Highly Potent and Selective Heptapeptide Antagonists of the Neurokinin NK-2 Receptor

Andrew B. McElroy,*,† Stephen P. Clegg, Martyn J. Deal, George B. Ewan, Russell M. Hagan,‡ Simon J. Ireland,‡ Christopher C. Jordan,‡ Barry Porter, Barry C. Ross, Peter Ward, and Andrew R. Whittington

Department of Medicinal Chemistry, Glaxo Group Research Ltd., Greenford Road, Greenford, Middlesex UB6 OHE, England, and Department of Neuropharmacology, Glaxo Group Research Ltd., Park Road, Ware, Hertfordshire SG12 ODP, England.
Received October 22, 1991

Incorporation of D-Pro[9] into substance P related peptides is known to enhance neurokinin NK-2 receptor agonist potency and selectivity with respect to other neurokinin receptors. We now report that replacement of D-Trp[9] by D-Pro[9] in the nonselective neurokinin antagonist [Arg[5],D-Trp[7,9], Nle[11]]-SP(5-11) gave a partial agonist with NK-2 receptor selectivity. Further incorporation of Pro[10] provided the weak but selective NK-2 antagonist Arg-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2 (compound 4; NK-2 pK_B = 5.9; NK-1 pK_B = 4.7; NK-3 pK_B < 4.6). Addition of a suitable lipophilic N-terminal substituent (e.g. Boc, PhCO, cyclohexylcarbonyl) to this compound greatly enhanced NK-2 antagonist activity (compound 10, GR 83074; NK-2 pK_B = 8.2), and combined with further optimization of the N-terminal amino acids, provided the extremely potent and selective NK-2 antagonist PhCO-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2 (compound 34, GR 94800; NK-2 pK_B = 9.6; NK-1 pK_B = 6.4; NK-3 pK_B = 6.0). Compounds of this class produced a potent inhibition of NK-2 agonist-induced bronchoconstriction in the anaesthetized guinea-pig.

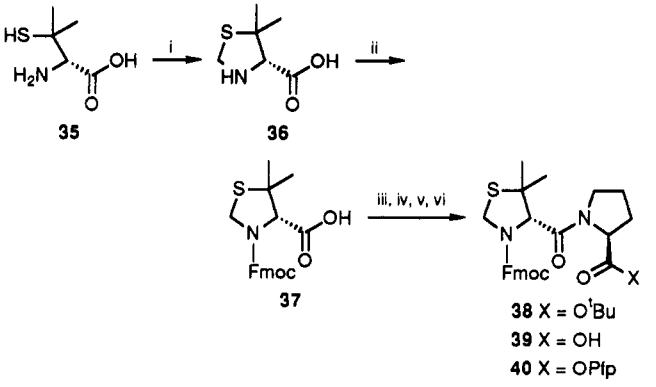
Introduction

The mammalian tachykinins, substance P (SP), neurokinin A (NKA), and neurokinin B (NKB) are the preferred endogenous agonists at three distinct neurokinin (NK) receptors termed NK-1, NK-2, and NK-3, respectively (Figure 1).[1,2] Neurokinins have been implicated in a wide range of physiological processes.[1,3] However, characterization of the receptor subtypes involved has previously been hampered by the limited availability of selective antagonists.[4-8] The earliest reported NK antagonists were D-Trp[7,9]-containing analogues of substance P.[5] Compounds of this class exhibit only moderate selectivity between NK receptor subtypes and are also known to antagonize bombesin, vasopressin, and cholecystokinin.[5,6] Similar incorporation of D-Trp into the NK-2 selective agonist NKA as in [Tyr[5],D-Trp[6,8,9],Arg[10]]-NKA(4-10) provided selective NK-2 antagonists,[7] whereas incorporation of D-Trp into the NK-3 selective agonist NKB as in [D-Pro[2],D-Trp[6,8],Nle[10]]-NKB, afforded antagonists with some degree of NK-3 receptor selectivity.[8] Replacement of Gly[9] by D-Pro in SP analogues is known to enhance NK-2 agonist activity while markedly reducing NK-1 agonist activity.[9] We now report that the inclusion of this D-Pro[9] modification into the poorly selective D-Trp[7,9]-containing antagonist 1, together with lipophilic N-terminal substitution, results in highly selective antagonists with subnanomolar affinity for NK-2 receptors in rat colon muscularis mucosae and guinea-pig trachea.

Chemistry

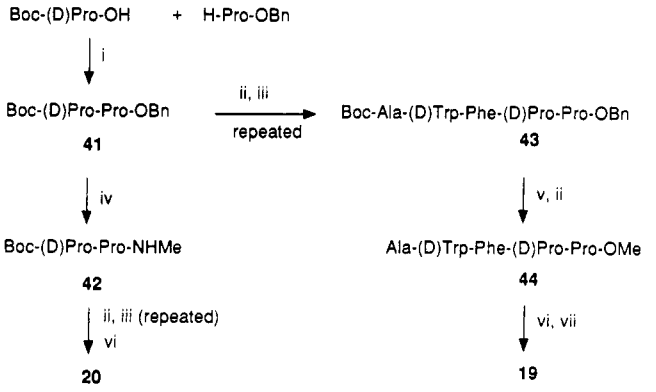
The heptapeptides 2, 4-18, and 21-34 (Tables I and II) were synthesized using the Fmoc-polyamide, automated continuous flow solid-phase methodology.[10] A 4-(hydroxymethyl)benzoyl peptide-resin linker group was employed to enable the formation of C-terminal amides by cleavage with concentrated amine solutions. First residues were attached to this linker by reaction with the appropriate N^{α}-(fluorenylmethoxycarbonyl) (Fmoc)-protected amino acid symmetrical anhydride, catalyzed by 4-(dimethylamino)pyridine. Subsequent Fmoc-protected amino acid residues were coupled as the symmetrical anhydride or as pentafluorophenyl (OPfp) esters, catalyzed in the latter case by 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine or 1-hydroxybenzotriazole. The coupling of Phe to D-Pro was found to be slow in early analogues, and

Scheme I[a]



[a] (i) aqueous CH_2O; (ii) Fmoc-OSu; (iii) Bop-Cl, Et_3N; (iv) Pro-O'Bu; (v) TFA, H_2O (19:1); (vi) PfpOH, DCC.

Scheme II[a]



[a] (i) DCC, HOBT, DMF; (ii) HCl, AcOH; (iii) Boc-Amino acid-OPfp; (iv) CH_3NH_2, EtOH; (v) n-$C_5H_{11}NH_2$, MeOH, 24 h; (vi) Boc-Arg-OH·HCl, DCC, DMF; (vii) n-$C_5H_{11}NH_2$, 48 h.

† Present address, Glaxo Research Institute, 5 Moore Drive, P.O. Box 13398, Research Triangle Park, NC 27709.

‡ Department of Neuropharmacology.

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0022-2623/92/1835-2582$03.00/0

	1	2	3	4	5	6	7	8	9	10	11	
Substance P (SP)	Arg	Pro	Lys	Pro	Gln	Gln	Phe	Phe	Gly	Leu	Met	NH_2
Neurokinin A (NKA)		His	Lys	Thr	Asp	Ser	Phe	Val	Gly	Leu	Met	NH_2
Neurokinin B (NKB)		Asp	Met	His	Asp	Phe	Phe	Val	Gly	Leu	Met	NH_2

Figure 1.

double coupling of Fmoc-Phe-OPfp was employed in later analogues. Coupling of the N-terminal Boc-Arg residue using the standard symmetrical anhydride methodology did not proceed to completion. This problem was overcome by repeated coupling of a large excess of freshly prepared symmetrical anhydride derived from Boc-ArgOH·HCl. In analogues 1–8, the N-terminal Boc group was removed by treatment with 95% aqueous trifluoroacetic acid or with a saturated solution of hydrogen chloride in acetic acid, after cleavage of the Boc-protected peptide from the solid support. Crude peptide products were

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Table I. pK_B Values for Neurokinin Antagonists Related to 1[a]

		5	6	7	8	9	10	11	GPI pK_B (NK-1)	RC pK_B (NK-2)	RPV pK_B (NK-3)
1		Arg	Gln	D-Trp	Phe	D-Trp	Leu	$NleNH_2$	6.4	5.7	NT
2		Arg	Ala	D-Trp	Phe	D-Trp	Leu	$NleNH_2$	6.0	6.2	<4.6
3		Arg	Ala	D-Trp	Phe	D-Pro	Leu	$NleNH_2$	(-)[b]	(-)[c]	NT
4		Arg	Ala	D-Trp	Phe	D-Pro	Pro	$NleNH_2$	4.7	5.9	<4.6
5		Arg	Ala	D-Trp	Phe	D-Pro	Leu	$D\text{-}MetNH_2$	<4.6	5.7	4.9
6		Arg	Ala	Phe	Phe	D-Pro	Pro	$NleNH_2$	<4.6	<4.6	<4.6
7		Arg	Ala	D-Trp	Phe	Gly	Pro	$NleNH_2$	4.8	<4.6	<4.6
8		Arg	Ala	D-Trp	Phe	D-DMTP[d]	Pro	$NleNH_2$	4.7	5.9	NT
9	Boc	Arg	Ala	D-Trp	Phe	D-DMTP[d]	Pro	$NleNH_2$	6.5	7.7	NT
10	Boc	Arg	Ala	D-Trp	Phe	D-Pro	Pro	$NleNH_2$	5.8	8.2	<4.6
11	Boc	Arg	Gln	D-Trp	Phe	D-Trp	Leu	$NleNH_2$	7.1	6.5	NT
12	Boc	Arg	Ala	D-Trp	Phe	D-Trp	Leu	$NleNH_2$	6.9	6.5	NT

[a] pK_B values determined from the antagonism of the contractile response of guinea-pig ileum longitudinal smooth muscle (GPI) to SP methyl ester, rat colon muscularis mucosae (RC) to neurokinin A, and rat portal vein (RPV) to senktide. pK_B (apparent affinity of the antagonist) = log (concentration-ratio - 1) - (molar concentration of antagonist), where the concentration-ratio is the ratio of equiactive molar concentrations of the agonist in the presence and absence of the antagonist. pK_B values for key compounds quoted in the text are mean ± SE of four to eight replicate determinations. [b] Partial agonist E_{max} = 80%. [c] Partial agonist E_{max} = 60%. [d] D-DMTP = (*S*)-5,5-dimethylthiaproline, NT = not tested, Boc = *tert*-butyloxycarbonyl.

purified by reverse-phase HPLC to greater than 95% purity. The peptide sequence and composition was confirmed by amino acid analysis and fast atom bombardment (FAB) mass spectrometry. Further details are given in the Experimental Section (Table III).

