3-Aryl-1,2-diacetamidopropane Derivatives as Novel and Potent NK-1 Receptor Antagonists

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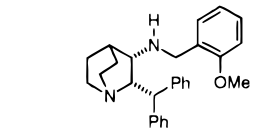
Early structure−activity studies on racemic tryptophan ester and amide NK-1 antagonists **5**−**7** led to the discovery that the potency of the series could be markedly increased by moving the carbonyl function in these molecules to an off-chain position as in the 3-aryl-1,2-diacetamidopropane **9**. Further medicinal chemistry incorporating this change resulted in the discovery of a novel series of highly potent aryl amino acid derived NK-1 antagonists of the *R* stereoisomeric series (IC_{50}'s = 100 pM to >5 μM). Compounds in this series were shown to be competitive antagonists using an *in vitro* NK-1 smooth muscle assay, and this data correlated well with observed human NK-1 binding affinities. Two of these agents, (*R*)-**25** and (*R*)-**32**, blocked intrathecal NK-1 agonist-driven [Ac-[Arg^6, Sar^9, Met(O_2)^{11}]-substance P 6−11 (Ac-Sar^9)] nociceptive behavior in mice. Both compounds potently blocked the neurogenic dural inflammation following trigeminal ganglion stimulation in the guinea pig after intravenous administration. Further, upon oral administration in this model, (*R*)-**32** was observed to be very potent (ID_{50} = 91 ng/kg) and have a long duration of action (>8 h at 1 μg/kg). Compound (*R*)-**32**, designated LY303870, is currently under clinical development as an NK-1 antagonist with a long duration of action.

Introduction

The undecapeptide neurotransmitter substance P (SP) is a member of the family of neuropeptides that includes neurokinin A and neurokinin B.[1] This family of peptides exert their effects through three recognized G-protein coupled receptor subtypes, NK-1, NK-2, and NK-3, respectively.[2] Besides potentially being a neuromediator of pain, SP, acting primarily at NK-1 receptors, stimulates smooth muscle contraction, vasodilation, plasma extravasation, and the release of inflammatory mediators.[3] These latter actions, known collectively as neurogenic inflammation, exacerbate the transmission of nociception as well as local inflammation. It has been proposed that SP antagonists may therefore be useful for the treatment of various human disease states including persistent pain,[4] migraine,[5] and asthma.[6] Numerous reports have appeared describing the discovery of nonpeptide, small molecule antagonists of the NK-1 receptor.[7] Among those compounds disclosed to date (CP-96345 (**1**),[8,9] RP-67580 (**2**),[10] SR140333 (**3**),[11] L-737,488 (**4**),[12] and others[13]) there is a remarkable level of structural variety. Further exemplifying this diversity, we report our efforts toward the discovery of a clinically useful SP antagonist.[14]

Following our own screening study based on the hypothesized importance of the Phe^7-Phe^8 substructure of SP for NK-1 receptor affinity and a subsequent medicinal chemistry effort, α-N-acetylated tryptophan amides and esters, such as **5** (4.9 μM),[15] **6** (0.75 μM), and **7** (0.15 μM), were uncovered.[16] Analysis of the dihedral angle about the C-1 to heteroatom single bond of compounds **5**−**7** indicated the potential importance of the ability of this bond to adopt an *s-cis* conformation for highest potency. To test this possibility, compounds **8** and **9** were synthesized. Whereas compound **8** (4.7 μM) is rigidly held *s-trans*, compound **9**[17] (0.068 μM) should ostensibly have maximum flexibility to adopt an *s-cis* conformation. Quite serendipitously, the diketopiperazine pseudodimer **10**[18] (0.028 μM) was isolated in an attempted formation of **8**. The most essential elements of **10** were determined, by sequential substructure deletion, to be embodied by **11** (0.030 μM). Further medicinal chemistry combined the elements of **11** with those of **9** and led to discovery of the indolyldiacetamidopropane **12** as a potent inhibitor of [^{125}I]SP binding to the human NK-1 receptor (IC_{50} = 1.14 ± 0.26 nM). Herein we describe our detailed studies centered on **12** to further define this novel series of potent NK-1 antagonists.



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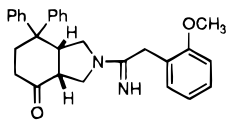

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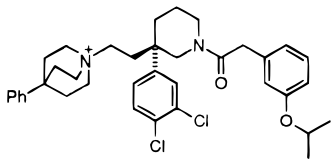

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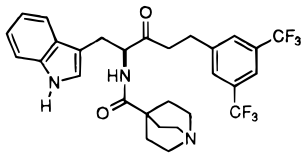

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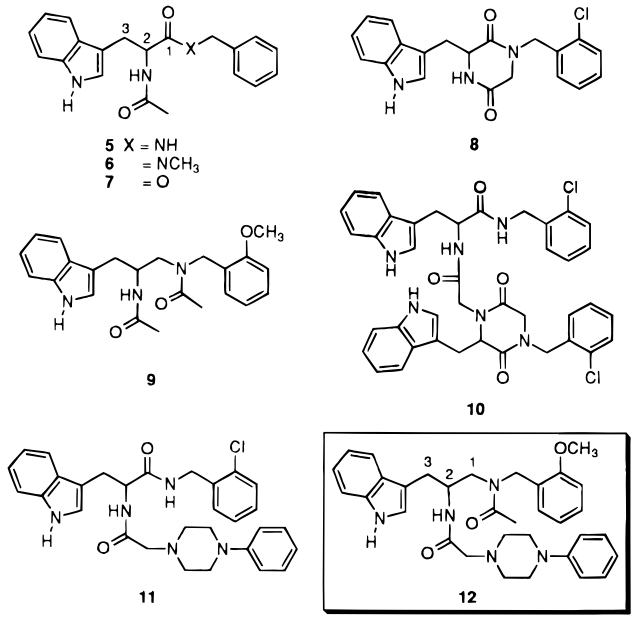
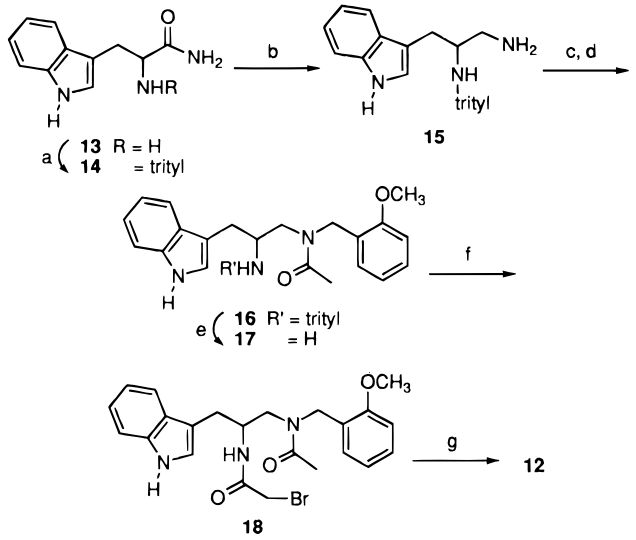


Chemistry

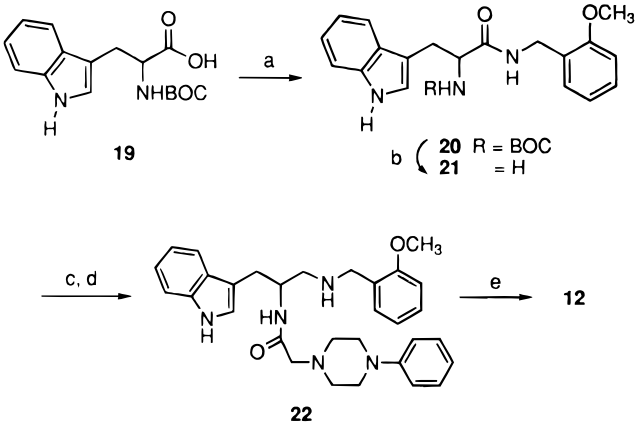
Initially, a structure−activity analysis based on racemic compounds was targeted. Replacements for the 2-(4-phenylpiperazinyl)acetamido and the 2-methoxybenzyl substituents of **12** were investigated using the racemic synthetic strategy illustrated in Scheme 1 (method A). Tritylation of (*RS*)-tryptophan amide **13** proceeded under standard conditions.[19] Next treatment of a tetrahydrofuran solution of **14** at reflux with borane dimethyl sulfide smoothly produced trityldiamine **15**. Without purification **15** was reductively alkylated with an appropriate aryl aldehyde, and the resulting amine was acylated with acetic anhydride to yield the crystalline tritylamine **16** in an 87% overall three-step yield. Straightforward deprotection of the trityl group to give aminoacetamide **17** was accomplished by treatment of **16** with formic acid in dichloromethane. The final 2-acetamido substituents were most conveniently appended using a two-step procedure: (1) acylation with bromoacetyl bromide to give **18** followed by (2) alkylation with the appropriate amine using powdered, anhydrous potassium carbonate in dichloromethane. This provided the racemic target structures exemplified in Scheme 1 as **12**. Compounds **9**, **27**, **30**, **52**, and **53** and the compounds found in Tables 1 and 3 were prepared using this general method A. Alternatively 2-acetamido substituents, several *N*-benzyl replacements, and indolyl methylene replacements for compound **12** were investigated as outlined in Scheme 2. *N*-BOC-tryptophan **19** (or other *N*-BOC-amino acid derivative as in the case of the compounds found in Table 5) was coupled with a variety of amines and then deprotected using standard methodology to give **21**. Borane dimethyl sulfide reduction followed by selective primary amine acylation with the acylimidazole of 2-(4-phenylpiperazinyl)acetic acid yielded **22**. Finally **22** was acylated, alkylated, or sulfonylated as necessary to produce the target compounds. Compounds **5**−**8** and **10**−**12** and the compounds found in Tables 2, 4, and 5 were made employing this general method B. Synthesis of enantiomerically pure materials from (*R*)-tryptophan has been described elsewhere.[20]

Scheme 1. General Method A[a]



[a] Reagents: (a) Ph_3CCl, Et_3N, CH_2Cl_2, room temperature; (b) BH_3·DMS, THF, reflux; (c) 2-$MeOC_6H_4CHO$, toluene, $-H_2O$; then $NaBH_4$, 1:1 THF/methanol, room temperature; (d) Ac_2O, Et_3N, THF, room temperature; (e) HCO_2H, CH_2Cl_2, 0 °C to room temperature; (f) *i*-Pr_2NEt, $BrCOCH_2Br$, THF, 0 °C to room temperature; (g) *N*-phenylpiperazine, K_2CO_3, CH_2Cl_2, room temperature.

Scheme 2. General Method B[a]

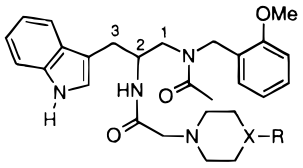


[a] Reagents: (a) CDI, 2-MeO-benzylamine, dioxane, room temperature; (b) aqueous TFA, anisole, 0 °C; (c) BH_3·DMS, THF, reflux; (d) CDI, (4-phenylpiperazin-1-yl)CH_2CO_2H, NEt_3, CH3CN, room temperature; (e) Ac_2O, *i*-Pr_2NEt, THF, room temperature.

Results and Discussion

Structure−Affinity Relationships. We first decided to investigate the role of the phenylpiperazine moiety in compound **12** (Table 1). Replacing the phenyl group with methyl (e.g. **23**) resulted in nearly a 5-fold loss in activity. Inserting one methylene between the nitrogen and phenyl groups of **12**, as in **24**, did not yield a significant improvement. Interestingly, saturation of the phenyl ring of **12** to the cyclohexyl analog **25** improved the potency 3-fold to 0.34 nM while increasing the basicity and water solubility. Direct exchange of the piperazine nitrogen attached to the phenyl ring of **12** for a carbon (**26**) resulted in a 4-fold loss in potency. Although this loss in activity could have been due to a conformational change in the side chain (ca. sp^2 nitrogen for sp^3 carbon), preparation of Δ^3-piperidine **27** (3.1 ± 0.4 nM) showed that this was not the entire explanation. The appendant phenyl group in **12**, **26**, and **27** would, in each case, have distinct conformational biases (rela-

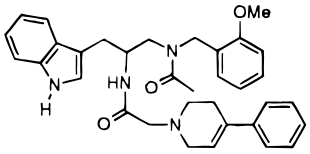
Table 1. *In Vitro* SAR: Variation of N_2 Acetamide



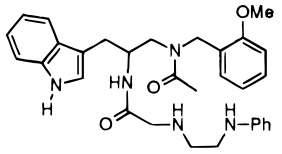
compd	X	R	IC_{50}[a]
1			0.35 ± 0.01
2			30 ± 4.9
12	N	Ph	1.1 ± 0.3
23	N	Me	5.3 ± 1.4
24	N	CH_2Ph	0.98 ± 0.1
25	N	cyclohexyl	0.34 ± 0.04
26	CH	Ph	4.5 ± 1.5
28	CH	H	33 ± 4
29	O		27 ± 9
31	CH	NMe_2	2.8 ± 1
32	CH	1-piperidinyl	0.23 ± 0.02

[a] Binding affinity for the NK-1 receptor in human IM-9 cells using ^{125}I-labeled Bolton−Hunter substance P as radioligand, given in nM units; see ref 15. IC_{50} values were determined from 11-point concentration−response curves with each concentration in triplicate. All values are the mean ± SEM, n = 3−14 determinations.

tive to the piperidine or piperazine ring) that would also have an effect on binding potency.



