1-naphthyl)	CH ₃	CH ₂ Ph	13.7 ± 3.6
4ad	2-Cl	S	H	CH ₂ (2-naphthyl)	CH ₃	CH ₂ Ph	0.7 ± 0.3
4ae	2-Cl	S	CH ₃	CH ₂ (2-naphthyl)	CH ₃	CH ₂ Ph	1.7 ± 0.7
4af	2-Cl	S	H	CH ₂ (benzothieryl)	CH ₃	CH ₂ Ph	7.4 ± 2.2
4ag	2-Cl	S	H	CH(Ph) ₂	CH ₃	CH ₂ Ph	29.4 ± 11.7
4ah	2-Cl	O	H	CH ₂ (2-naphthyl)	CH ₃	CH ₂ Ph	2.3 ± 0.6
4aj	2-Cl	NH	H	CH ₂ (2-naphthyl)	CH ₃	CH ₂ Ph	0.5 ± 0.2

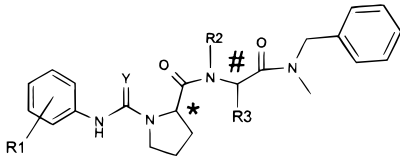
ent was therefore elaborated to the prolyl-phenylalanine amide **3n**. Phenylalanine benzyl amides overlaid the two aryl moieties in the model particularly well and, since *N*-methylbenzylamides had been found to confer oral activity on a series of structurally related analogues including FK 888,⁵ this substitution was therefore introduced. The *L*-phenylalanine *N*-methylbenzylamide analogue retained activity on the rabbit receptor with respect to **3a**, whereas the *D*-isomer, **3p**, was 5-fold less active than **3n**.

Benzylthioureas: Aromatic Ring Substitution in Prolyl-Phenylalanyl Amides. When the parent 2-methoxybenzylthiourea in the prolyl-phenylalanyl amide series, **3n**, was tested in the human NK₁ receptor binding assay, it was found to be slightly more potent than at the rabbit NK₁ receptor (Table 2). As was the case with the earlier studies on the rabbit receptor, the unsubstituted benzylthiourea (2-H, **4g**) was less potent than the 2-methoxy compound. Importantly, the 2-methoxy to halogen substitution which in the proline diphenylmethanamide series retained affinity, resulted in a large increase in affinity for the human receptor (compare **3v** with **4a–b**). 2-CF₃ aryl substitution (**4d**) gave similarly

high affinity, while as before, 2-F substitution (**4c**) was not so effective. Disubstitution was tolerated, as shown by the equipotency of the 2,6-dichloro compound with the 2-monochloro compound (compare **4h** and **3n**). 3,5-Bis-CF₃ substitution (**4j**), which is known to improve in vitro potency greatly in other structural series of NK₁ antagonists,⁸ gave only a modest increase in potency compared with **3n** in this series. 2-Nitro substitution, as in the diphenylmethanamides, was inferior to halogen though it was superior to methoxy in this series (compare **4e**, **3n**, and **4a**).

As with the prolyldiphenylmethyl amide series, the urea analogue of **4a** (the most potent thiourea), **4k**, was much less potent while the benzylguanidine, **4l**, retained or slightly increased activity and had improved solubility with respect to the thiourea.

Benzylthioureas: SAR of the Prolyl Phenylalanyl Amide Substituent. The optimum substitution of the phenylalanine amide was then explored. The *N*-methyl-*N*-benzyl amide could be substituted by other *N*-methyl-*N*-aralkyl substituents, e.g., 1- or 2-naphthylmethyl, **4q**, **4r** (Table 2). 3,5-Bis(trifluoromethyl) substitution of the benzyl ring in the *N*-methyl-*N*-benzyl

Table 3. Optimization of a Series of Phenylthiourea Proline Amides and Their Derivatives for Human NK₁ Receptor Binding Affinity


compd no.	R ¹	Y	*,#	R ²	R ³	human NK ₁ receptor binding K _i , nM, mean ± SEM (n ≥ 3) (IC ₅₀ , nM, n = 2)
5a	2-ClPh	S	<i>S,S</i>	H	CH ₂ Ph	(3200, 2700)
5b	2-HPH	S	<i>S,S</i>	H	CH ₂ Ph	80.0 ± 24.8
5c	2-NO ₂ Ph	S	<i>S,S</i>	H	CH ₂ Ph	100.0 ± 35.0
5d	2-BrPh	S	<i>S,S</i>	H	CH ₂ Ph	(600, 1000)
5e	2-NO ₂ Ph	S	<i>S,S</i>	H	CH ₂ -2-naphthyl	0.8 ± 0.3
5f	2-NO ₂ Ph	O	<i>S,S</i>	H	CH ₂ -2-naphthyl	0.16 ± 0.07
5g	2-NO ₂ Ph	O	<i>S,S</i>	CH ₃	CH ₂ -2-naphthyl	0.14 ± 0.04
5h	2-NO ₂ Ph	NH	<i>S,S</i>	H	CH ₂ -2-naphthyl	60.6 ± 20.6
5j	2-NO ₂ Ph	NCN	<i>S,S</i>	H	CH ₂ -2-naphthyl	2.7 ± 1.1
5k	2-NO ₂ Ph	O	<i>R,R</i>	H	CH ₂ -2-naphthyl	82.4 ± 12.4
5l	2-NO ₂ Ph	O	<i>S,R</i>	H	CH ₂ -2-naphthyl	7.1 ± 1.2
5m	2-NO ₂ Ph	O	<i>R,S</i>	H	CH ₂ -2-naphthyl	274 ± 63
5n	2-NO ₂ Ph	O	<i>S,S</i>	H	CH ₂ -3-indolyl	0.41 ± 0.15
5p	2-NO ₂ Ph	O	<i>S,S</i>	H	CH ₂ -3,4-dichlorophenyl	0.82 ± 0.20

amide **4s** was well tolerated but did not increase affinity with respect to **4a**. The incorporation of a heteroatom into the benzyl ring, to give the 4-pyridyl analogue, **4v**, caused a large loss of affinity. Shortening the aralkyl chain in such amides, e.g., benzyl to phenyl, **4n**, was not tolerated whereas lengthening this group to phenylethyl, **4m**, had less of a deleterious effect. The related indolyethyl group in this position restored affinity, **4t**, although the analogous secondary amide, **4u**, was much less active. In common with other *N*-methyl amides described in this paper, two rotameric forms of **4t** were observed by NMR (see the Experimental Section) but only one (presumably the trans amide) was observed with the secondary amide, **4u**. The much higher affinity observed with **4t** compared with **4u** suggests, therefore, that the conformer which binds to the NK₁ receptor was the cis amide. Constraint of the *N*-methyl-*N*-benzyl amide into a tetrahydroisoquinolyl amide, **4p**, also had a deleterious effect on affinity. No amide substitution which was markedly superior to the *N*-methyl benzylamide was identified and so this substructure was retained subsequently.

Benzylthioureas: Elaboration of the Central Amino Acid Moiety. Ring substitution of the phenylalanine side chain with hydrophobic substituents, as in **4y** and **4z**, gave a small increase in affinity. Modification of the benzyl methylene group by inserting an additional methylene group in the linker, as in **4x**, led to a decrease in affinity compared with **4a** whereas methylene branching (substitution with another phenyl ring), as in **4ag**, led to retention of potency. Bicyclic aromatic groups, including heterocycles (**4aa–af**), were tolerated, and some resulted in a large increase in affinity, especially the 2-naphthylalanine analogue, **4ad**. In this 2-naphthylalanine series, as noted previously for phenylalanine analogues, the benzylurea analogue, **4ah**, was somewhat less potent than the thiourea, **4ad**, whereas the benzylguanidine, **4aj**, was very potent and had increased solubility with respect to the thiourea. However, benzylguanidines of this type were acutely

toxic in vivo (lethal within 30 min of systemic administration) and so were not pursued further.

In both the parent phenylalanine and naphthylalanine compounds, *N*-methylation of the central amide NH was achieved without a significant loss of affinity (compare **4a** and **4w**; **4ad** and **4ae**, Table 2).

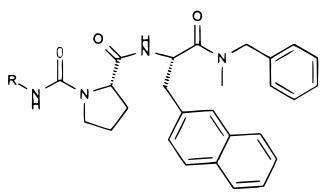
Phenylureas: Analogous Structural Modifications. The 2-chlorophenyl thiourea **5a** (Table 3, in which the benzyl methylene group has been omitted with respect to **4a**) was much less potent than the corresponding 2-chlorobenzyl compound. Interestingly, however, the unsubstituted compound, **5b**, retained high affinity. In the case of this unsubstituted compound, omission of the benzyl methylene group significantly *increased* affinity with respect to the unsubstituted benzyl compound, **4g**, but had the opposite effect in the halo-substituted coupled pairs, **4a** and **5a**, **4b** and **5d**. The 2-nitro-substituted compound **5c**, however, retained affinity with respect to the unsubstituted compound.

The SAR of the ring substitution is thus clearly different in the phenyl series to that observed in the benzyl series, with the rank order for potency being as follows:

benzyl: Cl = Br > OMe ≈ NO₂ > F > H

phenyl: NO₂ ≈ H > Cl, Br

In a series of 2-nitrophenyl analogues, the SAR of the central amino acid unit (phenylalanine in **5c**) was closely comparable to that observed in 2-chlorobenzylthioureas. Bicyclic hydrophobic amino acids increased affinity, particularly 2-naphthylalanine and tryptophan (**5e**, **5n**), all of which had sub-nanomolar affinity, as did 3,4-dichlorophenylalanine, **5p**. In a series of such analogues, where 2-naphthylalanine was chosen as the central amino acid substituent, the "linker group", e.g., thiourea in **5e**, was then varied. The *N*-cyanoguanidine, **5j**, was active, although slightly less potent than the

Table 4. Effect of Ring Substitution on in Vitro and in Vivo Activity of a Series of Phenylureas


compd no.	R	human NK ₁ receptor binding <i>K_i</i> , nM, mean ± SEM (<i>n</i> ≥ 3)	GPI NK ₁ IC ₅₀ , nM, mean ± SEM (<i>n</i> = 3)	% inhibn at 30 mg/kg po (3 h after dosing) (DR curve, <i>n</i> = 3)
5q	Ph	0.88 ± 0.29	5.0 ± 0.52	26
5r	3-pyridyl	6.7 ± 1.5	4.2 ± 2.56	14 (1 h)
5f	Ph-2-NO ₂	0.16 ± 0.07	1.6 ± 0.94	68.4 ± 1.3 (ED ₃₀ = 1.1 ± 0.5)
5s	Ph-3-NO ₂	2.3 ± 0.53	NT	NT
5t	Ph-4-NO ₂	1.0 ± 0.38	NT	NT
5u	Ph-2-CN	1.1 ± 0.37	1.0 ± 0.3	42 (ED ₃₀ = 2.1)
5v	Ph-3-CN	4.2 ± 1.5	2.6 ± 0.9	38
5w	Ph-4-CN	1.1 ± 0.4	2.4 ± 0.7	32
5x	Ph-2-F	3.5 ± 0.5	NT	NT
5y	Ph-3-F	0.7 ± 0.1	6.8 ± 3.0	10
5z	Ph-4-F	2.2 ± 0.4	17.3 ± 5.1	NT
5aa	Ph-2-CF ₃	18.2 ± 2.2	NT	NT
5ab	Ph-2-CO ₂ CH ₃	0.3 ± 0.09	6.0 ± 3.0	NT
5ac	Ph-2-CO ₂ H	172 ± 58	NT	NT
5ad	Ph-2-CO ₂ NH ₂	0.8 ± 0.2	1.9 ± 0.42	35 (ED ₃₀ = 15.7)
5ae	Ph-3-CO ₂ NH ₂	5.0 ± 1.2	NT	NT
5af	Ph-2-CO ₂ NHCH ₃	3.8 ± 0.6	3.0 ± 1.0	NT
5ag	Ph-2-CO ₂ N(CH ₃) ₂	6.1 ± 1.5	NT	NT
5ah	Ph-2-CH ₂ OCH ₃	1.0 ± 0.2	1.2 ± 0.4	67.0 ± 1.7 (ED ₃₀ = 1.2 ± 0.1)
5aj	Ph-2-CH ₂ OH	1.7 ± 0.6	NT	NT
5ak	Ph-2-OH	2.6 ± 0.9	NT	NT
5al	Ph-2-NH ₂	19.7 ± 3.9	21.0 ± 7.2	NT
5am	Ph-2-NHCOCH ₃	9.6 ± 3.1	7.5 ± 3.2	NT

thiourea, but the guanidine, **5h**, was much less potent. The urea **5f**, however, had outstandingly high human NK₁ receptor binding affinity. This contrasts with the benzyl series where the urea is less potent than the thiourea and guanidine (compare **4ah**, **4ad**, and **4aj**). This rank order, like that for aromatic ring substitution, is completely different from that seen in the benzylthiourea series:

benzyl:

guanidine ≈ thiourea > urea = NCN guanidine

phenyl:

urea > thiourea ≈ NCN guanidine > guanidine

5f has a high "eudysmic ratio" with the (*S,S*) diastereomer being the eutomer. Its enantiomer (**5k**, the *R,R* diastereomer) is >500-fold less potent, and the other two diastereoisomers also have lower affinity. In common with the analogous substitution in the benzylthiourea series, the *N*-methyl analogue of **5f**, **5g**, retains similarly high affinity.

Discussion

Clear differences in the preferred ring substitution and linking group (e.g., thiourea) are evident between the two (benzyl and phenyl) series.

The difference in affinity between the phenyl- and benzylguanidines, **5h** and **4aj**, is remarkable. The two guanidines would be expected to have markedly differ-

ent basicity and preferred tautomeric forms, but the most important feature responsible for modulating NK₁ receptor binding affinity is not clearly understood at present.

