2-Nitrophenylcarbamoyl-(*S*)-prolyl-(*S*)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methylamide (SDZ NKT 343), a Potent Human NK_1 Tachykinin Receptor Antagonist with Good Oral Analgesic Activity in Chronic Pain Models

C. Walpole,*,† S. Y. Ko,‡ M. Brown,§ D. Beattie, E. Campbell, F. Dickenson,⊥ S. Ewan,⊥ G. A. Hughes, M. Lemaire,|| J. Lerpiniere, S. Patel, and L. Urban

Novartis Institute for Medical Sciences, 5 Gower Place, London WC1E 6BN, U.K.

Received July 30, 1997

A lead compound which had sub-micromolar affinity for the rabbit NK_1 receptor but negligible affinity for rat NK_1 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 NK_1 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 NK_1 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-NO_2 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 NK_1 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 NK_1, NK_2, and NK_3 receptors.[1] SP is the preferred agonist at NK_1 receptors.[2]

Several non-peptide antagonists at NK_1 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 NK_1 tachykinin receptors are instumental in the development and maintenance of the central component of pain and hyperalgesia. Substance P is an important mediator in nociceptive pathways.[11] NK_1 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, NK_1 receptors become increasingly important.[12] The role of the NK_1 receptor in neuropathic pain is less clear, but recent evidence in animal models suggests that NK_1 antagonists might be effective.[13,14] One published report suggests that NK_1 antagonists may show clinical efficacy in certain pain states.[15]

Besides pain, the main indications forseen for these antagonists are emesis, migraine, inflammation, and chronic bronchitis.[16−18]

Chemistry

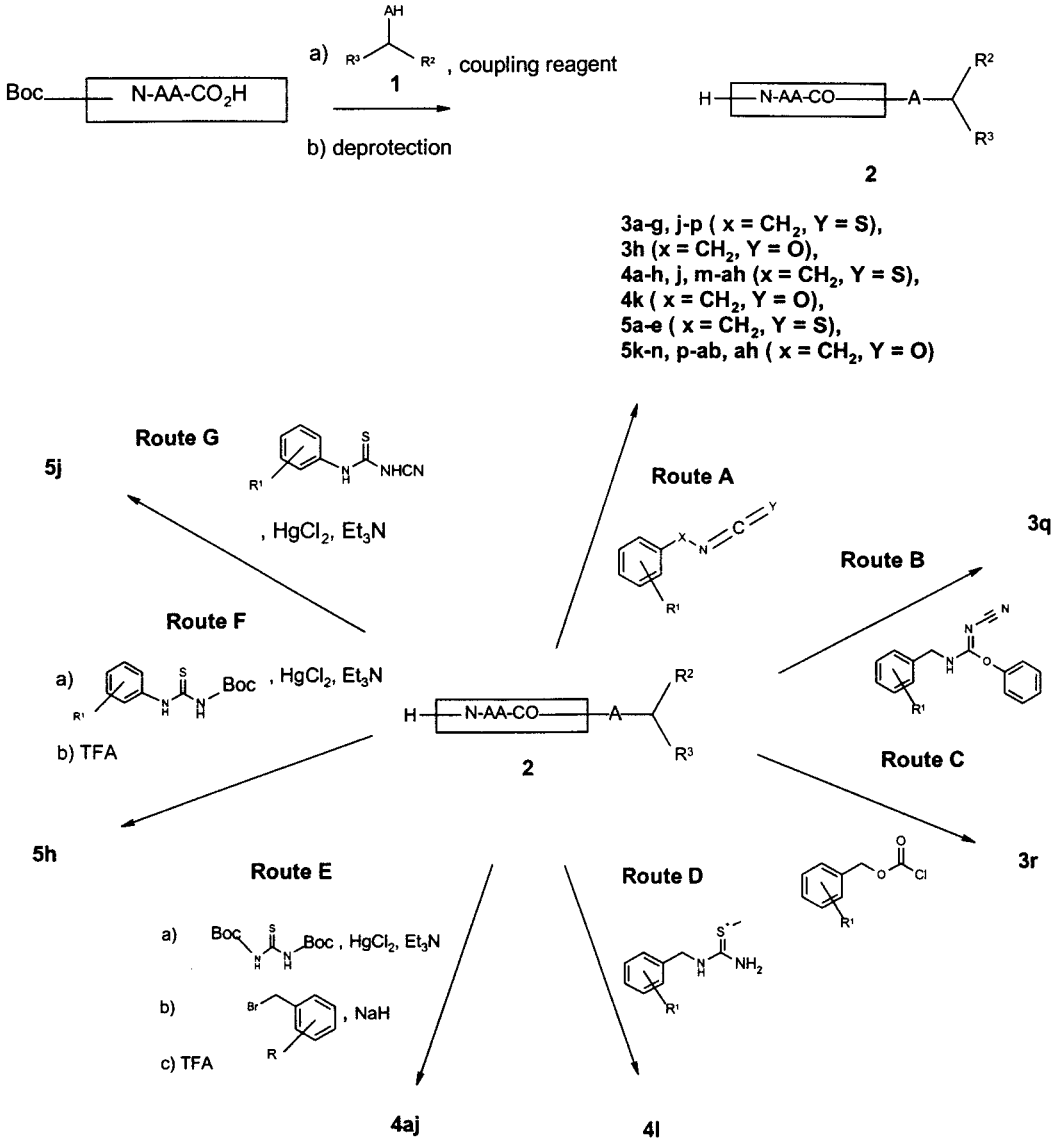
The benzyl- and phenylthioureas and their derivatives were prepared by reaction of the central intermediates **2** with a variety of reagents outlined in Scheme 1 by routes A−G. The intermediates **2** were prepared by reaction of an N-protected amino acid with the amines **1** using a variety of standard 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-

† Current address: Astra Research Centre Montreal, 7171 Frederick Banting, Saint-Laurent, Quebec H4S 1Z9, Canada.
‡ Current address: Samsung Advanced Institute of Technology, 103-112 Moonji-Dong, Yusong-Gu, Taejon, Korea 305-380.
§ Current address: New England Nuclear, Dupont UK Ltd., Wedgewood Way, Stevenage, Hertfordshire SG1 4QN, U.K.
⊥ Current address: Pfizer Central Research, Sandwich, Kent CT13 9NJ, U.K.
|| Novartis Pharma, Basel, Switzerland.

S0022-2623(97)00499-8 CCC: $15.00 © 1998 American Chemical Society
Published on Web 07/21/1998

Scheme 1

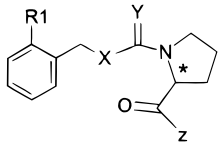


mental Section as exemplified by one example of the class. Full experimental details for the preparation of the individual intermediates is included as Supporting Information. Likewise the repetitive procedures, e.g., the formation of thioureas and ureas from these intermediates, are exemplified by a 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 ($R^1 = NH_2$, Table 1) was prepared by catalytic reduction of **3f** ($R^1 = NO_2$) using H_2/Pd on C. **5ac** (R = Ph-CO_2H, Table 4) was prepared by saponification of **5ab** (R = Ph-CO_2CH_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** (R = Ph-2-CO_2NH_2, Ph-3-CO_2NH_2, Ph-2-CO_2NHCH_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** (Ph-2-$CO_2N(CH_3)_2$, Table 4). **5aj** and **5ak** (Ph-2-CH_2OH 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** (R = Ph-2-NH_2, Table 4) was prepared by catalytic reduction of **5f** (R^1 = Ph-2-NO_2) using H_2/Pd on C. **5am** (R = Ph-2-$NHCOCH_3$, Table 4) was prepared by acetylation of **5al** using acetic anhydride.

The benzyl *N*-cyanoguanidine compound **3q** (X = NH, Y = NCN, Table 1) was prepared by reaction of **2** with the appropriately ring-substituted *N*-cyano-*O*-phenylisourea (route B). The benzyl carbamate, **3r** (X = O, Y = O, Table 1), and guanidine, **4l** (Y = NH, Table 2), were prepared by reaction of **2** with the appropriate chloroformate and *N*-benzyl-*S*-methylthiouronium salt (routes C and D respectively). The guanidines **4aj** (Y = NH, R^1 = Cl, Table 2) and **5h** ($R^1 = NO_2$Ph, Y = 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 NK$_1$ Receptor Binding Affinity of Simple Structural Modifications to **3a**



compd no.	R^1	X	Y	*	Z	rabbit NK$_1$ receptor binding K_i, μM, mean ± SEM ($n \geq 3$) (IC$_{50}$, μM, $n = 2$)
3a	OMe	NH	S	*S*	NHCH(Ph)$_2$	0.33 ± 0.11
3b	H	NH	S	*S*	NHCH(Ph)$_2$	(>5)
3c	Cl	NH	S	*S*	NHCH(Ph)$_2$	0.17 ± 0.06
3d	F	NH	S	*S*	NHCH(Ph)$_2$	1.27 ± 0.53
3e	Br	NH	S	*S*	NHCH(Ph)$_2$	0.17 ± 0.08
3f	NO$_2$	NH	S	*S*	NHCH(Ph)$_2$	1.56 ± 0.61
3g	NH$_2$	NH	S	*S*	NHCH(Ph)$_2$	(>50)
3h	OMe	NH	O	*S*	NHCH(Ph)$_2$	(>50)
3j	OMe	NH	S	*R*	NHCH(Ph)$_2$	(>5)
3k	OMe	NH	S	*S*	NHCH (CH$_2$Ph)$_2$	(>50)
3l	OMe	NH	S	*S*	NHCH$_2$CH(Ph)$_2$	(>5)
3m	OMe	NH	S	*S*	N(CH$_2$Ph)$_2$	(>50)
3n	OMe	NH	S	*S*	NHCH(CH$_2$Ph)CON(CH$_3$)CH$_2$Ph-(*S*)	0.26 ± 0.09
3p	OMe	NH	S	*S*	NHCH(CH$_2$Ph)CON(CH$_3$)CH$_2$Ph-(*R*)	1.28 ± 0.37
3q	OMe	NH	NCN	*S*	NHCH(Ph)$_2$	(>50)
3r	OMe	O	O	*S*	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 NK$_1$ receptor. Guinea pig ileum (GPI) contractions were evoked by the potent NK$_1$ selective peptide agonist [Sar^{9}Met(O$_2$)^{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 pig[21] was used to assess the effects of NK$_1$ 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.

