Conformationally Constrained Tachykinin Analogues: Potent and Highly Selective Neurokinin NK-2 Receptor Agonists

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The design and synthesis of potent and selective neurokinin NK-2 receptor agonists 12 (GR64349) and 31 are described, together with structure–activity relationships for related analogues. Compound 12 (EC_{50} = 3.7 nM at NK-2 receptors in the rat colon; selectivity >1000- and >300-fold with respect to NK-1 and NK-3 receptors, respectively) was derived by incorporation of a Gly-Leu γ-lactam conformational constraint into the C-terminal region of the neurokinin A octapeptide analogue [Lys³]-NKA(3–10). Compound 31 (EC_{50} = 15 nM in rat colon) contains a novel fused-bicyclic constraint at the corresponding site in the substance P hexapeptide analogue [Ava⁶]-SP(6–11).

The naturally occuring mammalian tachykinins substance P (SP), neurokinin A (NKA), and neurokinin B (NKB) are the preferred endogenous agonists at the NK-1, NK-2 and NK-3 receptors, respectively (Figure 1, Table II).[1,2] However, the low degree of receptor selectivity combined with the metabolic instability of these compounds has limited their use as pharmacological tools.[3] Hence, more selective and metabolically stable neurokinin receptor agonists are sought. Selective agonists for the NK-1, NK-2, and NK-3 receptors have been obtained by modifying the naturally occurring peptides.[2,4-7] With particular relevance to this work, it has been reported that replacement of the Gly⁹ residue in SP-related peptides by Pro enhances NK-1 receptor selectivity,[5] whereas

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. Mammalian tachykinin sequences.

introduction of D-Pro or an *R*-γ-lactam constraint at this position enhances NK-2 receptor selectivity.[6,7] In a previous paper[8] we described a similar structure–activity dependence in analogues of [Ava⁶]-SP(6–11) (Ava = δ-aminovaleric acid). We now report further studies leading to the *R*-γ-lactam peptide GR 64349, a potent and highly selective NK-2 agonist. In addition, we have extended this conformational approach to receptor selectivity with the design of a novel bicyclic constraint according to the rationale described below.

The D-Pro and *R*-γ-lactam modifications constrain only one of the two freely rotatable backbone bonds (φ and ψ) of the Gly⁹ residue as depicted on a conformational energy contour map for *N*-acetyl-*N'*-methylglycinamide (Figure 2).[9] The parallel broken lines represent the maximum torsional limits for proline (φ) and *R*-γ-lactam ring constraints estimated from a survey of relevant structures in the Cambridge Structural Database.[10] For NK-2 agonist activity, there is only a small region of conformational space common to both the D-Pro and *R*-γ-lactam constraints. This conformation is embodied in the D-Pro-[γ-Lac]-Leu fused-lactam constraint (φ = +75° ± 20°, ψ = +140° ± 10°) (Figure 2), which should therefore approximate the required conformation at Gly⁹ for NK-2 agonist activity. We report herein the synthesis of this bicyclic conformational constraint, together with three stereoisomers, and the biological activity of tachykinin-related sequences containing these structures.

† Neuropharmacology Department.

(1) Henry, J. L.; Couture, R.; Cuello, A. C.; Pelletier, G.; Quirion, R.; Regoli, D. (Eds) *Substance P and Neurokinins*; Springer-Verlag: New York, 1987.

(2) Regoli, D.; Dion, S.; Rhaleb, N.-E.; Rouissi, N.; Tousignant, C.; Jukic, D.; D'Orleans-Juste, P.; Drapeau, G. Selective Agonists for Receptors of Substance P and Related Neurokinins. *Biopolymers* 1989, *28*, 81–90.

(3) (a) Ireland, S. J.; Jordan, C. C.; Stephens-Smith, M. L.; Ward, P. Receptors Mediating the Contractile Response to Neurokinin Agonists in the Guinea-Pig Trachea. *Regul. Pept.* 1988, *22*, 93. (b) Stephens-Smith, M. L.; Ireland, S. J.; Jordan, C. C. Influence of Peptidase Inhibitors on Responses to Neurokinin Receptor Agonists in the Guinea-Pig Trachea. *Regul. Pept.* 1988, *22*, 177.