A modified procedure was employed to prepare the D-dimethylthiaproline (D-DMPT) containing analogues **8** and **9**, whereby the Fmoc-protected dipeptide-OPfp ester **40** was employed to introduce the D-DMTP-Pro segment in one step during the peptide assembly. This enabled the difficult coupling between the sterically hindered D-DMPT and Pro residues to be carried out under more forcing conditions in solution (Scheme I).

In the attempted solid-phase peptide assembly of peptides **19** and **20**, rapid loss of D-Pro-Pro dipeptide from the solid support, presumably by diketopiperazine formation, occurred after Fmoc-deprotection of the D-Pro residue. These analogues were prepared by an alternative solution-phase strategy (Scheme II) employing Boc-protected amino acids, coupled as pentafluorophenyl esters, or as the symmetrical anhydride for the N-terminal Boc-Arg residue, and deprotected at intermediate stages by treatment with HCl/acetic acid. In the solution-phase synthesis of **19**, diketopiperazine formation was minimized by deprotecting the intermediate Boc-D-Pro-Pro-OBn (**41**) with HCl/acetic acid and only regenerating the free base of the resulting amine at reduced temperature in the subsequent coupling reaction mixture. Attempted displacement of the benzyl ester in **43** with 1-amylamine gave instead the methyl ester **44** after Boc-deprotection. Substitution of the methyl ester with neat 1-amylamine was successful as the final step after coupling of the N-terminal arginine residue to yield **19**. Diketopiperazine formation was avoided in the solution-phase synthesis of **20** by formation of the methylamide **42** from intermediate **41** before Boc-deprotection (Scheme II).

Biological Results and Structure–Activity Discussion

Neurokinin antagonist activities were determined on NK-1, NK-2, and NK-3 receptor-containing tissues in vitro for a series of analogues related to the literature[5] antagonist [Arg^5,D-$Trp^{7,9}$,Nle^{11}]-SP(5–11) (**1**) (Table I). An early analogue, **2**, in which Gln^6 was replaced by Ala^6, showed a slight trend towards selectivity for the NK-2 versus the NK-1 receptor and so this modification was retained in further analogues. It was found that introduction of D-Pro^9, which enhances NK-2 receptor selectivity in agonist sequences,[9] gave a partial agonist (**3**) in both NK-1 and NK-2 in vitro preparations. However, further introduction of Pro^{10} abolished the agonist activity and gave antagonist **4** (pK_B = 5.86 ± 0.07) with over an order of magnitude selectivity for NK-2 versus both the NK-1 and NK-3 receptors. Replacement of Nle^{11} in the partial agonist **3** with D-Met^{11} also abolished agonist activity to give the antagonist **5**, though this compound was slightly less potent than **4** at NK-2 receptors and had slightly lower selectivity with respect to NK-3 receptors.

The importance of the D-Trp^7 and D-Pro^9 residues for imparting NK-2 antagonist potency to **4** was emphasized by the inactivity of analogues **6** and **7** in which these residues were replaced by Phe^7 and Gly^9, respectively. Further modifications of the important D-Pro^9 residue in **4** were investigated and D-dimethylthiaproline was introduced at this position to restrict the conformational freedom around the D-Pro-Pro amide bond. This yielded analogue **8** with similar activity to the parent compound. However, the Boc-protected intermediate **9** prepared during the synthesis of **8**, exhibited markedly increased NK-2 antagonist potency (pK_B = 7.68 ± 0.11). The N-terminal Boc group clearly has a substantial beneficial effect on the NK-2 antagonist activity of this compound, though NK-1 antagonist activity is also enhanced such that NK-2/NK-1 selectivity is not significantly improved. Reverting from D-$DMTP^9$ to D-Pro^9 in this N-terminally Boc-substituted (*N*-Boc) series gave the more potent NK-2 antagonist **10** (GR 83074; pK_B = 8.23 ± 0.08) which was inactive as an NK-3 antagonist and exhibited a 340-fold NK-2/NK-1 selectivity.

The effect of introducing an *N*-Boc substituent into the D-$Trp^{7,9}$ compounds **1** and **2** was investigated by preparing the *N*-Boc analogues **11** and **12**. The *N*-Boc substitution enhances NK-1 antagonist activity in these compounds by 0.5–1.0 log units but interestingly, NK-2 antagonist activity is increased to a lesser extent such that the resulting antagonists **11** and **12** have some selectivity for the NK-1 versus the NK-2 receptor (Table I). Hence it appears that *N*-Boc substitution can enhance either NK-1 or NK-2 antagonist activity depending on the substitution elsewhere in the molecule, whereas the replacement of D-Trp^9 by D-Pro^9 is a key modification for introducing potent and selective NK-2 antagonist potency. It is of interest in this regard that the D-Pro^9 substitution enhances NK-2 antagonist potency while markedly reducing NK-1 antagonist activity. This closely resembles the effect of D-Pro^9 substitution on the NK-2 agonist activity and selectivity in SP(6–11) hexapeptide analogues.[9]

The structure–activity requirements of the lead NK-2 antagonist **10** were further investigated by modifying in-

Table II. Neurokinin Antagonist pK_B Values for Close Analogues of **10**

	5	6	7	8	9	10	11	GPI pK_B (NK-1)	RC pK_B (NK-2)	RPV pK_B (NK-3)
10 Boc	Arg	Ala	D-Trp	Phe	D-Pro	Pro	$NleNH_2$	5.8	8.2	<4.6
13 –	–	–	D-Ala	–	–	–	–	4.8	5.6	NT
14 –	–	–	D-Pro	–	–	–	–	4.5	5.2	NT
15 –	–	–	D-Phe	–	–	–	–	NT[c]	6.4	NT
16			L-Trp					<5.0	6.4	NT
17 –	–	–	D-Trp	–	–	–	D-$MetNH_2$	<4.5	6.2	NT
18 –	–	–	–	–	–	–	$GlyNH_2$	5.3	7.0	NT
19 –	–	–	–	–	–	–	$NH(CH_2)_4CH_3$	5.8	7.6	NT
20 –	–	–	–	–	–	–	$NHCH_3$	5.1	7.5	NT
21 –	D-Arg	–	–	–	–	–	$NleNH_2$	5.7	5.2	NT
22 –	D-Glu(OBn)	–	–	–	–	–	–	6.0	7.1	NT
23 H	D-Glu(OBn)	–	–	–	–	–	–	<5.5	7.2	NT
24 Boc	$Arg(NO_2)$	–	–	–	–	–	–	5.5	8.2	5.2
25 –	Gln	–	–	–	–	–	–	5.6	7.9	5.1
26 –	Lys	–	–	–	–	–	–	4.8	7.1	NT
27 –	Glu	–	–	–	–	–	–	<4.5	6.5	NT
28 –	Glu(OBn)	–	–	–	–	–	–	6.6	7.4	NT
29 –	Ala	–	–	–	–	–	–	5.5	9.3	4.7
30 –	$-NH(CH_2)_4CO-$		–	–	–	–	–	NT	7.6	NT
31 Ac[a]	Arg	Ala	–	–	–	–	–	4.6	5.6	NT
32 Cbz[b]	–	–	–	–	–	–	–	6.7	7.5	NT
33 PhCO	Gln	–	–	–	–	–	–	6.6	8.8	5.6
34 PhCO	Ala	Ala	D-Trp	Phe	D-Pro	Pro	$NleNH_2$	6.4	9.6	6.0

[a] Ac = acetyl. [b] Cbz = benzyloxycarbonyl. [c] NT = not tested.

dividual amino acid residues (Table II). A requirement for specific interaction of the D-Trp^7 side chain with the NK-2 receptor was suggested by the low activity of the corresponding D-Phe^7 and L-Trp^7 analogues **15** and **16**. Removal of the indole moiety of D-Trp^7 as in the D-Ala^7 analogue **13** gave a massive fall in potency. Conformational constraint at this position by D-Pro^7 as in **14** was also incompatible with NK-2 antagonist activity.

Modification of the C-terminal residue of **10** also reduced NK-2 antagonist activity. Inversion at this position, as in the D-Met^{11} analogue **17** was more detrimental than removing the side chain to give the Gly^{11} analogue **18**. Removal of the C-terminal amide group from this Gly^{11} analogue, as in the methylamide **20**, increased potency. However, a similar amide deletion from the parent Nle^{11} analogue **10**, as in analogue **19**, reduced activity.