ED$_{30}$ 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

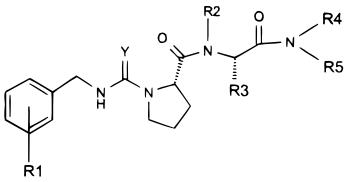
A lead compound, **3a** (Table 1), was discovered by directed screening of a proprietary library of amino acid amides in a rabbit NK$_1$ binding assay. **3a** had submicromolar affinity for the rabbit NK$_1$ receptor (K_i = 0.33 μM) but negligible affinity for the rat receptor.

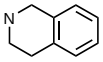
SAR of the Lead Structure, 3a. Simple variation of the aromatic ring substitution in this early lead structure established that 2-substitution was important (compare **3a** and **3b**, Table 1) for sub-micromolar affinity at the rabbit NK$_1$ receptor. 2-Substitution with halogens (Cl and Br but not 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 NK$_1$ 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 NK$_1$ 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 NK$_1$ selective peptide ligands, was used as a basis for design. The proline amide substitu-

Table 2. Optimization of a Series of Benzylthiourea Proline Amides for Human NK_1 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 ($n \geq 3$) (IC_{50}, nM, $n = 2$)
3n	2-OMe	S	H	CH_2Ph	CH_3	CH_2Ph	123.0 ± 34.0
4a	2-Cl	S	H	CH_2Ph	CH_3	CH_2Ph	29.9 ± 5.0
4b	2-Br	S	H	CH_2Ph	CH_3	CH_2Ph	29.7 ± 15.0
4c	2-F	S	H	CH_2Ph	CH_3	CH_2Ph	(290, 480)
4d	2-CF_3	S	H	CH_2Ph	CH_3	CH_2Ph	27.2 ± 10.9
4e	2-NO_2	S	H	CH_2Ph	CH_3	CH_2Ph	77.6 ± 23.2
4f	2-CH_3	S	H	CH_2Ph	CH_3	CH_2Ph	(410, 330)
4g	2-H	S	H	CH_2Ph	CH_3	CH_2Ph	1010 ± 47
4h	2,6-diCl	S	H	CH_2Ph	CH_3	CH_2Ph	25.9 ± 9.6
4j	3,5-diCF_3	S	H	CH_2Ph	CH_3	CH_2Ph	78.6 ± 32.2
4k	2-Cl	O	H	CH_2Ph	CH_3	CH_2Ph	(590, 660)
4l	2-Cl	NH	H	CH_2Ph	CH_3	CH_2Ph	18.5 ± 4.5
4m	2-Cl	S	H	CH_2Ph	CH_3	CH_2CH_2Ph	(2500, 1100)
4n	2-Cl	S	H	CH_2Ph	CH_3	Ph	(100,000, 11700)
4p	2-Cl	S	H	CH_2Ph			8000 ± 250
4q	2-Cl	S	H	CH_2Ph	CH_3	CH_2-1-naphthyl	17.1 ± 4.1
4r	2-Cl	S	H	CH_2Ph	CH_3	CH_2-2-naphthyl	36.0 ± 17.0
4s	2-Cl	S	H	CH_2Ph	CH_3	CH_2(3,5-diCF_3Ph)	26.9 ± 13.0
4t	2-Cl	S	H	CH_2Ph	CH_3	CH_2CH_2(3-indolyl)	48 ± 22
4u	2-Cl	S	H	CH_2Ph	H	CH_2CH_2(3-indolyl)	(3700, 6000)
4v	2-Cl	S	H	CH_2Ph	CH_3	CH_2(4-pyridyl)	(10000, 2500)
4w	2-Cl	S	CH_3	CH_2Ph	CH_3	CH_2Ph	38 ± 11
4x	2-Cl	S	H	CH_2CH_2Ph	CH_3	CH_2Ph	(206, 543)
4y	2-Cl	S	H	CH_2(4-Ph)Ph	CH_3	CH_2Ph	7.2 ± 1.9
4z	2-Cl	S	H	CH_2(3,4-diCl)Ph	CH_3	CH_2Ph	5 ± 1
4aa	2-Cl	S	H	CH_2(3-indolyl)	CH_3	CH_2Ph	16.7 ± 5.3
4ab	2-Cl	S	H	CH_2(N-Me 3-indolyl)	CH_3	CH_2Ph	4.7 ± 1.1
4ac	2-Cl	S	H	CH_2-(1-naphthyl)	CH_3	CH_2Ph	13.7 ± 3.6
4ad	2-Cl	S	H	CH_2-(2-naphthyl)	CH_3	CH_2Ph	0.7 ± 0.3
4ae	2-Cl	S	CH_3	CH_2-(2-naphthyl)	CH_3	CH_2Ph	1.7 ± 0.7
4af	2-Cl	S	H	CH_2-(benzothienyl)	CH_3	CH_2Ph	7.4 ± 2.2
4ag	2-Cl	S	H	$CH(Ph)_2$	CH_3	CH_2Ph	29.4 ± 11.7
4ah	2-Cl	O	H	CH_2-(2-naphthyl)	CH_3	CH_2Ph	2.3 ± 0.6
4aj	2-Cl	NH	H	CH_2-(2-naphthyl)	CH_3	CH_2Ph	0.5 ± 0.2

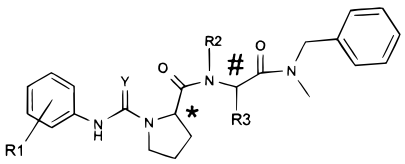
ent was therefore elaborated to the prolyl-phenylalanine amide **3n**. Phenylalanine benzyl amides overlayed the two aryl moieties in the model particulary well and, since *N*-methylbenzylamides had been found to confer oral activity on a series of structurally related analogues including FK 888,[5] 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-Phenylalanylamides. When the parent 2-methoxybenzylthiourea in the prolyl-phenylalanylamide series, **3n**, was tested in the human NK_1 receptor binding assay, it was found to be slightly more potent than at the rabbit NK_1 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 diphenylmethyamide series retained affinity, resulted in a large increase in affinity for the human receptor (compare **3v** with **4a**−**b**). 2-CF_3 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-CF_3 substitution (**4j**), which is known to improve in vitro potency greatly in other structural series of NK_1 antagonists,[8] gave only a modest increase in potency compared with **3n** in this series. 2-Nitro substitution, as in the diphenylmethylamides, 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 NK$_1$ Receptor Binding Affinity



compd no.	R^1	Y	*,#	R^2	R^3	human NK$_1$ receptor binding K_i, nM, mean ± SEM ($n \geq 3$) (IC_{50}, nM, $n = 2$)
5a	2-ClPh	S	*S*,*S*	H	CH_2Ph	(3200, 2700)
5b	2-HPh	S	*S*,*S*	H	CH_2Ph	80.0 ± 24.8
5c	2-NO_2Ph	S	*S*,*S*	H	CH_2Ph	100.0 ± 35.0
5d	2-BrPh	S	*S*,*S*	H	CH_2Ph	(600, 1000)
5e	2-NO_2Ph	S	*S*,*S*	H	CH_2-2-naphthyl	0.8 ± 0.3
5f	2-NO_2Ph	O	*S*,*S*	H	CH_2-2-naphthyl	0.16 ± 0.07
5g	2-NO_2Ph	O	*S*,*S*	CH_3	CH_2-2-naphthyl	0.14 ± 0.04
5h	2-NO_2Ph	NH	*S*,*S*	H	CH_2-2-naphthyl	60.6 ± 20.6
5j	2-NO_2Ph	NCN	*S*,*S*	H	CH_2-2-naphthyl	2.7 ± 1.1
5k	2-NO_2Ph	O	*R*,*R*	H	CH_2-2-naphthyl	82.4 ± 12.4
5l	2-NO_2Ph	O	*S*,*R*	H	CH_2-2-naphthyl	7.1± 1.2
5m	2-NO_2Ph	O	*R*,*S*	H	CH_2-2-naphthyl	274 ± 63
5n	2-NO_2Ph	O	*S*,*S*	H	CH_2-3-indolyl	0.41 ± 0.15
5p	2-NO_2Ph	O	*S*,*S*	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 indolylethyl 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 rotomeric 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 NK$_1$ 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.

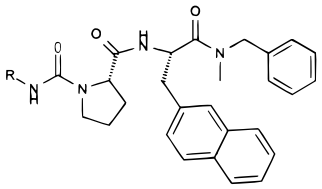
The SAR of the ring substitution is thus clearly different in the phenyl series to that observed in the benzyl series, with the rank order for potency being as follows:

benzyl: $Cl = Br > OMe \approx NO_2 > F > H$

phenyl: $NO_2 \approx H > Cl, Br$

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 K_i, nM, mean ± SEM ($n \geq 3$)	GPI NK_1 IC_{50}, nM, mean ± SEM ($n = 3$)	% inhibn at 30 mg/kg po (3 h after dosing) (DR curve, $n = 3$)
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 ($ED_{30} = 1.1 \pm 0.5$)
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_2CH_3	0.3 ± 0.09	6.0 ± 3.0	NT
5ac	Ph-2-CO_2H	172 ± 58	NT	NT
5ad	Ph-2-CO_2NH_2	0.8 ± 0.2	1.9 ± 0.42	35 ($ED_{30} = 15.7$)
5ae	Ph-3-CO_2NH_2	5.0 ± 1.2	NT	NT
5af	Ph-2-CO_2NHCH_3	3.8 ± 0.6	3.0 ± 1.0	NT
5ag	Ph-2-$CO_2N(CH_3)_2$	6.1 ± 1.5	NT	NT
5ah	Ph-2-CH_2OCH_3	1.0 ± 0.2	1.2 ± 0.4	67.0 ± 1.7 ($ED_{30} = 1.2 \pm 0.1$)
5aj	Ph-2-CH_2OH	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 NK_1 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 different basicity and preferred tautomeric forms, but the most important feature responsible for modulating NK_1 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 ($IC_{50} < 1$ 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

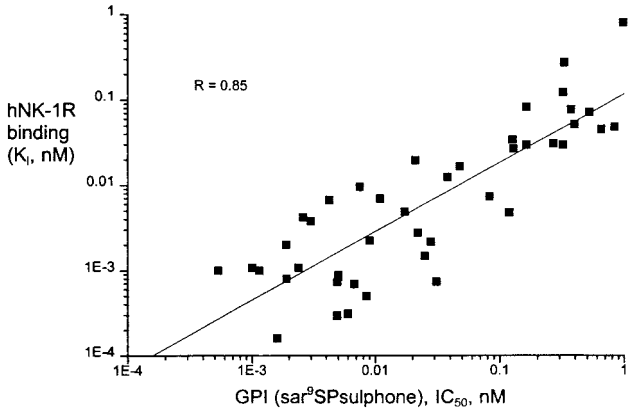


Figure 1. Correlation between hNK$_1$ receptor binding and guinea pig ileum bioassay potencies.