(4) (a) Drapeau, G.; D'Orleans-Juste, P.; Dion, S.; Rhaleb, N.-E.; Rouissi, N.-E.; Regoli, D. Selective Agonists for Substance P and Neurokinin Receptors. *Neuropeptides* 1987, *10*, 43–54. (b) Rovero, P.; Pestellini, V.; Rhaleb, N.-E.; Dion, S.; Rouissi, N.; Tousignant, C.; Telemaque, S.; Drapeau, G.; Regoli, D. Structure-Activity Studies of Neurokinin A. *Neuropeptides* 1989, *13*, 263–270. (c) Rovero, P.; Pestellini, V.; Patacchini, K.; Giuliani, S.; Santicioli, P.; Maggi, C. A.; Meli, A.; Giachetti, A. A Potent and Selective Agonist for the NK-2 Tachykinin Receptor. *Peptides* 1989, *10*, 593–595.

(5) (a) Laufer, R.; Gilon, C.; Chorev, M.; Selinger, Z. [pGlu⁶,Pro⁹] SP-(6-11), A Selective Agonist for the Substance P P-Receptor Subtype. *J. Med. Chem.* 1986, *29*, 1284–1288. (b) Haro, I.; Torres, J. L.; Valencia, G.; Garcia-Anton, J. M.; Reig, F. Synthesis and Purification of two SP(6-11) Analogues with Enhanced Selectivity for the NK-1 Receptor. *Int. J. Pept. Protein Res.* 1990, *35*, 123–127. (c) Wormser, V.; Laufer, R.; Hart, Y.; Chorev, M.; Gilon, C.; Selinger, Z. Highly Selective Agonists for Substance P Receptor Subtypes. *EMBO J.* 1986, *5*, 2805–2808. (d) Elgnero, J.; Garcia-Anton, J. M.; Goya, P.; Haro, I.; Martinez, A.; Reig, F. Conformation-Activity Relationship in Hexapeptides Related to Substance P. *Arzneim-Forsch/Drug Res.* 1989, *39(II)*, 835.

(6) Cascieri, M. A.; Chicchi, G. G.; Freidinger, R. M.; Colton, C. D.; Perlow, D. S.; Williams, B.; Curtis, N. R.; McKnight, A. T.; Maguire, J. J.; Veber, D. F.; Liang, T. Conformationally Constrained Tachykinin Analogues Which Are Selective Ligands for the Eledoisin Receptor. *Mol. Pharmacol.* 1986, *29*, 34–38.

(7) Lee, C.-M.; Campbell, N. J.; Williams, B. J.; Iversen, L. L. Multiple Tachykinin Binding Sites in Peripheral Tissues and in Brain. *Eur. J. Pharmacol.* 1986, *130*, 209.

(8) (a) Brown, J. R.; Clegg, S. P.; Ewan, G. B.; Hagan, R. M.; Ireland, S. J.; Jordan, C. C.; Porter, B.; Ross, B. C.; Ward, P. A Conformational Approach to the Discovery of Potent Selective Neurokinin Receptor Antagonists. *Molecular Recognition: Chemical and Biochemical Problems*, Royal Society of Chemistry Special Publication No. 78; Roberts, S. M., Ed.; Royal Society of Chemistry: Letchworth. (b) Ward, P.; Ewan, G. B.; Jordan, C. C.; Ireland, S. J.; Hagan, R. M.; Brown, J. R. Potent and Highly Selective Neurokinin Antagonists. *J. Med. Chem.* 1990, *33*, 1848.

(9) Lewis, P. N.; Momany, F. A.; Scheraga, H. A. Energy Parameters in Polypeptides. VI. Conformational Energy Analysis of the N-Acetyl N'-Methyl Amides of the Twenty Naturally Occurring Amino Acids. *Israel J. Chem.* 1973, *11*, 121–152.

(10) Allen, F. H.; Kennard, O.; Taylor, R. Systematic Analysis of Structural Data as a Research Technique in Organic Chemistry. *Acc. Chem. Res.* 1983, *16*, 146.