Inversion of Arg^5, as in the D-Arg^5 analogue **21**, abolished NK-2 antagonist activity but interestingly, some activity could be restored by introducing an inverted residue [D-Glu(OBn), analogue **22**] containing a pendant lipophilic group. It is possible that the benzyl group in **22** accesses the same lipophilic binding site occupied by the Boc group in analogues not inverted at residue-5. Indeed, analogue **23** in which the Boc group has been removed, maintains the same activity as the parent D-$Glu(OBn)^5$ analogue **22**. Compounds **25–28**, in which the functionality in the Arg^5 side chain was modified, had similar or reduced activity relative to **10**. However, reducing the size of this side chain, as in the Ala^5 analogue **29**, markedly enhanced NK-2 antagonist potency (pK_B = 9.28 ± 0.06) and imparted excellent selectivity of ca. 4 orders of magnitude versus NK-1 and NK-3 receptors.

Analogues **31–34** in which the N-terminal Boc group was modified, suggest that there is a specific requirement for the bulky lipophilic group at this position. Thus, replacement of Boc by the much smaller acetyl group markedly reduces NK-2 antagonist activity and the corresponding benzyloxycarbonyl (Cbz) analogue is also significantly less potent than **10**, indicating some specificity for the lipophilic interaction. Replacement of Boc by an N-terminal benzoyl group was found to enhance activity slightly and yielded the most potent NK-2 antagonist in this series (**34**) with a pK_B of 9.56 ± 0.08. This compound (GR 94800) is also the most potent peptide NK-2 antagonist yet reported.

The biological activity profile of the prototype potent NK-2 antagonist **10** (GR83074) was investigated further. In the isolated rat colon and guinea-pig trachea, GR 83074 was a potent competitive antagonist of the contractions induced by the selective NK-2 agonist GR64349[9d] (pK_B values 8.75 ± 0.1 and 8.5 ± 0.1, respectively). These values are in good agreement with the pK_B value in the rat colon using NKA as agonist and indicate that the antagonist has similar affinities for the NK-2 receptors in these two preparations.

Compound **10** was also tested for its ability to block NK-2 receptors in vivo. Intravenous administration of **10** to anaesthetized, artificially-respired guinea-pigs (0.3 μmol/kg) caused a marked antagonism of the increase in tracheal insufflation pressure induced by the NK-2 agonist, GR64349. Thus, the antagonist caused a maximum 91-fold shift in the dose–response curve to GR64349 after 11 min, and a dose-ratio shift of greater than 5-fold was maintained for approximately 90 min (Figure 2). In contrast, compound **10** was much less effective at blocking responses to NKA or the selective NK-1 agonist, SP methyl ester, in this model; at 0.3 μmol/kg, **10** produced dose-ratio shifts of less than 5 against NKA, and even at a considerably higher dose (5 μmol/kg), **10** did not antagonize SPOMe. Both NK-1 and NK-2 receptors are thought to mediate tachykinin-induced bronchoconstriction in the guinea-pig (although apparently not in man).[17] Thus it is possible that the relative lack of effect of **10** against NKA resulted from the activation of both receptor types by NKA. Indeed when the NK-1 antagonist GR82334[18] was coadministered with **10**, there was a much greater antagonism of NKA-induced bronchoconstriction than was observed with either compound alone (Figure 2). These results

(17) Maggi, C. A. Tachykinin Receptors in the Airways and Lung: What Should We Block? *Pharmacol. Res.* **1990**, *22*, 527–540.
(18) Hagan, R. M.; Ireland, S. J.; Bailey, F.; McBride, C.; Jordan, C. C.; Ward, P. A Spirolactam Conformationally-Constrained Analogue of Physalaemin Which is a Peptidase-Resistant, Selective Neurokinin NK-1 Receptor Antagonist. *Br. J. Pharmacol.* **1990**, *102*, 168P.

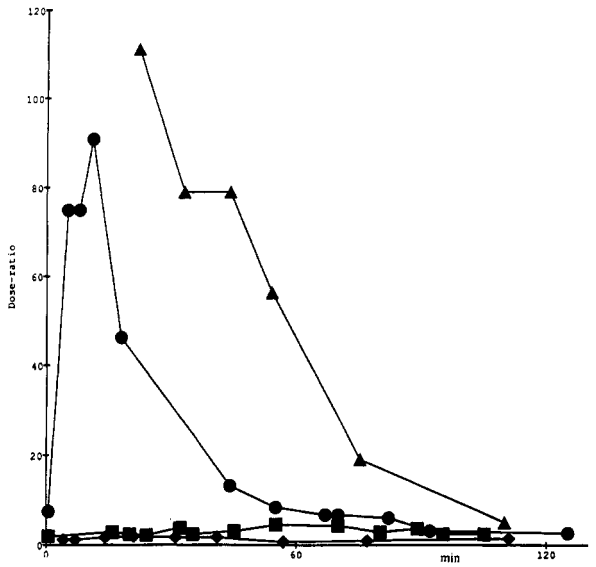

Figure 2. Antagonism of neurokinin agonist-induced bronchoconstriction by the NK-2 receptor antagonist GR83074 (compound **10**). GR 83074 was given as an intravenous bolus prior to bronchoconstrictor repeated challenges with intravenously administered neurokinin agonists. The antagonism of agonist-induced bronchoconstriction was estimated as an apparent dose-ratio (see Experimental Section) and was followed to recovery. Where the NK-1 receptor antagonist, GR82334, was used, this was given at a dose of 0.5 μmol/kg at 20-min intervals throughout the experiment in order to maintain maximal blockade of NK-1 receptors. Using this dose regime, GR82334 had no effect (dose-ratio less than 5) on NKA-induced bronochoconstriction (data omitted for clarity). Key: (●) GR83074 (0.3 μmol/kg) versus the selective NK-2 agonist GR64349, (■) GR83074 (0.3 μmol/kg) versus NKA, (♦) GR83074 (5.0 μmol/kg) versus the NK-1 selective agonist SPOMe, (▲) GR83074 (0.3 μmol/kg) versus NKA, in the presence of the NK-1 antagonist GR82334.

demonstrate that **10** is a highly potent and selective NK-2 antagonist in vivo.

Conclusions

Neurokinin NK-2 antagonists **10** (GR 83074), **29**, and **34** (GR 94800) of unparalleled potency and selectivity have been developed from weakly active and nonselective D-Trp^{7,9}-containing antagonists (e.g. **1**) through the application of known agonist structure–activity relationships and empirical modifications. The key discoveries leading to these compounds were (i) the D-Pro^9-Pro^{10} replacement which imparts NK-2 receptor selectivity, and (ii) the substantial potency increase which results from lipophilic N-terminal substitution (Boc-, PhCO-) in this series (but not in D-Trp^{7,9} antagonists, e.g. **11** and **12**), presumably via the location of an auxiliary binding site in the NK-2 receptor. These structural features together with D-Trp^7 are essential for high potency as demonstrated by further replacement studies.

A conformational analysis of the dipeptide Ac-D-Pro-Pro-NHMe (data not shown) indicates a high propensity for a Type II′ β-turn conformation. In a previous publication,[9c] we described a potent and selective neurokinin NK-1 antagonist, GR 71251, derived by incorporation of a spiro-bicyclic Type II′ β-turn constraint at positions 9,10 in a SP-related sequence. The present study therefore further illustrates the utility of conformational constraint in the design of peptide antagonists. However, despite an apparent conformational homology in the C-terminal region, the distinction between the two series is clearly highlighted by the specific requirement for a D-residue at position 7 (D-Trp^7) in the NK-2 antagonists, and an L-residue (Phe^7) at the corresponding site in the NK-1 antagonist.

Interestingly, the D-Trp^7 requirement also distinguishes the NK-2 antagonists described herein from the previously reported selective NK-2 antagonists L-659,877 [cyclo(Gln-**Trp**-Phe-Gly-Leu-Met)] and L-659,874 (Ac-Leu-Met-Gln-**Trp**-Phe-Gly-NH_2)[19] which contain L-Trp at the apparently corresponding site. In view of the conformational restraint inherent in the cyclic structure of L-659,877 and in the D-Pro-Pro moiety of the new antagonists, it is tempting to apply molecular modeling techniques in the construction of a receptor binding model accommodating both ligand structural types. However, a more definitive understanding of receptor–ligand interactions is likely to result from photoaffinity labeling and other structural studies now facilitated by the cloning and recombinant expression of the NK-2 receptor.

These new potent and selective antagonists are valuable tools for further defining the pharmacology of NK-2 receptors in vitro and in vivo. Although GR 83074 (**10**) is extensively peptidic in character, it is potent and reasonably long-acting in vivo, presumably reflecting metabolic stability afforded by N-terminal substitution (Boc-) and the presence of D-residues (D-Trp^7, D-Pro^9) at potentially endopeptidase-labile sites. GR 83074 will assist investigation of the pathophysiological roles of the tachykinins, and hence the prediction of therapeutic potential for neurokinin NK-2 antagonists.