In **5f**, the most potent of this series of compounds on the human receptor, the position of the nitro group is important for very high affinity (IC₅₀ < 1 nM), compare **5f**, **5s**, and **5t** (Table 4). The difference between the affinities of the corresponding CN or F-substituted positional isomers, **5u–z**, however, was less apparent, with all isomers having moderate to high affinity. The 2-methoxycarbonyl compound, **5ab**, and the related primary amide, **5ad**, had high affinity, comparable to **5f**, but the related carboxylic acid, **5ac**, had 500-fold lower affinity. As with the nitro compounds, previously discussed, the 3-positional isomer of the 2-primary amido compound **5ad**, **5ae**, was less potent. This SAR suggested to us that H-bond acceptor function in the 2-position had some influence on affinity at the human receptor, perhaps by stabilizing an intramolecular H-bond with the anilide-like NH and a consequent influence on conformation.

In general the correlation between the human receptor binding affinities and the guinea pig ileum bioassay (GPI) potencies was good (*R* = 0.85, Figure 1). Minor species differences were, however, evident within this data set, e.g., **5f**, which is outstanding in its human receptor affinity but is relatively less potent in the GPI. The lower potency of the methyl ester, **5ab**, in GPI may

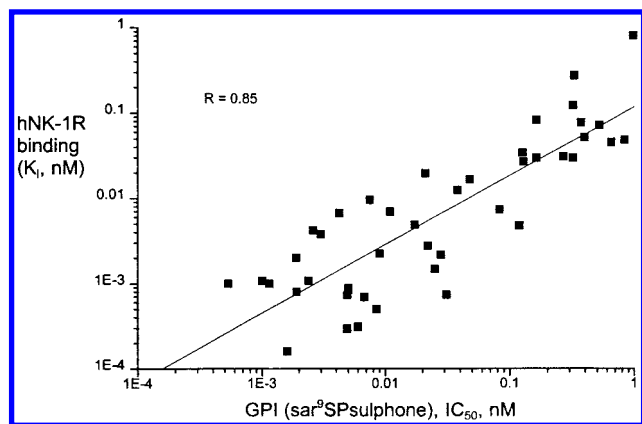


Figure 1. Correlation between hNK₁ receptor binding and guinea pig ileum bioassay potencies.

not, however, represent a species difference but, rather, some degree of enzymatic hydrolysis in the organ bath.

The guinea pig was, therefore, chosen as the species in which to assess anti-hyperalgesic activity *in vivo*, since activity in this model should predict efficacy in humans. The compounds with highest potency in the human assay *in vitro* and which retained acceptable potency in the GPI were therefore tested in a guinea pig model of persistent inflammatory hyperalgesia. Compounds which gave significant reversal of hyperalgesia 3 h after oral administration at 30 mg/kg po were tested further, and dose-response data was generated. The 2-nitro compound, **5f**, gave a good reversal of hyperalgesia ($68.4 \pm 1.3\%$) and had high potency 3 h after oral administration ($ED_{30} = 1.1 \pm 0.5$ mg/kg, $n = 3$). Its enantiomer, **5k**, was inactive in this model, in agreement with its much lower *in vitro* potency. For comparison, at the same time point, subcutaneously administered morphine had a $ED_{30} = 0.5$ mg/kg and an efficacy of $79.8 \pm 12.2\%$ and orally administered aspirin had a $ED_{30} = 71.6$ mg/kg and an efficacy of $55.0 \pm 6.8\%$.

Apart from the methyl ester **5ab** (which was assumed to be susceptible to rapid enzymatic hydrolysis *in vivo* despite having reasonable potency in GPI), the most promising compound other than **5f**, **5ad**, had rather poor *in vivo* activity. We assumed this might relate to the high polarity and H-bonding capacity of the primary amide since such properties are known to restrict access across the blood-brain barrier (BBB).²⁴

The postulated ability of, for example, **5f** to form an intramolecular H-bond can be offered as an explanation for the high analgesic potency and CNS penetration after oral administration of this compound,²⁵ in comparison with other closely related congeners, e.g., the unsubstituted or carboxamido-substituted phenylurea compounds, **5q** and **5ad**. Such an H-bond (shown schematically in Scheme 2) has the effect of "masking" the anilide-like NH. Despite the potential for **5ad** to form an intramolecular H-bond, the carboxamide NH₂ is still exposed. Likewise, since **5q** possesses no H-bond acceptor functionality in the 2-position, the urea NH is unmasked. The reversed phase HPLC retention time, which is qualitatively related to overall hydrophobicity,²⁶ shows that **5f** is less polar than **5q** and that **5ad** is by far the most polar compound (Table 5).

We have no direct evidence to support the above hypothesis at present; however, NMR chemical shift

Scheme 2

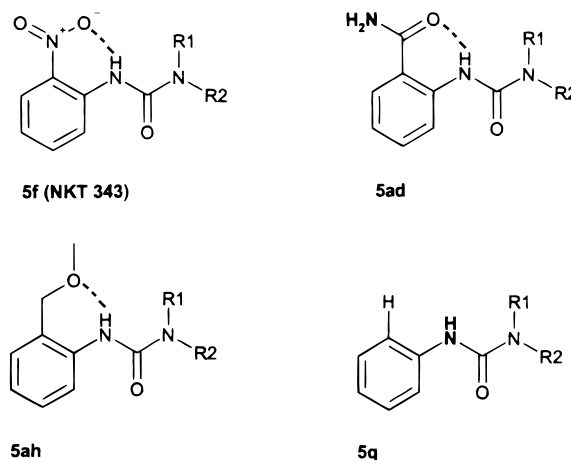


Table 5. Effect of Ring Substitution on Physicochemical Properties of a Series of Phenylureas

compd no.	2-substituent	chemical shift of urea NHAr (δ , ppm, CDCl ₃)	HPLC t_R (min)
5f	2-NO ₂	9.95	30.91
5ah	2-CH ₂ OCH ₃	8.82	30.42
5ad	2-CONH ₂	10.75	26.75
5q	2-H	6.40	29.56

data are suggestive of an H-bonded aromatic urea NH group in **5f**, **5ad**, and **5ah**, but not **5q** (Table 5). Other analogues with 2-substituents possessing H-bond acceptor function but lacking overtly polar groups were therefore synthesized. The 2-methoxymethylurea, **5ah**, retained high affinity in the human and guinea pig assays and had comparable anti-hyperalgesic activity to **5f** by oral administration (Table 4). The downfield shift observed with **5ah** (2-methoxymethyl, an electron-donating group) argues against these effects on chemical shift being due, rather, to an inductive electron-withdrawing effect.

Brain Uptake of 5f and 5ah. The locus of action of NK₁ antagonists as anti-hyperalgesic agents lies in the dorsal horn of the spinal cord,²⁷ and so CNS penetration is prerequisite for prospective drug candidates.

The BBB passage of **5f** and **5ah** was measured by the brain uptake index (BUI) method in the rat.²⁸ The brain extraction of the drugs was determined after a rapid carotid artery injection of [³H]-**5f** or [³H]-**5ah** together with the diffusible reference [¹⁴C]butanol.

The brain extraction ratio (BER) of [³H]-**5f** is plasma protein concentration dependent; $32.4 \pm 2.7\%$ in the presence and $16.0 \pm 5.0\%$ in the absence of plasma proteins. For comparison, **5ah** has a BER of $20.2 \pm 4.0\%$, in the absence of plasma proteins.

These values are lower than the extraction of highly diffusible substances, e.g., diazepam (56.3%)²⁹ and [³H]-propranolol (66.4%)³⁰ but are higher than the brain extraction of lovastatin (7%)³¹, a drug showing a low, but still significant brain penetration.

These results thus indicate a relatively good brain penetration of **5f** and **5ah**.

5f has fairly good oral bioavailability (25% in the rat³²) and good brain penetration. An observation that **5f** is a potent inhibitor of P Glycoprotein³³ (a transport protein which is heavily expressed in membranes of endothelial cells at the BBB and normally excludes

hydrophobic molecules from the CNS) might explain its unexpectedly good brain penetration.

In conclusion, **5f** is a highly potent antagonist of human NK₁ receptors which has good oral activity in animal models of persistent inflammatory pain and penetrates the CNS. **5f** is currently under clinical evaluation as a novel therapy for chronic pain conditions. The detailed pharmacology of this compound in vitro and in vivo will be reported elsewhere.³⁴

Experimental Section

General Information. Routine NMR spectra were recorded using a Varian Gemini 200 instrument. All spectra were recorded using tetramethylsilane (TMS) as an internal standard, and chemical shifts are reported in parts per million (δ) downfield from TMS. Coupling constants are reported in hertz. Mass spectra were recorded in Novartis, Basle, using a VG 70-SE spectrometer. A Perkin-Elmer 781 spectrophotometer was used to record IR spectra, either as liquid films or as KBr disks. Elemental analyses were performed in Novartis, Basel, and were within 0.4% of theory. Melting points were determined using a Reichert hot stage microscope and are uncorrected. TLC was performed using Merck Kieselgel 60 F₂₅₄ silica plates, and components were visualized using UV light, iodine vapor, or ammonium molybdate. Compounds were purified by crystallization, flash column chromatography using Merck Kieselgel 60 (230–400 mesh), or preparative HPLC using a Waters Delta Prep 3000 preparative chromatography system equipped with a Dynamax 300A C18 12 μ m particle size column (83–243-C), dimensions 41.4 \times 250 mm. Solvents were HPLC grade and were used without further purification. Chemical yields were not optimized.

Benzylloxycarbonyl-L-proline Diphenylmethylamide. Benzylloxycarbonyl-L-proline (10 g, 40.2 mmol) was stirred in CH₂Cl₂ (50 mL) under a N₂ atmosphere. DCCI (9.13 g, 44.2 mmol) in CH₂Cl₂ (25 mL) and aminodiphenylmethane (7.4 g, 40.2 mmol) in CH₂Cl₂ (10 mL) were then added, and the reaction mixture was stirred for 5 h. The solution was filtered and the solvent evaporated to give a colorless solid which was purified by flash column chromatography (cyclohexane/EtOAc 2:1). Pure fractions were concentrated to give a colorless solid, 12.5 g (75%). TLC (cyclohexane/EtOAc 2:1) *R*_f 0.25.

L-Proline Diphenylmethylamide Hydrochloride. Benzylloxycarbonyl-L-proline diphenylmethylamide (15 g, 36.2 mmol) was dissolved in methanol (200 mL), and Pd/C (10%) was added under a N₂ atmosphere. The flask was then filled with H₂, and the mixture was stirred for 24 h, after which time the reaction was complete by TLC. The catalyst was removed by filtration and the filtrate concentrated. The residue was redissolved in diethyl ether/EtOAc 4:1. HCl gas was introduced into the stirred solution, and the precipitated salt was collected by filtration and washed with dry ether, yielding a colorless solid, 8.0 g (70%), which was used without further purification.

N-(2-Methoxybenzyl)-aminothiocarbonyl-(S)-proline Diphenylmethylamide, 3a. L-Proline diphenylmethylamide hydrochloride (2.26 g, 7.15 mmol) in dry DMF (10 mL) containing triethylamine (1.12 mL, 7.9 mmol) was stirred at room temperature. A solution of 2-methoxybenzyl isothiocyanate (1.28 g, 7.15 mmol) in DMF (5 mL) was added and the solution stirred for 12 h. After removal of solvent in vacuo, the crude product was purified by flash column chromatography (cyclohexane/EtOAc 1:1). Pure fractions were concentrated to give a colorless glass which was recrystallized from ethylene glycol dimethyl ether/water to give colorless needles: 2.61 g (79.6%); mp 105–106 °C; TLC (cyclohexane/EtOAc 1:1) *R*_f 0.22; ¹H NMR (CDCl₃) δ 1.80–2.45 (4H, m, proline β - and γ -CH₂), 3.28–3.55 (2H, m, proline δ), 3.82 (3H, s, ArOCH₃), 4.86 (1H, dd_{ABX}, *J*_{AX} = 5.4 Hz, *J*_{AB} = 15 Hz, PhCH_AH_B-NH), 4.96 (1H, dd_{ABX}, *J*_{BX} = 5.4 Hz, *J*_{BA} = 15 Hz, PhCH_AH_B-NH), 5.22 (1H, br d, proline α -CH) 6.10–6.22 (1H, br, thiourea NH), 6.15 (1H, d, *J* = 8.6 Hz, NHCH(Ph)₂), 6.88

(1H, d, *J* = 8 Hz, ArH^F), 6.95 (1H, d, *J* = 8 Hz, ArH^F), 7.20–7.40 (12H, m, ArH), 8.26 (1H, m, NHCH(Ph)₂); FAB MS *m/e* 460 (MH⁺). Anal. (C₂₇H₂₉N₃O₂S) C, H, N.

N-(2-Aminobenzyl)-aminothiocarbonyl-(S)-proline Diphenylmethylamide, Trifluoroacetate, 3g. 3f (1.4 g, 2.95 mmol) was dissolved in MeOH (100 mL), and 10% Pd/C (100 mg) was added under an inert atmosphere. The atmosphere was replaced with H₂, and the mixture was stirred for 24 h, after which time the reaction was complete by TLC. The reaction mixture was evaporated and purified by flash column chromatography (CH₂Cl₂/MeOH 100:1) and then by preparative reversed phase HPLC (CH₃CN/0.1% aqueous TFA gradient 20–60%) to yield a colorless solid. The solid was recrystallized from hexane/EtOAc and dried to give a colorless solid (46 mg, 3%): mp 103–108 °C (glass); TLC (cyclohexane/EtOAc 1:1) *R*_f 0.12; ¹H NMR (CDCl₃) δ 1.80–2.45 (4H, m, proline β - and γ -CH₂), 3.28–3.48 (2H, m, proline δ -CH₂), 3.91 (2H, br, aniline NH₂) 4.57 (1H, dd_{ABX}, *J*_{AX} = 5.4 Hz, *J*_{AB} = 15 Hz, PhCH_AH_B-NH), 5.13 (1H, dd_{ABX}, *J*_{BX} = 5.4 Hz, *J*_{BA} = 15 Hz, PhCH_AH_B-NH), 5.28 (1H, m, proline α -CH), 5.57 (1H, br m, X of ABX) 6.18 (1H, d, *J* = 6.8 Hz, NHCH(Ph)₂), 6.57 (1H, d, *J* = 8 Hz, ArH^F), 6.68 (1H, m, ArH^F), 7.05–7.10 (2H, m, ArH^{F,6}), 7.20–7.40 (10H, m, diphenylmethyl ArH), 7.96 (1H, m, NH-CH(Ph)₂); FAB MS *m/e* 445, 10% (MH⁺). Anal. (C₂₈H₂₉N₄F₃O₃S·1.6H₂O) C, H, N.