27
IC_{50} = 3.1 ± 0.4 nM

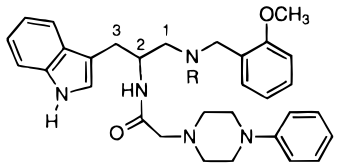


30
IC_{50} = 8.4 ± 2.8 nM

Simple substitution of piperidine (**28**) or morpholine (**29**) for the entire 4-phenylpiperazine side chain present in **12** produced compounds of substantially reduced potency. Cleavage of two methylene units out of the piperazine ring of **12**, as in acyclic compound **30** (8.4 ± 2.8 nM), yielded similar results. In contrast, reintroduction of a more basic, distal amine, as in the 4-(dimethylamino)piperidine derivative **31** (2.8 nM), increased the potency 12-fold with respect to the unsubstituted piperidine analog **28**. Further, reestablishing some hydrophobic character to the diamine motif as in the 4-piperidinylpiperidine analog **32** resulted in the most potent racemic compound in the series. Overall these data established the importance of the distal, basic nitrogen for activity (compound **28**, 33 nM vs **31**, 2.8 nM) while the exact nature of the groups attached to this distal nitrogen (hydrophobicity) also played a key role in obtaining compounds with subnanomolar activity (**23**, 5.3 nM vs **25**, 0.34 nM and **31**, 2.8 nM vs **32**, 0.23 nM).

The effect of varying the acetamide substituents at the C-1 nitrogen while holding the 2-methoxybenzyl group fixed was investigated (Table 2). All compounds with amide or amide like character (**33**−**41**) were active with IC_{50}'s less than 10 nM. Changing the amide to a sulfonamide resulted in a modest loss of activity (**41**, 4.0 nM vs **12**, 1.1 nM). Removing the amide character of the C-1 nitrogen (**22**, **42**, and **43**) had a substantially deleterious effect on activity. A wide variety of size is tolerated as demonstrated by the benzoyl (**35**, 2.0 nM)

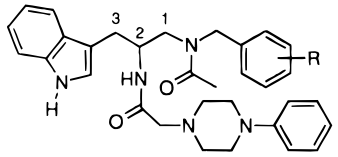
Table 2. *In Vitro* SAR: Variation of N_1 Acetamide



compd	R	IC_{50}[a]
12	$COCH_3$	1.1 ± 0.3
22	H	51 ± 14
33	CHO	0.80 ± 0.3
34	COEt	0.81 ± 0.1
35	COPh	2.0 ± 0.6
36	$COCH_2COOCH_3$	1.28 ± 0.3
37	$COCH_2COOH$	9.2 ± 1.2
38	$COCH_2N(CH_3)_2$	1.54 ± 0.1
39	COOEt	5.5 ± 2.8
40	$CONHCH_3$	3.0 ± 1.2
41	SO_2CH_3	4.0 ± 0.4
42	Et	85 ± 5.8
43	CH_2COOCH_3	31 ± 14

[a] Binding affinity for the NK-1 receptor in human IM-9 cells using ^{125}I-labeled Bolton−Hunter substance P as radioligand, given in nM units; see ref 15. IC_{50} values were determined from 11-point concentration response curves with each concentration in triplicate. All values are the mean ± SEM, n = 3 determinations.

Table 3. *In Vitro* SAR: Variation of N_1 Benzyl Substituent



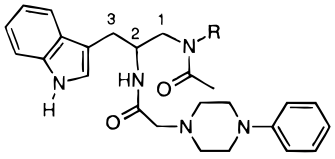
compd	R	IC_{50}[a]
12	2-MeO	1.1 ± 0.3
44	3-MeO	4.6 ± 0.06
45	4-MeO	2.5 ± 0.3
46	H	2.2 ± 0.4
47	2-SMe	7.2 ± 3
48	2-Cl	1.9 ± 0.4
49	2-Me	3.2 ± 0.1
50	2-CF_3	3.2 ± 0.9
51	2-NO_2	3.0 ± 0.6

[a] Binding affinity for the NK-1 receptor in human IM-9 cells using ^{125}I-labeled Bolton−Hunter substance P as radioligand, given in nM units; see ref 15. IC_{50} values were determined from 11-point concentration−response curves with each concentration in triplicate. All values are the mean ± SEM, n = 3 determinations.

and formyl (**33**, 0.8 nM) substituents. Also acidic and basic groups could be installed on the acetamide substituent while maintaining reasonable activity (**37**, **38**). As expected, all compounds with an additional basic group, such as **38**, showed enhanced water solubility at pH = 7.

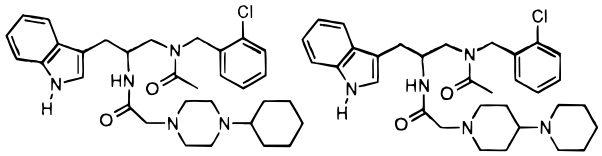
A limited survey of benzyl substituent variations was also undertaken. In this study the core 3-(indol-3-yl)-1,2-diacetamidopropane backbone of **12** was held constant (Table 3). 2-Methoxy- and 2-chlorobenzyl groups were modestly preferred over others examined. This proved to be a general trend with other C-2 acetamido substituents; additional 2-chlorobenzyl derivatives (**52**, 0.56 ± 0.1 nM and **53**, 0.35 ± 0.07 nM) were prepared and were found to be essentially equipotent to the corresponding 2-methoxy derivatives (**25** and **32**).[21] Further, the synthesis of several benzyl replacements was undertaken (Table 4). A substantial loss of affinity

Table 4. *In Vitro* SAR: N_1 Benzyl Replacements



compd	R	IC_{50} (nM)[a]
12	2-MeO-benzyl	1.1 ± 0.3
46	benzyl	2.2 ± 0.4
54	Me	>5000
55	*n*-Bu	149 ± 17
56	(cyclohexyl)-CH_2	21.9 ± 8.3
57	Ph	>5000
58	$PhCH_2CH_2$	126 ± 19

[a] Binding affinity for the NK-1 receptor in human IM-9 cells using ^{125}I-labeled Bolton−Hunter substance P as radioligand, given in nM units; see ref 15. IC_{50} values were determined from 11-point concentration response curves with each concentration in triplicate. All values are single determinations or the mean ±SEM, $n = 3$ determinations.



52 IC_{50} = 0.56 ± 0.1 nM

53 IC_{50} = 0.35 ± 0.07 nM

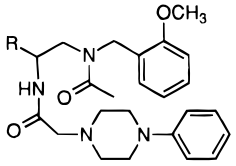
was observed in all cases where the aryl ring was deleted (compounds **54** and **55**) or repositioned (compounds **57** and **58**). Only in the case of the cyclohexylmethylene derivative, **56**, was the activity maintained within 1 order of magnitude of the parent **46**.

Modifications to the 3-indolylmethylene were also undertaken (Table 5). Of those examined, compounds that held to the arylmethylene type substructure (**59** to **62**) maintained reasonable potency (IC_{50}'s less than 12 nM). Those deviating from this general substructure (**64**−**67**) were inactive. Even slight deviations, as that found in the 3-indolinyl compound **63**, resulted in a marked decrease in potency. The data presented in Tables 4 and 5 underscore the need for a distal display of aryl−aryl functionality, separated by exactly five atoms in the main chain of this series of compounds.

Next the enantiospecificity of the series was examined (Table 6). Four pairs of isomers were synthesized, and in each case the *R* isomer was clearly the most active by 300−2800-fold. With the recent appearance of similar, *but optically antipodal* NK-1 receptor antagonists derived from tryptophan,[22] we were curious to see how compounds such as (*S*)-**4** would compare to (*R*)-**12** using molecular modeling techniques.[23] Both molecules exhibited a clear preference for $\pi-\pi$-face stacking,[24] but when the appropriate aryl groups of the two molecules were overlaid, the analysis was inconclusive for an overlap of the corresponding distal basic nitrogen. Further attempts to force an aryl−aryl−basic nitrogen (or nitrogen lone pair) three point overlay inevitably resulted in conformers of unreasonably high energy. One explanation for this differential stereochemistry could be that these two series are interacting at a different site on the receptor surface or at sites that only partially overlap.

Species Differences in Receptor Binding. A significant species difference in the affinity of nonpeptide antagonists for NK-1 receptors has been previously noted.[25] Compounds in this study likewise exhibited species differences (Table 7). In all cases tested, the compounds had greater NK-1 receptor affinity in human (IM-9 cells) and guinea pig brain membrane homogenates when compared to rodent receptors (rat and mouse brain membrane homogenates). Among those compounds tested, **38** and **45** showed the largest difference.

Table 5. *In Vitro* SAR: Indole Replacements



compd	R	IC_{50}[a]
12	3-indolyl-CH_2	1.1 ± 0.3
59	Ph-CH_2	8.3 ± 3
60	1-naphthyl-CH_2	2.73 ± 0.1
61	2-naphthyl-CH_2	11
62	3-benzo[*b*]thiophenyl-CH_2	1.18 ± 0.36
63	3-indolinyl-CH_2	27.4 ± 18
64	Ph	474 ± 122
65	3,4-dichlorophenyl	338 ± 25
66	H	>5000
67	*i*-Pr	>5000

[a] Binding affinity for the NK-1 receptor in human IM-9 cells using ^{125}I-labeled Bolton−Hunter substance P as radioligand, given in nM units; see ref 15. IC_{50} values were determined from 11-point concentration−response curves with each concentration in triplicate. All values are a single determination or the mean ± SEM, $n = 3$ determinations.

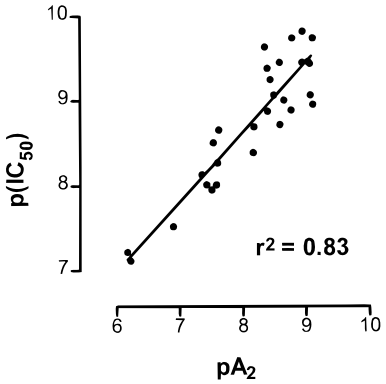
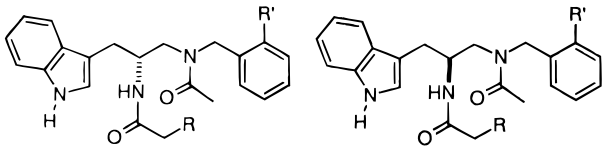


Figure 1. Correlation of IM-9 cell binding data (pIC_{50}) with rabbit vena cava smooth muscle functional activity (pA_2).

Functional Activity and Specificity. The ability of the compounds to block NK-1 receptor mediated contraction of the rabbit vena cava (RVC) tissue was examined.[26] The data obtained correlated well with the observed binding to SP receptors expressed in IM-9 cells. This is evidenced by a reasonable correlation ($r^2 = 0.83$) of RVC responses (pA_2) to IM-9 NK-1 affinities (IC_{50}) over an extensive list of antagonists from this series (Figure 1). A more extensive functional activity and specificity analysis of three enantiomerically pure compounds was performed (Table 8). Inhibition of SP-induced inositol phosphate accumulation in human UC-11 MG astrocytoma cells was assayed as previously described.[27] Block of the SP induced interleukin-6 secretion from human U-373 MG astrocytoma cells was measured also as previously described.[28] Assay of NK-1 specificity (vs NK-2 and NK-3) in smooth muscle systems was carried out using standard procedures.[29]

Table 6. *In Vitro* SAR: Enantiospecificity



compd	R	R′	IC_{50}[a] (*R*)-isomer	IC_{50}[a] (*S*)-isomer
12	4-phenylpiperazin-1-yl	2-MeO	0.85 ± 0.1	358 ± 53
25	4-cyclohexylpiperazin-1-yl	2-MeO	0.18 ± 0.04	80.9 ± 5.9
32	4-(piperidin-1-yl)piperidin-1-yl	2-MeO	0.15 ± 0.01	420 ± 62
52	4-(piperidin-1-yl)piperidin-1-yl	2-Cl	0.18 ± 0.03	159 ± 9.9

[a] Binding affinity for the NK-1 receptor in human IM-9 cells using ^{125}I-labeled Bolton-Hunter substance P as radioligand, given in nM units; see ref 15. IC_{50} values were determined from 11-point concentration−response curves with each concentration in triplicate. All values are the mean ± SEM, $n = 3$ determinations.

Table 7. Species Differences in Affinities: *in Vitro* NK-1 Binding Experiments

compd	IC_{50} human (IM-9)[a]	IC_{50} guinea pig brain[b]	IC_{50} rat brain[b]	IC_{50} mouse brain[b]
1	0.35	0.40	32	24
2	30	32	6.7	8.7
(*R*)-**12**	0.85	0.26	4.5	2.5
24	0.98	0.88	16	ND[c]
(*R*)-**25**	0.18	ND	1.5	1.3
27	3.1	3.8	14	ND
(*R*)-**32**	0.15	0.50	9.3	7.7
38	1.54	ND	480	200
45	2.5	ND	1100	ND

[a] Binding affinity for the NK-1 receptor in human IM-9 cells using ^{125}I-labeled Bolton−Hunter substance P as radioligand, given in nM units; see ref 15. IC_{50} values were determined from 11-point concentration−response curves with each concentration in triplicate. All values are the mean, $n = 1-3$ determinations. [b] Binding affinity for the NK-1 receptor in guinea pig brain, rat brain, or mouse brain homogenates using ^{125}I-labeled Bolton−Hunter substance P as radioligand, given in nM units; see ref 15. IC_{50} values were determined from six-point concentration−response curves with each concentration in triplicate. All values are the mean, $n = 1-3$ determinations. [c] ND = not determined.