Experimental Section

Abbreviations. Abbreviations follow the recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature for amino acids and peptides: *Eur. J. Biochem.* **1984**, *158*, 9–31. Additional abbreviations are defined in the text, or as follows: AcOH, acetic acid; Bn, benzyl; Boc, *tert*-butyloxycarbonyl; Bop-Cl, bis(2-oxo-3-oxazolidinyl)phosphinic chloride; ^{t}Bu, *tert*-butyl; CRB, Cambridge Research Biochemicals; DCC, dicyclohexylcarbodiimide; DCU, dicyclohexylurea; DHBT, 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine; DIPC, diisopropylcarbodiimide; DIPEA, N,N-diisopropylethylamine; DMAP, 4-(dimethylamino)pyridine; DMF, dimethylformamide; Et_3N, triethylamine; FAB, fast atom bombardment; HOBT, 1-hydroxybenzotriazole; HPLC, high pressure liquid chromatography; IMS, industrial methylated spirit (denatured alcohol); IR, infra red; Mtr, 4-methoxy-2,3,6-trimethylbenzenesulphonyl; Nle, nor-leucine; HOSu, N-hydroxysuccinimide; TFA, trifluoroacetic acid; TNBSA, 2,4,6-trinitrobenzenesulphonic acid.

General Methods. All compounds for biological evaluation were characterized by mass spectroscopy using fast atom bombardment ionization on a Finnigan 8400, or a VG ZAB-2SE, double focussing mass spectrometer operated at 1000 resolution. Fragment ions were used to confirm the peptide sequence. Peptide homogeneity was determined by analytical reverse-phase HPLC using a Brownlee wide pore RP-300(octyl) aquapore column using eluants A: H_2O, 0.1% TFA and B: acetonitrile, 0.05% TFA, with gradient elution from 85% A:15% B to 10% A:90% B over 25 min at a flow rate of 1.5 mL per min. Peptide purification was effected by preparative reverse-phase HPLC using a 2-in. diameter Dynamax ODS-2 column using the same eluants described for analytical HPLC with an appropriately varied gradient at a flow rate of 45 mL per min. Amino acid analyses were carried out on an LKB 4400 or a Biotronik LC5001 sequencer.

Nonpeptide intermediates were characterized by NMR using a Varian XL200, Varian VXR400, or a Bruker AM250 spec-

(19) (a) McKnight, A. T.; Maguire, J. J.; Williams, B. J.; Foster, A. C.; Tridgett, R.; Iversen, L. L. Pharmacological Specificity of Synthetic Peptides as Antagonists at Tachykinin Receptors. *Regul. Pept.* **1988**, *22*, 127. (b) Williams, B. J.; Curtis, N. R.; McKnight, A. T.; Maguire, J.; Foster, A.; Tridgett, R. Development of NK-2 Selective Antagonists. *Regul. Pept.* **1988**, *22*, 189.

trometer. IR spectra were recorded on Nicolet 5SXC, Nicolet 20SXB interferometers or Perkin Elmer 580B and 177 dispersive spectrometers. Melting points were recorded on a Reichert heated-block apparatus and are uncorrected.

General Methods for Peptide Synthesis. With the exception of **19** and **20**, peptides were prepared by the Fmoc-polyamide continuous flow method using a CRB Pepsynthesizer II semiautomatic synthesizer (peptides **2**, **4–9**, **30** and **34**), LKB Biolynx 4170 fully automated peptide synthesizer (peptides **11–18** and **33**) or batchwise using a Biosearch 9500 automated peptide synthesizer (peptides **10**, **21–39**, **31**, and **32**) following standard protocols supplied by the manufacturers. Peptides **1** and **3** were supplied by Ferring Pharmaceuticals and Peninsula Laboratories, respectively.

Preparation of 4-(Hydroxymethyl)benzoyl-Substituted Resin. Kieselguhr-supported dimethylacrylamide-ethylenebis-(acrylamide)-acryloylsarcosine methyl ester copolymer (Pepsyn K, CRB, sarcosine content 0.25 mmol/g, 16.5 g) was loaded into a 25 cm × 25 mm column, treated by recirculation on the CRB Pepsynthesizer with ethylenediamine for 20 h, and then washed with DMF (1.5 h), 10% DIPEA in DMF (0.75 h), and DMF (1.5 h). A solution of DHBT (2.22 g) in DMF (7.5 mL) was applied to the column, followed by a solution of Fmoc-Nle-OPfp (6.43 g, 12.3 mmol, 3 equiv) in DMF (20 mL). After recirculation for 1 h, the reagents were washed out (DMF, 1 h), and the Fmoc was deprotected by recirculation of piperidine (20% in DMF, 0.25 h) followed by washing with DMF (0.25 h). A solution of 4-(hydroxymethyl)benzoic acid pentafluorophenyl ester (3.94 g, 12.3 mmol) in DMF (20 mL) was added to the reaction column and recirculated for 18 h. The functionalized resin was then washed with DMF and diethyl ether and dried in vacuo (17.3 g, 0.23 mmol/g by amino acid analysis). This material was used for the synthesis of peptides **2** and **4–9**. Later peptides were prepared using an equivalent commercially available functionalized kieselguhr resin (Pepsin KB, 0.08 mmol/g). Peptides **10**, **21–29**, **31**, and **32** were assembled using the batch coupling methodology on a 4-(hydroxymethyl)benzoyl-substituted polystyrene resin.

General Procedure for Coupling of First Amino Acid Residues to the Resin. In all preparations the first residue was coupled using the preformed symmetrical anhydride, generated by the addition of DIPC to 2 mol equiv of the Fmoc-protected amino acid in DMF (6-fold excess of anhydride relative to the functionalized resin) and recirculated in the presence of a catalytic amount of DMAP (0.1–0.2 mmol) for 1–2 h.

General Procedure for Coupling of Subsequent Residues. Unless otherwise stated, protected amino acids were coupled using a 3-fold excess of the corresponding OPfp ester, premixed in DMF with an equivalent amount of HOBT or DHBT (peptides **2**, **4–9**, **34**) as catalyst. Protected amino acids were also coupled where indicated using a ca. 3-fold excess of the preformed symmetrical anhydride. In this method, DIPC (0.5 mol equiv) was added to a solution of the protected amino acid in DMF immediately prior to addition of the mixture to the functionalized resin; the batch synthesizer employed this methodology exclusively. Alternatively, where indicated, DCC was used in place of DIPC to generate the symmetrical anhydride, and after mixing for 10 min the precipitated DCU was removed by filtration prior to addition of the mixture to the resin. Unless otherwise stated, Boc-Arg-OH·HCl was double-coupled with 6 equiv of freshly prepared symmetrical anhydride using the CRB pepsynthesizer. Completion of the coupling reactions was confirmed by TNBSA and Kaiser color tests.[13]

General Procedure for Deprotection during Peptide Assembly. The continuous flow methodology employed a 15-min wash with 20% piperidine in DMF followed by washing of the resin with DMF to remove all traces of piperidine. The batch methodology employed two washes of 3 min and 7 min with 20% piperidine in DMF followed by DMF washing. In several peptides, the N-terminal residue was Boc-protected. Where indicated, this group was removed after cleavage of the peptide from the resin by stirring with either TFA/H_2O (95:5) or a saturated solution of HCl in AcOH (HCl/AcOH) for 1 h, followed by concentration of the reaction mixture in vacuo.

General Procedure for Cleavage of the Assembled Peptide from the Resin. The preferred method for cleaving the peptide from the solid support (NH_3/cat. AcOH) was by treatment with AcOH (120 μL) followed by liquid ammonia (ca. 6 mL/g solid) in a steel pressure vessel at 20 °C for 18 h. The ammonia was allowed to evaporate, and the residual solid was extracted with methanol (3 × 80 mL/g) (in earlier peptide cleavages, where indicated (NH_3), the AcOH catalyst was omitted or alternatively the cleavage was achieved by agitating the resin in a saturated solution of ammonia in methanol (NH_3/MeOH) at 20 °C for 24–48 h). The methanol extract was evaporated and the residue purified to >95% homogeneity by reverse-phase HPLC to afford the peptide amide which was characterized by amino acid analysis and FAB mass spectrometry (Table III).

Arg-Ala-D-Trp-Phe-D-Trp-Leu-Nle-NH_2 (2). All the protected amino acid residues were coupled using the DCC-generated symmetrical anhydride methodology. The peptide was cleaved with NH_3/cat. AcOH and the Boc protecting group removed with TFA/H_2O. Yield 55%.

Arg-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2 (4). Fmoc-D-Trp-OH was coupled via the DIPC-generated symmetrical anhydride and Boc-Arg-OH·HCl was double-coupled via the DIPC-generated symmetrical anhydride in the presence of an equivalent amount of HOBT, at which stage negative TNBSA and Kaiser color tests were obtained. The Boc-protected peptide was cleaved from the resin using NH_3/cat. AcOH and then deprotected with TFA/H_2O. Yield 26%.

Arg-Ala-D-Trp-Phe-D-Pro-Leu-D-Met-NH_2 (5). Fmoc-D-Pro-OH, Fmoc-Phe-OH, and Fmoc-D-Trp-OH were coupled via the corresponding DIPC-generated symmetrical anhydrides. Boc-Arg-OH·HCl was coupled via the DCC-generated symmetrical anhydride in the presence of an equivalent amount of DHBT as catalyst. The Boc-protected peptide was cleaved from the resin with NH_3/cat. AcOH and then deprotected using HCl/AcOH. Yield 64%.

Arg-Ala-Phe-Phe-D-Pro-Pro-Nle-NH_2 (6). Fmoc-D-Pro-OH and the first Fmoc-Phe-OH coupling employed the DIPC-generated symmetrical anhydride methodology. Boc-Arg-OH·HCl was coupled via the DCC-generated symmetrical anhydride in the presence of an equivalent amount of DHBT. The Boc-protected peptide was cleaved from the resin using NH_3/cat. AcOH and then deprotected with HCl/AcOH. Yield 75%.