Boc-(R)-proline Diphenylmethylamide. Boc-(R)-proline (4.3 g, 20 mmol) was dissolved in dichloromethane (50 mL). *N*-Methylmorpholine (2.03 g, 20 mmol) was added, and the solution was cooled to ca. –10 °C on a salt–ice bath. Isobutyl chloroformate (3.41 g, 25 mmol) was then added at such a rate that the temperature did not rise. Once the addition was complete the reaction mixture was stirred at –10 °C for 15 min. Aminodiphenylmethane (3.67 g, 20 mmol) was then added, and the reaction mixture was stirred at room temperature. The reaction was shown to be complete by TLC after 3 h. The solution was washed with 0.1M HCl(aq) (50 mL), water (50 mL), and finally brine (50 mL). The organic layer was dried over MgSO₄ and filtered, and the solvent was removed in vacuo. The product was purified by flash column chromatography (cyclohexane/ethyl acetate 4:1) (5.14 g, 68%). TLC (cyclohexane/ethyl acetate 1:1, *R*_f = 0.36).

(R)-Proline Diphenylmethylamide. Boc-(R)-proline diphenylmethylamide (5.00 g, 13.1 mmol) was dissolved in 4 M HCl/dioxan (40 mL), and the reaction mixture was stirred at room temperature for 1 h. The solvent was then removed in vacuo, and the product was dissolved in water (50 mL). Then, 2 M NaOH(aq) (5 mL) was added, and the product was extracted into ethyl acetate. The organic layer was dried over MgSO₄ and filtered, and the solvent was removed in vacuo to give (R)-proline diphenylmethylamide (3.46 g, 94%); TLC (cyclohexane/ethyl acetate 1:1, *R*_f = 0.36); ¹H NMR (CDCl₃) δ 1.71 (2H, m, CH₂ pyrrolidine), 1.98 (1H, m, CH₂ pyrrolidine), 2.15 (1H, m, NH pyrrolidine), 2.97 (2H, m, NCH₂ pyrrolidine), 3.80 (1H, m, CH pyrrolidine), 6.22 (1H, d, *J* = 8.5 Hz, Ph₂-CH), 7.15–7.38 (10H, m, ArH), 8.41 (1H, d, *J* = 8.5 Hz, CONH).

2-Methoxybenzylthiocarbonyl-(R)-prolyl-N-(diphenylmethyl)amide, 3j. (R)-Prolyl-N-(diphenylmethyl)-amide (0.39 g, 1.40 mmol) and 2-methoxybenzyl isothiocyanate (0.25 g, 1.40 mmol) were dissolved in dioxan (50 mL), and the reaction mixture was stirred at room temperature overnight. The reaction was shown to be complete by TLC. The solvent was removed in vacuo, and the product was purified by recrystallization (*n*-hexane/ethyl acetate) to give a white solid, mp 112–113 °C (75%); TLC (cyclohexane/ethyl acetate 1:1, *R*_f = 0.17); ¹H NMR (CDCl₃) δ 1.80–2.47 (4H, m, 2 \times CH₂ pyrrolidine), 3.41 (2H, m, NCH₂ pyrrolidine), 3.85 (3H, s, ArOCH₃), 4.80 (1H, dd_{ABX}, *J*_{AB} = 15.0 Hz, *J*_{AX} = 5.0 Hz, ArCH_AH_BNH), 4.95 (1H, dd_{ABX}, *J*_{AB} = 15.0 Hz, *J*_{BX} = 5.0 Hz, ArCH_AH_BNH), 5.24 (1H, broad, CH pyrrolidine), 6.15 (2H, broad, Ph₂CH and thiourea NH), 6.91 (2H, m, ArH), 7.15–7.40 (12H, m, ArH), 8.25 (1H, broad, CONH); FAB MS *m/e* 460 (MH⁺). Anal. (C₂₇H₂₉N₃O₂S) C, H, N.

***N*-[*N*(2)-Cyano-*N*(1)-(2-methoxybenzyl)amidino]-(*S*)-proline Diphenylmethylamide, 3q.** *o*-Methoxybenzylamine (0.65 mL, 5 mmol) was added to a suspension of diphenyl cyanocarbonimidate (1.19 g, 5 mmol) in 2-propanol (15 mL). The mixture was stirred at room temperature overnight. A white precipitate formed. TLC (hexane/EtOAc 2:1) indicated the complete disappearance of the starting materials and the formation of a new spot (*R*_f 0.18). The white precipitate was filtered and washed with additional 2-propanol. It was identified to be the newly formed compound (808 mg, 57%).

O-[*N*(2)-Cyano-*N*(1)-(2-methoxybenzyl)amidino]phenol (400 mg, 1.4 mmol) thus obtained was treated with proline diphenylmethylamide in 2-propanol (20 mL) under reflux for 5 days. The mixture was concentrated and the crude product purified by flash chromatography (hexane/EtOAc 1:7) to yield the title compound (89 mg, 13%): mp 79–81 °C; TLC (hexane/EtOAc 1:7) *R*_f 0.5; ¹H NMR (CDCl₃) δ 1.7–2.26 (4H, m, CH₂CH₂), 3.28–3.54 (2H, m, NCH₂), 3.81 (3H, s, OCH₃), 4.62–4.7 (1H, m, α-H), 4.73 (2H, d, *J* = 5.8 Hz, ArCH₂N), 5.50 (1H, br t, NH), 6.13 (1H, d, *J* = 7.4 Hz, CHPh₂), 6.86–6.97 (2H, m, NH and ArH), 7.2–7.41 (13H, m, ArH); IR 3269 (br), 2168 (s), 1669 (m), 1577 (s), 1543 (s), 1242 (s) cm⁻¹; MS *m/e* (FAB) 468 (MH⁺), 167 (100%). Anal. (C₂₈H₂₉N₅O₂) C, H, N.

***N*-(2-Methoxybenzylloxycarbonyl)-(S)-proline Diphenylmethylamide, 3r.** A solution of *o*-methoxybenzyl alcohol (0.4 mL, 3 mmol) in toluene (15 mL) was treated with phosgene (1.93 M in toluene, 2.5 mL, 4.3 mmol) at 0 °C. The mixture was stirred at 0 °C for 0.5 h and then warmed to room temperature, where it was stirred for a further 2 h. The mixture was concentrated to yield *o*-methoxybenzyl chloroformate.

o-Methoxybenzyl chloroformate (1 mmol) thus prepared was treated with proline diphenylmethylamide (280 mg, 1 mmol) in CH₂Cl₂ (10 mL) in the presence of Et₃N (0.17 mL, 1.2 mmol) at 0 °C. The mixture was warmed to room temperature and stirred for an additional 1.5 h. It was concentrated. The residue was dissolved in EtOAc (100 mL) and washed with aqueous NaHCO₃ (50 mL) and then with brine (50 mL). Drying (MgSO₄) was followed by flash chromatographic purification (hexane/EtOAc 2:3). The product fractions were concentrated and the product recrystallized (hexane/EtOAc) to yield the title compound (230 mg, 52%): mp 99–101 °C; TLC (hexane/EtOAc 1:1) *R*_f 0.37; ¹H NMR (CDCl₃) δ 1.8–2.5 (4H, m, CH₂CH₂), 3.4–3.6 (2H, m, NCH₂), 3.82 (3H, s, OCH₃), 4.4–4.5 (1H, m, α-H), 5.05–5.33 (2H, m, ArCH₂), 6.20 (1H, d, *J* = 8.4 Hz, CHPh₂), 6.97–7.4 (14H, m, ArH), 7.77 (1H, br, NH); IR 3330 (m), 1711 (s), 1692 (s), 1666 (s), 1539 (s), 1496 (s), 1416 (s), 1353 (s), 1243 (s) cm⁻¹; MS *m/e* (FAB) 445 (MH⁺), 167 (100%). Anal. (C₂₇H₂₈N₂O₄) C, H, N.

[Amino(2-chloro-benzylamino)methylene]methylsulfonium Iodide. To (2-chlorobenzyl)thiourea³⁵ (476 mg, 2.37 mmol) in ethyl acetate (20 mL) was added methyl iodide (0.6 mL, 9.5 mmol). The reaction mixture was stirred at room temperature for 1 h. A white solid was formed which was isolated by filtration and washed with diethyl ether (50 mL), to obtain [amino(2-chlorobenzylamino)methylene]methylsulfonium iodide as a white solid (755 mg, 2.20 mmol, 93%): ¹H NMR (CDCl₃) δ 2.69 (3H, s, SCH₃), 4.70 (2H, s, CH₂Ar), 4.71 (2H, s, br, NH), 7.36–7.48 (3H, m, ArH), 7.47–7.56 (1H, m, ArH); IR (KBr) 3202 m, 3049 s, 1633 s, 1589 s cm⁻¹; MS *m/z* (CI, CH₄) 215 (MH⁺) (92%), 125 (100%).

***N*-(2-Chlorobenzylamidino)-(S)-prolyl-(S)-phenylalanyl-N-benzylmethylamide, 4l.** To [amino(2-chlorobenzylamino)methylene]methylsulfonium iodide (342 mg, 2.14 mmol) in ethanol (10 mL) was added (*S*)-prolyl-(*S*)-phenylalanyl-*N*-benzyl-*N*-methylamide. The reaction mixture was stirred at room temperature for 16 days. Although the reaction had not gone to completion, the reaction mixture was concentrated in vacuo and purified by preparative HPLC to afford *N*-(2-chlorobenzylamidino)-(S)-prolyl-(S)-phenylalanyl-*N*-benzylmethylamide as a white solid (55 mg, 0.1 mmol, 5%): mp 68–71 °C; ¹H NMR (CDCl₃) mixture of two rotamers δ 1.78–2.33 (4H, m, CH₂ pyrrolidine), 2.72 (0.7 × 3H, s, NCH₃ major), 2.81 (0.3 × 3H, s, NCH₃ minor), 2.96–3.10 (2H, m, CHCH₂Ph),

3.48–3.65 (1H, m, NCH₂ pyrrolidine), 3.69–3.86 (1H, m, NCH₂ pyrrolidine), 4.15–4.60 (4H, NCH₂Ph, NCH₂Ar), 4.66–4.80 (1H, m, CH-pyrrolidine), 4.93–5.09 (1H, m, CHBn), 7.01–7.50 (15H, m, ArH), 7.73–7.94 (1H, m, ArH), 8.22–8.34 (1H, m, ArH); IR (film) 3425–3106 m, br, 3064 m, 1672 s, 1633 s, 1202 s cm⁻¹; MS *m/e* (FAB) 532 (MH⁺) (100%). Anal. (C₃₀H₃₄ClN₅O₂·0.5H₂O·1.2C₂H₅F₃O₂) C, H, N, F.

2-Chlorobenzylthiocarbamoyl-(S)-prolyl-(S)-phenylalanyl-1,2,3,4-tetrahydroisoquinol-2-ylamide, 4p. Boc-(*S*)-prolyl-(*S*)-phenylalanyl-1,2,3,4-tetrahydroisoquinol-2-ylamide³⁶ was deprotected using the method exemplified in the formation of (*R*)-prolyl-*N*-(diphenylmethyl)amide to give (*S*)-phenylalanyl-*N*-dibenzylamide. The product was not purified further.

(*S*)-Prolyl-(*S*)-phenylalanyl-1,2,3,4-tetrahydroisoquinol-2-ylamide and 2-chlorobenzylisothiocyanate were coupled using the method exemplified exemplified for **3a**. The product was purified by recrystallization (*n*-hexane/ethyl acetate) to give a white solid, mp 87–89 °C (67%); TLC (cyclohexane/ethyl acetate 1:1, *R*_f = 0.09); ¹H NMR (CDCl₃) mixture of 2 rotamers δ 1.95–2.22 (4H, m, 2 × CH₂ pyrrolidine), 2.42 (0.3 × 2H, m, ArCH₂CH₂N THIQ minor), 2.75 (0.7 × 2H, m, ArCH₂CH₂N THIQ major), 3.06 (2H, m, PhCH₂CH), 3.23 (0.3 × 2H, m, ArCH₂CH₂N THIQ minor), 3.38–3.90 (0.7 × 2H + 2H, m, ArCH₂CH₂N THIQ and NCH₂ pyrrolidine), 4.05 (0.3H, d_{AB}, *J*_{AB} = 15.0 Hz, ArCH_ACH_BN THIQ minor), 4.52 (0.3H, d_{AB}, *J*_{AB} = 15.0 Hz, ArCH_ACH_BN THIQ minor), 4.58 (0.7H, d_{AB}, *J*_{AB} = 15.0 Hz, ArCH_ACH_BN THIQ major), 4.74 (0.7H, d_{AB}, *J*_{AB} = 15.0 Hz, ArCH_ACH_BN THIQ major), 4.90 (1H, dd_{ABX}, *J*_{AB} = 15.0 Hz, *J*_{AX} = 5.0 Hz, ArCH_AH_BNHC(S)N), 4.98 (1H, broad, CH pyrrolidine), 5.02 (1H, dd_{ABX}, *J*_{AB} = 15.0 Hz, *J*_{BX} = 5.0 Hz, ArCH_AH_BNHC(S)N), 5.19 (1H, m, PhCH₂CH), 6.00 (1H, broad t, *J* = 5 Hz, thiourea NH), 6.85–7.27 (10H, m, ArH and CONH), 7.37 (2H, m, ArH), 7.52 (1H, m, ArH); FAB MS *m/e* 561 (MH⁺). Anal. (C₃₁H₃₃N₄O₂SCl) C, H, N.