Table 8. *In Vitro* Functional Activities in Cell Lines and Monoreceptor Smooth Muscle Assays

compd	K_i PI turnover[a]	K_i IL-6 release[b]	pA_2 NK-1 (RVC[c])	pA_2 NK-2 (RPA[d])	pA_2 NK-3 (RPV[e])
1	5.2	4.0	9.5	4.3	5.6
(*R*)-**12**	0.48	1.2	9.0	NA[f]	NA
(*S*)-**12**	630	3500	6.3	4.7	NA
(*R*)-**32**	1.3	5.5	9.4	4.7	4.7
(*S*)-**32**	5600	NA	5.3	4.7	6.7

[a] Inhibition of substance P induced phosphotidyl inositol turnover in UC-11 MG astrocytoma cells, given in nM units; see ref 27. Values represent mean of two determinations. [b] Inhibition of substance P induced IL-6 secretion in U-373 MG astrocytoma cells, given in nM units; see ref 28. Values represent mean of two determinations. [c] Inhibition of substance P induced contraction of rabbit vena cava (RVC) tissue; see ref 26. [d] Inhibition of neurokinin A induced contraction of rabbit pulmonary artery (RPA) tissue; see ref 29. [e] Inhibition of neurokinin B induced contraction of rat portal vein (RPV) tissue; see ref 29. [f] NA = not active to 10^{-5} M.

In summary, all compounds tested were competitive antagonists with high specificity for NK-1 (vs NK-2, NK-3, and others[30]) systems.

***In Vivo* Activities.** Compounds with high NK-1 receptor binding affinity were evaluated further using *in vivo* models of NK-1 activity. Central nervous system NK-1 antagonist efficacy was assessed using a behavioral response (biting and scratching) induced by an intrathecal (it) injection of the selective NK-1 receptor agonist Ac-[Arg^6, Sar^9, Met(O$_2$)^{11}]-SP 6−11 (Ac-Sar^9).[31] In this model, (*R*)-**25** and (*R*)-**32** were found to enantiospecifically block the it NK-1 agonist induced nociceptive behavioral response in the mouse with ED_{50}'s of 0.31 ± 0.22 nmol, it, and 0.21 ± 0.037 nmol, it, respectively. Additionally, a less potent compound in mouse brain binding assays, (*R*)-**53** was found to be active but less potent ($ED_{50} \approx 3$ nmol, it). For comparison, RP67580 was also tested in this model and an ED_{50} of 0.92 ± 0.16 nmol, it, was observed. Intraperitoneal administration of (*R*)-**25** and (*R*)-**32** also antagonized the effects of the intrathecal injection of the NK-1 specific agonist (ED_{50}'s of ca. 8.40 ± 3.6 mg/kg, ip, and 1.73 ± 0.74 mg/kg, ip, respectively). This suggests that compounds within this series penetrate the blood−brain barrier and block central NK-1 receptors following systemic administration.

Compounds were also tested for their ability to block the neurogenic dural inflammation and associated plasma extravasation in the guinea pig induced by direct ipsilateral electrical stimulation of the trigeminal ganglion.[32] Stimulation of this nerve bundle is purported to cause a release of inflammatory neuropeptides, including SP, from the primary sensory terminals in the dural tissue surrounding the brain.[33] Therefore, in this model of peripheral NK-1 activity, an NK-1 antagonist should block the resulting inflammation and extravasation. Experimentally, antagonism of plasma extravasation was quantitated as the ratio of the leakage of a plasma protein bound fluorescent dye (Evans blue) on the stimulated versus unstimulated side of the dural tissue. In this model (*R*)-**25** and (*R*)-**32** enantiospecifically blocked the neurogenic dural inflammation induced by electrical stimulation. Both compounds were extremely potent with ED_{50}'s of 1.2 ng/kg, iv, and 15 ng/kg, iv, respectively. Their enantiomers, (*S*)-**25** and (*S*)-**32**, were much less active with ED_{50}'s of >50 000 and 1300 ng/kg, respectively.[34] The effects of (*R*)-**32** were examined following oral administration and the compound displayed an ED_{50} of 91 ng/kg. Duration of action was estimated using various doses of (*R*)-**32**. Compound (*R*)-**32** had a long duration of action; at 1 μg/kg po (ca. ED_{100} at 35 min post oral administration) full efficacy was observed for more than 8 h.

Conclusions

Our own early structure−activity studies on tryptophan amide and ester NK-1 antagonists led to the discovery that the carbonyl function in these molecules could be moved to an off-chain position. This resulted in highly potent tryptophan derived compounds of the *R* stereoisomeric series. This series was found to have higher affinity for human and guinea pig over rodent NK-1 receptors, although compounds were found with K_i's < 10 nM in rodent binding assays. Compounds in this series were shown to be competitive antagonists in three different functional assays, and their potency in a functional NK-1 assay correlated well with observed receptor binding affinities for the NK-1 receptor. Two of these agents were demonstrated to be antagonists upon systemic administration in a central nervous system *in vivo* NK-1 agonist-driven model. In a guinea pig neurogenic dural inflammation model, a putative model of endogenous release of substance P, both (*R*)-**25** and (*R*)-**32** were highly active upon intravenous administration, and further, upon oral administration, (*R*)-**32** was observed to be very potent and have a long duration of action. Therefore compounds from this series show potential to be therapeutically useful in disease states where an excess of SP is implicated.

Experimental Section

All chemical experiments were run under a positive pressure of dry nitrogen or argon. All solvents and reagents were used as obtained. Generally, materials were obtained as dry foams; however, for crystalline materials, melting points were obtained on a Hoover melting point apparatus and are uncorrected. Proton NMR were obtained at 300.15 MHz on a General Electric QE300 spectrometer with tetramethylsilane as an internal standard. High-resolution mass spectra were recorded on a VG Analytical ZAB2-SE instrument. Elemental analyses were carried out by the Physical Chemistry Department of Lilly Research Laboratories and are within ±0.4% of theory unless otherwise noted. The following procedures serve to exemplify the methods used to prepare the compounds in the text above. Compounds not specifically detailed may be prepared by analogy with these methods.

Method A. Step 1. (*RS*)-3-(1*H*-Indol-3-yl)-2-(*N*-(triphenylmethyl)amino)propanamide (14). **13** (26.43 g, 0.130 mol) was suspended in 260 mL of methylene chloride, and this mixture was flushed with nitrogen and then put under argon. Trityl chloride (38.06 g, 0.136 mol) was dissolved in 75 mL of methylene chloride. The trityl chloride solution was added slowly to the tryptophan amide solution which sat in an ice bath, the addition taking about 25 min. The reaction mixture was allowed to stir overnight. The reaction mixture was poured into a separation funnel and was washed with 250 mL of water, followed by 250 mL of brine. As the organic layer was filtering through sodium sulfate to dry, a solid precipitated. The filtrate was collected and the solvent was evaporated. Ethyl acetate was added to the pooled solid, and this mixture was stirred and then refrigerated overnight. The next day the resulting solid was washed several times with cold ethyl acetate and then dried *in vacuo*: yield 49.76 g (86%); mp 209−210 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.50 (d_{AB}, J = 13.8, 7.2 Hz, 1H), 2.89 (d, J = 6.6 Hz, 1H), 3.12 (d_{AB}, J = 13.8, 7.2 Hz, 1H), 3.49 (dd, J = 7.2, 6.6 Hz, 1H), 5.45 (br s, 1H), 6.92 (d, J = 1.8 Hz, 1H), 7.00 (t, J = 7.2 Hz, 1H), 7.14 (m, 10H), 7.34 (m, 7H), 7.54 (d, J = 7.2 Hz, 1H), 9.83 (br s, 1H). Anal. ($C_{30}H_{27}N_3O$) C, H, N.

Step 2. (*RS*)-1-Amino-3-(1*H*-indol-3-yl)-2-(*N*-(triphenylmethyl)amino)propane (15). Under argon **14** (48.46 g, 0.108 mol) was suspended in 270 mL of tetrahydrofuran. This mixture was heated to reflux. Borane methyl sulfide complex (41.3 g, 0.543 mol) was slowly added to the reaction mixture. All of the starting amide dissolved during the addition of the borane methyl sulfide complex. This solution was stirred overnight in an 83 °C oil bath. After cooling to room temperature, a 1:1 mixture of tetrahydrofuran:water (75 mL total) was added to the solution. Sodium hydroxide (5 N, 230 mL) was added to the mixture, and the reaction was heated to reflux for about 30 min. After the biphasic mixture was cooled to room temperature, the aqueous and organic layers were separated and the organic layer was collected. The aqueous layer was reextracted with tetrahydrofuran. The organic layers were combined, and the solvents were removed by evaporation. The resulting liquid was partitioned between ethyl acetate and brine and was washed a second time with brine. The solution was dried over sodium sulfate, and the solvents were removed *in vacuo* to yield 48.68 g of the desired intermediate **15**. This material was used directly in the next step without further purification. High-resolution mass spectral analysis calculated for $C_{30}H_{30}N_3$ 432.2439, found 432.2420.

Step 3. (*RS*)-3-(1*H*-Indol-3-yl)-1-[*N*-(2-methoxybenzyl)-*N*-acetylamino]-2-(*N*-(triphenylmethyl)amino)propane (16). To a solution of crude **15** (48.68 g, 0.109 mol) dissolved in toluene (1.13 L) was added 2-methoxybenzaldehyde (23.12 g, 0.169 mol), the 2-methoxybenzaldehyde having been previously purified by base wash. The reaction mixture was stirred overnight. The solvents were removed *in vacuo*. The recovered solid was dissolved in 376 mL of a 1:1 tetrahydrofuran: methanol mixture. To this solution was added sodium borohydride (6.83 g, 0.180 mol). This mixture was stirred on ice for about 4 h. The solvents were removed by evaporation. The remaining liquid was partitioned between 1200 mL of ethyl acetate and 1000 mL of a 1:1 brine/20 N sodium hydroxide solution. This was extracted twice with 500 mL of ethyl acetate each, and then the organic layer was dried over sodium sulfate. The solvents were removed by evaporation, yielding 67.60 g (>99% yield) of the desired intermediate crude material, (*RS*)-3-(1*H*-indol-3-yl)-1-[*N*-(2-methoxybenzyl)amino]-2-(*N*-(triphenylmethyl)amino)propane, which was carried on directly. To a stirring solution of this crude (0.109 mol) in anhydrous tetrahydrofuran (325 mL) under a nitrogen atmosphere at 0 °C was added triethylamine (17.8 mL, 0.129 mol) and acetic anhydride (12.2 mL, 0.129 mol). After 4 h, the mixture was concentrated on a rotary evaporator, redissolved in methylene chloride and ethyl acetate, washed with water (2×) and brine (2×), dried over anhydrous sodium sulfate, filtered, and concentrated to a solid on a rotary evaporator. The resulting solid was dissolved in chloroform and loaded onto a silica gel 60 (230−400 mesh) column and eluted with a 1:1 mixture of ethyl acetate and hexanes. Fractions containing desired material were concentrated *in vacuo*. Finally the product was crystallized from an ethyl acetate/hexanes mixture, yielding, over three crops, 56 g of **16** (87% two-step yield): ^{1}H NMR (300 MHz, $CDCl_3$) 3:1 mixture of rotamers δ 1.92 (s, $^3/_4$·3H), 1.96 (s, $^1/_4$·1H), 2.30−2.48 (m, $^1/_4$·3H), 2.58−2.78 (m, $^3/_4$·3H), 3.02−3.33 (m, 3H), 3.57 (s, $^1/_4$·3H), 3.69 (s, $^3/_4$·3H), 3.72 (d_{AB}, J = 17 Hz, $^3/_4$·3H), 4.03 (d_{AB}, J = 17 Hz, $^3/_4$·1H), 4.42 (d_{AB}, J = 16 Hz, $^1/_4$·1H), 4.66 (d_{AB}, J = 16 Hz, $^1/_4$·1H), 6.38 (d, J = 7 Hz, 1H), 6.58−6.78 (m, 2H), 6.85−7.34 (m, 15H), 7.43−7.58 (m, 6H), 8.12 (s, $^3/_4$·1H), 8.22 (s, $^1/_4$·1H). Anal. ($C_{40}H_{39}N_3O_2$) C, H, N.