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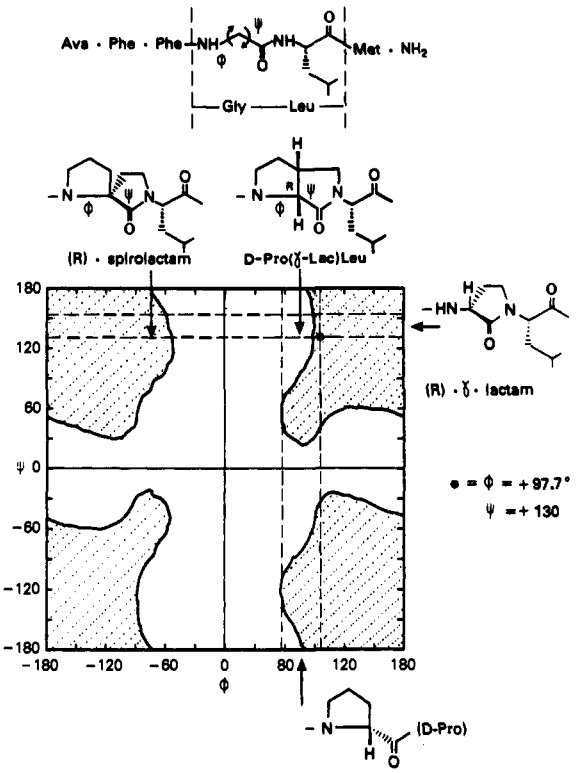
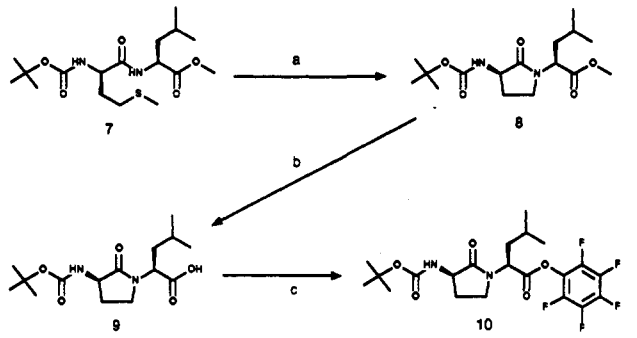


Figure 2. Conformational ϕ,ψ energy map for *N*-acetyl-*N'*-methylglycinamide (2 kcal mol^{-1} contour; low-energy areas are shaded). When incorporated in [Ava^6]-SP(6–11) as indicated, the conformational constraints, D-Pro, *R*-γ-lactam, and D-Pro-[γ-Lac]-Leu fused lactam are compatible with high agonist activity at the NK-2 receptor. The ϕ,ψ torsion angles calculated from X-ray crystallographic data for intermediate **19a** are indicated by the asterisk.

Scheme I[a]

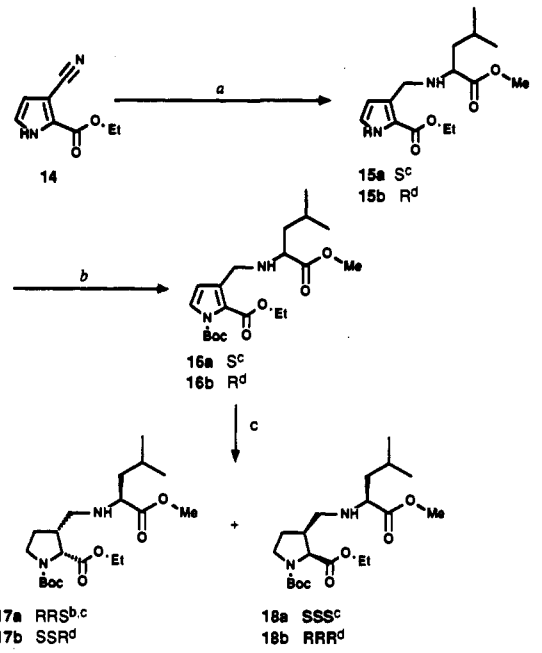


[a] (a) MeI; NaH, DMF, −50 °C. (b) NaOH, H_2, MeOH. (c) DCC, PfpOH, DMF.