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 aministration 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 ± 1.3%) and had high potency 3 h after oral administration (ED_{30} = 1.1 ± 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 ± 12.2% and orally administered aspirin had a ED_{30} = 71.6 mg/kg and an efficacy of 55.0 ± 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 NH_2 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

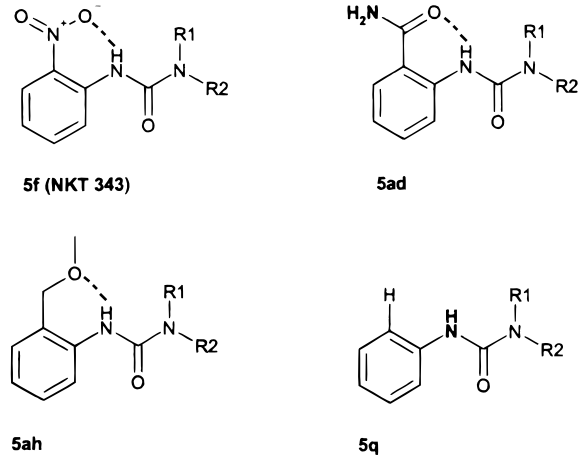


Table 5. Effect of Ring Substitution on Physicochemical Properties of a Series of Phenylureas

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_2OCH_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 NK$_1$ 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 [^{3}H]-**5f** or [^{3}H]-**5ah** together with the diffusible reference [^{14}C]butanol.

The brain extraction ratio (BER) of [^{3}H]-**5f** is plasma protein concentration dependent; 32.4 ± 2.7% in the presence and 16.0 ± 5.0% in the absence of plasma proteins. For comparison, **5ah** has a BER of 20.2 ± 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 [^{3}H]-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 rat[32]) and good brain penetration. An observation that **5f** is a potent inhibitor of P Glycoprotein[33] (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 NK_1 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 elesewhere.[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 F_{254} 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 μm particle size column (83−243-C), dimensions 41.4 × 250 mm. Solvents were HLPC grade and were used without further purification. Chemical yields were not optimized.

Benzyloxycarbonyl-L-proline Diphenylmethylamide. Benzyloxycarbonyl-L-proline (10 g, 40.2 mmol) was stirred in CH_2Cl_2 (50 mL) under a N_2 atmosphere. DCCI (9.13 g, 44.2 mmol) in CH_2Cl_2 (25 mL) and aminodiphenylmethane (7.4 g, 40.2 mmol) in CH_2Cl_2 (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. Benzyloxycarbonyl-L-proline diphenylmethylamide (15 g, 36.2 mmol) was dissolved in methanol (200 mL), and Pd/C (10%) was added under a N_2 atmosphere. The flask was then filled with H_2, 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; ^{1}H NMR ($CDCl_3$) δ 1.80−2.45 (4H, m, proline β- and γ-CH_2), 3.28−3.55 (2H, m, proline δ), 3.82 (3H, s, ArOCH_3), 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$_A$$H_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$), 6.88 (1H, d, J = 8 Hz, ArH^3), 6.95 (1H, d, J = 8 Hz, ArH^5), 7.20−7.40 (12H, m, ArH), 8.26 (1H, m, NHCH(Ph)$_2$); FAB MS *m/e* 460 (MH^+). Anal. ($C_{27}H_{29}N_3O_2S$) 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 H_2, 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 (CH_2Cl_2/MeOH 100:1) and then by preparative reversed phase HPLC (CH_3CN/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; ^{1}H NMR ($CDCl_3$) δ 1.80−2.45 (4H, m, proline β- and γ-CH_2), 3.28−3.48 (2H, m, proline δ-CH_2), 3.91 (2H, br, aniline NH_2) 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$_A$$H_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$), 6.57 (1H, d, J = 8 Hz, ArH^3), 6.68 (1H, m, ArH^5), 7.05−7.10 (2H, m, Ar$H^{4,6}$), 7.20−7.40 (10H, m, diphenylmethyl ArH), 7.96 (1H, m, NH-CH(Ph)$_2$); FAB MS *m/e* 445, 10% (MH^+). Anal. ($C_{28}H_{29}N_4F_3O_3S\cdot1.6H_2O$) 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 $MgSO_4$ 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 $MgSO_4$ 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); ^{1}H NMR ($CDCl_3$) δ 1.71 (2H, m, CH_2 pyrrolidine), 1.98 (1H, m, CH_2 pyrrolidine), 2.15 (1H, m, NH pyrrolidine), 2.97 (2H, m, NCH_2 pyrrolidine), 3.80 (1H, m, CH pyrrolidine), 6.22 (1H, d, J = 8.5 Hz. Ph_2-CH), 7.15−7.38 (10H, m, ArH), 8.41 (1H, d, J = 8.5 Hz. CONH).

2-Methoxybenzylthiocarbamoyl-(*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, mp112−113 °C (75%): TLC (cyclohexane/ethyl acetate 1:1, R_f = 0.17); ^{1}H NMR ($CDCl_3$) δ 1.80−2.47 (4H, m, 2 × CH_2 pyrrolidine), 3.41 (2H, m, NCH_2 pyrrolidine), 3.85 (3H, s, ArOCH_3), 4.80 (1H, dd_{ABX}, J_{AB} = 15.0 Hz, J_{AX} = 5.0 Hz, ArCH_AH$_B$NH), 4.95 (1H, dd_{ABX}, J_{AB} = 15.0 Hz, J_{BX} = 5.0 Hz, ArCH$_A$$H_B$NH), 5.24 (1H, broad, CH pyrrolidine), 6.15 (2H, broad, Ph_2CH and thiourea NH), 6.91 (2H, m, ArH), 7.15−7.40 (12H, m, ArH), 8.25 (1H, broad, CONH); FAB MS *m/e* 460 (MH^+). Anal. ($C_{27}H_{29}N_3O_2S$) 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; ^{1}H NMR($CDCl_3$) δ 1.7−2.26 (4H, m, CH_2CH_2), 3.28−3.54 (2H, m, NCH_2), 3.81 (3H, s, OCH_3), 4.62−4.7 (1H, m, α-H), 4.73 (2H, d, J = 5.8 Hz, ArC*H*$_2$N), 5.50 (1H, br t, NH), 6.13 (1H, d, J = 7.4 Hz, C*H*Ph$_2$), 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. ($C_{28}H_{29}N_5O_2$) C, H, N.

***N*-(2-Methoxybenzyloxycarbonyl)-(*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 CH_2Cl_2 (10 mL) in the presence of Et_3N (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 $NaHCO_3$ (50 mL) and then with brine (50 mL). Drying ($MgSO_4$) 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; ^{1}H NMR($CDCl_3$) δ 1.8−2.5 (4H, m, CH_2CH_2), 3.4−3.6 (2H, m, NCH_2), 3.82 (3H, s, OCH_3), 4.4−4.5 (1H, m, α-H), 5.05−5.33 (2H, m, ArC*H*$_2$), 6.20 (1H, d, J = 8.4 Hz, C*H*Ph$_2$), 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. ($C_{27}H_{28}N_2O_4$) C, H, N.