Step 4. (*RS*)-2-Amino-3-(1*H*-indol-3-yl)-1-[*N*-(2-methoxybenzyl)-*N*-acetylamino]propane (17). Formic acid (9.0 mL, 240 mmol) was added to a stirring solution of **16** (14.11 g, 23.8 mmol) in 240 mL of anhydrous methylene chloride under a nitrogen atmosphere at 0 °C. After 4 h, the reaction mixture was concentrated to an oil on a rotary evaporator and redissolved in diethyl ether and 1.0 N hydrochloric acid. The aqueous layer was washed twice with diethyl ether and basified with sodium hydroxide to a pH greater than 12. The product was extracted with methylene chloride (4×). The organic extracts were combined, dried over anhydrous sodium sulfate, filtered, and concentrated on a rotary evaporator to a white foam (7.52 g) identified to be **17** (90% yield). No further purification was necessary, and the material was used directly in the next step; however an analytical sample (as the dihydrochloride salt) was prepared by dissolving the crude material in dichloromethane and adding an excess of gaseous, anhydrous HCl. Data for this material is as follows: ^{1}H (300 MHz, DMSO-d_6) 3:2 mixture of amide rotamers δ 1.94 (s, $^3/_5$·-

3H), 2.08 (s, $^2/_5$·3H), 2.88 (dd, 1H, J = 14, 9 Hz), 2.96 (dd, 1H, J = 14, 4 Hz), 3.42−3.49 (m, 3H), 3.49 (s, 3H), 3.69 (broad doublet, 1H, J = 5 Hz), 4.38 (d, 1H, J = 17 Hz), 4.48, (d, 1H, J = 17 Hz), 6.76−6.80 (m, 2H), 6.94 (m, 2H), 7.05 (t, 1H, J = 7 Hz), 7.18 (m, 2H), 7.36 (d, 1H, J = 8 Hz), 7.56 (d, 1H, J = 8 Hz), 8.45 (br s, $^3/_5$·2H), 8.60 (br s, $^2/_5$·2H), 11.1 (broad singlet, 2H). Anal. ($C_{21}H_{27}Cl_2N_3O_2$) H, N; C: calcd, 59.44; found, 59.86.

Step 5. (*RS*)-2-[(2-Bromoacetyl)amino]-3-(1*H*-indol-3-yl)-1-[*N*-(2-methoxybenzyl)-*N*-acetylamino]propane (18). To a stirring solution of **17** (7.51 g, 21.4 mmol) (free base form) in anhydrous tetrahydrofuran (100 mL) under a nitrogen atmosphere at 0 °C were added diisopropylethylamine (4.1 mL, 23.5 mmol) and bromoacetyl bromide (2.05 mL, 23.5 mmol). After 2 h, ethyl acetate was added and the reaction mixture washed with water twice, 1.0 N hydrochloric acid (2×), saturated sodium bicarbonate solution (2×), and brine. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated to a tan foam on a rotary evaporator. In this manner **18** was obtained in quantitative yield. No further purification was necessary: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.15 (s, 3H), 2.81−2.96 (m, 2H), 3.15 (AB_q, J = 4.3 Hz, $\Delta\nu$ = 14.6 Hz, 1H), 3.72 (s, 3H), 3.79 (s, 2H), 4.06−4.15 (m, 1H), 4.30 (m, 1H), 4.38 (AB_q, J = 16.7 Hz, $\Delta\nu$ = 49.0 Hz, 2H), 6.72−6.81 (m, 3H), 7.01 (s, 1H), 7.13−7.30 (m, 3H), 7.35−7.41 (m, 2H), 7.71 (d, J = 7.8 Hz, 1H), 8.04 (m, 1H). Anal. ($C_{23}H_{26}N_3O_3$-Br) C, H, N.

Step 6. (*RS*)-2-[*N*-(2-(4-Cyclohexylpiperazin-1-yl)acetyl)amino]-3-(1*H*-indol-3-yl)-1-[*N*-(2-methoxybenzyl)-*N*-acetylamino]propane (25). 1-Cyclohexylpiperazine (3.65 g, 22.5 mmol) was added to a stirring solution of **18** (21.4 mmol) and powdered potassium carbonate (3.56 g, 25.8 mmol) in methylene chloride under a nitrogen atmosphere. The reaction mixture was stirred overnight at room temperature. The salts were filtered, and the solution was concentrated to a brown foam on a rotary evaporator. The desired product was purified on a Prep 500 column using a 10 L gradient starting with 100% methylene chloride and ending with 5% methanol/94.5% methylene chloride/0.5% ammonium hydroxide. Impure fractions were combined and purified further by reverse phase preparative high-performance liquid chromatography (methanol/acetonitrile/water/ammonium acetate). After combining the material from both chromatographic purifications, the title compound **25** (10.4 g, 18.7 mmol) was isolated (87% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05−1.34 (m, 6H), 1.55−1.95 (m, 4H), 2.09 (s, 3H), 2.20−2.60 (m, 9H), 2.90 (s, 2H), 2.85−3.16 (m, 3H), 3.77 (s, 3H), 4.02 (dd, J = 11, 13 Hz, 1H), 4.47 (AB q, J = 16 Hz, $\Delta\nu$ = 44 Hz, 2H), 4.54 (m, 1H), 6.77−6.88 (m, 3H), 7.05−7.25 (m, 4H), 7.31−7.42 (m, 2H), 7.66 (d, J = 7 Hz, 1H), 8.08 (br s, 1H). Anal. ($C_{33}H_{45}N_5O_3$) C, H, N.

The following compounds were prepared from **18**, by analogy to **25** using the above method A, steps 1−6, and substituting the appropriate amine for 1-cyclohexylpiperazine.

(*RS*)-2-(*N*-Acetylamino)-1-[*N*-acetyl-*N*-(2-methoxybenzyl)amino]-3-(1*H*-indol-3-yl)propane (9): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.95 (s, 3H), 2.13 (s, 3H), 2.81 (dd, J = 8, 16 Hz, 1H), 2.89 (dd, J = 4, 14 Hz, 1H), 3.72 (s, 3H), 3.99 (t, J = 10 Hz, 1H), 4.35 (m, 1H), 4.37 (AB q, J = 16 Hz, $\Delta\nu$ = 58 Hz, 2H), 7.65−7.82 (m, 4H), 6.99 (s, 1H), 7.01−7.22 (m, 3H), 7.37 (d, J = 7 Hz, 1H), 7.66 (d, J = 8 Hz, 1H), 9.19 (br s, 1H). Anal. ($C_{23}H_{27}N_3O_3$) C, H, N.

(*RS*)-3-(1H-Indol-3-yl)-1-[*N*-(2-methoxybenzyl)-*N*-acetylamino]-2-[*N*-(2-(4-methylpiperazin-1-yl)acetyl)amino]propane (23): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.10 (s, 3H), 2.26 (s, 3H), 2.33−2.40 (m, 8H), 2.86−3.14 (m, 5H), 3.75 (s, 3H), 4.01 (dd, J = 10, 14 Hz, 1H), 4.54 (br s, 1H), 4.48 (AB q, J = 17 Hz, $\Delta\nu$ = 45 Hz, 2H), 6.82 (m, 2H), 7.04 (d, J = 2 Hz, 1H), 7.08−7.28 (m, 3H), 7.35 (d, J = 8 Hz, 1H), 7.43 (d, J = 9 Hz, 1H), 7.66 (d, J = 8 Hz, 1H), 8.49 (br s, 1H); high-resolution mass spec calcd for $C_{28}H_{37}N_5O_3$ 492.2975, found 492.2977.

(*RS*)-2-[*N*-(2-(4-Benzylpiperazin-1-yl)acetyl)amino]-3-(1*H*-indol-3-yl)-1-[*N*-(2-methoxybenzyl)-*N*-acetylamino]propane (24): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.08 (s, 3H), 2.16−2.62 m, 8H), 2.82−2.97 (m, 3H), 2.99−3.18 (m, 2H), 3.41−3.62 (m, 2H), 3.76 (s, 3H), 4.02 (dd, J = 10, 13 Hz, 1H), 4.49 (AB q, J = 18 Hz, $\Delta\nu$ = 48 Hz, 2H), 4.53 (m, 1H), 6.76−6.88 (m, 3H), 7.06 (d, J = 3 Hz, 1H), 7.06−7.45 (m, 10H), 7.68 (d, J = 8 Hz, 1H), 8.06 (br s, 1H). Anal. ($C_{34}H_{41}N_5O_3$) C, H, N.

(*RS*)-3-(1*H*-Indol-3-yl)-1-[*N*-(2-methoxybenzyl)-*N*-acetylamino]-2-[*N*-(2-(4-phenylpiperidin-1-yl)acetyl)amino]propane (26): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50−1.91 (m, 4H), 2.08 (s, 3H), 2.06−2.22 (m, 2H), 2.40 (m, 1H), 2.64 (br d, J = 11 Hz, 1H), 2.80 (br d, J = 12 Hz, 1H), 2.86−2.98 (m, 3H), 3.04−3.18 (m, 2H), 3.73 (s, 3H), 4.01 (dd, J = 10, 14 Hz, 1H), 4.46 (AB q, J = 17 Hz, $\Delta\nu$ = 45 Hz, 2H), 4.54 (m, 1H), 6.76−6.85 (m, 3H), 7.02−7.36 (m, 10H), 7.54 (d, J = 8 Hz, 1H), 7.70 (d, J = 8 Hz, 1H), 8.01 (br s, 1H). Anal. ($C_{34}H_{40}N_4O_3$) C, H, N.

(*RS*)-3-(1*H*-Indol-3-yl)-1-[*N*-(2-methoxybenzyl)-*N*-acetylamino]-2-[*N*-(2-(4-phenyl-$\Delta^{3,4}$-piperidin-1-yl)acetyl)amino]propane (27): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.12 (s, 3H), 2.21−2.70 (m, 4H), 2.90−3.25 (m, 7H), 3.77 (s, 1H), 3.95 (dd, J = 10, 14 Hz, 1H), 4.52 (AB q, J = 17 Hz, $\Delta\nu$ = 38 Hz, 2H), 4.61 (m, 1H), 5.95 (br s, 1H), 6.85 (m, 3H), 7.00−7.54 (m, 11H), 7.67 (d, J = 8 Hz, 1H), 8.08 (br s, 1H). Anal. ($C_{34}H_{38}N_4O_3$) C, H, N.

(*RS*)-3-(1*H*-Indol-3-yl)-1-[*N*-(2-methoxybenzyl)-*N*-acetylamino]-2-[*N*-(2-(piperidin-1-yl)acetyl)amino]propane (28): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37−1.56 (m, 6H), 2.09 (s, 3H), 2.30 (br s, 4H), 2.80−3.19 (m, 5H), 3.75 (s, 3H), 3.95 (dd, J = 11, 13 Hz, 1H), 4.46 (AB q, J = 17 Hz, $\Delta\nu$ = 44 Hz, 2H), 4.53 (m, 1H), 6.75−6.88 (m, 3H), 7.04−7.24 (m, 5H), 7.34 (d, J = 8 Hz, 1H), 7.68 (d, J = 7 Hz, 1H), 8.04 (br s, 1H). Anal. ($C_{28}H_{36}N_4O_3$) C, H, N.

(*RS*)-3-(1*H*-Indol-3-yl)-1-[*N*-(2-methoxybenzyl)-*N*-acetylamino]-2-[*N*-(2-(morpholin-4-yl)acetyl)amino]propane (29): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.07 (s, 3H), 2.20−2.29 (m, 2H), 2.31−2.41 (m, 2H), 2.85−2.97 (m, 3H), 3.01−3.13 (m, 2H), 3.46−3.67 (m, 4H), 3.77 (s, 3H), 4.15 (dd, J = 10, 13 Hz, 1H), 4.47 (AB q, J = 17 Hz, $\Delta\nu$ = 48 Hz, 2H), 4.52 (m, 1H), 6.77−6.89 (m, 3H), 7.02−7.28 (m, 4H), 7.36 (d, J = 6 Hz, 1H), 7.46 (d, J = 8 Hz, 1H), 7.68 (d, J = 7 Hz, 1H), 8.02 (br s, 1H). Anal. ($C_{27}H_{34}N_4O_4$) C, H, N.

(*RS*)-3-(1*H*-Indol-3-yl)-1-[*N*-(2-methoxybenzyl)-*N*-acetylamino]-2-[*N*-(2-(*N*-(2-(phenylamino)ethyl)amino)-acetyl)amino]propane (30): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.11 (s, 3H), 2.72−2.95 (m, 4H), 3.00−3.34 (m, 6H), 3.72 (s, 3H), 4.14 (dd, J = 13 Hz, J = 11 Hz, 1H), 4.40 (AB q, J = 17 Hz, $\Delta\nu$ = 63 Hz, 2H), 4.42 (m, 1H), 4.78 (br s, 1H), 6.65−6.84 (m, 6H), 6.95 (d, J = 3 Hz, 1H), 7.05−7.35 (m, 6H), 7.67 (d, J = 8 Hz, 1H), 7.80−7.91 (m, 2H). Anal. ($C_{31}H_{37}N_5O_3$) C, H, N.

(*RS*)-2-[*N*-(2-(4-(Dimethylamino)piperidin-1-yl)acetyl)-amino]-3-(1*H*-indol-3-yl)-1-[*N*-(2-methoxybenzyl)-*N*-acetylamino]propane (31): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 (m, 1H), 1.48−1.76 (m, 3H), 1.90−2.11 (m, 3H), 2.09 (s, 3H), 2.25 (s, 6H), 2.51 (br d, J = 13 Hz, 1H), 2.73 (br d, J = 12 Hz, 1H), 2.85 (s, 2H), 2.85−3.23 (m, 3H), 3.75 (s, 3H), 3.94 (dd, J = 10, 14 Hz, 1H), 4.47 (AB q, J = 17 Hz, $\Delta\nu$ = 43 Hz, 2H), 4.51 (m, 1H), 6.77−6.88 (m, 3H), 7.01−7.28 (m, 4H), 7.35 (d, J = 8 Hz, 1H), 7.41 (d, J = 9 Hz, 1H), 7.66 (d, J = 7 Hz, 1H), 8.09 (br s, 1H). Anal. ($C_{30}H_{41}N_5O_3$) C, H, N.