Arg-Ala-D-Trp-Phe-Gly-Pro-Nle-NH_2 (7). Fmoc-D-Trp-OH was coupled via the DIPC-generated symmetrical anhydride and Boc-Arg-OH·HCl was double coupled via the DIPC-generated symmetrical anhydride in the presence of an equivalent amount of HOBT, at which stage negative TNBSA and Kaiser color tests were obtained. The Boc-protected peptide was cleaved from the resin using NH_3/cat. AcOH and then deprotected with HCl/AcOH. Yield 31%.

Arg-Ala-D-Trp-Phe-D-DMTP-Pro-Nle-NH_2 (8). The Boc-protected peptide (**9**) was deprotected with TFA/H_2O. Yield 67%.

Boc-Arg-Ala-D-Trp-Phe-D-DMTP-Pro-Nle-NH_2 (9). Fmoc-D-DMTP-Pro-OPfp (**40**) was coupled to the Nle-substituted resin with DHBT catalysis following the general methodology for amino acid OPfp esters. Fmoc-Phe-OH was coupled via the DCC-generated symmetrical anhydride and Fmoc-D-Trp-OH via the DIPC-generated symmetrical anhydride. Boc-Arg-OH·HCl was double coupled using the DIPC-generated symmetrical anhydride methodology. The peptide was cleaved from the resin using NH_3/cat. AcOH. Yield 26%.

Boc-Arg-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2 (10). For the preparation of peptides **10**, **21–29**, **30**, and **31** a large batch (5.0 g, 0.29 mmol/g) of an intermediate resin-supported peptide, Fmoc-Ala-D-Trp-Phe-D-Pro-Pro-Nle-O-Resin was prepared using the batch-coupling methodology on a 4-(hydroxymethyl)-benzoyl-substituted polystyrene resin. All couplings in this assembly employed the DIPC-generated symmetrical anhydride methodology. The integrity of this intermediate resin was confirmed by amino acid analysis (Ala, 0.29; Phe, 0.34; Pro, 0.68; Nle, 0.36 mmol/g). This resin was stored at −20 °C in the Fmoc-protected form and portions were deprotected immediately prior to further coupling reactions. The above resin (0.25 g) was washed with 20% piperidine in DMF (3 min, 7 min) and DMF (10 × 3 mL) and was then agitated for 45 min with a mixture of Boc-Arg-OH·HCl (0.186 g, 0.6 mmol) and DIPC (45 μL, 0.3 mmol) in DMF (2 mL). The resin was then washed with DMF (3 mL) and the coupling repeated. A negative TNBSA test was obtained after 45 min. The resin was washed with DMF (5 × 3 mL), *tert*-amyl

Table III. Analytical Data

	amino acid analysis											HPLC	MS
	Arg	Glu	Phe	Leu	Nle	Ala	Pro	Met	Lys	Gly	Ava	RT(min)	[MH] m/z
2	0.93		1.00	1.00	0.95	0.88							990
4	1.07		1.00		1.03	0.97	2.01						885
5	1.08		1.00	1.11		1.03	0.96	1.01				11.56	919
6	1.08		2.00		1.05	1.04	2.05					11.19	846
7	1.09		1.00		1.05	1.00	1.02			0.93			845
8	1.19		1.00		1.11	1.07	1.05						931
9	1.09		1.00		1.04	1.01	1.00						1031
10	1.06		1.00		1.04	0.74	1.94					14.55	985
11	1.09	0.96	1.00	1.00	1.06							15.62	1147
12	0.92		1.00	0.91	0.92	0.86						16.26	1090
13	1.08		1.00		1.09	2.00	2.00					12.51	870
14	1.08		1.00		1.10	1.00	2.84					12.95	896
15	1.09		2.00		1.02	1.00	1.91					14.92	946
16	1.09		1.00	1.08	1.10	2.10						14.41	985
17	1.11		1.00	1.02		1.02	1.00	1.05				14.11	1019
18	1.12		1.00			1.05	1.98			1.00		12.47	929
19	1.11		1.00			1.06	2.06						942
20	1.09		1.00			1.01	2.01						886
21	1.09		1.00		1.03	0.99	2.00					14.68	985
22		1.18	1.00		1.06	0.96	2.01					19.29	1048
23		1.05	1.00		1.06	0.98	2.02					15.58	948
24	0.77		1.00		1.06	1.02	2.05					15.73	1030
25		1.01	1.00		1.06	1.00	2.07					15.24	957
26			1.00		1.08	0.99	1.91		1.00			14.60	957
27		1.03	1.00		1.04	1.03	2.00					15.93	958
28		1.06	1.00		1.07	1.03	2.05					19.38	1048
29			1.00		1.07	2.04	2.00					16.30	900
30			1.00		1.09		1.94				1.05	17.01	857
31	1.11		1.00		1.11	1.05	2.03					13.37	927
32	1.09		1.00		1.09	1.00	1.98					15.40	1019
33		1.05	1.00		1.10	1.04	2.09					14.88	961
34			1.00		1.03	1.97	1.97					15.97	904

alcohol (2 × 3 mL), AcOH (3 × 3 mL), *tert*-amyl alcohol (2 × 3 mL), DMF (2 × 3 mL), and diethyl ether (3 × 3 mL) and then dried in vacuo. The peptide was cleaved from the resin using NH_3/cat. AcOH. Yield 69%.

Boc-Arg-Gln-D-Trp-Phe-D-Trp-Leu-Nle-NH_2 (11). Fmoc-D-Trp-OH was coupled via the DIPC-generated symmetrical anhydride. Boc-Arg-OH·HCl was coupled using the CRB pepsynthesizer with freshly prepared DIPC-generated symmetrical anhydride (5 equiv). The peptide was cleaved from the resin with NH_3. Yield 27%.

Boc-Arg-Ala-D-Trp-Phe-D-Trp-Leu-Nle-NH_2 (12). Fmoc-D-Trp-OH were coupled via the DIPC-generated symmetrical anhydride. Boc-Arg-OH·HCl was coupled using the CRB pepsynthesizer with freshly prepared DIPC-generated symmetrical anhydride (5 equiv). The peptide was cleaved from the resin with NH_3. Yield 34%.

Boc-Arg-Ala-D-Ala-Phe-D-Pro-Pro-Nle-NH_2 (13). Fmoc-D-Ala-OH was coupled via the DIPC-generated symmetrical anhydride. Boc-Arg-OH was double coupled on the CRB pepsynthesizer using freshly prepared DCC-generated symmetrical anhydride (2 × 9 equiv). The peptide was cleaved from the resin with NH_3/MeOH. Yield 62%.

Boc-Arg-Ala-D-Pro-Phe-D-Pro-Pro-Nle-NH_2 (14). Synthesis similar to that described for **13** with Fmoc-D-Pro-OH in place of Fmoc-D-Ala-OH. Yield 71%.

Boc-Arg-Ala-D-Phe-Phe-D-Pro-Pro-Nle-NH_2 (15). Synthesis similar to that described for **13** with Fmoc-D-Phe-OH in place of Fmoc-D-Ala-OH. Yield 55%.

Boc-Arg-Ala-Trp-Phe-D-Pro-Pro-Nle-NH_2 (16). Fmoc-Phe-OPfp was double coupled with HOBT catalysis. Boc-Arg-OH·HCl was treble coupled on the CRB pepsynthesizer using freshly prepared DCC-generated symmetrical anhydride (3 × 5 equiv). The peptide was cleaved from the resin with NH_3. Yield 47%.

Boc-Arg-Ala-D-Trp-Phe-D-Pro-Pro-D-Met-NH_2 (17). Fmoc-Phe-OPfp was double coupled with HOBT catalysis. Fmoc-D-Pro-OH and Fmoc-D-Trp-OH were coupled via the corresponding DIPC-generated symmetrical anhydrides. Boc-Arg-OH·HCl was double coupled on the CRB pepsynthesizer using freshly prepared DIPC-generated symmetrical anhydride (2 × 6 equiv). The peptide was cleaved from the resin with NH_3/MeOH. Yield 58%.

Boc-Arg-Ala-D-Trp-Phe-D-Pro-Pro-Gly-NH_2 (18). Synthesis similar to that described for **17** with Fmoc-Gly-OH replacing Fmoc-D-Met-OH on the first coupling onto the resin. Yield 66%.

General Methods for the Solution Phase Synthesis of Peptides 19 and 20. Intermediate Boc-protected peptides were deprotected by treatment with a saturated solution of HCl in AcOH (10 mL/mmol) at room temperature under nitrogen for 1 h. The reaction mixtures were concentrated in vacuo, triturated with diethyl ether, and thoroughly dried under high vacuum to remove all traces of acetic acid. Unless otherwise stated, the resulting amine salts were coupled without purification by treating with a solution of the Boc-protected amino acid OPfp ester (2 mmol/mmol of amine) and triethylamine (140 μL, 1 mmol/mmol) in DMF (2 mL/mmol). *N*-(2-aminoethyl)morpholine (300 μL/mmol) was added after stirring for a further 20 min, the reaction mixture was diluted with ethyl acetate (50 mL/mmol), and the resulting solution washed with 10% aqueous citric acid (3 × 20 mL/mmol), 10% aqueous Na_2CO_3 (3 × 20 mL/mmol), and water (20 mL/mmol) and then dried (MgSO4) and concentrated in vacuo.