[Amino(2-chloro-benzylamino)methylene]methylsulfonium Iodide. To (2-chlorobenzyl)thiourea[35] (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%): ^{1}H NMR ($CDCl_3$) δ 2.69 (3H, s, SCH_3) 4.70 (2H, s, CH_2Ar), 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, CH_4) 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; ^{1}H NMR ($CDCl_3$) mixture of two rotamers δ 1.78−2.33 (4H, m, CH_2 pyrrolidine), 2.72 (0.7 × 3H, s, NCH_3 major), 2.81 (0.3 × 3H, s, NCH_3 minor), 2.96−3.10 (2H, m, CHC*H*$_2$Ph), 3.48−3.65 (1H, m, NCH_2 pyrrolidine), 3.69−3.86 (1H, m, NCH_2 pyrrolidine), 4.15−4.60 (4H, NC*H*$_2$Ph, NHC*H*$_2$Ar), 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. ($C_{30}H_{34}ClN_5O_2\cdot0.5H_2O\cdot1.2C_2HF_3O_2$) 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-ylamide[36] 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); ^{1}H NMR ($CDCl_3$) mixture of 2 rotamers δ 1.95−2.22 (4H, m, 2 × CH_2 pyrrolidine), 2.42 (0.3 × 2H, m, ArC*H*$_2$CH$_2$N THIQ minor), 2.75 (0.7 × 2H, m, ArC*H*$_2$CH$_2$N THIQ major), 3.06 (2H, m, PhC*H*$_2$CH), 3.23 (0.3 × 2H, m, ArCH$_2$C*H*$_2$N THIQ minor), 3.38−3.90 (0.7 × 2H + 2H, m, ArCH$_2$C*H*$_2$N THIQ and NCH_2 pyrrolidine), 4.05 (0.3H, d$_{AB}$, J_{AB} = 15.0 Hz, ArC*H*$_A$CH$_B$N THIQ minor), 4.52 (0.3H, d$_{AB}$, J_{AB} = 15.0 Hz, ArCH$_A$C*H*$_B$N THIQ minor), 4.58 (0.7H, d$_{AB}$, J_{AB} = 15.0 Hz, ArC*H*$_A$CH$_B$N THIQ major), 4.74 (0.7H, d$_{AB}$, J_{AB} = 15.0 Hz, ArCH$_A$C*H*$_B$N THIQ major), 4.90 (1H, dd$_{ABX}$, J_{AB} = 15.0 Hz, J_{AX} = 5.0 Hz, ArC*H*$_A$H$_B$NHC(S)N), 4.98 (1H, broad, CH pyrrolidine), 5.02 (1H, dd$_{ABX}$, J_{AB} = 15.0 Hz, J_{BX} = 5.0 Hz, ArCH$_A$H$_B$N*H*C(S)N), 5.19 (1H, m, PhCH$_2$C*H*), 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. ($C_{31}H_{33}N_4O_2SCl$) 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*-[2-(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); ^{1}H NMR ($CDCl_3$) δ 1.80−2.22 (4H, m, 2 × CH_2 pyrrolidine), 2.60−3.10 (7H, m, C*H*$_2$CH$_2$NCH$_3$, PhC*H*$_2$CH and CH$_2$CH$_2$NC*H*$_3$), 3.30−3.70 (4H, m, CH$_2$C*H*$_2$NCH$_3$ and NCH_2 pyrrolidine), 4.85−5.20 (4H, m, ArC*H*$_2$NHC(S)N, CH pyrrolidine, and PhCH$_2$C*H*), 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. ($C_{33}H_{36}N_5O_2SCl$) C, H, N.

2-Chlorobenzylthiocarbamoyl-(*S*)-prolyl-(*S*)-phenylalanyl-*N*-[2-(indol-3-yl)ethyl]amide, 4u. 2-Chlorobenzylthiocarbamoyl-(*S*)-prolyl-(*S*)-phenylalanyl-*N*-[2-(1-Boc-indol-3-yl-)ethyl]amide 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); ^{1}H NMR ($CDCl_3$) δ 1.40−2.10 (4H, m, 2 × CH_2 pyrrolidine), 2.78−3.33 (6H, m, C*H*$_2$CH$_2$NH, PhC*H*$_2$CH and NCH_2 pyrrolidine), 3.57 (2H, m, CH$_2$C*H*$_2$NH), 4.70 (1H, m, PhCH$_2$C*H*), 4.93 (3H, m, ArC*H*$_2$NHC(S)N and CH pyrrolidine), 5.99 (1H, m, thiourea NH), 6.40 (1H, m, CH$_2$CH$_2$N*H*), 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. ($C_{32}H_{34}N_5O_2SCl$) 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 isocyanate[37] (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; ^{1}H NMR ($CDCl_3$) mixture of two rotamers

δ 1.63−1.94 (3H, m, CH_2 pyrrolidine), 2.08−2.25 (1H, m, CH_2 pyrrolidine), 2.72 (0.6 × 3H, s, NCH_3 major), 2.90 (0.4 × 3H, s, NCH_3 minor), 3.00−3.31 (4H, m, CH_2Nap, NCH_2 pyrrolidine), 4.28 (0.6 × 1H,, d_{AB}, J_{AB} = 14 Hz, NCH_AH_BPh major), 4.25−4.56 (2.8 H, m, $ArCH_2NH$, NCH_AH_BPh minor), 4.76 (0.6 × 1H, d_{AB}, J_{AB} = 14 Hz, NCH_AH_BPh major), 4.82−4.98 (1H, m, pyrrolidine), 5.16−5.32 (1H, m, $CHCH_2Nap$), 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. ($C_{34}H_{35}N_4O_3Cl\cdot0.25H_2O$) 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 (138m mg, 0.5 mmol), and Et_3N (0.17 mL, 1.25 mmol) in DMF (2 mL) cooled in ice was added $HgCl_2$ (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 × 50 mL). The combined organic extracts were dried (Na_2SO_4) and filtered through silica. The colorless filtrate was concentrated to afford a white solid (348 mg, 0.5 mmol, 100%): ^{1}H NMR ($CDCl_3$) mixture of two rotamers δ 1.46 (0.3 × 18H, s, *t*-Bu major), 1.51 (0.7 × 18H, s, *t*-Bu minor), 1.68−2.01 (4H, m, CH_2 pyrrolidine), 2.64 (0.7 × 3H, s, NCH_3 major), 2.85 (0.3 × 3H, s, NCH_3 minor), 3.17−3.36 (2H, m, NCH_2 pyrrolidine), 3.36−3.63 (2H, m, CH_2Nap), 4.20−4.37 (1H, m, CH pyrrolidine), 4.55−4.81 (2H, m, CH_2Ph), 5.12−5.38 (1H, m, $CHCH_2$Nap), 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 × 50 mL). The combined organic extracts were washed with brine, dried ($MgSO_4$), 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%): ^{1}H NMR-($CDCl_3$) mixture of two rotamers δ 1.29−1.63 (18H, m, *t*-Bu), 1.63−1.97 (4H, m, CH_2 pyrrolidine), 2.67 (0.3 × 3H, s, NCH_3 minor), 2.82 (0.7 × 3H, s, NCH_3 major), 2.97−3.48 (4H, m, NCH_2 pyrrolidine, CH_2Nap), 4.15−4.37 (4H, m, $NHCH_2Ar$, NCH_2Ph), 4.42−4.69 (1H, m, CH pyrrolidine), 4.88−5.29 (1H, m, $CHCH_2$Nap), 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 CH_2Cl_2 (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 H_2O:acetonitrile) to afford a cream-colored foam (63 mg, 0.1 mmol, 47%): mp 73−76 °C; ^{1}H NMR ($CDCl_3$) mixture of two rotamers δ 1.84−2.32 (4H, m, CH_2 pyrrolidine), 2.72 (0.7 × 3H, s, NCH_3 major), 2.82 (0.3 × 3H, s, NCH_3 minor), 3.03−3.25 (2H, m, CH_2Nap), 3.38−3.81 (2H, m, NCH_2 pyrrolidine), 4.20−4.81 (5H, m, $NHCH_2Ar$, CH pyrrolidine, NCH_2Ph), 5.02−5.25 (1H, m, $CHCH_2$Nap), 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. ($C_{34}H_{36}N_5O_2\cdot1.0H_2O\cdot1.3C_2HF_3O_2$) C, H, N, F.

2-Chlorophenylthiocarbamoyl-(*S*)-prolyl-(*S*)-phenylalanyl-*N*-benzylmethylamide, 5a. H-Pro-Phe-benzylmethylamide[36] was treated with 2-chlorophenyl isothiocyanate as described above for **3a**. The title compound was obtained in 63% yield after crystallization (hexane/EtOAc): TLC (CH_2Cl_2/MeOH 14:1) R_f 0.76; ^{1}H NMR ($CDCl_3$) mixture of two rotamers δ 2.05−2.27 (4H, m, CH_2CH_2), 2.67 (0.7 × 3H, s, NCH_3, major), 2.87 (0.3 × 3H, s, NCH_3, minor), 3.07 (0.3 × 2H, d, J = 9.5 Hz, $PhCH_2$), 3.09 (0.7 × 2H, d, J = 8.0 Hz, $PhCH_2$), 3.65−3.83 (2H, m, NCH_2), 4.30 (0.3 × 1H, d_{AB}, J = 17.4 Hz, $PhCH_AH_BN$, minor), 4.39 (0.7 × 1H, d_{AB}, J = 14.7 Hz, $PhCH_AH_BN$, major), 4.60 (1H, d_{AB}, J = 14.7 Hz, $PhCH_AH_BN$, 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. ($C_{29}H_{31}N_4O_2SCl$) C, H, N.

2-Nitrophenylcarbamoyl-(*S*)-prolyl-(*S*)-3-(2-naphthyl)-alanyl-*N*-benzyl-*N*-methylamide, 5f. (*S*)-Prolyl-(*S*)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methylamide[38] 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; ^{1}H NMR ($CDCl_3$) mixture of two rotamers δ 1.71−2.05 (3H, m, CH_2 pyrrolidine), 2.15−2.33 (1H, m, CH_2 pyrrolidine), 2.78 (0.6 × 3H, s, NCH_3 major), 2.94 (0.4 × 3H, s, NCH_3 minor), 3.14−3.41 (4H, m, CH_2Nap, NCH_2 pyrrolidine), 4.32−4.75 (3H, m, NCH_2Ph. CH pyrrolidine), 5.36 (1H, q, J = 7.55 Hz, $CHCH_2$Nap), 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 × 1H, s, NH minor), 10.00 (0.6 × 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%); $[\alpha]_D^{23}$ −60.6° (*c* 0.53, $CHCl_3$). Anal. ($C_{33}H_{33}N_5O_5\cdot0.5H_2O$) 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 $NaHCO_3$ (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 $MgSO_4$. 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; ^{1}H NMR ($CDCl_3$) 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 Na_2SO_4, 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; ^{1}H NMR ($CDCl_3$) mixture of two rotamers δ 1.34 and 1.43 (9H, s, Boc), 1.6−2.2 (4H, m, CH_2CH_2), 2.85 and 2.9 (3H, s, NCH_3), 3.2−3.7 (4H, m, $ArCH_2$, NCH_2), 4.3−4.7 (3H, m, $PhCH_2$ 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*-benzylmethylamide, 5h.** To *N*,*N*′-bisbutyloxycarbonyl-*N*-(2-nitrophenylamidino)-(*S*)-prolyl-(*S*)-3-(2-naphthyl)alanyl-*N*-benzylmethylamide (293 mg, 0.43 mmol) in CH_2Cl_2 (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 $NaHCO_3$ (30 mL) The organic phase was removed, and the aqueous phase was reextracted twice with ethyl acetate. The combined organic fractions were dried ($MgSO_4$) and concentrated. The residue was purified by flash column chromatography (5% methanol in CH_2Cl_2), to afford the title compound as an orange foam (198 mg, 0.34 mmol, 80%): mp 64−70 °C; ^{1}H NMR ($CDCl_3$) mixture of two rotamers δ 1.62−2.30 (4H, m, CH_2 pyrrolidine), 2.78 (0.6 × 3H, s, NCH_3 major), 2.87 (0.4 × 3H, s, NCH_3 minor), 3.18−3.45 (4H, m, CH_2Nap, NCH_2 pyrrolidine), 4.21−4.73 (5H, m, NCH_2Ph, CH pyrrolidine, 2 × NH), 5.22 (1H, q, J = 7.3 Hz, C*H*CH_2Nap), 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. ($C_{33}H_{34}N_6O_4$·0.5H_2O) C, H, N.