(*RS*)-3-(1*H*-Indol-3-yl)-1-[*N*-(2-methoxybenzyl)-*N*-acetylamino]-2-[*N*-(2-(4-(piperidin-1-yl)piperidin-1-yl)-acetyl)amino]propane (32): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30−1.72 (m, 6H), 1.90−2.20 (m, 6H), 2.10 (s, 3H), 2.33−2.60 (m, 6H), 2.68−3.20 (m, 6H), 3.76 (s, 3H), 3.99 (dd, J = 11.4, 14.4 Hz, 1H), 4.48 (AB q, J = 17 Hz, $\Delta\nu$ = 42 Hz, 2H), 4.55 (m, 1H), 6.78−6.89 (m, 3H), 7.04−7.25 (m, 4H), 7.35 (d, J = 7.8 Hz, 1H), 7.46 (d, J = 8.5 Hz, 1H), 7.67 (d, J = 7.9 Hz, 1H), 8.26 (br s, 1H). Anal. ($C_{33}H_{45}N_5O_3$) C, H, N.

The following compounds were prepared from **18**, by analogy to **25** using the above method A, steps 1−6, and substituting the appropriate aldehyde for 2-methoxybenzaldehyde.

(*RS*)-3-(1*H*-Indol-3-yl)-1-[*N*-(3-methoxybenzyl)-*N*-acetylamino]-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane (44): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.08 (s, 3H), 2.15−2.63 (m, 4H), 2.72−3.27 (m, 8H), 3.75 (m, 1H), 3.78 (s, 3H), 4.04 (m, 1H), 4.51 (AB q, J = 16 Hz, $\Delta\nu$ = 46 Hz, 2H), 4.56 (m, 1H), 6.60−6.70 (m, 2H), 6.72−6.94 (m, 5H), 7.04−7.46 (m, 7H), 7.65 (d, J = 8 Hz, 1H), 8.04 (br s, 1H). Anal. ($C_{33}H_{39}N_5O_3$) C, H, N.

(*RS*)-3-(1*H*-Indol-3-yl)-1-[*N*-(4-methoxybenzyl)-*N*-acetylamino]-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane (45): ^{1}H NMR (300 MHz, DMSO-d_6) 1:1 mixture of amide rotamers δ 2.01 (s, $^1/_2$·3H), 2.05 (s, $^1/_2$·3H), 2.23−2.60 (m, 4H), 2.74−3.30 (m, 8H), 3.69 (m, 1H), 3.72 (s, $^1/_2$·3H), 3.74 (s, $^1/_2$·3H), 4.23 (AB q, J = 16 Hz, $\Delta\nu$ = 42 Hz, $^1/_2$·2H), 4.52 (m, 1H), 4.36 (AB *q*, J = 14 Hz, $\Delta\nu$ = 164 Hz, $^1/_2$·2H), 6.70−7.16 (m, 10H), 7.24 (m, 2H), 7.35 (m, 1H), 7.55 (m, $^1/_2$·1H + 1H), 7.73 (m, $^1/_2$·1H), 10.84 (br s, 1H). Anal. ($C_{33}H_{39}N_5O_3$) C, H, N.

(*RS*)-1-(*N*-Benzyl-*N*-acetylamino)-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane (46): ^{1}H NMR (300 MHz, DMSO-d_6) 1:1 mixture of amide rotamers δ 1.99 (s, $^1/_2$·3H), 2.07 (s, $^1/_2$·3H), 2.20−2.50 (m, 4H), 2.69−2.95 (m, 4H), 2.95−3.12 (m, 4H), 3.12−3.52 (m, $^1/_2$·1H + 1H), 3.63 (m, $^1/_2$·1H), 4.40 (m, 1H), 4.51 (AB q, J = 16 Hz, $\Delta\nu$ = 140 Hz, $^1/_2$·2H), 4.54 (AB q, J = 16 Hz, $\Delta\nu$ = 30 Hz, $^1/_2$·2H), 6.78 (t, J = 8 Hz, 1H), 6.86−6.94 (m, 2H), 6.98 (m, 1H), 7.03−7.15 (m, 4H), 7.15−7.38 (m, 6H), 7.50−7.60 (m, 1.5H), 7.74 (d, J = 8 Hz, $^1/_2$·1H), 10.93 (br s, 1H). Anal. ($C_{32}H_{37}N_5O_2$) C, H, N.

(*RS*)-3-(1*H*-Indol-3-yl)-1-[*N*-(2-(methylthio)benzyl)-*N*-acetylamino]-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane (47): mp 138 °C dec; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.09 (s, 3H), 2.1−2.6 (m, 3H), 2.46 (s, 3H), 2.8−3.1 (m, 8H), 3.30 (m, 1H), 3.55 (m, 1H), 3.98 (m, 1H), 4.47 (AB q, J = 12 Hz, $\Delta\nu$ = 52 Hz, 2H), 4.58 (m, 1H), 6.8−6.9 (m, 3H), 6.95 (d, J = 8 Hz, 2H), 7.0−7.4 (m, 9H), 7.66 (d, J = 8 Hz, 1H), 8.08 (br s, 1H). Anal. ($C_{33}H_{39}N_5O_2S$) C, H, N.

(*RS*)-1-[*N*-(2-Chlorobenzyl)-*N*-acetylamino]-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane (48): ^{1}H NMR (300 MHz, DMSO-d_6) 3:2 mixture of amide rotamers δ 1.93 (s, $^2/_5$·3H), 2.09 (s, $^3/_5$·3H), 2.25−2.50 (m, 4H), 2.70−2.96 (m, 4H), 2.96−3.19 (m, 4H), 3.20−3.64 (m, 2H), 4.50 (m, 1H), 4.59 (AB q, J = 16 Hz, $\Delta\nu$ = 70 Hz, $^3/_5$·2H), 4.64 (s, $^2/_5$·2H), 6.78 (t, J = 7 Hz, 1H), 6.91 (d, J = 8 Hz, 2H), 6.98 (t, J = 7 Hz, 1H), 7.02−7.10 (m, 2H), 7.12 (m, 1H), 7.16−7.37 (m, 5H), 7.44 (m, 1H), 7.50−7.62 (m, $^2/_5$·1H + 1H), 7.75 (d, J = 8 Hz, $^3/_5$·1H), 10.83 (br s, 1H). Anal. ($C_{32}H_{36}ClN_5O_2$) C, H, N.

(*RS*)-3-(1*H*-Indol-3-yl)-1-[*N*-(2-methylbenzyl)-*N*-acetylamino]-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane (49): ^{1}H NMR (300 MHz, CDCl3) δ 2.06 (s, 3H), 2.21 (s, 3H), 2.1−2.6 (m, 2H), 2.9−3.3 (m, 12H), 3.58 (m, 1H), 4.4−4.6 (m, 2H), 6.8−7.0 (m, 5H), 7.0−7.4 (m, 9H), 7.62 (d, J = 7 Hz, 1H), 8.15 (br s, 1H). Anal. ($C_{33}H_{39}N_5O_2$) C, H, N.

(*RS*)-3-(1*H*-Indol-3-yl)-2-[*N*-(2-(4-phenylpiperazin-1-yl)-acetyl)amino]-1-[*N*-(2-(trifluoromethyl)benzyl)-*N*-acetylamino]propane (50); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.03 (s, 3H), 2.15−2.80 (m, 5H), 2.80−3.73 (m, 8H), 3.88 (m, 1H), 4.47−4.93 (m, 3H), 6.72−7.03 (m, 4H), 7.03−7.45 (m, 7H), 7.45−7.76 (m, 4H), 8.22 (br s, 1H). Anal. ($C_{33}H_{36}F_3N_5O_2$) C, H, N.

(*RS*)-3-(1*H*-Indol-3-yl)-1-[*N*-(2-nitrobenzyl)-*N*-acetylamino]-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane (51): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.05 (s, 3H), 2.28 (m, 1H), 2.3−2.7 (m, 4H), 2.8−3.2 (m, 8H), 3.2−3.9 (m, 2H), 4.58 (m, 1H), 4.97 (m, 1H), 6.8−7.0 (m, 2H), 7.0−7.5 (m, 10H), 7.5−7.7 (m, 2H), 8.12 (d, J = 7 Hz, 1H), 8.15 (br s, 1H). Anal. ($C_{32}H_{36}N_6O_4$) C, H, N.

The following compounds were prepared by analogy to **25** using the above method A, steps 1−6, and substituting 2-chlorobenzaldehyde for the 2-methoxybenzaldehyde.

(*RS*)-1-[*N*-(2-Chlorobenzyl)-*N*-acetylamino]-2-[*N*-(2-(4-cyclohexylpiperazin-1-yl)acetyl)amino]-3-(1*H*-indol-3-yl)propane (52): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.0−1.4 (m, 6H), 1.6 (m, 1H), 1.7−1.9 (m, 4H), 2.08 (s, 3H), 2.1−2.6 (m, 9H), 2.8−3.1 (m, 4H), 4.0 (m, 1H), 4.5−4.7 (m, 3H), 7.0−7.4 (m, 9H), 7.63 (d, J = 6 Hz, 1H), 8.18 (br s, 1H). Anal. ($C_{32}H_{42}ClN_5O_2$) C, H, N.

(*RS*)-1-[*N*-(2-Chlorobenzyl)-3-(1*H*-indol-3-yl)-*N*-acetylamino]-2-[*N*-(2-(4-(piperidin-1-yl)piperidin-1-yl)-acetyl)amino]propane (53): ^{1}H NMR (300 MHz, CDCl3) δ 1.17−1.80 (m, 10H), 1.90−2.27 (m, 3H), 2.03 (s, 3H), 2.35−2.59 (m, 5H), 2.67−3.23 (m, 6H), 3.97 (dd, J = 10, 15 Hz, 1H), 4.53 (m, 1H), 4.58 (AB q, J = 17 Hz, $\Delta\nu$ = 21 Hz, 21 Hz, 2H), 6.95−7.29 (m, 6H), 7.34 (d, J = 8 Hz, 2H), 7.42 (d, J = 9 Hz, 1H), 7.63 (d, J = 8 Hz, 1H), 8.19 (br s, 1H); high-resolution mass spectral data, calcd for $C_{32}H_{42}ClN_5O_2$ 564.3105, found 564.3135. Anal. ($C_{32}H_{42}ClN_5O_2$·0.5H_2O) C, H, N.

Method B. Step 1. 2-[(*tert*-Butoxycarbonyl)amino]-3-(1*H*-indol-3-yl)-*N*-(2-methoxybenzyl)propanamide (20). To a solution of **19** (46.4 g, 152.6 mmol) in 500 mL of dioxane was added carbonyldiimidazole (25.4 g, 156 mmol) in a portionwise manner. The resulting mixture was stirred for about 2.5 h at room temperature and then stirred at 45 °C for 30 min. Next, 2-methoxybenzylamine (20.7 mL, 158.7 mmol) was added to the reaction mixture which was stirred for 16 h at room temperature. The dioxane was removed under reduced pressure. The product was partitioned between ethyl acetate and water and was washed successively with 1 N hydrochloric acid, saturated sodium bicarbonate solution, water, and brine, followed by drying over sodium sulfate and removal of the solvent. Final crystallization from methanol yielded 52.2 g of homogeneous product **20** as yellow crystals: yield 80.8%; mp 157−160 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (s, 9H), 3.10 (m, 1H), 3.29 (m, 1H), 3.58 (3, 3H), 4.26 (m, 2H), 4.40 (m, 1H), 5.24 (m, 1H), 6.08 (m, 1H), 6.67−6.93 (m, 3H), 6.97−7.40 (m, 5H), 7.67 (d, J = 8 Hz, 1H), 7.79 (br s, 1H). Anal. ($C_{24}H_{29}N_3O_4$) C, H, N.

Step 2. 2-Amino-3-(1*H*-indol-3-yl)-*N*-(2-methoxybenzyl)propanamide (21). To a mixture of **20** (25.1 g, 59.2 mmol) and anisole (12 mL, 110.4 mmol) at 0 °C was added dropwise an aqueous solution of trifluoroacetic acid (118 mL, 1.53 moles) in 50 mL of water. This mixture was stirred for 1 h at 0 °C, followed by stirring for about 2.5 h at ambient temperature. The volatile materials were removed under reduced pressure. The product was partitioned between ethyl acetate and saturated sodium bicarbonate solution and was washed with water followed by brine and then dried over sodium sulfate. The solvents were removed *in vacuo*. Recrystallization from a 1:1 diethyl ether/cyclohexane solution yielded 18.0 g (94.2%) of **21** as an off-white powder: mp 104−108 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.09 (m, 2H), 3.18 (AB q, J = 16 Hz, $\Delta\nu$ = 120 Hz, 2H), 3.77 (s, 3H), 3.79 (m, 1H), 4.40 (m, 2H), 6.76−6.95 (m, 2H), 6.99 (s, 1H), 7.05 (t, J = 8 Hz, 1H), 7.12−7.31 (m, 3H), 7.34 (d, J = 9 Hz, 1H), 7.54 (m, 1H), 7.63 (d, J = 9 Hz, 1H), 8.07 (br s, 1H). Anal. ($C_{19}H_{21}N_3O_2$) C, H, N.