Chemistry

N-terminally modified peptide analogues of NKA and SP, **1–6**, were prepared using the Fmoc-based solid-phase methodology developed by Atherton et al.[11] The *R*-γ-lactam constraint in peptides **11–13** was prepared using a modification of literature procedures[12] (Scheme I). Methylation of the D-methionine side chain in **7** with methyl iodide, cyclization using sodium hydride at −5 °C for 45 min, and quenching with ammonium chloride allowed the isolation of the ester **8** with less than 10% racemization at the leucine α-carbon. Extended reaction times caused a greater degree of racemization. Recrystallization of **8** gave the major diastereoisomer in 39% yield, but more conveniently, the crude ester product was hydrolyzed and the resulting acid recrystallized to give **9** as a single diastereoisomer in 70% overall yield from **7**. The acid **9** was converted into the corresponding pentafluorophenyl ester **10** for use in solid-phase peptide synthesis. The *R*-γ-lactam-constrained peptides, **11–13**, were prepared using solid-phase methodology as described in the Experimental Section.

Scheme II[a,b]



[a] (a) L- or D-LeuOMe, H_2, Raney Ni. (b) Boc_2O, DMAP. (c) H_2, Rh/Al_2O_3. [b] First two descriptors for fused lactam ring junction, third descriptor for adjacent Leu. [c] 50% enantiomeric excess. [d] 80% enantiomeric excess.

Synthesis of all four stereoisomers of the D-Pro-[γ-Lac]-Leu fused-lactam constraint (Figure 1) was achieved starting from the known 2,3-disubstituted pyrrole **14**[13] (Scheme II). Reductive amination of **14** with either L- or D-leucine methyl ester using Raney nickel catalysis gave the corresponding amine adducts **15a** and **15b**, respectively, in moderate yield. However, NMR studies on **15a** or **15b** using the chiral shift reagent tris[3-[(heptafluoropropyl)hydroxymethylene]-(+)-camphorato]europium-(III), revealed that racemization of the leucine residue had occurred during this reaction. Accurate integrations of the α- and β-pyrrole protons enabled determination of the enantiomeric composition. The extent of racemization was variable between 10 and 25% and was minimized by vigorous stirring of the hydrogenation reaction. This indicated that the racemization was occurring in competition with the hydrogenation and so probably proceeds via deprotonation of the intermediate imine (Figure 3). It was subsequently found that performing this reaction in a buffered solution at pH = 7 reduced the extent of racemization to ca. 8%, i.e. producing a 12:1 mixture of enantiomers. The remainder of the synthesis was carried

(11) Atherton, E.; Fox, H.; Harkiss, D.; Logan, C. J.; Sheppard, R. C.; Williams, B. J. A Mild Procedure for Solid Phase Peptide Synthesis: Use of Fluorenylmethoxycarbonylamino Acids. *J. Chem. Soc., Chem. Commun.* **1978**, 537.

(12) (a) Freidinger, R. M. Synthesis of γ-Lactam-Constrained Tryptophyllysine Derivatives. *J. Org. Chem.* **1985**, *50*, 3631. (b) Freidinger, R. M.; Perlow, D. S.; Veber, D. F. Protected Lactam-Bridged Dipeptides for use as Conformational Constraints in Peptides. *J. Org. Chem.* **1982**, *47*, 104.

(13) Husingen, R.; Laschtuvka, E. Eine Neue Synthese von Derivaten des Pyrrols. *Chem. Ber.* **1960**, *93*, 65.