***N*-(*N*-2-Nitrophenyl-*N*′-cyanoamidino)-(*S*)-prolyl-(*S*)-3-(2-naphthyl)alanyl-*N*-benzylmethylamide, 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*-methylamide (415 mg, 1 mmol) and $HgCl_2$ (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 ($MgSO_4$) 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; ^{1}H NMR ($CDCl_3$) mixture of two rotamers δ 1.86−2.37 (4H, m, CH_2 pyrrolidine), 2.68 (0.7 × 3H, s, NCH_3 major), 2.88 (0.3 × 3H, s, NCH_3 minor), 3.13−3.29 (2H, m, CH_2Nap), 3.49−3.71 (2H, m, NCH_2 pyrrolidine), 4.14 (0.3 × 1H, d_{AB}, J_{AB} = 15.82 Hz, NC$H_A$$H_B$Ph minor), 4.37 (0.7 × 1H, d_{AB}, J_{AB} = 14.57 Hz, NC$H_A$$H_B$Ph major), 4.43 (0.3 × 1H, d_{AB}, J_{AB} = 15.78 Hz, NCH$_A$$H_B$Ph minor), 4.56−4.75 (1H, m, CH pyrrolidine), 4.66 (0.7 × 1H, d_{AB}, J_{AB} = 14.49 Hz, NCH$_A$$H_B$Ph major), 5.21−5.38 (1H, m, C*H*CH_2Nap), 6.88−7.50 (10H, m, ArH), 7.51−7.82 (6H, m, ArH), 8.14 (1H, d, J = 8.16 Hz, ArH), 9.38 (0.3 × 1H, s, NH minor), 9.41 (0.7 × 1H, s, NH major); IR (KBr) 3610−3130 w, br, 2177 m, 1647 m, 1578 s, 1496 m cm^{-1}; MS *m/z* (FAB) 604 (MH)$^+$ (100%), 319 (24%), 286 (32%). Anal. ($C_{34}H_{33}N_7O_4$·1.5H_2O) C, H, N.

3-Pyridylcarbamoyl-(*S*)-prolyl-(*S*)-3-(2-naphthyl)alanyl-*N*-benzylmethylamide, 5r. To (*S*)-prolyl-(*S*)-3-(2-naphthyl)-alanyl-*N*-benzyl-*N*-methylamide (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/CH_2Cl_2, 1:10) to afford a white foam (173 mg, 0.32 mmol, 21%): mp 84−87 °C; ^{1}H NMR ($CDCl_3$) mixture of two rotamers δ 1.69−2.27 (4H, m, CH_2 pyrrolidine), 2.78 (0.7 × 3H, s, NCH_3 major), 2.93 (0.3 × 3H, s, NCH_3 minor), 3.03−3.52 (2H, m, CH_2Nap), 4.30−4.61 (3.3H, m, NCH_2 pyrrolidine, NC$H_A$$H_B$Ph), 4.70 (0.7 × 1H, d_{AB}, J_{AB} = 15 Hz, NCH$_A$$H_B$Ph major), 5.18−5.35 (2H, m, CH pyrrolidine, C*H*CH_2Nap), 6.95−7.12 (3H, m, ArH), 7.12−7.33 (4H, m, ArH), 7.33−7.56 (4H, m, ArH), 7.56−8.09 (4H, m, ArH), 7.97−8.09 (1H, m, ArH), 8.21−8.33 (1H, m, ArH), 8.48−8.58 (1H, m, ArH); IR (KBr) 3293 w, 2928 w, 1645 s, 1593 w, 1533 s, 1482 m, 1420 s cm^{-1}; MS *m/e* (FAB) 536 (MH)$^+$ (100%). Anal. ($C_{32}H_{33}N_5O_3$·HCl·0.25H_2O) C, H, N, Cl.

2-Carboxyphenylcarbamoyl-(*S*)-prolyl-(*S*)-naphthylalanyl-*N*-benzyl-*N*-methylamide, 5ac. 2-Methoxycarbonylphenylcarbamoyl-(*S*)-prolyl-(S)-naphthylalanyl-*N*-benzyl-*N*-methylamide (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; ^{1}H NMR ($CDCl_3$) mixture of two rotamers δ 1.25−2.28 (4H, m, proline $\beta\gamma$-CH_2), 2.86 (0.67 × 3H, s, NCH_3 major), 2.97 (0.33 × 3H, s, NCH_3 minor), 3.08−3.46 (4H, m, naphthylAla-CH_2CH, proline δ), 4.22 (0.66 × 1H, d_{AB}, J = 14.5 Hz, ArC$H_A$$H_BNCH_3$ major), 4.44−4.66 (1.33 × 1H, ArCH$_A$$H_BNCH_3$ minor, proline α-C*H*), 4.92 (0.66 × 1H, m, d_{AB}, J = 14.5 Hz, ArC$H_A$$H_BNCH_3$ major), 5.46 (1H, br m, naphthylalanine α-C*H*), 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, ArH_3), 10.90−11.00 (1H, br s, urea NH); FABMS *m/e* 579, 45% (MH$^+$). Anal. ($C_{34}H_{34}N_4O_5$·0.2H_2O) C, H, N.