Step 3. 2-Amino-3-(1*H*-indol-3-yl)-1-[*N*-(2-methoxybenzyl)amino]propane. To a refluxing solution of **21** (9.81 g, 30.3 mmol) in 100 mL of anhydrous tetrahydrofuran was added dropwise a 10 M borane methyl sulfide complex (9.1 mL, 91.0 mmol). The resulting mixture was refluxed for about 2 h. The mixture was cooled to room temperature, and the excess borane was quenched by the dropwise addition of 160 mL of methanol. The resulting mixture was refluxed for 15 min, and the methanol was removed under reduced pressure. The residue was dissolved in a saturated methanol solution of hydrochloride acid (250 mL) and the solution refluxed for about 1 h. The methanol was removed *in vacuo*, and the product was isolated by the addition of 5 N sodium hydroxide followed by extraction with diethyl ether (2×). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (silica gel, 10:100:0.5 methanol/methylene chloride/ammonium hydroxide) provided 7.1 g of a mixture of the intermediate 2-amino-3-(1*H*-indol-3-yl)-1-[*N*-(2-methoxybenzyl)amino]propane (75%) and its indoline derivative (25%) as an amber oil. This mixture was used directly in the next step.

Step 4. 3-(1*H*-Indol-3-yl)-1-[*N*-(2-methoxybenzyl)amino]-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane (22). A mixture of 2-(4-phenylpiperazin-1-yl)acetic acid, sodium salt (1.64 g, 6.8 mmol), and triethylamine hydrobromide (1.24 g, 6.8 mmol) in 35 mL of anhydrous dimethylformamide was prepared and heated to 50 °C and held at that temperature for about 35 min. The mixture was allowed to cool to room temperature. 1,1-Carbonyldiimidazole (1.05 g, 6.5 mmol) and 10 mL of anhydrous dimethylformamide were added to the mixture. The resulting mixture was stirred for about 3 h at room temperature. Next, a solution of the above prepared mixture of 2.17 g of 2-amino-3-(1*H*-indol-3-yl)-1-[*N*-(2-methoxybenzyl)amino]propane (75%) and its indoline derivative (25%) (ca. 6.2 mmol) in 10 mL of anhydrous dimethylforma-

mide was added to the activated acylimidazole mixture prepared above. The resulting mixture was stirred for about 16 h at room temperature. The dimethylformamide was removed under reduced pressure. The products were partitioned between ethyl acetate and water, then washed with brine, and dried over sodium sulfate. The solvents were removed *in vacuo*. This process yielded 3.2 g of a mixture of the title compound **22** and its indoline derivative as a yellow oil. These two compounds were separated using high-performance liquid chromatography using a reverse phase column followed by a silica gel column to give the title product **22**, (266 mg, ca. 5.2% overall two-step yield) as a yellow foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.30−2.43 (m, 2H), 2.43−2.54 (m, 2H), 2.70−3.10 (m, 11H), 3.82 (s, 3H), 3.84 (m, 2H), 4.44 (m, 1H), 6.74−6.94 (m, 6H), 7.04 (m, 1H), 7.07−7.36 (m, 7H), 7.64 (d, J = 8 Hz, 1H), 8.09 (br s, 1H). Anal. ($C_{31}H_{37}N_5O_2$) C, H, N.

The above intermediate **22** was used in the following steps 5a−d.

Step 5a. 1-[*N*-(Ethoxycarbonyl)-*N*-(2-methoxybenzyl)-amino]-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane (39). Ethyl chloroformate (89 μL, 0.93 mmol) was added slowly to a 0 °C solution of 3-(1*H*-indol-3-yl)-1-[*N*-(2-methoxybenzyl)amino]-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane (**22**) (0.43 g, 0.85 mmol) and triethylamine (130 μL, 0.93 mmol) in anhydrous tetrahydrofuran (5 mL). The resulting mixture was stirred for 16 h at room temperature. The tetrahydrofuran was removed under reduced pressure. The residue was partitioned between ethyl acetate and 0.2 N NaOH, washed with water and brine successively, and then dried over sodium sulfate. Flash chromatography (silica gel, methanol:methylene chloride, 2.5:97.5) provided chromatographically homogeneous product **39** as a white foam: yield 390 mg (79%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.05 (t, J = 8 Hz, 3H), 2.37 (br s, 4H0, 2.83 (br s, 4H), 3.03 (br s, 4H), 3.22−3.48 (m, 2H), 3.66 (s, 3H), 3.87−4.03 (m, 2H), 4.26−4.55 (m, 3H), 6.77 (t, J = 7 Hz, 1H), 6.80−7.00 (m, 6H), 7.05 (t, J = 8 Hz, 1H), 7.11 (br s, 1H), 7.20 (t, J = 9 Hz, 3 H), 7.32 (d, J = 10 Hz, 1H), 7.52 (br d, J = 6 Hz, 2H). Anal. ($C_{34}H_{41}N_5O_4$) C, H, N.

The following compounds were prepared from **22**, by analogy to **39** using the above method B, step 5a, and substituting with the appropriate acylating (or sulfonylating) agent.

(*RS*)-1-[*N*-Acetyl-*N*-(2-methoxybenzyl)amino]-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]-propane (12): ^{1}H NMR (300 MHz, DMSO-d_6) 3:2 mixture of amide rotamers δ 1.97 (s, 1.8H), 2.07 (s, 1.2H), 2.26−2.50 (m, 4H), 2.70−2.96 (m, 4H), 2.96−3.16 (m, 4H), 3.16−3.65 (m, 2H), 3.72 (s, $^2/_5$·3H), 3.74 (s, $^3/_5$·3H), 4.40 (m, 1H), 4.42 (AB q, J = 18 Hz, $\Delta\nu$ = 30 Hz, $^3/_5$·2H), 4.46 (AB q, J = 16 Hz, $\Delta\nu$ = 62 Hz, $^2/_5$·2H), 6.70−7.03 (m, 7H), 7.03−7.13 (m, 2H), 7.13−7.29 (m, 3H), 7.34 (d, J = 8 Hz, 1H), 7.49−7.62 (m, $^3/_5$H·1H), 7.72 (d, J = 6 Hz, $^2/_5$H), 10.93 (br s, 1H). Anal. ($C_{33}H_{39}N_5O_3$) C, H, N.

3-(1*H*-Indol-3-yl)-1-[*N*-(2-methoxybenzyl)-*N*-propionylamino]-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]-propane (34): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12 (t, J = 9 Hz, 3H), 2.38 (q, J = 9 Hz, 2H), 2.33−2.60 (m, 4H), 2.83−3.13 (m, 8H), 3.22 (br d, J = 15 Hz, 1H), 3.80 (s, 3H), 4.03 (br t, J = 13 Hz, 1H), 4.55 (AB q, J = 20 Hz, $\Delta\nu$ = 40 Hz, 2H), 4.60 (m, 1H), 6.83−6.97 (m, 6H), 7.10−7.57 (m, 8H), 7.68 (d, J = 8 Hz, 1H), 8.24 (br s, 1H). Anal. ($C_{34}H_{41}N_5O_3$) C, H, N.

1-[*N*-Benzoyl-*N*-(2-methoxybenzyl)amino]-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane (35): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.28−2.57 (m, 4H), 2.77−3.17 (m, 9H), 3.65 (s, 3H), 4.22 (t, J = 13 Hz, 1H), 4.60 (AB q, J = 15 Hz, $\Delta\nu$ = 30 Hz, 2H), 4.82 (m, 1H), 6.70−6.92 (m, 5H), 7.02−7.55 (m, 14H), 7.68 (d, J = 7 Hz, 1H), 8.22 (br s, 1H). Anal. ($C_{38}H_{41}N_5O_3$) C, H, N.

1-[*N*-(Carbomethoxyacetyl)-*N*-(2-methoxybenzyl)-amino]-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane (36): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.37−2.47 (m, 2H), 2.50−2.60 (m, 2H), 2.82−3.18 (m, 9H), 3.57 (s, 2H), 3.72 (s, 3H), 3.78 (s, 3H), 4.02 (dd, J = 10, 14 Hz, 1H), 4.47 (AB q, J = 20 Hz, $\Delta\nu$ = 40 Hz, 2H), 4.60 (m, 1H), 6.77−6.92 (m, 6H), 7.03−7.30 (m, 6H), 7.37 (d, J = 7 Hz, 1H), 7.45 (d, J = 10 Hz, 1H), 7.68 (d, J = 9 Hz, 1H), 8.12 (s, 1H). Anal. ($C_{35}H_{41}N_5O_5$) C, H, N.

3-(1*H*-Indol-3-yl)-1-[*N*-((methylamino)carbonyl)-*N*-(2-methoxybenzyl)amino]-2-[*N*-(2-(4-phenylpiperazin-1-yl)-acetyl)amino]propane (40): ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.32−2.46 (m, 4H), 2.55 (d, J = 5 Hz, 3H), 2.78−2.90 (m, 4H), 2.96−3.10 (m, 4H), 3.18 (dd, J = 5, 14 Hz, 1H), 3.44 (dd, J = 8, 13 Hz, 1H), 3.70 (s, 3H), 4.30 (m, 1H), 4.37 (AB q, J = 18 Hz, $\Delta\nu$ = 42 Hz, 2H), 6.32 (br d, J = 5 Hz, 1H), 6.77 (t, J = 7 Hz, 1H), 6.82−7.00 (m, 6H), 7.05 (t, J = 8 Hz, 1H), 7.11 (d, J = 3 Hz, 1H), 7.16−7.25 (m, 3H), 7.32 (d, J = 9 Hz, 1H), 7.53 (d, J = 8 Hz, 1H), 7.61 (d, J = 9 Hz, 1H), 10.82 (br s, 1H). Anal. ($C_{33}H_{40}N_6O_3$) C, H, N.

3-(1*H*-Indol-3-yl)-1-[*N*-(2-methoxybenzyl)-*N*-(methylsulfonyl)amino]-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)-amino]propane (41): ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.28−2.46 (m, 4H), 2.83 (d, J = 7 Hz, 4H), 2.90 (s, 3H), 2.98−3.04 (m, 4H), 3.26−3.34 (m, 2H), 3.67 (s, 3H), 4.30 (m, 1H), 4.36 (d, J = 5 Hz, 2H), 6.77 (t, J = 8 Hz, 1H), 6.84−6.92 (m, 3H), 6.92−7.00 (m, 2H), 7.03−7.09 (m, 2H), 7.18−7.30 (m, 4H), 7.33 (d, J = 8 Hz, 1H), 7.46 (d, J = 8 Hz, 1H), 7.54 (d, J = 9 Hz, 1H), 10.82 (br s, 1H). Anal. ($C_{32}H_{39}N_5O_4S$) C, H, N.

The following compounds were prepared from **19** using the above method B, steps 1, 2, 4, and 5a (skipping step 3), and substituting the appropriate amine for 2-methoxybenzylamine.

(*RS*)-2-(*N*-Acetylamino)-3-(1*H*-indol-3-yl)-*N*-benzylpropanamide (5): ^{1}H NMR (300 MHz, DMSO) δ 1.79 (s, 3H), 2.92 (dd_{AB}, J = 8, 15 Hz, 1H), 3.12 (dd_{AB}, J = 6, 15 Hz, 1H), 4.25 (d, J = 6 Hz, 2H), 4.56 (m, 1H), 6.97 (t, J = 7 Hz, 1H), 7.06 (t, J = 7 Hz, 1H), 7.08−7.29 (m, 6H), 7.33 (d, J = 8 Hz, 1H), 7.61 (d, J = 8 Hz, 1H), 8.08 (d, J = 8 Hz, 1H), 8.47 (t, J = 6 Hz, 1H), 10.80 (br s, 1H). Anal. ($C_{20}H_{21}N_3O_2$) C, H, N.

(*RS*)-2-(*N*-Acetylamino)-3-(1*H*-indol-3-yl)-*N*-benzyl-*N*-methylpropanamide (6): ^{1}H NMR (300 MHz, DMSO-d_6) 2:1 mixture of amide rotamers δ 1.75 (s, $^1/_3$·3H), 1.83 (s, $^2/_3$·3H), 2.70 (s, $^1/_3$·3H), 2.72 (s, $^2/_3$·3H), 2.92 (m, 1H), 3.23 (m, 1H), 4.40 (AB q, J = 15 Hz, $\Delta\nu$ = 55 Hz, $^2/_3$·2H), 4.50 (AB q, J = 18 Hz, $\Delta\nu$ = 45 Hz, $^1/_3$·2H), 4.95 (m, $^1/_3$·1H), 5.14 (m, $^2/_3$·1H), 6.80−7.40 (m, 8H), 7.60 (d, J = 8 Hz, 1H), 8.41 (d, J = 7 Hz, $^2/_3$·1H), 8.46 (d, J = 7 Hz, $^1/_3$·1H), 10.8 (s, $^1/_3$·1H), 10.9 (s, $^2/_3$·1H). Anal. ($C_{21}H_{23}N_3O_2$) C, H, N.

(*RS*)-Benzyl 2-(*N*-acetylamino)-3-(1*H*-indol-3-yl)propanoate (7): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.65 (s, 3H), 3.18 (m, 2H), 4.55 (m, 1H), 5.06 (AB q, J = 12 Hz, $\Delta\nu$ = 18 Hz, 2H), 6.94−7.44 (m, 8H), 7.5 (d, J = 8 Hz, 1H), 8.35 (d, J = 9 Hz, 1H), 10.8 (s, 1H). Anal. ($C_{20}H_{20}N_2O_3$) C, H, N.