Boc-Arg-Ala-D-Trp-Phe-D-Pro-Pro-NH$(CH_2)_4CH_3$ (19). Boc-D-Pro-Pro-OBn (**41**, 340 mg, 0.85 mmol) was deprotected with HCl/AcOH and then sequentialy coupled with Boc-Phe-OPfp, Boc-D-Trp-OPfp, and Boc-Ala-OPfp with intervening HCl/AcOH Boc deprotections. The resulting peptide, Boc-Ala-D-Trp-Phe-D-Pro-Pro-OBn (120 mg), was dissolved in a mixture of amylamine (0.5 mL) and methanol (2 mL) and allowed to stand for 24 h, before concentrating in vacuo. The residue was purified by reverse-phase HPLC, and the major isolated product was the methyl ester, Boc-Ala-D-Trp-Phe-D-Pro-Pro-OMe (60 mg). This compound was Boc-deprotected using HCl/AcOH. The resulting amine salt was treated with a mixture of Boc-Arg-OH·HCl (250 mg, 0.8 mmol), DCC (82.5 mg, 0.4 mmol), and DMF (1 mL) (premixed for 10 min). Triethylamine (11 μL, 0.8 mmol) was added and the mixture stirred for 2 h at 20 °C. The solvent was removed in vacuo, and the residual oil was submitted to the HCl/AcOH Boc-deprotection conditions. The resultant solid was partially dissolved in water (5 mL) and filtered. The filtrate was

purified by reverse-phase HPLC to give the intermediate peptide Arg-Ala-D-Trp-Phe-D-Pro-Pro-OMe (61 mg); FAB mass spectrum, $MH^+ = 787$, fragment ions confirm sequence. This peptide was dissolved in amylamine (1 mL) for 48 h at 20 °C and then concentrated in vacuo. The residual oil was treated with a solution of Boc_2O (200 mg) and triethylamine (50 μL) in DMF (2 mL) for 2 h. The reaction mixture was concentrated in vacuo and purified by reverse-phase HPLC to give **19** (31 mg, 4% overall yield).

Boc-Arg-Ala-D-Trp-Phe-D-Pro-Pro-NHCH$_3$ (20). A solution of Boc-D-Pro-Pro-OBn (260 mg, 0.65 mmol) in methylamine/IMS was allowed to stand under nitrogen at 20 °C for 18 h before concentrating in vacuo. The residual oil was Boc-deprotected with HCl/AcOH and then sequentially coupled with Boc-Phe-OPfp, Boc-D-Trp-OPfp and Boc-Ala-OPfp with Boc-deprotection using HCl/AcOH after each coupling. The resultant peptide, Ala-D-Trp-Phe-D-Pro-Pro-$NHCH_3$·HCl was coupled with Boc-Arg-OH·HCl via the DCC-generated symmetrical anhydride using a similar method to that described in the synthesis of **19**. Purification of the crude product by reverse-phase HPLC gave **20** (23 mg, 4% overall yield) as a white amorphous freeze-dried solid.

Boc-D-Arg-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH$_2$ (21). Synthesis similar to that described for **10** with Boc-D-Arg-OH replacing Boc-Arg-OH. Yield 66%.

Boc-D-Glu(OBn)-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH$_2$ (22). Fmoc-Ala-D-Trp-Phe-D-Pro-Pro-Nle-O-Resin (1.0 g, 0.29 mmol/g), obtained as an intermediate in the synthesis of **10**, was treated with AcOH (50 μL) and condensed liquid NH_3 in a steel pressure vessel at 20 °C for 22 h. The pressure vessel was cooled in dry ice/acetone prior to opening, and the NH_3 was allowed to evaporate. The resin was extracted with methanol (3 × 40 mL), the combined extracts were concentrated, and the residue was purified by preparative reverse-phase HPLC to give the intermediate peptide Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2 as a white solid (0.208 g). Amino acid analysis relative to Phe, 1.00; Pro, 1.96; Nle, 1.06; Ala, 0.96. A solution of this peptide (0.138 g, 0.124 mmol) in DMF (2 mL) was treated with Boc-D-Glu(OBn)-OSu (0.164 g, 0.378 mmol, Bachem) and triethylamine (52 μL, 0.378 mmol), and stirring was continued at 20 °C under nitrogen for 2 h. *N*-(2-aminoethyl)morpholine (46 μL, 0.35 mmol) was added. After a further 15 min, the reaction mixture was diluted with ethyl acetate (100 mL), washed with 10% aqueous citric acid (3 × 50 mL), 10% aqueous Na_2CO_3 (3 × 50 mL), water (3 × 50 mL), and brine (100 mL), dried ($MgSO_4$), and concentrated in vacuo to give a white solid. Yield 95%.

D-Glu(OBn)-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH$_2$ (23). The Boc-protected peptide **22** was deprotected using HCl/AcOH. Yield 88%.

Boc-Arg(NO$_2$)-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH$_2$ (24). Synthesis similar to that described for **22** except for the final coupling reaction Boc-Arg(NO_2)-OH (0.115 g, 0.362 mmol) in CH_2Cl_2 (3 mL) was treated with DCC (37 mg, 0.181 mmol) in CH_2Cl_2 (3 mL) and after 15 min the reaction mixture was filtered and the filtrate concentrated in vacuo. The residue and Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2 were dissolved in DMF and stirred for 1 h at 20 °C. *N*-(2-aminoethyl)morpholine (23 μL, 0.180 mmol) was added, and the reaction was worked up as described for **22**. Yield 49%.

Boc-Gln-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH$_2$ (25). Synthesis similar to that described for **10** except that Boc-Gln-OH was coupled, in place of Boc-Arg-OH·HCl, as the DIPC-generated symmetrical anhydride. The peptide was cleaved from the resin using NH_3/cat. AcOH. Yield 67%.

Boc-Lys-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH$_2$ (26). Synthesis similar to that described for **10** except that Boc-Lys(Fmoc)-OH was coupled, in place of Boc-Arg-OH·HCl, as the DIPC-generated symmetrical anhydride. The resultant functionalized resin was treated with 20% piperidine in DMF (3 min, 7 min) and then washed with DMF (10 × 3 mL), *tert*-amyl alcohol (3 × 3 mL), acetic acid (3 × 3 mL), *tert*-amyl alcohol (3 × 3 mL), DMF (3 × 3 mL), and diethyl ether (4 × 4 mL) and then dried in vacuo. The peptide was cleaved from the resin using NH_3/MeOH. Yield 27%.

Boc-Glu-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH$_2$ (27). The benzyl ester-protected peptide **28** (43 mg, 0.045 mmol) in methanol (30 mL) was hydrogenated at room temperature and pressure with 10% palladium on carbon for 3 h. The reaction mixture was filtered through hyflo (Celite) and the solids washed with methanol. The combined filtrates were concentrated in vacuo to give a foam. Yield 60%.

Boc-Glu(OBn)-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH$_2$ (28). Synthesis similar to that described for **22** except Boc-Glu-(OBn)-OSu (Bachem) was used in the final coupling step in place of Boc-D-Glu(OBn)-OSu. Yield 33%.

Boc-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH$_2$ (29). Synthesis similar to that described for **10** except for the final coupling to the resin which was achieved by treatment of the deprotected hexapeptide-substituted resin (0.25 g, 0.29 mmol/g) with a solution of Boc-Ala-OPfp (90 mg, 0.25 mmol) in DMF (2 mL) for 1 h at 20 °C. The resultant functionalized resin was washed with DMF (10 × 3 mL) and diethyl ether (3 × 3 mL) and then dried in vacuo. The peptide was cleaved from the resin using NH_3/cat. AcOH. Yield 41%.

Boc-NH(CH$_2$)$_4$CO-D-Trp-Phe-D-Pro-Pro-Nle-NH$_2$ (30). The functionalized resin Boc-Ava-D-Trp-Phe-D-Pro-Pro-Nle-O-resin was assembled, coupling Fmoc-D-Pro-OH, Fmoc-Phe-OH, and Fmoc-D-Trp-OH via the DIPC-generated symmetrical anhydrides and coupling Fmoc-Pro-OPfp and Boc-Ava-OPfp using standard methodology for the LKB peptide synthesizer. The Boc group was removed from this resin by treating with degassed TFA/water (19:1) for 1 h followed by thorough washing with DMF and diethyl ether. The resultant functionalized resin was cleaved using NH_3/cat. AcOH and the crude peptide obtained was purified by reverse-phase HPLC to give Ava-D-Trp-Phe-D-Pro-Pro-Nle-NH_2 (44 mg). A portion of this peptide (20 mg) was treated with a mixture of Boc_2O (17 mg, 3 equiv) and catalytic DMAP (2 mg) in acetonitrile (1 mL) for 1 h. The reaction mixture was concentrated and the residue purified. Yield 55%.

Ac-Arg-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH$_2$ (31). Synthesis similar to that described for **10** except Fmoc-Arg(Mtr)-OH was used in the final coupling step as the DIPC-generated symmetrical anhydride, in place of Fmoc-Arg-OH·HCl. The resultant functionalized resin was treated with 20% piperidine in DMF (3 min, 7 min) and then thoroughly washed with DMF before treating with 10% acetic anhydride in DMF (45 min). The resin was then washed with DMF (5 × 3 mL), *tert*-amyl alcohol (3 × 3 mL), acetic acid (3 × 3 mL), *tert*-amyl alcohol (3 × 3 mL), DMF (3 × 3 mL), and diethyl ether (4 × 3 mL) and dried in vacuo. The resultant resin was agitated with TFA/Phenol/H_2O (95:4:1) for 20 h to remove the Mtr protecting group and then subjected to the same washing and drying cycle described above. The peptide was cleaved from the solid support using NH_3/cat. AcOH. Yield 14%.