2-Carboxamidophenylcarbamoyl-(*S*)-prolyl-(*S*)-naphthylalanyl-*N*-benzyl-*N*-methylamide, 5ad. 5ac (500 mg, 0.86 mmol) was stirred in dry EtOAc (25 mL), under N_2, 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; ^{1}H NMR ($CDCl_3$) mixture of two rotamers δ 1.60−2.29 (4H, m, proline $\beta\gamma$-CH_2), 2.72 (0.67 × 3H, s, NCH_3 major), 2.91 (0.33 × 3H, s, NCH_3 minor), 3.08−3.55 (4H, m, naphthylala-CH_2CH, proline δ), 4.30 (0.66 × 1H, d_{AB}, J = 14.7 Hz, ArC$H_A$$H_BNCH_3$ major), 4.36−4.61 (1.33 × 2H, proline α-C*H*, ArC$H_A$$H_BNCH_3$ minor), 4.71 (0.66 × 1H, d_{AB}, J = 14.7 Hz, ArCH$_A$$H_BNCH_3$ major), 5.41 (1H, br m, naphthylalanine α-C*H*), 6.00−6.20 (1H, br, CON*H*), 6.90−7.86 (16H, m, ArH), 8.54 (1H, d, J = 8 Hz, ArH_3), 10.11 (1H, s, CON*H*), 8.38 8.30 (0.66 × 1H, br d, CONH major), 8.57 (1H, d, J = 8 Hz, ArH_3), 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. ($C_{34}H_{35}N_5O_4$·0.75H_2O) 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*-methylamide. The title compound was prepared from (*S*)-prolyl-(*S*)-phenylalanyl-*N*-benzyl-*N*-methylamide 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; ^{1}H NMR ($CDCl_3$) mixture of two rotamers δ 1.75−2.20 (4H, m, 2 × CH_2 pyrrolidine), 2.79 (0.67 × 3H, s, NCH_3 major), 2.92 (0.33 × 3H, s, NCH_3 minor), 3.0−3.4 (4H, m, ArCH_2CH + CH_2N pyrrolidine), 3.88 (3H, s, $ArCO_2$-CH_3), 4.34 (0.67 × 1H, d_{AB}, J_{AB} = 14.5 Hz, ArC$H_A$$H_BNCH_3$ major), 4.48 (1.66H, m, $ArCH_2NCH_3$ 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; ^{1}H 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 pyrollidine), 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; ^{1}H 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. ($C_{34}H_{35}N_5O_4$·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; ^{1}H 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-C*H*), 4.71 (0.66 × 1H, d_{AB}, J = 14.7 Hz, $ArCH_AH_BNCH_3$ major), 5.29 (1H, br m, naphthylalanine α-C*H*), 6.52 (1H, br, CON*H*CH_3), 6.90−7.84 (16H, m, 15 × ArH, CON*H*), 8.50 (1H, dd, J = 8 Hz, J' = 2 Hz ArH_3), 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; ^{1}H 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-C*H*), 4.71 (0.66 × 1H, d_{AB}, J = 15.1 Hz, $ArCH_AH_BNCH_3$ major), 5.29 (1H, br m, naphthylalanine α-C*H*), 6.90−7.82 (16H, m, 15 × ArH, CON*H*), 8.26 (1H, d, J = 8 Hz, ArH_3), 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. ($C_{36}H_{39}N_5O_4$·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; ^{1}H 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-C*H*), 4.70 (0.66 × 1H, d_{AB}, J = 13.5 Hz, $ArCH_AH_BNCH_3$ major), 5.29 (1H, br m, naphthylalanine α-C*H*), 6.90−7.80 (16H, m, 15 × ArH, CON*H*), 8.26 (1H, d, J = 8 Hz, ArH_3), 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-isocyanatobenzyloxy)dimethylsilane.** *tert*-Butyl(2-aminobenzyloxy)dimethylsilane[40] (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*-Butyldimethylsilanyloxymethyl)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-isocyanatobenzyloxy)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; ^{1}H 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, ^{t}BuSi), 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 pyrollidine), 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*-Butyldimethylsilanyloxymethyl)phenylcarbamoyl-(*S*)-prolyl-(*S*)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methylamide (300 mg, 0.000 44 mol) was dissolved in dry THF (6 mL) with tetrabutylammonium fluoride (155 mg, 0.000 49 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; ^{1}H 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 pyrollidine), 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. ($C_{34}H_{36}N_4O_4$·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 olvent	crystallization solvent	yield, %	mp, °C	TLC	FABMS (MH^+) *m/e*	anal.
3a	CHEX/EtOAc 1:1	EGDME/water	80	105−106	CHEX/EtOAc 1:1, R_f 0.22	460	($C_{27}H_{29}N_3O_2S$) C, H, N
3b	HEX/EtOAc 2:3		77	66−69	HEX/EtOAc 1:1, R_f 0.5	430	($C_{26}H_{27}N_3OS$) C, H, N
3c		HEX/EtOAc	41	154−156	CHEX/EtOAc 1:1, R_f 0.24	465	($C_{26}H_{26}N_3ClOS$) C, H, N
3d		HEX/EtOAc	31	136−138	CHEX/EtOAc 1:1, R_f 0.25	449	($C_{26}H_{26}N_3FOS$) C, H, N
3e	CHEX/EtOAc 2:1	HEX/EtOAc	45	152−155	CHEX/EtOAc 1:1, R_f 0.3	508	($C_{26}H_{26}N_3BrOS$) C, H, N
3f	CHEX/EtOAc 1:1	EtOAc	81	143−145	CHEX/EtOAc 1:1, R_f 0.14	475	($C_{26}H_{26}N_4O_3S$) C, H, N
3h		HEX/EtOAc	63	147−149	CHEX/EtOAc 1:2, R_f 0.4	444	($C_{27}H_{29}N_3O_3$) C, H, N
3k	CHEX/EtOAc 1:1	HEX/EtOAc	82	glass	CHEX/EtOAc 1:1, R_f 0.29	488	($C_{29}H_{33}N_3O_2S$) C, H, N
3l	CHEX/EtOAc 1:1	MeOH/water	77	66−67	CHEX/EtOAc 1:1, R_f 0.24	474	($C_{28}H_{31}N_3O_2S$) C, H, N
3m		HEX/EtOAc	63	166	CHEX/EtOAc 1:1, R_f 0.25	474	($C_{28}H_{31}N_3O_2S$) C, H, N
3n	CHEX/EtOAc 1:1	HEX/EtOAc	75	82−83	CHEX/EtOAc 1:1, R_f 0.14	545	($C_{31}H_{36}N_4O_3S$) C, H, N
3p	CHEX/EtOAc 1:1	HEX/EtOAc	92	68−69	CHEX/EtOAc 1:1, R_f 0.10	545	($C_{31}H_{36}N_4O_3S\cdot0.25H_2O$) C, H, N
4a	CHEX/EtOAc 1:1	HEX/EtOAc	64	165	CHEX/EtOAc 1:1, R_f 0.14	549	($C_{30}H_{33}N_4O_2SCl$) C, H, N
4b	CHEX/EtOAc 1:1	HEX/EtOAc	22	152−153	CHEX/EtOAc 1:1, R_f 0.16	593	($C_{30}H_{33}N_4O_2BrS$) C, H, N
4c	CHEX/EtOAc 1:1	HEX/EtOAc	77	174−175		533	($C_{30}H_{33}N_4FO_2S$) C, H, N
4d		HEX/EtOAc	31	152−153		583	($C_{31}H_{33}N_4F_3O_2S$) C, H, N
4e		HEX/EtOAc	40	173−175		560	($C_{30}H_{33}N_5O_4S$) C, H, N
4f	CHEX/EtOAc 2:1	HEX/EtOAc	67	160−161		529	($C_{31}H_{36}N_4O_2S\cdot0.25H_2O$) C, H, N
4 g		HEX/EtOAc	78	89−92	CH_2Cl_2/MeOH 9:1, R_f 0.88	515	($C_{30}H_{34}N_4O_2S$) C, H, N
4h	CHEX/EtOAc 1:1	HEX/EtOAc	51	156−157	CHEX/EtOAc 1:1), R_f 0.10	583	($C_{30}H_{32}N_4Cl_2O_2S$) C, H, N
4j		EtOAc	45	172−173	CHEX/EtOAc 1:1, R_f 0.07	651	($C_{32}H_{32}N_4F_6O_2S$) C, H, N
4k	MeOH/EtOAc, 1:20		36	54−56	MeOH/EtOAc 1:50, R_f 0.5	533	($C_{30}H_{33}ClN_4O_3\cdot0.25H_2O$) C, H, N
4m		HEX/EtOAc	43	132−134	CHEX/EtOAc 1:1, R_f = 0.05	563	($C_{31}H_{35}N_4O_2SCl$) C, H, N
4n		HEX/EtOAc	32	174−176	CHEX/EtOAc 1:1, R_f = 0.06	535	($C_{29}H_{31}N_4O_2SCl$) C, H, N
4q		HEX/EtOAc	39	154−156	CHEX/EtOAc 1:1, R_f = 0.08	599	($C_{34}H_{35}N_4O_2SCl\cdot0.2H_2O$) C, H, N
4r	CHEX/EtOAc 1:1	HEX/EtOAc	47	81−82	CHEX/EtOAc 1:1, R_f 0.13	599	($C_{34}H_{35}N_4O_2SCl\cdot0.33H_2O$) C, H, N
4s	CHEX/EtOAc 1:1		37	109−111	CHEX/EtOAc 1:1, R_f = 0.10	685	($C_{32}H_{31}N_4O_2SClF_6$) C, H, N
4v		HEX/EtOAc	63	95−97	CH_2Cl_2/MeOH 25:1, R_f = 0.23	550	($C_{29}H_{32}N_5O_2SCl\cdot0.6H_2O$) C, H, N
4w	CHEX/EtOAc 1:1		47	76−78	CHEX/EtOAc 1:1, R_f = 0.10	563	($C_{31}H_{35}N_4O_2SCl\cdot0.3H_2O$) C, H, N
4x		HEX/EtOAc	47	138−139	CH_2Cl_2/MeOH 25:1, R_f 0.58	563	($C_{31}H_{35}N_4O_2SCl$) C, H, N
4y		HEX/EtOAc	77	91−92	CHEX/EtOAc 1:1, R_f 0.34	625	($C_{36}H_{37}N_4O_2SCl$) C, H, N
4aa		HEX/EtOAc	56	101−103	CHEX/EtOAc 1:1, R_f 0.05	588	($C_{32}H_{34}N_5O_2SCl$) C, H, N
4ab		HEX/EtOAc	59	160−162	CHEX/EtOAc 1:1, R_f 0.06	602	($C_{33}H_{36}N_5O_2SCl\cdot0.5H_2O$) C, H, N
4ac		HEX/EtOAc	68	126−127	CH_2Cl_2/MeOH 25:1, R_f 0.58	599	($C_{34}H_{35}N_4O_2SCl\cdot0.33C_4H_8O_2$) C, H, N
4ad	CHEX/EtOAc 1:1	HEX/EtOAc	84	134−135	CHEX/EtOAc 1:4, R_f 0.44	599	($C_{34}H_{37}N_4O_2SCl$) C, H, N
4ae	CHEX/EtOAc 1:1	HEX/EtOAc	47	84−85	CHEX/EtOAc 1:1, R_f 0.19	613	($C_{35}H_{37}N_4O_2SCl$) C, H, N
4af	CHEX/EtOAc 1:1	HEX/EtOAc	66	194−195	CH_2Cl_2/MeOH 25:1, R_f 0.60	605	($C_{32}H_{31}N_4O_2S_2Cl$) C, H, N
4ag	CHEX/EtOAc 1:1	HEX/EtOAc	55	92−93	CH_2Cl_2/MeOH 25:1, R_f 0.59	613	($C_{36}H_{37}N_4O_2SCl\cdot0.33H_2O$) C, H, N
5a		HEX/EtOAc	63	glass	CH_2Cl_2/MeOH 14:1, R_f 0.76	535	($C_{29}H_{31}N_4O_2SCl$) C, H, N
5b		HEX/CH_2Cl_2	34	183−184		501	($C_{29}H_{32}N_4O_2S$) C, H, N, S
5c		HEX/EtOAc	66	86−88		546	($C_{29}H_{31}N_5O_4S\cdot0.75H_2O$) C, H, N
5d	CHEX/EtOAc 1:1		96	glass	HEX/EtOAc 1:1, R_f 0.2	579	($C_{29}H_{31}BrN_4O_2S\cdot H_2O$) C, H, N
5e		HEX/EtOAc	95	118−121	CHEX/EtOAc 1:2, R_f 0.45	596	($C_{33}H_{33}N_5O_4S\cdot0.5H_2O$) C, H, N
5f	HEX/EtOAc 1:2		83	79−82	HEX/EtOAc, 1:1, R_f 0.1	580	($C_{33}H_{33}N_5O_5\cdot0.5H_2O$) C, H, N
5g	CHEX/EtOAc 1:2		60	71−72	CHEX/EtOAc 1:4, R_f 0.60	594	($C_{34}H_{35}N_5O_5\cdot0.33H_2O$) C, H, N
5k	CHEX/EtOAc 1:2	HEX/EtOAc	58	73−76	CHEX/EtOAc 1:2, R_f 0.47	580	($C_{33}H_{33}N_5O_5\cdot0.33H_2O$) C, H, N
5l	CHEX/EtOAc 1:2	HEX/EtOAc	78	76−79	CHEX/EtOAc 1:4, R_f 0.53	580	($C_{33}H_{33}N_5O_5\cdot0.33H_2O$) C, H, N
5m	CHEX/EtOAc 1:2		79	76−82		580	($C_{33}H_{33}N_5O_5\cdot0.5H_2O$) C, H, N
5n	CHEX/EtOAc 2:3	CHEX/EtOAc	50	96−98		569	($C_{31}H_{32}N_6O_5\cdot0.25H_2O$) C, H, N
5p	CHEX/EtOAc 1:2	HEX/EtOAc	86	151−152	CHEX/EtOAc 1:1, R_f 0.16	598	($C_{29}H_{29}N_5O_5Cl_2$) C, H, N
5q	CHEX/EtOAc 1:1		97	72−74		535	($C_{33}H_{34}N_4O_3\cdot1.3H_2O$) C, H, N
5s	HEX/EtOAc 1:4	HEX/CH_2Cl_2	69	98−101	HEX/EtOAc 1:2, R_f 0.24	580	($C_{33}H_{33}N_5O_5$) C, H, N
5t	HEX/EtOAc 1:4	HEX/EtOAc	48	126−129		580	($C_{33}H_{33}N_5O_5\cdot0.25H_2O$) C, H, N
5u	CHEX/EtOAc 1:2		22	124−126	CH_2Cl_2/ MeOH 25:1 R_f 0.36	560	($C_{34}H_{33}N_5O_3\cdot0.2H_2O$) C, H, N
5v	CHEX/EtOAc 1:4		22	105−107	CH_2Cl_2/MeOH 25:1, R_f 0.32	560	($C_{34}H_{33}N_5O_3$) C, H, N
5w	CHEX/EtOAc 1:4		52	150−152	CH_2Cl_2/MeOH 25:1, R_f 0.21	560	($C_{34}H_{33}N_5O_3\cdot0.25H_2O$) C, H, N
5x	HEX/EtOAc 1:2	HEX/EtOAc	47	67−70	CHEX/EtOAc 1:1, R_f 0.08	553	($C_{33}H_{33}N_4O_3F\cdot0.25H_2O$) C, H, N
5y	HEX/EtOAc 1:2	HEX/EtOAc	65	82−85	CHEX/EtOAc 1:4, R_f 0.68	553	($C_{33}H_{33}N_4O_3F\cdot0.25H_2O$) C, H, N
5z	HEX/EtOAc 1:2	HEX/EtOAc	67	85−87	CHEX/EtOAc 1:2, R_f 0.30	553	($C_{33}H_{33}N_4O_3F\cdot0.25H_2O$) C, H, N
5aa	HEX/EtOAc 1:3		64	66−69	HEX/EtOAc 1:4, R_f 0.6	603	($C_{34}H_{33}F_3N_4O_3\cdot0.25H_2O$) C, H, N
5ab	CHEX/EtOAc 2:3		57	52−56	CHEX/EtOAc 1:2, R_f 0.22	593	($C_{35}H_{36}N_4O_5\cdot1.2H_2O$) C, H, N