(*RS*)-*N*-(2-Chlorobenzyl)-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propanamide (11): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.45 (m, 4H), 2.91 (m, 4H), 2.97 (d, J = 9 Hz, 2H), 3.21 (dd_{AB}, J = 8, 14 Hz, 1H), 3.34 (dd_{AB}, J = 7, 14 Hz, 1H), 4.44 (d, J = 7 Hz, 2H), 4.82 (q, J = 8 Hz, 1H), 6.37 (t, J = 6 Hz, 1H), 6.81−6.94 (m, 4H), 7.08−7.38 (m, 9H), 7.68 (m, 2H), 7.99 (br s, 1H). Anal. ($C_{30}H_{32}N_5O_2Cl$) C, H, N.

The following compounds were prepared from **19**, by analogy to **12** using the above method B, steps 1−4 and 5a, and substituting the appropriate amine for 2-methoxybenzylamine.

(*RS*)-1-(*N*-Acetyl-*N*-methylamino)-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane (54): mp 128−129 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.07 (s, 3H), 2.38−2.78 (m, 3H), 2.8−3.3 (m, 11H), 3.42 (m, 1H), 3.67 (m, 1H), 3.95 (m, 1H), 4.58 (m, 1H), 6.8−7.0 (m, 3H), 7.1−7.4 (m, 7H), 7.68 (d, J = 7 Hz, 1H), 8.21 (br s, 1H). Anal. ($C_{26}H_{33}N_5O_2$) C, H, N.

(*RS*)-1-(*N*-Acetyl-*N*-butylamino)-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane (55): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 6 Hz, 3H), 1.1−1.40 (m, 2H), 1.4−1.6 (m, 2H), 2.08 (s, 3H), 2.2−2.4 (m, 4H), 2.8−3.1 (m, 8H), 3.1−3.4 (m, 3H), 3.9 (m, 1H), 4.5 (br s, 1H), 6.8−7.0 (m, 3H), 7.0−7.5 (m, 7H), 7.68 (d, J = 6 Hz, 1H), 8.31 (br s, 1H). Anal. ($C_{29}H_{39}N_5O_2$) C, H, N.

(*RS*)-1-[*N*-acetyl-*N*-(cyclohexylmethyl)amino]-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]-propane (56): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.65−1.02 (m, 2H), 1.02−1.36 (m, 3H), 1.36−1.87 (m, 9H), 2.07 (s, 3H), 2.15−3.70 (m, 12H), 3.95 (m, 1H), 4.57 (m, 1H), 6.70−7.03 (m, 4H),

7.03−7.23 (m, 4H), 7.31−7.44 (m, 2H), 7.69 (d, J = 10 Hz, 1H), 8.16 (br s, 1H). Anal. ($C_{32}H_{43}N_5O_2$) C, H, N.

(*RS*)-1-(*N*-Acetyl-*N*-phenylamino)-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane (57): mp 183−184 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.71 (s, 3H), 2.23−2.43 (m, 4H), 2.71−2.94 (m, 4H), 2.94−3.10 (m, 4H), 3.61 (m, 1H), 4.03 (m, 1H), 4.24 (m, 1H), 6.77 (t, J = 8 Hz, 1H), 6.92−6.99 (m, 3H), 6.99−7.12 (m, 2H), 7.21 (t, J = 8 Hz, 2H), 7.24−7.35 (m, 3H), 7.4 (m, 1H), 7.40−7.54 (m, 4H), 10.92 (br s, 1H). Anal. ($C_{31}H_{35}N_5O_2$) C, H, N.

(*RS*)-1-(*N*-Acetyl-*N*-phenethylamino)-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino)amino]propane (58): ^{1}H NMR (300 MHz, DMSO-d_6) 3:2 mixture of amide rotamers δ 1.69 (s, $^3/_5$·3H), 2.00 (s, $^2/_5$·3H), 2.50−2.60 (m, 5H), 2.70−3.05 (m, 5H), 3.05−3.19 (m, 4H), 3.19−3.36 (m, 2H), 3.36−3.64 (m, 2H), 4.32 (m, 1H), 6.76 (t, J = 8 Hz, 1H), 6.90 (d, J = 8 Hz, 2H), 6.95−7.39 (m, 11H), 7.56 (m, 1H), 7.76 (m, $^2/_5$·1H), 7.92 (m, $^3/_5$·1H), 10.81 (br s, $^2/_5$·1H), 10.85 (br s, $^3/_5$·1H). Anal. ($C_{33}H_{39}N_5O_2$) C, H, N.

The following compounds were prepared from the appropriate amino acid starting material, by analogy to **12** using the above method B, steps 1−4 and 5a.

(*RS*)-1-[*N*-(2-Methoxybenzyl)-*N*-acetylamino]-3-phenyl-2-[*N*-(2-(4-phenylpiperazin-1yl)acetyl)amino]propane (59): ^{1}H NMR (300 MHz, DMSO-d_6) 3:2 mixture of amide rotamers δ 1.93 (s, $^3/_5$·3H), 2.09 (s, $^2/_5$·3H), 2.23−2.46 (m, 4H), 2.60−2.90 (m, 4H), 3.00−3.20 (m, 2H), 3.30−3.53 (m, 4H), 3.75 (s, 3H), 4.20−4.60 (m, 3H), 6.70−7.04 (m, 7H), 7.04−7.30 (m, 7H), 7.57 (d, J = 9 Hz, $^3/_5$·1H), 7.71 (d, J = 9 Hz, $^2/_5$·1H). Anal. ($C_{31}H_{38}N_4O_3$) C, H, N.

(*RS*)-1-[*N*-(2-Methoxybenzyl)-*N*-acetylamino]-3-(1-naphthyl)-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane (60): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.13 (s, 3H), 2.38−2.70 (m, 4H), 2.82−3.07 (m, 4H), 3.07−3.30 (m, 4H), 3.56 (dd, J = 7, 14 Hz, 1H), 3.66 (s, 3H), 4.14 (m, 1H), 4.34 (AB q, J = 16 Hz, $\Delta\nu$ = 58 Hz, 2H), 4.47 (m, 1H), 6.52−6.67 (m, 2H), 6.73 (d, J = 8 Hz, 1H), 6.77−7.00 (m, 3H), 7.09−7.20 (m, 1H), 7.20−7.40 (m, 4H), 7.43−7.70 (m, 3H), 7.73 (d, J = 8 Hz, 1H), 7.86 (d, J = 8 Hz, 1H), 8.34 (d, J = 8 Hz, 1H). Anal. ($C_{35}H_{40}N_4O_3$) C, H, N.

(*RS*)-1-[*N*-(2-Methoxybenzyl)-*N*-acetylamino]-3-(2-naphthyl)-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane (61): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.12 (s, 3H), 2.26−2.50 (m, 4H), 2.59−3.30 (m, 9H), 3.78 (s, 3H), 3.98 (m, 1H), 4.51 (AB q, J = 17 Hz, $\Delta\nu$ = 30 Hz, 2H), 4.53 (m, 1H), 6.55−7.03 (m, 6H), 7.05−7.39 (m, 5H), 7.39−7.53 (m, 2H), 7.60 (m, 1H), 7.71−7.85 (m, 3H). Anal. ($C_{35}H_{40}N_4O_3$) C, H, N.

(*RS*)-3-(Benzo[*b*]thiophenyl)-1-[*N*-(2-methoxybenzyl)-*N*-acetylamino]-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)-amino]propane (62): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.15 (s, 3H), 2.44−2.60 (m, 4H), 2.89−3.26 (m, 9H), 3.73 (s, 3H), 4.07 (dd, J = 10.4, 13.9 Hz, 1H), 4.43 (AB q, J = 16.5 Hz, $\Delta\nu$ = 45.4 Hz, 2H), 4.50 (m, 1H), 6.74−6.92 (m, 6H), 7.15 (s, 1H), 7.18−7.30 (m, 3H), 7.39 (m, 2 H), 7.57 (d, J = 8.1 Hz, 1H), 7.87 (d, J = 7.4 Hz, 1H), 7.98 (d, J = 7.6 Hz, 1H). Anal. ($C_{33}H_{38}N_4O_3S$) C, H, N.

(*RS*)-3-(1*H*-Indolin-3-yl)-1-[*N*-(2-methoxybenzyl)-*N*-acetylamino]-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane (63). The titled compound was obtained starting from 3-(1*H*-indolin-3-yl)-1-[*N*-(2-methoxybenzyl)amino]-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane (obtained in method B, step 3 and proceeding as in method B, step 5a): mp 102−105 °C; ^{1}H NMR (300 MHz, $CDCl_3$) 1:1 mixture of diastereomers δ 1.57−2.08 (m, 2H), 2.15 (s, $^1/_2$·3H), 2.17 (s, $^1/_2$·3H), 2.75−3.60 (m, 13H), 3.65−4.00 (m, 2H), 3.82 (s, $^1/_2$·3H), 3.85 (s, $^1/_2$·3H), 4.18−4.48 (m, 2H), 4.58 (s, 2H), 6.70−7.40 (m, 13H), 7.67 (m, 1H); high-resolution mass spectral data calcd for $C_{33}H_{41}N_5O_3$ 556.3287, found 556.3280.

(*RS*)-1-[*N*-(2-Methoxybenzyl)-*N*-acetylamino]-2-phenyl-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]ethane (64): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.14 (s, 3H), 2.60−2.80 (m, 4H), 3.00−3.20 (m, 2H), 3.20−3.43 (m, 5H), 3.82 (s, 3H), 4.30 (m, 1H), 4.40−4.63 (m, 2H), 5.18 (m, 1H), 6.80−7.06 (m, 6H), 7.03−7.40 (m, 8H), 8.24 (br s, 1H). Anal. ($C_{30}H_{36}N_4O_2$) C, H, N.

(*RS*)-2-(3,4-Dichlorophenyl)-1-[*N*-(2-methoxybenzyl)-*N*-acetylamino]-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)-amino]ethane (65): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.19 (s, 3H), 2.63−2.83 (m, 2H), 2.93−3.20 (m, 4H), 3.20−3.50 (m, 3H), 3.50−3.70 (m, 2H), 3.85 (s, 3H), 4.23 (m, 1H), 4.30−4.60 (m, 2H), 5.00 (m, 1H), 6.85−7.06 (m, 5H), 7.13 (m, 1H), 7.20−7.45 (m, 6H), 8.41 (br s, 1H). Anal. ($C_{30}H_{34}Cl_2N_4O_3$) C, H, N.

(*RS*)-1-[*N*-(2-Methoxybenzyl)-*N*-acetylamino]-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]ethane (66): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.11 (s, 3H), 2.63−2.78 (m, 4H), 3.03 (s, 2H), 3.18−3.32 (m, 4H), 3.39−3.48 (m, 2H), 3.50−3.59 (m, 2H), 3.84 (s, 3H), 4.52 (s, 2H), 6.83−6.98 (m, 4H), 7.04 (br d, J = 8 Hz, 1H), 7.22−7.38 (m, 4H), 7.61 (br s, 1H). Anal. ($C_{24}H_{32}N_4O_3$) C, H, N.

(*RS*)-1-[*N*-(2-Methoxybenzyl)-*N*-acetylamino]-3-methyl-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]butane (67): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (d, J = 7 Hz, 6H), 1.80 (m, 1H), 2.08 (s, 3H), 2.70 (t, J = 6 Hz, 4H), 2.87 (br d, J = 11 Hz, 1H), 3.07 (d, J = 3 Hz, 2H), 3.20−3.38 (m, 4H), 3.85 (s, 3H), 4.04−4.18 (m, 2H), 4.55 (AB q, J = 17 Hz, $\Delta\nu$ = 39 Hz, 2H), 6.84−7.00 (m, 4H), 7.07 (d, J = 8 Hz, 1H), 7.24−7.39 (m, 5H). Anal. ($C_{27}H_{38}N_4O_3$) C, H, N.

Step 5b. 1-[*N*-((Dimethylamino)acetyl)-*N*-(2-methoxybenzyl)amino]-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane (38). 1-(3-(Dimethylamino)-propyl)-3-ethylcarbodiimide hydrochloride (131 mg, 0.68 mmol) was added to a mixture of a secondary amine **22** (350 mg, 0.68 mmol), *N*,*N*-dimethylglycine (70 mg, 0.68 mmol), and 1-hydroxybenzotriazole hydrate (92 mg, 0.68 mmol) in anhydrous tetrahydrofuran (25 mL). The resulting mixture was stirred at room temperature for 16 h. The tetrahydrofuran was removed under reduced pressure. The residue was dissolved in ethyl acetate, washed successively with saturated sodium bicarbonate solution, water, and brine, and dried over sodium sulfate. Flash chromatography (silica gel, methanol:methylene chloride:ammonium hydroxide, 4:96:0.5) provided homogeneous product **38** as a yellow foam: yield 300 mg (74%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.30 (s, 6H), 2.32−2.50 (m, 4H), 2.87−3.05 (m, 8H), 3.20 (s, 2H), 3.33 (dd, J = 6, 9 Hz, 1H), 3.78 (s, 3H), 3.85 (m, 1H), 4.58 (m, 1H), 4.65 (AB q, J = 18 Hz, $\Delta\nu$ = 42 Hz, 2H), 6.81−6.93 (m, 6H), 7.10−7.40 (m, 8H), 7.65 (d, J = 11 Hz, 1H), 8.17 (br s, 1H). Anal. ($C_{35}H_{44}N_6O_3$) C, H, N.