Cbz-Arg-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH$_2$ (32). Synthesis similar to that described for **10** except Cbz-Arg-OH·HCl was used in the final coupling reaction in place of Boc-Arg-OH·HCl. The resin was cleaved from the solid support using NH_3/cat. AcOH. Yield 61%.

PhCO-Gln-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH$_2$ (33). Fmoc-D-Pro-OH and Fmoc-D-Trp-OH were coupled using the DIPC-generated symmetrical anhydride methodology and the Phe, D-Trp, Ala, and Gln residues were double coupled. The resultant solid-supported peptide Gln-Ala-D-Trp-Phe-D-Pro-Pro-Nle-O-Resin (830 mg, 0.08 mmol/g) was agitated with a mixture of DMF (3 mL), benzoyl chloride (1 mL), and aqueous NaOH (2 M, 1 mL) at −10 °C for 1 h. Further benzoyl chloride (1 mL) was added and agitation continued for a further 1 h. The resin was then washed with DMF and diethyl ether and dried in vacuo. The peptide was cleaved from the solid support using NH_3/cat. AcOH. Yield 25%.

PhCO-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH$_2$ (34). In the peptide assembly, D-Pro-OPfp was double coupled using the standard methodology for the CRB pepsynthesizer. The benzoyl group was coupled on the CRB machine as the DIPC-generated symmetrical anhydride. The required peptide was cleaved from the solid support using NH_3/cat. AcOH. Yield 71%.

D-5,5-Dimethylthiaproline (36). D-Penicillamine (5.0 g, 33.5 mmol) was dissolved in hot water then cooled to 0 °C and treated with formaldehyde (5 mL, 37% aqueous). Stirring was continued at 0 °C for 1 h and then at 20 °C for 22 h. The reaction mixture

was concentrated in vacuo to give **36** (4.36 g, 81%) as two crops: mp 189–190 °C (lit.[14] 193–194 °C; lit.[15] 201–202 °C) (from MeOH), IR (Nujol) 3600–2300 (br, NH, CO_2H), 1560 cm^{-1} (br, CO_2^-); NMR (DMSO-d_6) δ 1.19 (3 H, s, CH_3), 1.56 (3 H, s, CH_3), 3.62 (1 H, s, CH), 4.05 (1 H, d, J = 9 Hz, CH_AH_B), 4.25 (1 H, d, J = 9 Hz, CH_AH_B), 8.0 (broad s, NH).

Fmoc-D-5,5-dimethylthiaproline (37). To a stirred solution of **36** (3.48 g, 21.5 mmol) in aqueous NaOH (0.4 N, 50 mL) at 0 °C was added Fmoc-OSu (8.00 g, 23.7 mmol) and stirring was continued for 2 h and then at 20 °C for 20 h. The reaction mixture was partitioned between water (200 mL) and diethyl ether (200 mL). The aqueous layer was acidified with 2 N HCl, and the precipitated oil was extracted into diethyl ether (2 × 200 mL). The combined extracts were dried ($MgSO_4$) and concentrated in vacuo to give **37** (7.00 g, 83%) as a colorless foam: IR ($CHBr_3$) 3600–2300 (br, CO_2H), 1720 (shoulder) and 1700 cm^{-1} (2 × C=O); NMR (DMSO-d_6, doubling due to rotameric mixture) δ 1.39 and 1.51 (3 H, 2 × s, CH_3), 1.42 and 1.54 (3 H, 2 × s, CH_3), 4.10–4.70 (6 H, m), 7.25–7.85 (8 H, m).

Fmoc-D-DMTP-Pro-O^{t}Bu (38). To a solution of **37** (1.93 g, 5 mmol) in CH_2Cl_2 (20 mL) at −15 °C under nitrogen, was added Et_3N (0.77 mL, 5.54 mmol) followed by Bop-Cl (1.42 g, 5.54 mmol). The mixture was stirred for 20 min before adding Pro-O^{t}Bu·HCl (0.95 g, 5.54 mmol) and triethylamine (0.77 mL, 5.54 mmol), then warmed to 0 °C, reducing the volume to 5 mL using a nitrogen stream, and stirred for a further 4 h. The reaction mixture was separated between EtOAc (70 mL) and water (70 mL). The organic phase was washed with 10% aqueous citric acid (2 × 50 mL), 10% aqueous Na_2CO_3 (2 × 50 mL), and water (50 mL), then dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by gravity chromatography on silica (100 g) with EtOAc/hexane (1:3) as eluant to give **38** (1.63 g, 60%) as a colorless foam: IR ($CHBr_3$) 1720, 1680, 1650 cm^{-1} (3 × C=O); NMR (DMSO-d_6, 100 °C to simplify rotameric mixture) δ 1.30–1.40 (6 H, m, 2 × CH_3), 1.36 (9 H, s, $C(CH_3)_3$), 1.75–2.09 (4 H, m), 3.32 (2 H, m), 4.58 (1 H, s, CHC=O), 4.59 (2 H, AB system, CH_2S), 4.44 (2 H, m, CH_2O), 4.32 (1 H, m, $CHCH_2O$), 7.3–7.9 (8 H, m); FAB (resol 10000) MH^+ = 537.2452 ($C_{30}H_{37}N_2O_5S$ = 537.2423).

Fmoc-D-DMTP-Pro-OH (39). The ester **38** (1.55 g, 2.9 mmol) was dissolved in TFA/H_2O (20 mL; 19:1) and stirred for 1 h at 20 °C under nitrogen. The reaction mixture was concentrated in vacuo and the residue triturated with diethyl ether and dried to give **39** (1.3 g, 94%) as a white solid mp 194–196 °C. IR (Nujol) 1730, 1690, and 1610 cm^{-1} (3 × C=O); NMR (DMSO-d_6, 100 °C to simplify rotameric mixture) δ 1.33 (3 H, s, CH_3), 1.41 (3 H, s, CH_3), 1.83 (3 H, m), 2.10 (1 H, m), 3.33 (2 H, m), 4.32 (1 H, broad t, J = 4 Hz, $CHCH_2O$), 4.43 (2 H, m, CH_2O), 4.60 (2 H, AB system, CH_2S), 4.60 (1 H, s, $CHC(CH_3)_2S$), 7.3–7.9 (8 H, m); FAB (resol 10000) MH^+ = 481.1771 ($C_{28}H_{29}N_2O_5S$ = 481.1797).

Fmoc-D-DMTP-Pro-OPfp (40). To a solution of the acid **39** (1.25 g, 2.6 mmol) and pentafluorophenol (0.48 g, 2.6 mmol) in a mixture of EtOAc (50 mL) and DMF (2 mL) at 5 °C under nitrogen was added DCC (0.54 g, 2.6 mmol), and the resulting mixture was stirred for 1 h before warming to 20 °C and stirring for a further 2 h. The reaction mixture was filtered, and the solids were washed with EtOAc (20 mL). The combined filtrates were concentrated in vacuo, the residue was triturated with diethyl ether (20 mL) and filtered, and the filtrate was concentrated to give the OPfp-ester **40** (1.78 g, 106%), contaminated with DCU, which was used without further purification.

Boc-D-Pro-Pro-OBn (41). Boc-D-Pro-OH (215 mg, 1 mmol) was dissolved in DMF (2 mL) and cooled in ice. HOBT (153 mg, 1 mmol) was added followed by DCC (206 mg, 1 mmol). After stirring for 15 min, Pro-OBn·HCl (242 mg, 1 mmol) was added followed by triethylamine (140 μL, 1 mmol). The mixture was maintained at 5 °C for 6 days then diluted with EtOAc (100 mL) and the resulting solution washed with 10% aqueous citric acid (2 × 30 mL), 10% aqueous Na_2CO_3 (2 × 30 mL), and water (30 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was triturated with diethyl ether (30 mL) for 20 h, the resulting mixture filtered, and the filtrate concentrated to give the required protected dipeptide[16] (381 mg, 95%). IR (Nujol) 1745, 1700–1640 cm^{-1} (br); NMR (DMSO-d_6, 100 °C to simplify rotameric mixture) δ 1.39 (9 H, s, $C(CH_3)_3$), 1.75 (2 H, m), 1.91 (2 H, m), 1.95 (2 H, m), 2.19 (2 H, m), 3.36 (2 H, m), 3.54 (2 H, m), 4.3–4.5 (2 H, m), 5.10 (1 H, broad d, J = 8 Hz, CH_ACH_BPh), 5.17 (1 H, d, J = 8 Hz, CH_ACH_BPh), 7.3–7.4 (5 H, m, Ph); FAB (resol 10000) MH^+ = 403.2219 ($C_{22}H_{31}N_2O_5S$ = 403.2233).

Pharmacological Methods. Compounds were tested in vitro for activity at NK-1, NK-2, and NK-3 receptors on isolated guinea-pig ileum (GPI), rat colon muscularis mucosae (RC), and rat everted portal vein (RPV), respectively. Selected compounds were tested for NK-2 antagonist activity in guinea-pig trachea (GPT). Tissues were prepared and agonist dose–response curves constructed as described previously.[9d,11] Tests in GPT were carried out in the presence of phosphoramidon (1 μM) and bestatin (100 μM) (see ref 9d).

Animals. Male guinea-pigs (300–600 g, Dunkin-Hartley strain, Porcellus) and male rats (250–500 g, AHA strain, Glaxo) were used. Animals were killed by concussion followed by exsanguination; required tissues were excized immediately.