2-Chlorobenzylthiocarbamoyl-(S)-prolyl-(S)-phenylalanyl-*N*-[2-(indol-3-yl)ethyl]-*N*-methylamide, 4t. 2-Chlorobenzylthiocarbamoyl-(*S*)-prolyl-(*S*)-phenylalanyl-*N*-(1-Boc-indol-3-yl)ethyl-*N*-methylamide was heated in a flask at ~160 °C. The reaction was shown to be complete by TLC after 1 h. The product was purified by flash column chromatography (cyclohexane/ethyl acetate 1:1) to give a cream-colored solid, mp 97–99 °C (65%); TLC (cyclohexane/ethyl acetate 1:4, *R*_f = 0.38); ¹H NMR (CDCl₃) δ 1.80–2.22 (4H, m, 2 × CH₂ pyrrolidine), 2.60–3.10 (7H, m, CH₂CH₂NCH₃, PhCH₂CH and CH₂CH₂NCH₃), 3.30–3.70 (4H, m, CH₂CH₂NCH₃ and NCH₂ pyrrolidine), 4.85–5.20 (4H, m, ArCH₂NHC(S)N, CH pyrrolidine, and PhCH₂CH), 6.00 (1H, m, thiourea NH), 6.90–7.70 (15H, m, ArH and CONH), 8.15 (1H, broad, ArH); FAB MS *m/e* 602 (MH⁺). Anal. (C₃₃H₃₆N₅O₂SCl) C, H, N.

2-Chlorobenzylthiocarbamoyl-(S)-prolyl-(S)-phenylalanyl-*N*-[2-(indol-3-yl)ethyl]amide, 4u. 2-Chlorobenzylthiocarbamoyl-(*S*)-prolyl-(*S*)-phenylalanyl-*N*-(1-Boc-indol-3-yl)ethylamide was heated in a flask at ~160 °C. The reaction was shown to be complete by TLC after 1 h. The product was purified by flash column chromatography (cyclohexane/ethyl acetate 1:1) to give a cream-colored solid, mp 93–95 °C (43%); TLC (cyclohexane/ethyl acetate 1:4, *R*_f = 0.28); ¹H NMR (CDCl₃) δ 1.40–2.10 (4H, m, 2 × CH₂ pyrrolidine), 2.78–3.33 (6H, m, CH₂CH₂NH, PhCH₂CH and NCH₂ pyrrolidine), 3.57 (2H, m, CH₂CH₂NH), 4.70 (1H, m, PhCH₂CH), 4.93 (3H, m, ArCH₂NHC(S)N and CH pyrrolidine), 5.99 (1H, m, thiourea NH), 6.40 (1H, m, CH₂CH₂NH), 6.88 (1H, m, indole NH), 7.00–7.68 (14H, m, ArH and CONH), 8.06 (1H, broad, ArH); FAB MS *m/e* 588 (MH⁺). Anal. (C₃₂H₃₄N₅O₂SCl) C, H, N.

2-Chlorobenzylcarbamoyl-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methylamide, 4ah. To (*S*)-prolyl-(*S*)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methylamide (168 mg, 0.4 mmol) in dioxan (3 mL) was added crude 2-chlorobenzyl isocyanate³⁷ (354 mg, 1 mmol). The reaction mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated in vacuo and purified by flash column chromatography (hexane/EtOAc 1:4) to afford 145 mg (0.24 mmol, 62%): mp 69–73 °C; ¹H NMR (CDCl₃) mixture of two rotamers

δ 1.63–1.94 (3H, m, CH₂ pyrrolidine), 2.08–2.25 (1H, m, CH₂ pyrrolidine), 2.72 (0.6 \times 3H, s, NCH₃ major), 2.90 (0.4 \times 3H, s, NCH₃ minor), 3.00–3.31 (4H, m, CH₂Nap, NCH₂ pyrrolidine), 4.28 (0.6 \times 1H, d_{AB}, J_{AB} = 14 Hz, NCH₂H_BPh major), 4.25–4.56 (2.8 H, m, ArCH₂NH, NCH₂H_BPh minor), 4.76 (0.6 \times 1H, d_{AB}, J_{AB} = 14 Hz, NCH₂H_BPh major), 4.82–4.98 (1H, m, pyrrolidine), 5.16–5.32 (1H, m, CHCH₂Nap), 6.92–7.05 (2H, m, ArH), 7.08–7.52 (11H, m, ArH), 7.54–7.83 (5H, m, ArH); IR (KBr) 3630–3170s, br, 1636 s, 1533 m cm⁻¹; MS *m/e* (FAB) 583 (MH)⁺ (53%), 265 (93%), 122 (100%). Anal. (C₃₄H₃₅N₄O₃Cl \cdot 0.25H₂O) C, H, N.

***N*-(*N,N*-Di-*tert*-butoxycarbonylamidino)-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-*N*-benzylmethylamide.** To (S)-prolyl-(S)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methylamide (207 mg, 0.5 mmol), 1,3-bis-*tert*-butoxycarbonylthiourea (138 mg, 0.5 mmol), and Et₃N (0.17 mL, 1.25 mmol) in DMF (2 mL) cooled in ice was added HgCl₂ (150 mg, 0.55 mmol). The reaction mixture was stirred in ice for 2 h. The reaction mixture was poured into water and extracted with EtOAc (3 \times 50 mL). The combined organic extracts were dried (Na₂SO₄) and filtered through silica. The colorless filtrate was concentrated to afford a white solid (348 mg, 0.5 mmol, 100%): ¹H NMR (CDCl₃) mixture of two rotamers δ 1.46 (0.3 \times 18H, s, *t*-Bu major), 1.51 (0.7 \times 18H, s, *t*-Bu minor), 1.68–2.01 (4H, m, CH₂ pyrrolidine), 2.64 (0.7 \times 3H, s, NCH₃ major), 2.85 (0.3 \times 3H, s, NCH₃ minor), 3.17–3.36 (2H, m, NCH₂ pyrrolidine), 3.36–3.63 (2H, m, CH₂Nap), 4.20–4.37 (1H, m, CH pyrrolidine), 4.55–4.81 (2H, m, CH₂Ph), 5.12–5.38 (1H, m, CHCH₂Nap), 6.83–6.96 (2H, m, ArH), 7.03–7.19 (3H, m, ArH), 7.46–7.52 (3H, m, ArH), 7.52–7.65 (1H, m, ArH), 7.65–7.84 (4H, m), 10.12–10.28 (1H, s, NH); IR (KBr) 3350–3200 s, br, 2978 m, 2931 s, 1749 s, 1634 s, 1605 s cm⁻¹; MS *m/e* (FAB) 658 (MH)⁺ (32%), 458 (100%).

***N*-(*N,N*-Di-*tert*-butoxycarbonylamidino)-*N*-[2-chlorobenzyl]-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-*N*-benzylmethylamide.** To *N*-(*N,N*-di-*tert*-butoxycarbonylamidino)-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-*N*-benzylmethylamide (165 mg, 0.25 mmol) dissolved in DMF (5 mL) was added NaH (12 mg, 0.3 mmol). The reaction was stirred at room temperature for 5 min before 2-chlorobenzyl bromide (37 mL, 0.28 mmol) was added. The reaction mixture was stirred at room temperature for 2 h. The reaction mixture was poured into water and extracted with EtOAc (3 \times 50 mL). The combined organic extracts were washed with brine, dried (MgSO₄), and concentrated. The residue was purified by flash column chromatography (hexane/EtOAc 1:2) to afford *N*-(*N,N*-di-*tert*-butoxycarbonylamidino)-*N*-[2-chlorobenzyl]-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-*N*-benzylmethylamide (98 mg, 50%): ¹H NMR (CDCl₃) mixture of two rotamers δ 1.29–1.63 (18H, m, *t*-Bu), 1.63–1.97 (4H, m, CH₂ pyrrolidine), 2.67 (0.3 \times 3H, s, NCH₃ minor), 2.82 (0.7 \times 3H, s, NCH₃ major), 2.97–3.48 (4H, m, NCH₂ pyrrolidine, CH₂Nap), 4.15–4.37 (4H, m, NHCH₂Ar, NCH₂Ph), 4.42–4.69 (1H, m, CH pyrrolidine), 4.88–5.29 (1H, m, CHCH₂Nap), 6.66–7.15 (6H, m, ArH), 7.15–7.31 (2H, m, ArH), 7.31–7.56 (5H, m, ArH), 7.56–7.83 (4H, m, ArH); IR (KBr) 2977 m, 1722 s, 1680 s, 1653 s, 1600 s cm⁻¹; MS *m/e* (FAB) 782 (MH)⁺ (98%), 582 (100%).

***N*-Amidino-*N*-[2-chlorobenzyl]-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-*N*-benzylmethylamide, 4aj.** *N*-(*N,N*-di-*tert*-butoxycarbonylamidino)-*N*-[2-chlorobenzyl]-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-*N*-benzylmethylamide (181 mg, 0.23 mmol) was treated with a mixture of trifluoroacetic acid (2 mL) and CH₂Cl₂ (2 mL). The reaction mixture was stirred at room temperature for 4 h. The reaction mixture was concentrated and purified by preparative HPLC (gradient run (90:10–0:100 H₂O:acetonitrile) to afford a cream-colored foam (63 mg, 0.1 mmol, 47%): mp 73–76 °C; ¹H NMR (CDCl₃) mixture of two rotamers δ 1.84–2.32 (4H, m, CH₂ pyrrolidine), 2.72 (0.7 \times 3H, s, NCH₃ major), 2.82 (0.3 \times 3H, s, NCH₃ minor), 3.03–3.25 (2H, m, CH₂Nap), 3.38–3.81 (2H, m, NCH₂ pyrrolidine), 4.20–4.81 (5H, m, NHCH₂Ar, CH pyrrolidine, NCH₂Ph), 5.02–5.25 (1H, m, CHCH₂Nap), 6.77–7.82 (18H, m, ArH, NH), 8.30–8.45 (1H, ArH); IR (KBr) 3510–3100 w, br, 1663 s, 1632 s cm⁻¹;

MS *m/e* (FAB) 582 (MH)⁺ (100%). Anal. (C₃₄H₃₆N₅O₂ \cdot 1.0H₂O \cdot 1.3C₂HF₃O₂) C, H, N, F.

2-Chlorophenylthiocarbamoyl-(S)-prolyl-(S)-phenylalanyl-*N*-benzylmethylamide, 5a. H-Pro-Phe-benzylmethylamide³⁶ was treated with 2-chlorophenyl isothiocyanate as described above for 3a. The title compound was obtained in 63% yield after crystallization (hexane/EtOAc): TLC (CH₂Cl₂/MeOH 14:1) *R*_f 0.76; ¹H NMR (CDCl₃) mixture of two rotamers δ 2.05–2.27 (4H, m, CH₂CH₂), 2.67 (0.7 \times 3H, s, NCH₃, major), 2.87 (0.3 \times 3H, s, NCH₃, minor), 3.07 (0.3 \times 2H, d, *J* = 9.5 Hz, PhCH₂), 3.09 (0.7 \times 2H, d, *J* = 8.0 Hz, PhCH₂), 3.65–3.83 (2H, m, NCH₂), 4.30 (0.3 \times 1H, d_{AB}, *J* = 17.4 Hz, PhCH₂H_BN, minor), 4.39 (0.7 \times 1H, d_{AB}, *J* = 14.7 Hz, PhCH₂H_BN, major), 4.60 (1H, d_{AB}, *J* = 14.7 Hz, PhCH₂H_BN, both), 5.02–5.27 (2H, m, α -H, α -H), 6.98–7.52 (14H, m, ArH, NH, NH), 8.23 (1H, d, *J* = 9 Hz, ArH); IR 3327 (m), 3239 (br), 1629 (s), 1520 (s), 1383 (m), 1344 (m) cm⁻¹; MS *m/e* (FAB) 535 (MH)⁺, 269 (100%). Anal. (C₂₉H₃₁N₄O₂SCl) C, H, N.

2-Nitrophenylthiocarbamoyl-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methylamide, 5f. (S)-Prolyl-(S)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methylamide³⁸ was treated with 2-nitrophenyl isocyanate as described for 3a. Flash chromatographic purification (ethyl acetate/hexane 2:1) yielded the title compound in 83% yield: TLC (EtOAc/hexane 1:1) *R*_f 0.1; mp 79–82 °C; ¹H NMR (CDCl₃) mixture of two rotamers δ 1.71–2.05 (3H, m, CH₂ pyrrolidine), 2.15–2.33 (1H, m, CH₂ pyrrolidine), 2.78 (0.6 \times 3H, s, NCH₃ major), 2.94 (0.4 \times 3H, s, NCH₃ minor), 3.14–3.41 (4H, m, CH₂Nap, NCH₂ pyrrolidine), 4.32–4.75 (3H, m, NCH₂Ph, CH pyrrolidine), 5.36 (1H, q, *J* = 7.55 Hz, CHCH₂Nap), 7.05–7.74 (15H, m, ArH), 8.22 (1H, d, *J* = 8.5 Hz, ArH), 8.75 (1H, d, *J* = 8.79 Hz, ArH), 9.90 (0.4 \times 1H, s, NH minor), 10.00 (0.6 \times 1H, s, NH minor); IR (KBr) 3600–3190 w, br, 1681 s, 1645 s, 1610 m, 1586 s, 1501 s, 1452 m, 1437 s, 1355 m cm⁻¹; MS *m/e* (FAB) 580 (MH)⁺ (79%), 459 (8%), 416 (24%), 319 (24%), 262 (80%), 234 (91%), 170 (100%); [α]_D²³ –60.6° (*c* 0.53, CHCl₃). Anal. (C₃₃H₃₃N₅O₅ \cdot 0.5H₂O) C, H, N.