Step 5c. 1-[*N*-Ethyl-*N*-(2-methoxybenzyl)amino]-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]-propane (42). 3-(1*H*-Indol-3-yl)-1-[*N*-(2-methoxybenzyl)-amino]-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane (**22**) (0.41 g, 0.80 mmol), ethyl iodide (120 μL, 1.5 mmol), and potassium carbonate (120 mg, 0.80 mmol) in anhydrous *N*,*N*-dimethylformamide (5 mL) were heated to 50 °C for 4 h and then stirred at room temperature for 16 h. The *N*,*N*-dimethylformamide was removed under reduced pressure. The residue was dissolved in ethyl acetate, washed with water and brine successively, and then dried over sodium sulfate. Radial thin-layer chromatography (silica gel, methanol:methylene chloride:ammonia atmosphere, 1:99) provided homogeneous product **43** as a yellow foam: yield 360 mg (83%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.04 (t, J = 8 Hz, 3H), 2.32−2.43 (m, 2H), 2.43−2.66 (m, 6H), 2.83−2.91 (m, 4H), 2.94 (d, J = 5 Hz, 2H), 3.08 (t, J = 6 Hz, 2H), 3.65 (AB q, J = 14 Hz, $\Delta\nu$ = 22 Hz, 2H), 3.77 (s, 3H), 4.41 (q, J = 6 Hz, 1H), 6.78−6.96 (m, 6H), 7.06−7.29 (m, 6H), 7.33 (d, J = 8 Hz, 1H), 7.40 (d, J = 7 Hz, 1H), 7.64 (d, J = 8 Hz, 1H), 7.99 (br s, 1H). Anal. ($C_{33}H_{41}N_5O_2$) C, H, N.

The following compounds were prepared from **22**, by analogy to **42** using the above method B, steps 1−4 and 5c, and substituting with the appropriate alkylating agent.

(*RS*)-1-[*N*-(Carboxyacetyl)-*N*-(2-methoxybenzyl)amino]-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)-amino]propane (37): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.68−2.90 (m, 4H), 2.90−3.37 (m, 9H), 3.57 (br s, 2H), 3.78 (s, 3H), 3.93 (t, J = 12 Hz, 1H), 4.53 (AB q, J = 17 Hz, $\Delta\nu$ = 47 Hz, 2H), 4.70 (m, 1H), 6.77−6.97 (m, 6H), 7.07−7.33 (m, 7H), 7.37 (d, J = 8 Hz, 1H), 7.63 (d, J = 8 Hz, 1H), 7.85 (br s, 1H), 8.33 (br s, 1H). High-resolution mass spectral data calcd for

$C_{34}H_{39}N_5O_5$ 598.3029, found 598.3046. Anal. ($C_{34}H_{39}N_5O_5 \cdot CH_3COOH$) C, H, N.

(*RS*)-1-[*N*-(Carbomethoxymethyl)-*N*-(2-methoxybenzyl)amino]-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane (43): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.37−2.47 (m, 2H), 2.50−2.58 (m, 2H), 2.78−2.98 (m, 6H), 3.00 (s, 2H), 3.12 (t, J = 6 Hz, 2H), 3.37 (AB q, J = 18 Hz, $\Delta\nu$ = 26 Hz, 2H), 3.65 (s, 3H), 3.77 (s, 3H), 3.83 (s, 2H), 4.45 (m, 1H), 6.80−6.92 (m, 5H), 7.00 (s, 1H), 7.10−7.40 (m, 8H), 7.70 (d, J = 9 Hz, 1H), 8.08 (s, 1H). Anal. ($C_{34}H_{41}N_5O_4$) C, H, N.

Step 5d. 1-[*N*-Formyl-*N*-(2-methoxybenzyl)amino]-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)-amino]propane (33). Acetic anhydride (240 mL, 2.5 mmol) and formic acid (98%, 120 μL, 3.1 mmol) were heated at 50 °C for 2 h. This solution was cooled to room temperature, and anhydrous tetrahydrofuran (5 mL) was added. A solution of secondary amine **22** (500 mg, 0.98 mmol) and triethylamine (1.5 mL, 10.7 mmol) in anhydrous tetrahydrofuran (5 mL) was added to the acetic anhydride/formic acid solution. The resulting solution was stirred for 1 h at room temperature. The reaction mixture was diluted with ice/water and extracted with ethyl acetate. The extract was washed with water and brine and dried over sodium sulfate. Liquid chromatography (silica gel, methanol:methylene chloride, 5:95) provided homogeneous product **33** as a white foam: yield 440 mg (84%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.33−2.47 (m, 2H), 2.50−2.65 (m, 2H), 2.87−3.10 (m, 9H), 3.75 (s, 3H), 3.77 (m, 1H), 4.40 (AB q, J = 15 Hz, $\Delta\nu$ = 35 Hz, 2H), 4.65 (m, 1H), 6.75−6.95 (m, 6H), 7.03−7.42 (m, 8H), 7.67 (d, J = 9 Hz, 1H), 8.20 (br s, 1H), 8.33 (s, 1H). Anal. ($C_{32}H_{37}N_5O_3$) C, H, N.

The following compounds were also prepared from **19** using the following procedures:

(*RS*)-*N*-(2-Chlorobenzyl)-2,5-dioxo-3-(indol-3-ylmethyl)-piperazine (8). Triethylamine (2.78 mL, 20 mmol) was added to a slurry of **19** (6.08 g, 20 mmol) in acetone (40 mL) under nitrogen at ice bath temperature, and the reaction was stirred for $^3/_4$ h. Ethyl chloroformate (2.02 mL, 21.2 mmol) was added, and the reaction mixture was stirred for 1 h at ice bath temperature. Ethyl *N*-(2-chlorobenzyl)glycinate (4.83 g, 21.2 mmol) was added, the reaction mixture was stirred at room temperature for 18 h, and the reaction mixture was evaporated. The residue dissolved in ethyl acetate was washed successively with dilute hydrochloric acid, water, dilute sodium hydroxide solution, water, and saturated aqueous sodium chloride. The ethyl acetate extract was dried over sodium sulfate, filtered, and evaporated to yield, after crystallization, an intermediate amide (4.52 g, 9.17 mmol). This amide (4.52 g, 9.17 mmol) was treated with 70% aqueous trifluoroacetic acid (30 mL) containing anisole (3 mL) for 18 h at ambient temperature. The reaction mixture was evaporated, and the residue was dissolved in ether and washed with a sodium hydroxide solution (100 mL, 1 N), water, and saturated aqueous sodium chloride. This ethereal solution was dried over sodium sulfate, filtered, and evaporated to give an oil which could be crystallized slowly from ether containing a small amount of ethyl acetate to give 2.31 g of **8** (31% overall): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.32 (AB q, J = 18 Hz, $\Delta\nu$ = 141 Hz, 2H), 3.38 (d, J = 6 Hz, 2H), 4.39 (m, 1H), 4.53 (AB q, J = 15 Hz, $\Delta\nu$ = 149 Hz, 2H), 6.45 (br s, 1H), 7.02 (m, 1H), 7.03 (s, 1H), 7.12−7.24 (m, 4H), 7.25 (d, J = 6 Hz, 1H), 7.39 (d, J = 6 Hz, 1H), 7.65 (d, J = 9 Hz, 1H), 8.29 (br s, 1H). Anal. ($C_{20}H_{18}N_3O_2Cl$) C, H, N.

(*RS*)-*N*-(2-Chlorobenzyl)-3-(1*H*-indol-3-yl)-2-[*N*-(2-(*N*$_4$-(2-chlorobenzyl)-3,6-dioxo-2-(indol-3-ylmethyl)piperazin-1-yl)acetyl)amino]propanamide (10). Under nitrogen at ice bath temperature, a solution of lithium diisopropylamide (2.86 mmol) was added dropwise to a solution of **8** (0.99 g, 2.7 mmol) and *tert*-butyl bromoacetate (0.40 mL, 2.72 mmol) in tetrahydrofuran (18 mL). The reaction mixture was permitted to warm to room temperature and stirred for 18 h. The reaction mixture was evaporated, and the residue was dissolved in ether and washed succesively with water, dilute aqueous hydrochloric acid, water, dilute aqueous sodium hydroxide solution, water, and a saturated aqueous sodium chloride solution. The resulting ethereal solution was dried over sodium sulfate, filtered, and evaporated to give a *tert*-butyl acetate intermediate as a foam (1.29 g, 2.68 mmol). This ester was treated with 70% aqueous trifluoroacetic acid (5 mL) containing anisole (0.5 mL) for 18 h at room temperature. The reaction mixture was evaporated. Acetonitrile was added and evaporated twice. The residue was partitioned with ether and a dilute aqueous sodium hydroxide solution. The basic aqueous extract was extracted with ether, acidified with 1 N hydrochloric acid, and extracted three times with ethyl acetate. The combined ethyl acetate extract was dried over sodium sulfate, filtered, and evaporated to give an intermediate acetic acid (1.0 g, 2.35 mmol) as a foam. This intermediate (0.40 g, 0.94 mmol) was treated in acetone (11 mL) with triethylamine (0.13 mL, 0.94 mmol) at ice bath temperature under nitrogen with stirring for $^1/_6$ h. Ethyl chloroformate (0.10 mL, 1.03 mmol) was added, and the reaction mixture was stirred at ice bath temperature for $^5/_6$ h. (*RS*)-*N*-(2-Chlorobenzyl)tryptophanamide[35] (0.34 g, 1.03 mmol) was added, and the reaction was stirred at room temperature for 16 h and then heated briefly to reflux and evaporated. The residue was dissolved in ethyl acetate and washed successively with dilute aqueous hydrochloric acid, water, dilute aqueous sodium hydroxide solution, water, and a saturated aqueous sodium chloride solution. This ethyl acetate extract was dried over sodium acetate, filtered, and evaporated to give, after silica gel chromatography, 0.459 g of **10** (57% overall three step yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.62 (AB q, J = 18 Hz, $\Delta\nu$ = 275 Hz, 2H), 3.10−3.66 (m, 3H), 3.94 (AB q, J = 16 Hz, $\Delta\nu$ = 60 Hz, 2H), 4.12 (AB q, J = 15 Hz, $\Delta\nu$ = 336 Hz, 2H), 4.25 (m, 1H), 4.40 (m, 3H), 4.83 (m, 1H), 6.65 (m, 1H), 6.70 (d, J = 8 Hz, 1H), 6.76 (d, J = 10 Hz, 1H), 6.91 (d, J = 3 Hz, 1H), 7.02 (m, 2H), 7.05−7.48 (m, 12H), 7.59 (d, J = 9 Hz, 1H), 7.68 (d, J = 6 Hz, 1H), 7.99 (br s, 1H), 8.03 (br s, 1H). Anal. ($C_{40}H_{36}N_6O_4Cl_2 \cdot 1.4H_2O$) C, H, N.

Biological Methods. Substance P Induced Contraction of Rabbit Vena Cava Tissue. Procedures used were essentially as was reported by Regoli *et al.* with the following modifications.[36] Compounds were evaluated using loops of rabbit vena cava and performing noncumulative dose−response curves with antagonists and substance P. The pA_2 values were estimated on the basis of nonlinear curve fitting.

Trigeminal Electrical Stimulation Induced Neurogenic Dural Extravasation. Male guinea pigs from Charles River Laboratories (225−325 g) were anesthetized with sodium pentobarbital (45 mg/kg). Two pairs of bilateral holes were drilled through the skull, and stainless steel stimulating electrodes, insulated except at the tip, were implanted. The femoral vein was exposed, and a dose of the test compound was injected intravenously (1 mL/kg). Approximately 7 min later, a 50 mg/kg dose of Evans Blue, a fluorescent dye, was also injected intravenously. Exactly 10 min after injection of the test compound, the left trigeminal ganglion was stimulated for 3 min at a current intensity of 1.0 mA (5 Hz, 4 ms duration). Fifteen minutes following the stimulation, the animals were euthanized by exsanguination. The dural membranes were removed from both hemispheres and mounted on microscope slides. A Zeiss fluorescence microscope equipped with a grating monochromator and a spectrophotometer was used to quantify the amount of Evans Blue dye in each sample. An excitation wavelength of approximately 535 nm was utilized, and the emission intensity at 600 nm was determined. The ratio of the amount of extravasation in the dura from the stimulated side compared to the unstimulated (control) side dura was calculated. A dose−response curve was generated and the dose that inhibited the extravasation by 50% (ID_{50}) was approximated. For oral studies, test compounds were administrated by gavage (2 mL/kg) to conscious fasted animals approximately 45 min prior to injection of the anesthetic. To determine the duration of action, the time between oral dosing and injection of anesthetic was lengthened.

Intrathecal NK-1 Agonist Induced Nociceptive Behavior. Conscious male mice from Charles River Laboratories (20−25 g) were coinjected intrathecally (it, into the spinal cord) between the L5/L6 intravertebral space according to previously published procedures with the test compound and Ac-[Sar^9-Met(O$_2$)^{11}]SP 6−11, [Ac-(Sar^9)] (0.5 pmol) in a volume of 5 μL using a 30 gauge needle connected to a 50-μL Hamilton

microsyringe. Animals were lightly restrained to maintain the position of the needle. Puncture of the dura was indicated by the flick of the tail, which was evident throughout the infusion of the agonist. Only animals that showed this response were included in the study. The number of caudally directed scratching and biting events induced by Ac-Sar^9 was scored for 5 min after the injections. A dose−response curve was generated, and the dose that blocked the selective NK-1 agonist induced scratching and biting events by 50% (ID_{50}) was approximated. Alternatively the test compound was administered intraperitoneally 15 min prior to agonist challenge.

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