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

2-(*tert*-butyldimethylsilanyloxy)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; ^{1}H NMR ($CDCl_3$) mixture of two rotamers δ 0.27 (3H, s, $SiCH_3$), 0.30 (3H, s, $SiCH_3$), 1.02 (9H, s, $SiC(CH_3)_3$), 1.51−2.03 (3H, m, CH_2 pyrrolidine), 2.10−2.32 (1H, m, CH_2 pyrrolidine), 2.73 (0.7 × 3H, s, NCH_3 major), 2.92 (0.3 × 3H, s, NCH_3 minor), 3.04−3.36 (4H, m, CH_2Nap, NCH_2 pyrrolidine), 4.22−4.63 (2.3H, m, NCH_AH_BPh, C*H* pyrrolidine), 4.72 (0.7 × 1H, d_{AB}, J_{AB} = 15 Hz, NCH_AH_BPh major), 5.18−5.37 (1H, m, C*H*CH_2Nap), 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. ($C_{39}H_{48}N_4O_4Si$) 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 NH_4Cl and extracted with EtOAc (3 × 50 mL). The combined organic extracts were dried ($MgSO_4$) 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; ^{1}H NMR ($CDCl_3$) mixture of two rotamers δ 1.73−2.22 (4H, m, CH_2 pyrrolidine), 2.73 (0.7 × 3H, s, NCH_3 major), 2.91 (0.3 × 3H, s, NCH_3 minor), 3.08−3.46 (4H, m, CH_2Nap, NCH_2 pyrrolidine), 4.28−4.49 (2.3H, m, NCH_AH_BPh, C*H* pyrrolidine), 4.68 (0.7 × 1H, d_{AB}, J_{AB} = 14 Hz, NCH_AH_BPh major), 5.18−5.34 (1H, m, C*H*CH_2Nap), 6.60 (0.3 × 1H, s, NH minor), 6.73 (0.7 × 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 s cm^{-1}; MS *m/e* (FAB) 551 $(MH)^+$ (100%). Anal. ($C_{33}H_{34}N_4O_4 \cdot 0.5H_2O$) 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; ^{1}H 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 × 3H, s, NCH_3 major), 2.88 (0.3 × 3H, s, NCH_3 minor), 3.05−3.47 (4H, m, CH_2Nap, NCH_2 pyrrolidine), 4.24−4.49 (2.3H, m, NCH_AH_BPh, C*H* pyrrolidine), 4.67 (0.7 × 1H, d_{AB}, J_{AB} = 14 Hz, NCH_AH_BPh major), 5.18−5.34 (1H, m, C*H*CH_2Nap), 6.18 (0.3 × 1H, s, NH minor), 6.23 (0.7 × 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 m cm^{-1}; MS *m/e* (FAB) 550 $(MH)^+$ (100%). Anal. ($C_{33}H_{35}N_5O_3 \cdot 0.5H_2O$) 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 × 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; ^{1}H 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 × 3H, s, NCH_3 major), 2.88 (0.3 × 3H, s, NCH_3 minor), 3.04−3.51 (4H, m, CH_2Nap, NCH_2 pyrrolidine), 4.22−4.53 (2.3H, m, NCH_AH_BPh, CH pyrrolidine), 4.69 (0.7 × 1H, d_{AB}, J_{AB} = 14 Hz, NCH_AH_BPh major), 5.18−5.35 (1H, m, C*H*CH_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 m cm^{-1}; MS *m/e* (FAB) 592 $(MH)^+$ (100%). Anal. ($C_{35}H_{36}N_5O_4 \cdot H_2O$) C, H, N.

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

Rabbit NK_1. Displacement of [^{3}H][Sar^{9}Met$(O_2)^{11}$]SP (1 nM) binding to rabbit whole brain membranes; K_D for SP = 0.14 ± 0.03 nM, B_{max} = 9.82 ± 1.47 fmol/mg of protein.

Rat NK_1. Displacement of [^{3}H][Sar^{9}Met$(O_2)^{11}$]SP (0.5 nM) binding to rat whole forebrain membranes: K_D for SP = 0.23 ± 0.09 nM, B_{max} = 14.6 ± 2.6 fmol/mg of protein.

Human NK_1. Displacement of [^{3}H]SP (0.6 nM) binding to membranes from Cos-7 cells transiently transfected with the hNK-1R. (K_D for SP = 85 ± 12 pM), B_{max} = 537 ± 139 fmol/mg of protein.

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

Functional Assays:[34,38] Guinea Pig Ileum Bioassay. Contractions were evoked by [Sar^{9}Met$(O_2)^{11}$]SP (4.0 nM). The application of the NK_1 receptor agonist was repeated at least five times to establish stable responses. Once the amplitude of the contraction became stable, the NK_1 agonist was administered in the presence of the selected NK_1 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. $\log_{10}$ concentration was plotted against calculated responses, and the IC_{50} value was determined from the curve. Data represent the mean IC_{50} values ± 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 NK_1 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.

ED_{30} 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): ED_{30} = 0.5 mg/kg, 79.8 ± 12.2% efficacy

aspirin (po): ED_{30} = 71.6 mg/kg, 55.0 ± 6.8% efficacy

The NK_1 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):
ED_{30} = 0.7 ± 0.3 mg/kg, 68.9 ± 3.88% efficacy

5f (microemulsion po):
ED_{30} = 1.1 ± 0.5 mg/kg, 68.4 ± 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.

References

(1) Nakanishi, S. Mammalian Tachykinin Receptors. *Annu. Rev. Neurosci.* **1991**, *14*, 123−136.
(2) Maggi, C. A.; Patacchini, R.; Rovero, P.; Giachetti, A. Tachykinin Receptors and Tachykinin Receptor Antagonists *J. Auton. Pharmacol.* **1993**, *13*, 23−93.
(3) Snider, R. M.; Constantine, S. J. W.; Lowe, J. A.; Longo, K. P.; Lebel, W. S.; Woody, H. A.; Drozda, S. E.; Desai, M. C.; Vinick, F. J.; Spencer, R. W.; Hess, H. J. A Potent Nonpeptide Antagonist of the Substance P (NK_1) Receptor. *Science* **1991**, *251*, 435−437.
(4) Garret, C.; Carruette, A.; Fardin, V.; Moussaoui, S.; Peyronel, J. F.; Blanchard, J. C.; Laduron, P. M. Pharmacological Properties of a Potent and Selective Nonpeptide Substance P Antagonist. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10208−10212.

(5) Hagiwara, D.; Miyake, H.; Igari, N.; Masako, K.; Maeda, Y.; Fujii, T.; Matsuo, M. Studies on Neurokinin Antagonists. 4. Synthesis and Structure-Activity Relationships of Novel Substance P Antagonists: N^2-[(4R)-Hydroxy-1-{(1-methyl-1-H-indol-3-yl)carbonyl]-L-prolyl]-*N*-methyl-*N*-(phenylmethyl)-3-(2-naphthyl)-L-alaninamide and Its Related Compounds. *J. Med. Chem.* **1994**, *37*, 2090−2099.