***N*-Boc-*N*-(2-nitrophenyl)thiourea.** To a solution of 2-nitrophenylthiourea (0.59 g, 3 mmol) in dry THF (60 mL) was added NaH (180 mg, 4.5 mmol), causing a red coloration. The mixture was cooled on ice, and di-*tert*-butyl dicarbonate (0.72 g, 3.3 mmol) in THF (10 mL) was added. After 12 h of stirring, the reaction mixture was evaporated in vacuo and then redissolved in EtOAc (100 mL). Then 2 M NaHCO₃ (10 mL) was added and the red aqueous layer removed. The aqueous layer was acidified to pH 4 with 10% citric acid and extracted with EtOAc (50 mL). The orange organic phase was separated, washed with brine, and dried over MgSO₄. The crude product was purified by flash column chromatography (hexane/EtOAc 5:1) to give a yellow solid, 340 mg (38%): TLC (hexane/EtOAc 1:1) *R*_f 0.8; ¹H NMR (CDCl₃) δ 1.54 (9H, s, Boc), 7.37 (1H, t, *J* = 8.2 Hz, ArH), 7.65 (1H, t, *J* = 8.2 Hz, ArH), 8.08 (2H, d, *J* = 8.2 Hz, ArH and NH), 8.43 (1H, d, *J* = 8.2 Hz, ArH), 12.50 (1H, br s, NH); IR 3178 (s), 1722 (s), 1609 (m), 1584 (m), 1552 (s), 1520 (s), 1369 (s), 1339 (s) cm⁻¹; MS *m/e* (FAB) 298 (MH)⁺, 242 (100%).

***N,N*-Bisbutyloxycarbonyl-*N*-(2-nitrophenylamidino)-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-*N*-benzylmethylamide.** *N*-Boc-*N*-(2-nitrophenyl)thiourea (130 mg, 0.44 mmol), triethylamine (0.15 mL, 1 mmol), and (S)-prolyl-(S)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methylamide (183 mg, 0.44 mmol) in DMF (5 mL) was cooled in an ice bath. Mercury(II) chloride (130 mg, 0.48 mmol) was added and the mixture stirred for 1 h. After this time the reaction mixture was diluted with EtOAc and washed with water. The organic phase was separated, washed with brine, dried over Na₂SO₄, and then filtered through a pad of Celite. Evaporation yielded a yellow solid which was purified by flash column chromatography (hexane/EtOAc 1:1) to give the title compound (269 mg, 90%): TLC (hexane/EtOAc 1:1) *R*_f 0.3; ¹H NMR (CDCl₃) mixture of two rotamers δ 1.34 and 1.43 (9H, s, Boc), 1.6–2.2 (4H, m, CH₂CH₂), 2.85 and 2.9 (3H, s, NCH₃), 3.2–3.7 (4H, m, ArCH₂, NCH₂), 4.3–4.7 (3H, m, PhCH₂ and α -H), 5.2 (1H, m, α -H), 6.9–7.2 (8H, m, ArH, NH), 7.3–7.5 (4H, m, ArH), 7.6–7.9 (6H,

m, ArH, NH); IR 3350 (br), 2978 (w), 1717 (m), 1643 (s), 1599 (s), 1520 (m), 1496 (m), 1453 (m), 1417 (m) cm⁻¹; MS *m/e* (FAB) 679 (MH⁺), 261 (100%).

***N*-(2-Nitrophenylamidino)-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-*N*-benzylmethanamide, 5h.** To *N,N*-bisbutyloxycarbonyl-*N*-(2-nitrophenylamidino)-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-*N*-benzylmethanamide (293 mg, 0.43 mmol) in CH₂Cl₂ (4 mL) was added TFA (2 mL). The reaction mixture was stirred at room temperature for 10 min. The reaction mixture was concentrated in vacuo. The pale yellow oil was partitioned between ethyl acetate (50 mL) and saturated NaHCO₃ (30 mL). The organic phase was removed, and the aqueous phase was reextracted twice with ethyl acetate. The combined organic fractions were dried (MgSO₄) and concentrated. The residue was purified by flash column chromatography (5% methanol in CH₂Cl₂), to afford the title compound as an orange foam (198 mg, 0.34 mmol, 80%); mp 64–70 °C; ¹H NMR (CDCl₃) mixture of two rotamers δ 1.62–2.30 (4H, m, CH₂ pyrrolidine), 2.78 (0.6 × 3H, s, NCH₃ major), 2.87 (0.4 × 3H, s, NCH₃ minor), 3.18–3.45 (4H, m, CH₂Nap, NCH₂ pyrrolidine), 4.21–4.73 (5H, m, NCH₂Ph, CH pyrrolidine, 2 × NH), 5.22 (1H, q, *J* = 7.3 Hz, CHCH₂Nap), 6.95–7.13 (7H, m, ArH), 7.32–7.48 (4H, m, ArH), 7.64–7.93 (6H, m, ArH); IR (KBr) 3628–3148 m, br, 1632 s, 1583 s, 1514 m, 1450 m, 1351 m cm⁻¹; MS *m/e* (FAB) 579 (MH⁺) (45%), 261 (100%). Anal. (C₃₃H₃₄N₆O₄·0.5H₂O) C, H, N.

***N*-(2-Nitrophenyl-*N*-cyanoamidino)-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-*N*-benzylmethanamide, 5j.** To cyanamide (44 mg, 1 mmol) in DMF (5 mL) was added potassium *tert*-butoxide (1 mL, 1 M in THF, 1 mmol). A white precipitate formed. The reaction mixture was stirred at room temperature for 20 min. 2-Nitrophenyl isothiocyanate (180 mg, 1 mmol) was added as a solid, and the reaction mixture was stirred at room temperature for 10 min. The solution became dark orange. The reaction mixture was cooled in ice, and triethylamine (0.42 mL, 3 mmol) was added followed by (S)-prolyl-(S)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methanamide (415 mg, 1 mmol) and HgCl₂ (300 mg, 1.1 mmol). After 3 h the reaction mixture was diluted with ethyl acetate (50 mL) and filtered through a pad of Celite. The filtrate was washed with water. The aqueous extract was reextracted with ethyl acetate (2 × 50 mL). The combined organic extracts were dried (MgSO₄) and concentrated in vacuo. The residue was purified by flash column chromatography (ethyl acetate/hexane, 4:1) to afford the title compound as a yellow foam (259 mg, 0.43 mmol, 43%); mp 92–96 °C; ¹H NMR (CDCl₃) mixture of two rotamers δ 1.86–2.37 (4H, m, CH₂ pyrrolidine), 2.68 (0.7 × 3H, s, NCH₃ major), 2.88 (0.3 × 3H, s, NCH₃ minor), 3.13–3.29 (2H, m, CH₂Nap), 3.49–3.71 (2H, m, NCH₂ pyrrolidine), 4.14 (0.3 × 1H, d_{AB}, *J*_{AB} = 15.82 Hz, NCH_AH_BPh minor), 4.37 (0.7 × 1H, d_{AB}, *J*_{AB} = 14.57 Hz, NCH_AH_BPh major), 4.43 (0.3 × 1H, d_{AB}, *J*_{AB} = 15.78 Hz, NCH_AH_BPh minor), 4.56–4.75 (1H, m, CH pyrrolidine), 4.66 (0.7 × 1H, d_{AB}, *J*_{AB} = 14.49 Hz, NCH_AH_BPh major), 5.21–5.38 (1H, m, CHCH₂Nap), 6.88–7.50 (10H, m, ArH), 7.51–7.82 (6H, m, ArH), 8.14 (1H, d, *J* = 8.16 Hz, ArH), 9.38 (0.3 × 1H, s, NH minor), 9.41 (0.7 × 1H, s, NH major); IR (KBr) 3610–3130 w, br, 2177 m, 1647 m, 1578 s, 1496 m cm⁻¹; MS *m/z* (FAB) 604 (MH⁺) (100%), 319 (24%), 286 (32%). Anal. (C₃₄H₃₃N₇O₄·1.5H₂O) C, H, N.

3-Pyridylcarbamoyl-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-*N*-benzylmethanamide, 5r. To (S)-prolyl-(S)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methanamide (645 mg, 1.55 mmol) in toluene (10 mL) was added 3-pyridyl azide (285 mg, 1.55 mmol). The reaction mixture was heated to 90 °C for 5 h and then stirred at room temperature overnight. The reaction mixture was cooled and concentrated in vacuo. The mixture was purified by flash column chromatography (MeOH/CH₂Cl₂, 1:10) to afford a white foam (173 mg, 0.32 mmol, 21%); mp 84–87 °C; ¹H NMR (CDCl₃) mixture of two rotamers δ 1.69–2.27 (4H, m, CH₂ pyrrolidine), 2.78 (0.7 × 3H, s, NCH₃ major), 2.93 (0.3 × 3H, s, NCH₃ minor), 3.03–3.52 (2H, m, CH₂Nap), 4.30–4.61 (3.3H, m, NCH₂ pyrrolidine, NCH_AH_BPh), 4.70 (0.7 × 1H, d_{AB}, *J*_{AB} = 15 Hz, NCH_AH_BPh major), 5.18–5.35 (2H, m, CH pyrrolidine, CHCH₂Nap), 6.95–7.12 (3H, m, ArH), 7.12–7.33

(4H, m, ArH), 7.33–7.56 (4H, m, ArH), 7.56–8.09 (4H, m, ArH), 7.97–8.09 (1H, m, ArH), 8.21–8.33 (1H, m, ArH), 8.48–8.58 (1H, m, ArH); IR (KBr) 3293 w, 2928 w, 1645 s, 1593 w, 1533 s, 1482 m, 1420 s cm⁻¹; MS *m/e* (FAB) 536 (MH⁺) (100%). Anal. (C₃₂H₃₃N₅O₃·HCl·0.25H₂O) C, H, N, Cl.

2-Carboxyphenylcarbamoyl-(S)-prolyl-(S)-naphthylalanyl-*N*-benzyl-*N*-methanamide, 5ac. 2-Methoxycarbonylphenylcarbamoyl-(S)-prolyl-(S)-naphthylalanyl-*N*-benzyl-*N*-methanamide (250 mg, 0.44 mmol) was stirred in MeOH (60 mL) while an aqueous solution of sodium hydroxide (4.4 mL of 5 M, 21.9 mmol) was added dropwise and thereafter for 12 h. After this time complete consumption of starting material was demonstrated by TLC. The solution was evaporated in vacuo and then dissolved in a minimum of water. The solution was acidified to pH 1, causing precipitation of a white solid which was collected by filtration and air-dried to give a colorless solid, 136 mg (56%); mp 128–129 °C; ¹H NMR (CDCl₃) mixture of two rotamers δ 1.25–2.28 (4H, m, proline βγ-CH₂), 2.86 (0.67 × 3H, s, NCH₃ major), 2.97 (0.33 × 3H, s, NCH₃ minor), 3.08–3.46 (4H, m, naphthylAla-CH₂CH, proline δ), 4.22 (0.66 × 1H, d_{AB}, *J* = 14.5 Hz, ArCH_AH_BNCH₃ major), 4.44–4.66 (1.33 × 1H, ArCH_AH_BNCH₃ minor, proline α-CH), 4.92 (0.66 × 1H, m, d_{AB}, *J* = 14.5 Hz, ArCH_AH_BNCH₃ major), 5.46 (1H, br m, naphthylalanine α-CH), 6.62–7.85 (15H, m, ArH), 8.30 (0.33 × 1H, br d, CONH minor), 8.38 8.30 (0.66 × 1H, br d, CONH major), 8.57 (1H, d, *J* = 8 Hz, ArH₃), 10.90–11.00 (1H, br s, urea NH); FABMS *m/e* 579, 45% (MH⁺). Anal. (C₃₄H₃₄N₄O₅·0.2H₂O) C, H, N.

2-Carboxamidophenylcarbamoyl-(S)-prolyl-(S)-naphthylalanyl-*N*-benzyl-*N*-methanamide, 5ad. 5ac (500 mg, 0.86 mmol) was stirred in dry EtOAc (25 mL), under N₂, at –15 °C, and *N*-methylmorpholine (NMM, 104 μL, 0.95 mmol) was added. A solution of isobutyl chloroformate (124 μL, 0.95 mmol) in dry EtOAc (25 mL) was added dropwise, such that the temperature did not exceed –10 °C. The reaction mixture was stirred for 15 min before dry ammonia gas was slowly bubbled through the solution with efficient stirring for 30 min. The reaction mixture was evaporated and the product purified by flash column chromatography (cyclohexane/EtOAc 1:4 to elute high *R_f* material, then changing to EtOAc/MeOH 1:1 to elute the product). Concentration of the pure fractions and then crystallization from cyclohexane/EtOAc gave a colorless solid, 270 mg (54%); mp 109–115 °C; TLC (silica, cyclohexane/EtOAc 1:4) *R_f* 0.10; ¹H NMR (CDCl₃) mixture of two rotamers δ 1.60–2.29 (4H, m, proline βγ-CH₂), 2.72 (0.67 × 3H, s, NCH₃ major), 2.91 (0.33 × 3H, s, NCH₃ minor), 3.08–3.55 (4H, m, naphthylAla-CH₂CH, proline δ), 4.30 (0.66 × 1H, d_{AB}, *J* = 14.7 Hz, ArCH_AH_BNCH₃ major), 4.36–4.61 (1.33 × 2H, proline α-CH, ArCH_AH_BNCH₃ minor), 4.71 (0.66 × 1H, d_{AB}, *J* = 14.7 Hz, ArCH_AH_BNCH₃ major), 5.41 (1H, br m, naphthylalanine α-CH), 6.00–6.20 (1H, br, CONH), 6.90–7.86 (16H, m, ArH), 8.54 (1H, d, *J* = 8 Hz, ArH₃), 10.11 (1H, s, CONH), 8.38 8.30 (0.66 × 1H, br d, CONH major), 8.57 (1H, d, *J* = 8 Hz, ArH₃), 10.78 (0.33 × 1H, br s, urea NH minor), 10.86 (0.66 × 1H, br s, urea NH major); FABMS *m/e* 578, 10% (MH⁺). Anal. (C₃₄H₃₅N₅O₄·0.75H₂O) C, H, N.