In Vitro Experimental Design. On each preparation, at least two concentration–response curves were constructed to the standard agonist (SPOMe on GPI, NKA on RC, senktide on RPV, or GR 64349 on GPT). Tissues were used in the study only if two consecutive curves were found to have EC_{50} values consistent to within a factor of two and maxima reproducible to within ± 15%.

All compounds were evaluated for agonist activity; where this was observed (compound 3 only), a full concentration–response curve was constructed to the test-compound.

Routinely, to test for antagonist activity, test compounds shown to be devoid of agonist activity were preequilibrated with the tissue for 15 min. After this period, agonist at a concentration inducing approximately 50% of the maximum response was applied at 15-min intervals until the response was constant. This was taken to indicate that equilibrium had been achieved. Each preparation was exposed to one concentration of test compound only. In control preparations not exposed to antagonist, EC_{50} values for agonist concentration–response curves were reproducible to within 2-fold. No measurements were made of reversibility of effect on washout of test compound.

Data Analysis. For agonists, EC_{50} values and maxima of concentration–response curves were estimated using the curve-fitting program ALLFIT.[12] Antagonist-induced parallel displacement of agonist concentration–response curves was quantified as the ratio of equiactive molar concentrations. These were estimated graphically at the level of the half-maximal response. The apparent affinity (pK_B) of the antagonist was then estimated using the equation: pK_B = log (concentration-ratio − 1) − log (molar concentration of antagonist). pK_B values quoted in the text are mean ± SE of single determinations in four to eight separate tissue preparations.

Measurement of Antagonist Activity in Vivo. Antagonist activity at NK-1 and NK-2 receptors in vivo was assessed in anaesthetized guinea-pigs; measurement was made of the inhibition of the bronchoconstriction response to rapid intravenous (iv) injection of an NK-1 agonist (SPOMe) or an NK-2 agonist (GR64349), respectively. Male Dunkin–Hartley guinea-pigs (300–500 g) were anaesthetized with a mixture of chloralose (Glaxo; 80 mg/kg) and phenobarbitone (May and Baker, 10 mg/kg). Supplementary doses of the anaesthetic mixture were given as required during the course of the experiment. The trachea was cannulated to allow mechanical ventilation (10 mL/kg per stroke, 60 stroke/min). Insufflation pressure was recorded from a side-arm attached to the tracheal cannula. A carotid artery was cannulated for measurement of blood pressure; heart rate was derived from this. A jugular vein was cannulated for the iv administration of drugs. Core temperature was measured via a rectal probe and maintained at 37 ± 1 °C using a thermostatically-controlled heating blanket. Animals were pretreated with mepyramine (5 mg/kg iv) to inhibit the effects of released histamine.

Dose–response curves to SPOMe (1.0–10.0 nmol/kg iv) or GR64349 (0.1–1.0 nmol/kg iv) were constructed using noncumulative addition and were repeated until the apparent EC_{50} values of successive curves differed by less than 2-fold. Antagonist was then administered as a single dose given either iv or subcutaneously (sc) and measurement made of the amount by which doses of agonist needed to be increased to obtain bronchoconstriction responses comparable to controls. Such dose-ratio measurements were repeated at suitable intervals.

Registry No. 1, 91234-81-0; 2, 102937-85-9; 3, 141636-37-5; 4, 141636-38-6; 5, 141636-39-7; 6, 141636-40-0; 7, 141636-41-1; 8, 141636-42-2; 9, 141636-43-3; 10, 141636-44-4; 11, 141636-45-5; 12, 141636-46-6; 13, 141636-47-7; 14, 141636-48-8; 15, 141663-84-5; 16, 141636-49-9; 17, 141636-50-2; 18, 141636-51-3; 19, 141636-52-4; 20, 141636-53-5; 21, 141636-54-6; 22, 141636-55-7; 23, 141663-85-6; 24, 141636-56-8; 25, 141636-57-9; 26, 141636-58-0; 27, 141636-59-1; 28, 141636-60-4; 29, 141663-86-7; 30, 141636-61-5; 31, 141636-62-6; 32, 141636-63-7; 33, 141636-64-8; 34, 141636-65-9; 35, 52-67-5; 36, 22916-26-3; 37, 141636-66-0; 38, 141636-67-1; 39, 141636-68-2; 40, 141636-69-3; 41, 141636-70-6; 42, 105959-87-3; 43, 141636-71-7; 44·HCl, 141663-87-8; Fmoc-D-Trp-OH, 86123-11-7; Boc-Arg-OH·HCl, 35897-34-8; Fmoc-D-Pro-OH, 101555-62-8; Fmoc-Phe-OH, 35661-40-6; Fmoc-D-Ala-OH, 35661-39-3; Boc-Arg-OH, 13726-76-6; Fmoc-D-Phe-OH, 86123-10-6; Fmoc-Phe-OPfp, 86060-92-6; Fmoc-Gly-OH, 29022-11-5; Fmoc-D-Met-OH, 112883-40-6; Boc-D-Trp-OPfp, 94778-76-4; Boc-Ala-OPfp, 50903-48-5; Boc-D-Glu(OBn)-OSu, 18800-76-5; Boc-Arg(NO_2)-OH, 2188-18-3; Boc-Gln-OH, 13726-85-7; Boc-D-Arg-OH, 78603-12-0; Boc-Lys(Fmoc)-OH, 84624-27-1; Fmoc-Pro-OPfp, 86060-90-4; Boc-Ava-OPfp, 129605-96-5; Fmoc-Arg(Mtr)-OH, 98930-01-9; Cbz-Arg-OH·HCl, 56672-63-0; H-Pro-OBu-*t*·HCl, 5497-76-7; Boc-D-Pro-OH, 37784-17-1; H-Pro-OBn·HCl, 16652-71-4; Boc-Ala-D-Trp-Phe-D-Pro-Pro-OMe, 141636-73-9; H-Arg-Ala-D-Trp-Phe-D-Pro-Pro-OMe, 141636-74-0; $CH_3(CH_2)_4NH_2$, 110-58-7; $MeNH_2$, 74-89-5; H-Ala-D-Trp-Phe-D-Pro-Pro-NHMe·HCl, 141636-75-1; H-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2, 141636-76-2; H-Ava-D-Trp-Phe-D-Pro-Pro-Nle-NH_2, 141636-77-3; Boc-Phe-OPfp, 50903-54-3; Boc-Glu(OBn)-OSu, 32886-40-1; H-D-Pro-OPfp, 141636-79-5.

The Discovery of (2*S*,3*S*)-*cis*-2-(Diphenylmethyl)-*N*-[(2-methoxyphenyl)methyl]-1-azabicyclo[2.2.2]-octan-3-amine as a Novel, Nonpeptide Substance P Antagonist

John A. Lowe, III,*,† Susan E. Drozda,† R. Michael Snider,† Kelly P. Longo,† Stevin H. Zorn,† Jean Morrone,† Elisa R. Jackson,† Stafford McLean,† Dianne K. Bryce,† Jon Bordner,† Atsushi Nagahisa,‡ Yoshihito Kanai,‡ Osamu Suga,‡ and Megumi Tsuchiya‡

Department of Exploratory Medicinal Chemistry, Central Research Division, Pfizer, Inc., Groton, Connecticut 06340, and Department of Medicinal Biology, Central Research Division, Pfizer, Inc., 5-2 Taketoyo, Aichi 470-23, Japan.
Received December 26, 1991

We describe the structure–activity relationship development of a series of quinuclidines which culminated in the first potent, selective, nonpeptide substance P (SP) antagonist, (2*S*,3*S*)-*cis*-2-(diphenylmethyl)-*N*-[(2-methoxyphenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine, **3** (CP-96,345). Compound **3** is a potent displacer of [^{3}H]SP binding in human IM-9 cells and blocks SP-induced and capsaicin-induced plasma extravasation, as well as SP-induced salivation in the rat in vivo. This compound may both help to further our understanding of the interactions of small molecules with peptide receptors and serve to evaluate the therapeutic potential of a SP antagonist.

Substance P (SP), a peptide neurotransmitter first discovered in 1931[1] and eventually characterized in 1970 as the undecapeptide Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2,[2] is a member of the tachykinin family of peptides, which includes neurokinins A and B (NKA and NKB). These peptides bind to a series of three neurokinin receptors, NK_1, NK_2, and NK_3, which have selective affinity for SP, NKA, and NKB, respectively.[3] SP has been shown to elicit a wide variety of physiological responses which suggest it plays a role in initiating the immune response and in transmitting pain and stress to the central nervous system (CNS). For example, SP has been reported to elicit IL-1 production in macrophages,[4] sensitize neutrophils,[5] and enhance dopamine release in the substantia nigra region in cat brain.[6] Its key position at the interface between the immune system and CNS suggests SP may be involved in a variety of important diseases. There is evidence, for example, for SP's role in rheumatoid arthritis,[7] ulcerative colitis,[8] and migraine.[9]

Recognizing that SP blockade represents a potentially important avenue for novel therapy, previous workers in the field have sought to discover NK_1 receptor antagonists by modifying SP's peptide structure. Two strategies were ultimately successful: the "linear" approach used by Folkers' group, which led to spantide II,[10] **1**, and the "conformational constraint" approach, which culminated in the recent announcement of GR71251,[11] **2**. Despite the impressive pA_2 value of 7.7 at the NK_1 receptor for both these compounds, their peptidic structure is expected to limit their oral bioavailability and hence their potential for investigating clinical applications of NK_1 antagonists.

† Department of Exploratory Medicinal Chemistry.
‡ Department of Medicinal Biology.

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0022-2623/92/1835-2591$03.00/0