(6) Emonds-Alt, X.; Doutremepuich, J.-D.; Heaulme, M.; Neliat, G.; Santucci, V.; Steinberg, R.; Vilain, P.; Bichon, D.; Ducoux, J.-P.; Proietto, V.; Van Broeck, D.; Soubrié, P.; Le Fur, G.; Brelière, J.-C. In vitro and in vivo Biological Activities of SR 140333, a Novel Potent Non-Peptide Tachykinin NK_1 Antagonist. *Eur. J. Pharmacol.* **1993**, *250*, 403−413.

(7) Schilling, W.; Bittiger, H.; Brugger, F.; Criscione, L.; Hauser, K.; Ofner, S.; Olpe, H.-R.; Vassout, A.; Veenstra, S. Approaches Towards the Design and Synthesis of Nonpeptidic Substance-P Antagonists. *Perspectives in Medicinal Chemistry*; Verlag Helvetica Chimica Acta: Basel, 1993; pp 207−220.

(8) Swain, C. J.; Seward, E. M.; Cascieri, M. A.; Fong, T. M.; Herbert, R.; MacIntyre, D. Euan; Merchant, K. J.; Owen, S. N.; Owens, A. P.; Sabin, V.; Teall, M.; VanNiel, M. Bodil; Williams, B. J.; Sadowski, S.; Strader, C.; Ball, R. G.; Baker, R. Identification of a Series of 3-(Benzyloxy)-1-azabicyclo[2,2,2]octane Human NK_1 Receptor Antagonists. *J. Med. Chem.* **1995**, *38*, 4793−4805.

(9) Natsugari, H.; Ikeura, Y.; Kiyota, Y.; Ishimaru, T.; Saga, O.; Shirafuji, H.; Tanaka, T.; Kamo, I.; Doi, T.; Otsuka, M. Novel, Potent and Orally Active Substance P Antagonists: Synthesis and Antagonist Activity of N-benzylcarboxamide Derivatives of Pyrido[3,4-b]pyridine. *J. Med. Chem.* **1995**, *38*, 3106−3120.

(10) Hipskind, P. A.; Howbert, J. J.; Bruns, R. F.; Cho, S. S. Y.; Crowell, T. A.; Foreman, M. M.; Gehlert, D. R.; Iyengar, S.; Johnson, K. W.; Krushinski, J. H.; Li, D. L.; Lobb, K. L.; Mason, N. R.; Muehl, B. S.; Nixon, J. A.; Phebus, L. A.; Regoli, D.; Simmons, R. M.; Threlkeld, P. G.; Waters, D. C.; Gitter, B. D. 3-Aryl-1,2-diacetamidopropane Derivatives as Novel and Potent NK_1 Receptor Antagonists. *J. Med. Chem.* **1996**, *39*, 736−748.

(11) McMahon, S. B.; Lewin, G. R.; Wall, P. D. Central hyperexcitability triggered by noxious inputs. *Curr. Opin. Neurobiol.* **1993,** *3*, 602−610.

(12) Thompson, S. W. N.; Dray, A.; Urban, L. Injury-induced Plasticity of Spinal Reflex Activity: NK_1 Neurokinin Receptor Activation and Enhanced A- and C-fiber Mediated Responses in the Rat Spinal Cord in vitro. *J. Neurosci.* **1994**, *14*, 3672−3687.

(13) Campbell, E. A.; Davis, A.; Dray, A.; Gentry, C.; Kane, S.; Patel, S.; Perkins, M N.; Urban, L. The Analgesic Activity of the NK_1 Receptor Antagonist CP 99,994 in Models of Inflammatory and Neuropathic Pain in the Rat. *Br. J. Pharmacol.* **1995**, *114*, 350P.

(14) Courteix, C.; Lavarenne, J.; Eschalier, A. RP-67580, a Specific Tachykinin NK_1 Receptor Antagonist Relieves Chronic Hyperalgesia in Diabetic Rats. *Eur. J. Pharmacol.* **1993, *241***, 267−270.

(15) Dionne, R. A.; Max, M. B.; Parada, S.; Gordon, S. M.; MacLean, D. B. *Clin. Pharmacol. Ther.* **1996**, *59*, 216.

(16) Longmore, J.; Swain, C. J.; Hill R. G. Neurokinin receptors. *Drug News Perspect.* **1995**, *8* (1), 5−23.

(17) Kucharczyk N. Tachykinin antagonists in development. *Exp. Opin. Invest. Drugs* **1995**, *4* (4), 299−311.

(18) Elliott, J.; Seward, E. M. Neurokinin receptor antagonists. *Exp. Opin. Ther. Pat.* **1997**, *7* (1), 43−54.

(19) Ko, S. Y.; Lerpiniere, J.; Christofi, M. An Efficient Synthesis of Internal Guanidines. *Synlett* **1995**, *8*, 815.

(20) Ko, S. Y.; Levallet, C.; Lerpiniere, J. The $HgCl_2$-promoted Guanylation Reaction: The Scope and Limitations. *Tetrahedron* **1997**, *53*, 5291−5304.

(21) Gentry, C.; Patel, S.; Ewan, S.; Brown, M.; Campbell, E. A. Oral Analgesic Efficacy of Neurokinin-1(NK_1) Receptor Antagonists in Models of Carrageenan and Substance P-induced Hyperalgesia in the Guinea Pig. *Br. J. Pharmacol.*, in press.

(22) Ganellin, R. Medicinal Chemistry and Dynamic Structure-Activity Analysis in the Discovery of Drugs Acting at Histamine H_2 Receptors. *J. Med. Chem.* **1981**, *24*, 913−920.

(23) Rosenkilde, M. M.; Cahir, M.; Gether, U.; Hjorth, S. A.; Schwartz, T. W. Mutations along transmembrane segment II of the NK_1 receptor affect substance P competition with non-peptide antagonists but not substance P binding. *J. Biol. Chem.* **1994**, *269* (45), 28160−4.

(24) Young, R. C.; Mitchell, R. C.; Brown, T. H.; Ganellin, C. R.; Griffiths, R.; Jones, M.; Rana, K. K.; Saunders: D.; Smith, I. R. Development of a New Physicochemical Model for Brain Penetration and its Application to the Design of Centrally Acting H_2 Receptor Histamine Antagonists. *J. Med. Chem.* **1988**, *31*, 656−71.

(25) Gray, R. A.; Vander velde, D. G.; Burke, C. J.; Manning, M. C.; Middaugh, C. R.; Borchardt, R. T. Delta-Sleep-Inducing Peptide: Solution Conformational Studies of a Membrane-Permeable Peptide. *Biochemistry.* **1994**, *33*, 1323−1331.

(26) Yamagami, C.; Takami, H.; Yamamoto, K.; Miyoshi, K.; Takao, N. Hydrophobic Properties of Anticonvulsant Phenylacetanilides. Relationships between Octanol-Water Partition Coefficient and Capacity Factor Determined by Reversed-Phase Liquid Chromatography. *Chem. Pharm. Bull.* **1984**, *32*, 4994−5002.

(27) Urban, L.; Thomson, S. W. N.; Dray, A. Modulation of Spinal Excitability: Co-operation Between Neurokinins and Excitatory Amino Acid Neurotransmitters. *Trends Neurosci.* **1994**, *17*, 432−438.

(28) Pardridge, W. M.; Eisenberg, J.; Cefalu, W. T. Absence of Albumin Receptor on Brain Capilliaries in vivo or in vitro. *Am. J. Physiol.* **1985**, *249*, E264−267.

(29) Lin, T. H.; Lin, J. H. Effects of Protein Binding and Experimental Disease States on Brain Uptake of Benzodiazepines in Rats. *J. Pharmacol. Exp. Ther.* **1990**, *253*, 45−50.

(30) Lin, T. H.; Sawada, Y.; Sugiyama, T. Iga; Hanano, M. Inhibition of Blood-Brain Barrier Permeability of DL-propranolol by serum from acute renal failure rats. *Biochem. Pharmacol.* **1987**, *36*, 3425−3431.

(31) Guillot, F.; Misslin, P.; LeMaire, M., Comparison of Fluvastatin and Lovastatin Blood-Brain Barrier Transfer using *in vitro* and *in vivo* Methods. *J. Cardiovascular Pharmacology*, **1993**, *21*, 339−346.

(32) Private communication, Novartis Pharma Drug Metabolism and Pharmacokinetics department.

(33) Private communication, Prof. F. Loor, Novartis Pharma, Basel, Switzerland.

(34) Walpole, C. S. J.; Brown, M. C. S.; James, I. F.; Campbell, E. A.; McIntyre, P.; Docherty, R.; Ko, S.; Hedley, L.; Ewan, S.; Buchheit, K.-H.; Urban, L. A. Comparative, General Pharmacology of SDZ NKT 343, a Novel, Selective NK_1 Receptor Antagonist. *Br. J. Pharmacol.* **1998**, *124*, 83−92.

(35) Girard, G. R.; Hempel, J.; Hill, D, T.; Samenen, J.; Weinstock, J. Preparation of imidazolylalkanoates as angiotensin II antagonists. WO 9202510 A1 920220.

(36) Masaaki, M.; Hagiwara, D.; Hiroshi, M. Preparation of acyldipeptide amides as tachykinin antagonists. Eur. Pat. Appl. EP 394989 A2 901031.

(37) Handa, S.; Tanaka, Y.; Nishibata, A.; Inamoto, Y. Halogen-containing organic isocyanates. DE 2434055 750206.

(38) Soo Y. Ko and Christopher Walpole, Tachykinin Antagonists, WO 96/18643 (June 20, 1996).

(39) Bermudez, J.; Dabbs, S.; King, F. D.5-Hydroxytryptamine (5-HT_3) receptor antagonists. 3. Ortho-substituted phenylureas. *J. Med. Chem.* **1990**, *33*, 1932−5.

(40) Just, G.; Zamboni, R. β-Lactams II. The synthesis of cis-N-(2′-carboxyphenyl)-3-phenylacetamido-4-methoxymethyl-2-azetidinone. *Can. J. Chem.* **1978**, *56* (21), 2720−4.

JM970499G