3-Methoxycarbonylphenyl Isocyanate. Methyl 3-aminobenzoate (1.0 g, 0.0066 mol) was dissolved in toluene (20 mL), containing phosgene (0.0265 mol), and stirred at 80 °C for 3 h. The solvent was removed in vacuo, and the product was used without characterization or purification.

3-Methoxycarbonylphenylcarbamoyl-(S)-prolyl-(S)-naphthylalanyl-*N*-benzyl-*N*-methanamide. The title compound was prepared from (S)-prolyl-(S)-phenylalanyl-*N*-benzyl-*N*-methanamide and 3-methoxycarbonylphenyl isocyanate, using the method exemplified in the formation of 3a. The product was purified by flash column chromatography (cyclohexane/EtOAc 1:2) to give a colorless glass, yield 50%; TLC (cyclohexane/EtOAc 1:2) *R_f* 0.25; ¹H NMR (CDCl₃) mixture of two rotamers δ 1.75–2.20 (4H, m, 2 × CH₂ pyrrolidine), 2.79 (0.67 × 3H, s, NCH₃ major), 2.92 (0.33 × 3H, s, NCH₃ minor), 3.0–3.4 (4H, m, ArCH₂CH + CH₂N pyrrolidine), 3.88 (3H, s, ArCO₂-CH₃), 4.34 (0.67 × 1H, d_{AB}, *J*_{AB} = 14.5 Hz, ArCH_AH_BNCH₃ major), 4.48 (1.66H, m, ArCH₂NCH₃ minor + CH pyrrolidine),

4.73 (0.67 × 1H, d_{AB} , J_{AB} = 14.6 Hz, $ArCH_AH_BNCH_3$ major), 5.26 (1H, m, $ArCH_2CH$), 7.00–8.00 (18H, m, 17 × ArH + CONH).

3-Carboxyphenylcarbamoyl-(S)-prolyl-(S)-naphthylalanyl-N-benzyl-N-methylamide. The title compound was prepared from 3-methoxycarbonylphenylcarbamoyl-(S)-prolyl-(S)-naphthylalanyl-N-benzyl-N-methylamide, in the method exemplified in the formation of 2-carboxyphenylcarbamoyl-(S)-prolyl-(S)-naphthylalanyl-N-benzyl-N-methylamide, to yield a colorless foam (76%): TLC (silica, $CH_2Cl_2/MeOH$ 10:1) R_f 0.45; 1H NMR ($CDCl_3$) mixture of two rotamers δ 1.60–2.10 (4H, m, 2 × CH_2 pyrrolidine), 2.80 (0.67 × 3H, s, NCH_3 major), 2.90 (0.33 × 3H, s, NCH_3 minor), 3.30 (2H, m, $ArCH_2CH$), 3.62 (2H, m, CH_2N pyrrolidine), 4.25 (0.67 × 1H, d_{AB} , J_{AB} = 14.5 Hz, $ArCH_AH_BNCH_3$ major), 4.36 (0.33 × 1H, d_{AB} , J_{AB} = 16.6 Hz, $ArCH_AH_BNCH_3$ minor), 4.58 (0.66H, m, CH pyrrolidine), 4.71 (0.67 × 1H, d_{AB} , J_{AB} = 14.6 Hz, $ArCH_AH_BNCH_3$ major), 4.73 (0.33 × 1H, d_{AB} , J_{AB} = 16.6 Hz, $ArCH_AH_BNCH_3$ minor), 5.40 (1H, m, $ArCH_2CH$), 7.00–7.85 (17H, m, 14 × ArH, NH, CO_2H + CONH), 8.07 (1H, m, ArH), 8.19 (1H, d, J = 9.7 Hz, ArH).

3-Carboxamidophenylcarbamoyl-(S)-prolyl-(S)-naphthylalanyl-N-benzyl-N-methylamide, 5ae. The title compound was prepared from 3-carboxyphenylcarbamoyl-(S)-prolyl-(S)-naphthylalanyl-N-benzyl-N-methylamide, using the method exemplified in the formation of the 2-carboxamidophenyl analogue **5ad**. The product was purified by crystallization from EtOAc to give a colorless crystalline solid, yield 42%: mp 140–141 °C; TLC (silica, $CH_2Cl_2/MeOH$ 10:1) R_f 0.75; 1H NMR ($CDCl_3$) mixture of two rotamers δ 1.70–2.20 (4H, m, 2 × CH_2 pyrrolidine), 2.76 (0.67 × 3H, s, NCH_3 major), 2.88 (0.33 × 3H, s, NCH_3 minor), 3.1–3.4 (4H, m, $ArCH_2CH$ + CH_2N pyrrolidine), 4.30 (0.67 × 1H, d_{AB} , J_{AB} = 15.2 Hz, $ArCH_AH_BNCH_3$ major), 4.46 (1.66H, m, CH pyrrolidine + $ArCH_2NCH_3$ minor), 4.67 (0.67 × 1H, d_{AB} , J_{AB} = 14.0 Hz, $ArCH_AH_BNCH_3$ major), 5.25 (1H, m, $ArCH_2CH$), 7.03 (2H, m, ArH), 7.15–7.80 (16H, m, 14 × ArH + 2 × NH); FABMS m/e 578 (MH^+). Anal. ($C_{34}H_{35}N_5O_4 \cdot 0.5H_2O$) C, H, N.

2-Carboxymethylamidophenylcarbamoyl-(S)-prolyl-(S)-naphthylalanyl-N-benzyl-N-methylamide, 5af. The title compound was prepared using the method exemplified in the formation of **5ad** from **5ac**, using dry methylamine gas. The reaction mixture was evaporated and the product purified by flash column chromatography (cyclohexane/EtOAc 1:4). Concentration of the pure fractions and then crystallization from cyclohexane/EtOAc gave a colorless solid, 60% yield: mp 96–102 °C; TLC (cyclohexane/EtOAc 1:4) R_f 0.21; 1H NMR ($CDCl_3$) mixture of two rotamers δ 1.68–2.28 (4H, m, proline $\beta\gamma$ - CH_2), 2.70 (0.67 × 3H, s, NCH_3 major), 2.91 (0.33 × 3H, s, NCH_3 minor), 2.96 (3H, d, J = 4 Hz, $NHCH_3$), 3.21 (2H, m, naphthylAla- CH_2CH), 3.45 (2H, m, proline δ), 4.29 (0.66 × 1H, d_{AB} , J = 14.7 Hz, $ArCH_AH_BNCH_3$ major), 4.32–4.58 (0.33 × 2H, $ArCH_AH_BNCH_3$ minor, 1H, proline α -CH), 4.71 (0.66 × 1H, d_{AB} , J = 14.7 Hz, $ArCH_AH_BNCH_3$ major), 5.29 (1H, br m, naphthylalanine α -CH), 6.52 (1H, br, $CONHCH_3$), 6.90–7.84 (16H, m, 15 × ArH, CONH), 8.50 (1H, dd, J = 8 Hz, J = 2 Hz ArH₃), 10.72 (0.33 × 1H, br s, urea NH minor), 10.79 (0.66 × 1H, br s, urea NH major); FABMS m/e 592, 75% (MH^+). Anal. ($C_{35}H_{37}N_5O_4$) C, H, N.

2-Carboxydimethylamidophenylcarbamoyl-(S)-prolyl-(S)-naphthylalanyl-N-benzyl-N-methylamide, 5ag. The title compound was prepared using the method exemplified in the formation of **5ad** from **5ac**, using anhydrous dimethylamine and isopropyl chloroformate. The reaction mixture was evaporated and the product purified by flash column chromatography (silica, EtOAc/MeOH 10:1). Evaporation of the pure gave a colorless solid, 75% yield: mp 71–73 °C; TLC (silica, cyclohexane/EtOAc 1:4) R_f 0.07; 1H NMR ($CDCl_3$) mixture of two rotamers δ 1.65–2.16 (4H, m, proline $\beta\gamma$ - CH_2), 2.67 (0.67 × 3H, s, NCH_3 major), 2.88 (0.33 × 3H, s, NCH_3 minor), 3.08 (6H, s, $N(CH_3)_2$), 3.22 (2H, m, naphthylAla- CH_2CH), 3.39 (2H, m, proline δ), 4.29 (0.66 × 1H, d_{AB} , J = 15.1 Hz, $ArCH_AH_BNCH_3$ major), 4.39 (0.33 × 1H, d_{AB} , J = 10.1 Hz $ArCH_AH_BNCH_3$ minor), 4.48 (0.33 × 1H, d_{AB} , J = 10.1 Hz $ArCH_AH_BNCH_3$ minor), 4.52 (1H, m, 1H, proline α -CH), 4.71 (0.66 ×

1H, d_{AB} , J = 15.1 Hz, $ArCH_AH_BNCH_3$ major), 5.29 (1H, br m, naphthylalanine α -CH), 6.90–7.82 (16H, m, 15 × ArH, CONH), 8.26 (1H, d, J = 8 Hz, ArH₃), 8.79 (0.33 × 1H, br s, urea NH minor); 8.85 (0.66 × 1H, br s, urea NH major); FABMS m/e 607, 70% (MH^+). Anal. ($C_{36}H_{39}N_5O_4 \cdot 0.9H_2O$) C, H, N.

2-Methoxymethylphenylcarbamoyl-(S)-prolyl-(S)-naphthylalanyl-N-benzyl-N-methylamide, 5ah. The title compound was prepared from (S)-prolyl-(S)-phenylalanyl-N-benzyl-N-methylamide and 2-methoxymethylphenyl isocyanate,³⁹ using the method exemplified in the formation of **3a**. The product was purified by flash column chromatography (cyclohexane/EtOAc 1:2) as a colorless glass, yield 71%: mp 62–66 °C; TLC (silica, cyclohexane/EtOAc 1:2) R_f 0.26; 1H NMR ($CDCl_3$) mixture of two rotamers δ 1.60–2.30 (4H, m, proline $\beta\gamma$ - CH_2), 2.71 (0.67 × 3H, s, NCH_3 major), 2.89 (0.33 × 3H, s, NCH_3 minor), 3.00–3.35 (7H, m, naphthylAla- CH_2CH , proline δ , $ArCH_2OCH_3$), 4.29 (0.66 × 1H, d_{AB} , J = 13.5 Hz, $ArCH_AH_BNCH_3$ major), 4.38–4.59 (0.33 × 2H, m, $ArCH_AH_BNCH_3$ minor, 3H, $ArCH_2OCH_3$, proline α -CH), 4.70 (0.66 × 1H, d_{AB} , J = 13.5 Hz, $ArCH_AH_BNCH_3$ major), 5.29 (1H, br m, naphthylalanine α -CH), 6.90–7.80 (16H, m, 15 × ArH, CONH), 8.26 (1H, d, J = 8 Hz, ArH₃), 8.79 (0.33 × 1H, br s, urea NH minor), 8.85 (0.66 × 1H, br s, urea NH major); FABMS m/e 579, 100% (MH^+). Anal. ($C_{35}H_{38}N_4O_4$) C, H, N.

tert-Butyl(2-isocyanatobenzoyloxy)dimethylsilane. *tert*-Butyl(2-aminobenzoyloxy)dimethylsilane⁴⁰ (400 mg, 0.0017 mol), was dissolved in toluene (5 mL), and 20% phosgene in toluene (3.5 mL) was added. The solution was stirred at 60 °C for 1 h, and was complete by TLC. The toluene was removed in vacuo, and the isocyanate was used without purification or characterization.

2-(tert-Butyldimethylsilyloxymethyl)phenylcarbamoyl-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-N-benzyl-N-methylamide. The title compound was prepared from (S)-prolyl-(S)-N-methyl-3-(2-naphthyl)alanyl-N-benzyl-N-methylamide and *tert*-butyl(2-isocyanatobenzoyloxy)dimethylsilane, using the method exemplified in the formation of **3a**. The product was purified by flash column chromatography (cyclohexane/EtOAc 1:1) to give a colorless amorphous solid, yield 42%: TLC (cyclohexane/EtOAc 1:1) R_f 0.30; 1H NMR ($DMSO-d_6$) mixture of two rotamers δ 0.07 (3H, s, $SiCH_3$), 0.09 (3H, s, $SiCH_3$), 0.88 (9H, s, $tBuSi$), 1.70–2.00 (4H, m, 2 × CH_2 pyrrolidine), 2.75 (0.33 × 3H, s, NCH_3 major), 2.85 (0.67 × 3H, s, NCH_3 minor), 3.15 (2H, m, $ArCH_2CH$), 3.45 (2H, m, CH_2N pyrrolidine), 4.30–4.80 (3H, m, $ArCH_2NCH_3$ + CH pyrrolidine), 5.10 (1H, m, $ArCH_2CH$), 7.00–7.80 (17H, m, 17 × ArH + NH), 8.22 (1H, m, NH).

2-Hydroxymethylphenylcarbamoyl-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-N-benzyl-N-methylamide, 5aj. 2-(*tert*-Butyldimethylsilyloxymethyl)phenylcarbamoyl-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-N-benzyl-N-methylamide (300 mg, 0.00044 mol) was dissolved in dry THF (6 mL) with tetrabutylammonium fluoride (155 mg, 0.00049 mol), and stirred for 15 min. The reaction was poured into saturated aqueous ammonium chloride and the product extracted into EtOAc (3 × 50 mL). The organic washes were combined, washed with brine (25 mL), then dried over $MgSO_4$, and filtered, and the solvent was removed in vacuo. The product was purified by flash column chromatography (silica, cyclohexane/EtOAc 1:4), then precipitated from EtOAc in *n*-hexane, to give a white powdered solid, yield 200 mg (80%): mp 83–86 °C; TLC (silica, cyclohexane/EtOAc 1:4) R_f 0.18; 1H NMR ($CDCl_3$) mixture of two rotamers δ 1.70–2.20 (4H, m, 2 × CH_2 pyrrolidine), 2.79 (0.67 × 3H, s, NCH_3 major), 2.94 (0.33 × 3H, s, NCH_3 minor), 3.20 (4H, m, $ArCH_2CH$ + CH_2N pyrrolidine), 4.37 (0.67 × 1H, d_{AB} , J_{AB} = 14.5 Hz, $ArCH_AH_BNCH_3$ major), 4.50 (1.66H, m, $ArCH_2NCH_3$ minor + CH pyrrolidine), 4.67 (0.67 × 1H, d_{AB} , J_{AB} = 14.7 Hz, $ArCH_AH_BNCH_3$ major), 5.20 (1H, m, $ArCH_2CH$), 7.00–7.80 (16H, m, ArH), 8.00 (1H, m, NH); FABMS m/e 565 (MH^+). Anal. ($C_{34}H_{36}N_4O_4 \cdot 0.33H_2O$) C, H, N.

2-(tert-Butyldimethylsilyloxy)phenylcarbamoyl-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-N-benzyl-N-methylamide. The title compound was prepared from (S)-prolyl-(S)-N-methyl-3-(2-naphthyl)alanyl-N-benzyl-N-methylamide and

Table 6. Characterization Data on Compounds Synthesized by Repetitive Methods^a

compd no.	chromatography solvent	crystallization solvent	yield, %	mp, °C	TLC	FABMS (MH ⁺) <i>m/e</i>	anal.
3a	CHEX/EtOAc 1:1	EGDME/water	80	105–106	CHEX/EtOAc 1:1, <i>R_f</i> 0.22	460	(C ₂₇ H ₂₉ N ₃ O ₂ S) C, H, N
3b	HEX/EtOAc 2:3		77	66–69	HEX/EtOAc 1:1, <i>R_f</i> 0.5	430	(C ₂₆ H ₂₇ N ₃ OS) C, H, N
3c		HEX/EtOAc	41	154–156	CHEX/EtOAc 1:1, <i>R_f</i> 0.24	465	(C ₂₆ H ₂₆ N ₃ CLOS) C, H, N
3d		HEX/EtOAc	31	136–138	CHEX/EtOAc 1:1, <i>R_f</i> 0.25	449	(C ₂₆ H ₂₆ N ₃ FOS) C, H, N
3e	CHEX/EtOAc 2:1	HEX/EtOAc	45	152–155	CHEX/EtOAc 1:1, <i>R_f</i> 0.3	508	(C ₂₆ H ₂₆ N ₃ BrOS) C, H, N
3f	CHEX/EtOAc 1:1	EtOAc	81	143–145	CHEX/EtOAc 1:1, <i>R_f</i> 0.14	475	(C ₂₆ H ₂₆ N ₄ O ₃ S) C, H, N
3h		HEX/EtOAc	63	147–149	CHEX/EtOAc 1:2, <i>R_f</i> 0.4	444	(C ₂₇ H ₂₉ N ₃ O ₃) C, H, N
3k	CHEX/EtOAc 1:1	HEX/EtOAc	82	glass	CHEX/EtOAc 1:1, <i>R_f</i> 0.29	488	(C ₂₉ H ₃₃ N ₃ O ₂ S) C, H, N
3l	CHEX/EtOAc 1:1	MeOH/water	77	66–67	CHEX/EtOAc 1:1, <i>R_f</i> 0.24	474	(C ₂₈ H ₃₁ N ₃ O ₂ S) C, H, N
3m		HEX/EtOAc	63	166	CHEX/EtOAc 1:1, <i>R_f</i> 0.25	474	(C ₂₈ H ₃₁ N ₃ O ₂ S) C, H, N
3n	CHEX/EtOAc 1:1	HEX/EtOAc	75	82–83	CHEX/EtOAc 1:1, <i>R_f</i> 0.14	545	(C ₃₁ H ₃₆ N ₄ O ₃ S) C, H, N
3p	CHEX/EtOAc 1:1	HEX/EtOAc	92	68–69	CHEX/EtOAc 1:1, <i>R_f</i> 0.10	545	(C ₃₁ H ₃₆ N ₄ O ₃ S·0.25H ₂ O) C, H, N
4a	CHEX/EtOAc 1:1	HEX/EtOAc	64	165	CHEX/EtOAc 1:1, <i>R_f</i> 0.14	549	(C ₃₀ H ₃₃ N ₄ O ₂ SCI) C, H, N
4b	CHEX/EtOAc 1:1	HEX/EtOAc	22	152–153	CHEX/EtOAc 1:1, <i>R_f</i> 0.16	593	(C ₃₀ H ₃₃ N ₄ O ₂ BrS) C, H, N
4c	CHEX/EtOAc 1:1	HEX/EtOAc	77	174–175		533	(C ₃₀ H ₃₃ N ₄ FO ₂ S) C, H, N
4d		HEX/EtOAc	31	152–153		583	(C ₃₁ H ₃₃ N ₄ F ₃ O ₂ S) C, H, N
4e		HEX/EtOAc	40	173–175		560	(C ₃₀ H ₃₃ N ₅ O ₄ S) C, H, N
4f	CHEX/EtOAc 2:1	HEX/EtOAc	67	160–161		529	(C ₃₁ H ₃₆ N ₄ O ₂ S·0.25H ₂ O) C, H, N
4g		HEX/EtOAc	78	89–92	CH ₂ Cl ₂ /MeOH 9:1, <i>R_f</i> 0.88	515	(C ₃₀ H ₃₄ N ₄ O ₂ S) C, H, N
4h	CHEX/EtOAc 1:1	HEX/EtOAc	51	156–157	CHEX/EtOAc 1:1, <i>R_f</i> 0.10	583	(C ₃₀ H ₃₂ N ₄ Cl ₂ O ₂ S) C, H, N
4j		EtOAc	45	172–173	CHEX/EtOAc 1:1, <i>R_f</i> 0.07	651	(C ₃₂ H ₃₂ N ₄ F ₆ O ₂ S) C, H, N
4k	MeOH/EtOAc, 1:20		36	54–56	MeOH/EtOAc 1:50, <i>R_f</i> 0.5	533	(C ₃₀ H ₃₃ ClN ₄ O ₃ ·0.25H ₂ O) C, H, N
4m		HEX/EtOAc	43	132–134	CHEX/EtOAc 1:1, <i>R_f</i> = 0.05	563	(C ₃₁ H ₃₅ N ₄ O ₂ SCI) C, H, N
4n		HEX/EtOAc	32	174–176	CHEX/EtOAc 1:1, <i>R_f</i> = 0.06	535	(C ₂₉ H ₃₁ N ₄ O ₂ SCI) C, H, N
4q		HEX/EtOAc	39	154–156	CHEX/EtOAc 1:1, <i>R_f</i> = 0.08	599	(C ₃₄ H ₃₅ N ₄ O ₂ SCI·0.2H ₂ O) C, H, N
4r	CHEX/EtOAc 1:1	HEX/EtOAc	47	81–82	CHEX/EtOAc 1:1, <i>R_f</i> 0.13	599	(C ₃₄ H ₃₅ N ₄ O ₂ SCI·0.33H ₂ O) C, H, N
4s	CHEX/EtOAc 1:1		37	109–111	CHEX/EtOAc 1:1, <i>R_f</i> = 0.10	685	(C ₃₂ H ₃₁ N ₄ O ₂ SCI ₆) C, H, N
4v		HEX/EtOAc	63	95–97	CH ₂ Cl ₂ /MeOH 25:1, <i>R_f</i> = 0.23	550	(C ₂₉ H ₃₂ N ₅ O ₂ SCI·0.6H ₂ O) C, H, N
4w	CHEX/EtOAc 1:1		47	76–78	CHEX/EtOAc 1:1, <i>R_f</i> = 0.10	563	(C ₃₁ H ₃₅ N ₄ O ₂ SCI·0.3H ₂ O) C, H, N
4x		HEX/EtOAc	47	138–139	CH ₂ Cl ₂ /MeOH 25:1, <i>R_f</i> 0.58	563	(C ₃₁ H ₃₅ N ₄ O ₂ SCI) C, H, N
4y		HEX/EtOAc	77	91–92	CHEX/EtOAc 1:1, <i>R_f</i> 0.34	625	(C ₃₆ H ₃₇ N ₄ O ₂ SCI) C, H, N
4aa		HEX/EtOAc	56	101–103	CHEX/EtOAc 1:1, <i>R_f</i> 0.05	588	(C ₃₂ H ₃₄ N ₅ O ₂ SCI) C, H, N
4ab		HEX/EtOAc	59	160–162	CHEX/EtOAc 1:1, <i>R_f</i> 0.06	602	(C ₃₃ H ₃₆ N ₅ O ₂ SCI·0.5H ₂ O) C, H, N
4ac		HEX/EtOAc	68	126–127	CH ₂ Cl ₂ /MeOH 25:1, <i>R_f</i> 0.58	599	(C ₃₄ H ₃₅ N ₄ O ₂ SCI·0.33C ₄ H ₉ O ₂) C, H, N
4ad	CHEX/EtOAc 1:1	HEX/EtOAc	84	134–135	CHEX/EtOAc 1:4, <i>R_f</i> 0.44	599	(C ₃₄ H ₃₇ N ₄ O ₂ SCI) C, H, N
4ae	CHEX/EtOAc 1:1	HEX/EtOAc	47	84–85	CHEX/EtOAc 1:1, <i>R_f</i> 0.19	613	(C ₃₅ H ₃₇ N ₄ O ₂ SCI) C, H, N
4af	CHEX/EtOAc 1:1	HEX/EtOAc	66	194–195	CH ₂ Cl ₂ /MeOH 25:1, <i>R_f</i> 0.60	605	(C ₃₂ H ₃₁ N ₄ O ₂ SCI) C, H, N
4ag	CHEX/EtOAc 1:1	HEX/EtOAc	55	92–93	CH ₂ Cl ₂ /MeOH 25:1, <i>R_f</i> 0.59	613	(C ₃₆ H ₃₇ N ₄ O ₂ SCI·0.33H ₂ O) C, H, N
5a		HEX/EtOAc	63	glass	CH ₂ Cl ₂ /MeOH 14:1, <i>R_f</i> 0.76	535	(C ₂₉ H ₃₁ N ₄ O ₂ SCI) C, H, N
5b		HEX/CH ₂ Cl ₂	34	183–184		501	(C ₂₉ H ₃₂ N ₄ O ₂ S) C, H, N, S
5c		HEX/EtOAc	66	86–88		546	(C ₂₉ H ₃₁ N ₅ O ₄ S·0.75H ₂ O) C, H, N
5d	CHEX/EtOAc 1:1		96	glass	HEX/EtOAc 1:1, <i>R_f</i> 0.2	579	(C ₂₉ H ₃₁ BrN ₄ O ₂ S·H ₂ O) C, H, N
5e		HEX/EtOAc	95	118–121	CHEX/EtOAc 1:2, <i>R_f</i> 0.45	596	(C ₃₃ H ₃₃ N ₅ O ₄ S·0.5H ₂ O) C, H, N
5f	HEX/EtOAc 1:2		83	79–82	HEX/EtOAc, 1:1, <i>R_f</i> 0.1	580	(C ₃₃ H ₃₃ N ₅ O ₅ ·0.5H ₂ O) C, H, N
5g	CHEX/EtOAc 1:2		60	71–72	CHEX/EtOAc 1:4, <i>R_f</i> 0.60	594	(C ₃₄ H ₃₅ N ₅ O ₅ ·0.33H ₂ O) C, H, N
5k	CHEX/EtOAc 1:2	HEX/EtOAc	58	73–76	CHEX/EtOAc 1:2, <i>R_f</i> 0.47	580	(C ₃₃ H ₃₃ N ₅ O ₅ ·0.33H ₂ O) C, H, N
5l	CHEX/EtOAc 1:2	HEX/EtOAc	78	76–79	CHEX/EtOAc 1:4, <i>R_f</i> 0.53	580	(C ₃₃ H ₃₃ N ₅ O ₅ ·0.33H ₂ O) C, H, N
5m	CHEX/EtOAc 1:2		79	76–82		580	(C ₃₃ H ₃₃ N ₅ O ₅ ·0.5H ₂ O) C, H, N
5n	CHEX/EtOAc 2:3	CHEX/EtOAc	50	96–98		569	(C ₃₁ H ₃₂ N ₆ O ₅ ·0.25H ₂ O) C, H, N
5p	CHEX/EtOAc 1:2	HEX/EtOAc	86	151–152	CHEX/EtOAc 1:1, <i>R_f</i> 0.16	598	(C ₂₉ H ₂₉ N ₅ O ₅ Cl ₂) C, H, N
5q	CHEX/EtOAc 1:1		97	72–74		535	(C ₃₃ H ₃₄ N ₄ O ₃ ·1.3H ₂ O) C, H, N
5s	HEX/EtOAc 1:4	HEX/CH ₂ Cl ₂	69	98–101	HEX/EtOAc 1:2, <i>R_f</i> 0.24	580	(C ₃₃ H ₃₃ N ₅ O ₅) C, H, N
5t	HEX/EtOAc 1:4	HEX/EtOAc	48	126–129		580	(C ₃₃ H ₃₃ N ₅ O ₅ ·0.25H ₂ O) C, H, N
5u	CHEX/EtOAc 1:2		22	124–126	CH ₂ Cl ₂ /MeOH 25:1, <i>R_f</i> 0.36	560	(C ₃₄ H ₃₃ N ₅ O ₃ ·0.2H ₂ O) C, H, N
5v	CHEX/EtOAc 1:4		22	105–107	CH ₂ Cl ₂ /MeOH 25:1, <i>R_f</i> 0.32	560	(C ₃₄ H ₃₃ N ₅ O ₃) C, H, N
5w	CHEX/EtOAc 1:4		52	150–152	CH ₂ Cl ₂ /MeOH 25:1, <i>R_f</i> 0.21	560	(C ₃₄ H ₃₃ N ₅ O ₃ ·0.25H ₂ O) C, H, N
5x	HEX/EtOAc 1:2	HEX/EtOAc	47	67–70	CHEX/EtOAc 1:1, <i>R_f</i> 0.08	553	(C ₃₃ H ₃₃ N ₄ O ₃ F·0.25H ₂ O) C, H, N
5y	HEX/EtOAc 1:2	HEX/EtOAc	65	82–85	CHEX/EtOAc 1:4, <i>R_f</i> 0.68	553	(C ₃₃ H ₃₃ N ₄ O ₃ F·0.25H ₂ O) C, H, N
5z	HEX/EtOAc 1:2	HEX/EtOAc	67	85–87	CHEX/EtOAc 1:2, <i>R_f</i> 0.30	553	(C ₃₃ H ₃₃ N ₄ O ₃ F·0.25H ₂ O) C, H, N
5aa	HEX/EtOAc 1:3		64	66–69	HEX/EtOAc 1:4, <i>R_f</i> 0.6	603	(C ₃₄ H ₃₃ F ₃ N ₄ O ₃ ·0.25H ₂ O) C, H, N
5ab	CHEX/EtOAc 2:3		57	52–56	CHEX/EtOAc 1:2, <i>R_f</i> 0.22	593	(C ₃₅ H ₃₆ N ₄ O ₅ ·1.2H ₂ O) C, H, N

^a Abbreviations: CHEX, cyclohexane; HEX, *n*-hexane; EGDME, ethylene glycol dimethyl ether.

2-(*tert*-butyldimethylsilyloxy)phenyl isocyanate (prepared as described above) using the method exemplified in the formation of **3a**. The product was purified by flash column chromatography (hexane/EtOAc 4:1) to give the title compound in 73% yield: mp 56–58 °C; ¹H NMR (CDCl₃) mixture of two rotamers δ 0.27 (3H, s, SiCH₃), 0.30 (3H, s, SiCH₃), 1.02 (9H, s, SiC(CH₃)₃), 1.51–2.03 (3H, m, CH₂ pyrrolidine), 2.10–2.32 (1H, m, CH₂ pyrrolidine), 2.73 (0.7 × 3H, s, NCH₃ major), 2.92 (0.3 × 3H, s, NCH₃ minor), 3.04–3.36 (4H, m, CH₂Nap, NCH₂ pyrrolidine), 4.22–4.63 (2.3H, m, NCH₂H_BPh, CH pyrrolidine), 4.72 (0.7 × 1H, d_{AB}, *J*_{AB} = 15 Hz, NCH₂H_BPh major), 5.18–5.37 (1H, m, CHCH₂Nap), 6.76–7.94 (17H, m, ArH), 8.28–8.40 (1H, m, ArH); IR (KBr) 2954 w, 2929 w, 1650 s, 1598 m,

1521 s, 1450 s cm⁻¹; MS *m/e* (FAB) 665 (MH)⁺ (100%). Anal. (C₃₉H₄₈N₄O₄Si) C, H, N.

2-Hydroxyphenylcarbamoyl-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-N-benzyl-N-methylamide, 5ak. To 2-(*tert*-butyldimethylsilyloxy)phenylcarbamoyl-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-N-benzyl-N-methylamide (305 mg, 0.46 mmol) in THF (5 mL) was added TBAF (158 mg, 0.5 mmol). The reaction mixture was stirred at room temperature for 30 min. The reaction mixture was poured into saturated NH₄Cl and extracted with EtOAc (3 × 50 mL). The combined organic extracts were dried (MgSO₄) and concentrated in vacuo. The residue was purified by flash column chromatography (silica, EtOAc/hexane 4:1) to afford a white foam (265 mg, 0.48 mmol,

100%): TLC (silica, EtOAc/Hexane, 2:1) R_f 0.1; mp 98–104 °C; ^1H NMR (CDCl_3) mixture of two rotamers δ 1.73–2.22 (4H, m, CH_2 pyrrolidine), 2.73 (0.7 \times 3H, s, NCH_3 major), 2.91 (0.3 \times 3H, s, NCH_3 minor), 3.08–3.46 (4H, m, CH_2Nap , NCH_2 pyrrolidine), 4.28–4.49 (2.3H, m, $\text{NCH}_A\text{H}_B\text{Ph}$, CH pyrrolidine), 4.68 (0.7 \times 1H, d_{AB} , J_{AB} = 14 Hz, $\text{NCH}_A\text{H}_B\text{Ph}$ major), 5.18–5.34 (1H, m, CHCH_2Nap), 6.60 (0.3 \times 1H, s, NH minor), 6.73 (0.7 \times 1H, s, NH major), 6.93–7.58 (14H, m, ArH), 7.58–7.83 (3H, m, ArH), 9.23 (1H, s, br, OH); IR(KBr) 3269 w, 1637 s, 1600 m, 1531 s, 1498 s, 1452 cm^{-1} ; MS m/e (FAB) 551 (MH) $^+$ (100%). Anal. ($\text{C}_{33}\text{H}_{34}\text{N}_4\text{O}_4 \cdot 0.5\text{H}_2\text{O}$) C, H, N.

2-Aminophenylcarbamoyl-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-N-benzyl-N-methylamide, 5al. To 2-nitrophenylcarbamoyl-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-N-benzyl-N-methylamide (1.15 g, 2 mmol) in ethanol (12 mL) and H_2O (3 mL) were added CaCl_2 (141 mg) and zinc dust (4.3 g). The reaction mixture was heated to reflux for 5 h. The reaction mixture was filtered through Celite to remove the zinc powder, and the filtrate was concentrated in vacuo. The residue was diluted with EtOAc (100 mL) and washed with water (50 mL) and brine (50 mL). The organic phase was dried (MgSO_4) and concentrated in vacuo. The yellow solid was purified by flash column chromatography (silica, EtOAc/MeOH 20:1) to afford 2-aminophenylcarbamoyl-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-N-benzyl-N-methylamide as a yellow foam (816 mg, 1.49 mmol, 74%): mp 92–96 °C; ^1H NMR (CDCl_3) mixture of two rotamers δ 1.71–1.96 (3H, m, CH_2 pyrrolidine), 1.98–2.22 (1H, m, CH_2 pyrrolidine), 2.74 (0.7 \times 3H, s, NCH_3 major), 2.88 (0.3 \times 3H, s, NCH_3 minor), 3.05–3.47 (4H, m, CH_2Nap , NCH_2 pyrrolidine), 4.24–4.49 (2.3H, m, $\text{NCH}_A\text{H}_B\text{Ph}$, CH pyrrolidine), 4.67 (0.7 \times 1H, d_{AB} , J_{AB} = 14 Hz, $\text{NCH}_A\text{H}_B\text{Ph}$ major), 5.18–5.34 (1H, m, CHCH_2Nap), 6.18 (0.3 \times 1H, s, NH minor), 6.23 (0.7 \times 1H, s, NH major), 6.69–6.86 (2H, m, ArH), 6.94–7.08 (3H, m, ArH), 7.08–7.37 (6H, m, ArH), 7.37–7.58 (4H, m, ArH), 7.58–7.83 (4H, m, ArH); IR(KBr) 3401 m, 1640 s, 1506 m, 1453 cm^{-1} ; MS m/e (FAB) 550 (MH) $^+$ (100%). Anal. ($\text{C}_{33}\text{H}_{35}\text{N}_5\text{O}_3 \cdot 0.5\text{H}_2\text{O}$) C, H, N.

2-Acetamidophenylcarbamoyl-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-N-benzyl-N-methylamide, 5am. To 2-aminophenylcarbamoyl-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-N-benzyl-N-methylamide (404 mg, 0.74 mmol) in CH_2Cl_2 (4 mL), cooled in ice were added pyridine (0.06 mL, 0.74 mmol) and acetic anhydride (0.07 mL, 0.74 mmol). The reaction mixture was allowed to warm to room temperature and stirred at room temperature overnight. The reaction mixture was poured into water and extracted with CH_2Cl_2 (3 \times 40 mL). The combined organic extracts were dried (MgSO_4) and concentrated in vacuo. The residue was purified by flash column chromatography (silica, EtOAc/MeOH 20:1) to afford an orange foam (150 mg, 0.25 mmol, 34%): mp 104–110 °C; ^1H NMR (CDCl_3) mixture of two rotamers δ 1.73–2.30 (4H, m, CH_2 pyrrolidine), 2.06 (3H, s, COCH_3), 2.71 (0.7 \times 3H, s, NCH_3 major), 2.88 (0.3 \times 3H, s, NCH_3 minor), 3.04–3.51 (4H, m, CH_2Nap , NCH_2 pyrrolidine), 4.22–4.53 (2.3H, m, $\text{NCH}_A\text{H}_B\text{Ph}$, CH pyrrolidine), 4.69 (0.7 \times 1H, d_{AB} , J_{AB} = 14 Hz, $\text{NCH}_A\text{H}_B\text{Ph}$ major), 5.18–5.35 (1H, m, CHCH_2Nap), 6.90–7.84 (18H, m, ArH), 8.14–8.53 (1H, m, NH); IR (KBr) 3280 m, 3057 w, 1735 w, 1645 s, 1600 m, 1521 s, 1450 m, 1371 cm^{-1} ; MS m/e (FAB) 592 (MH) $^+$ (100%). Anal. ($\text{C}_{35}\text{H}_{36}\text{N}_5\text{O}_4 \cdot \text{H}_2\text{O}$) C, H, N.

Biology. Receptor Binding Assays. Standard binding assays were performed using the following membrane preparations and radioligands.^{34,38}

Rabbit NK₁. Displacement of [^3H][Sar⁹Met(O₂)¹¹]SP (1 nM) binding to rabbit whole brain membranes; K_D for SP = 0.14 \pm 0.03 nM, B_{max} = 9.82 \pm 1.47 fmol/mg of protein.

Rat NK₁. Displacement of [^3H][Sar⁹Met(O₂)¹¹]SP (0.5 nM) binding to rat whole forebrain membranes: K_D for SP = 0.23 \pm 0.09 nM, B_{max} = 14.6 \pm 2.6 fmol/mg of protein.

Human NK₁. Displacement of [^3H]SP (0.6 nM) binding to membranes from Cos-7 cells transiently transfected with the hNK-1R. (K_D for SP = 85 \pm 12 pM), B_{max} = 537 \pm 139 fmol/mg of protein.

The software packages EBDA and LIGAND were used to estimate binding parameters from the experimental data. Data represent mean \pm SEM of at least three separate experiments.

Functional Assays.^{34,38} Guinea Pig Ileum Bioassay. Contractions were evoked by [Sar⁹Met(O₂)¹¹]SP (4.0 nM). The application of the NK₁ receptor agonist was repeated at least five times to establish stable responses. Once the amplitude of the contraction became stable, the NK₁ agonist was administered in the presence of the selected NK₁ receptor antagonist (added 2 min prior to the agonist application to the bath). Each antagonist concentration was tested three times. At least three different concentrations of the antagonist were used. log₁₀ concentration was plotted against calculated responses, and the IC₅₀ value was determined from the curve. Data represent the mean IC₅₀ values \pm SEM, calculated from three separate experiments.

In Vivo Models. A model of carrageenan-induced hyperalgesia in the guinea pig was used to assess the effects of NK₁ antagonists on inflammatory hyperalgesia in vivo.^{38,21} Carrageenan (1.0%, 100 μL) injected intraplantar into one hind paw induces both a mechanical and thermal hyperalgesia which persists over 24 h. To determine the analgesic effects of drugs, 1% carrageenan (100 μL intraplantar) was injected into the hind paw, and 24 h later mechanical hyperalgesia assessed as above. Drug was then administered, and further readings were taken at various times up to 6 h.

ED₃₀ was calculated as the dose required to produce a 30% inhibition of hyperalgesia, and the maximum effect achieved was expressed as percentage reversal of the predose hyperalgesia.

Aspirin was administered orally in 1% tragacanth, and morphine was injected subcutaneously in saline. Three hours after drug administration, morphine completely reversed the hyperalgesia, and the NSAID, aspirin, produced a partial reversal:

morphine (sc): ED₃₀ = 0.5 mg/kg, 79.8 \pm 12.2% efficacy

aspirin (po): ED₃₀ = 71.6 mg/kg, 55.0 \pm 6.8% efficacy

The NK₁ antagonists were given orally in 10% DMSO in tragacanth (1%) or as a microemulsion.³⁸ Three hours after oral administration, **5f** gave a good, though not complete, reversal of hyperalgesia and had potency comparable to subcutaneous morphine:

5f (10% DMSO/tragacanth po):

ED₃₀ = 0.7 \pm 0.3 mg/kg, 68.9 \pm 3.88% efficacy

5f (microemulsion po):

ED₃₀ = 1.1 \pm 0.5 mg/kg, 68.4 \pm 1.3% efficacy

Supporting Information Available: Synthetic procedures for the preparation of and characterization data for compounds included in Table 6 (compounds synthesized by repetitive procedures) (43 pages). Ordering information is given on any current masthead page.

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