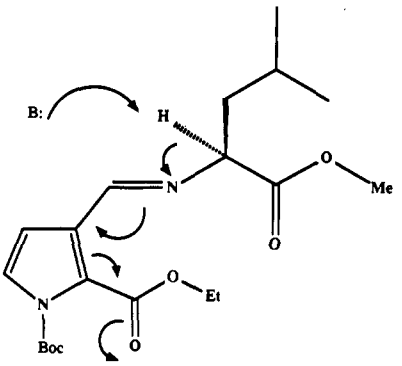
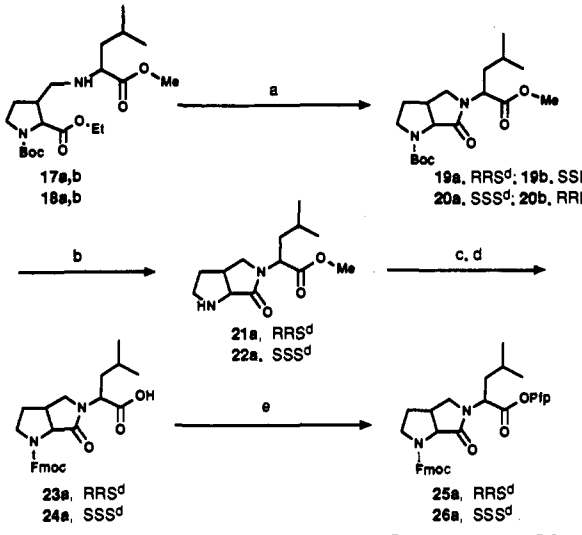


Figure 3. Deprotonation of the imine intermediate during reductive amination of nitrile **14** resulting in partial racemization (B: = basic function).

Scheme III[a-c]



[a] (a) Et_3N, Δ. (b) HCl, AcOH. (c) NaOH. (d) FmocOSu. (e) PfpOH, DCC. [b] Where Fmoc = fluorenylmethyloxycarbonyl and Pfp = pentafluorophenyl. [c] Where first two stereochemical descriptors refer to the ring fusion. [d] 50% enantiomeric excess. [e] 80% enantiomeric excess.

out using the initial mixture of enantiomers obtained from this reaction.

Boc protection of the pyrrole **15a**[14] and further hydrogenation with rhodium on alumina gave a 1:1 mixture of the diastereoisomeric 2,3-disubstituted pyrrolidines **17a** and **18a** with high cis stereoselectivity but with no diastereofacial selectivity (Scheme II). These diastereoisomers could be separated at this stage but were more conveniently separated by column chromatography after triethylamine-catalyzed cyclization to the corresponding mixture of fused-lactam diastereoisomers **19a** and **20a** (Scheme III). The proton NMR spectra of the diastereoisomers **19a** and **20a** showed significant differences, particularly in the chemical shifts of the lactam protons. The spectra were complicated by broadening due to rotameric isomerization of the Boc group with a coalescence temperature around 25 °C. However low-temperature NMR at −40 °C resulted in sharpening of the 6a-bridgehead proton in the diastereoisomer **19a** into two doublets, in a ratio 2:1 due to the two rotameric forms, both doublets having a coupling constant of 8 Hz consistent with a cis ring fusion. High-temperature NMR at 100 °C in DMSO

(14) Grehn, L.; Ragvarsson, U. A Convenient Method for the Preparation of 1-(tert-butyloxycarbonyl) Pyrroles. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 296.

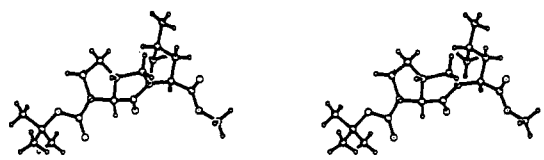


Figure 4. X-ray crystal structure of compound **19a**.

gave a sharp doublet, $J = 8$ Hz, for the 6a-proton of both diastereoisomers **19a** and **20a**, with chemical shifts of δ 4.43 and 4.47, respectively. An X-ray crystallographic structure obtained for **19a** enabled complete assignment of the relative stereochemistry (Figure 4). The enantiomeric series starting from D-leucine yielded the other two fused-lactam isomers **19b** and **20b** with 90% enantiospecificity.

Initial fused-lactam-containing analogues of the peptide Ava-SP(7–11) were prepared using the diastereoisomers **19a** and **20a** prepared from L-leucine (Scheme IV). Hydrolysis of the methyl ester groups and coupling to L-methionine amide gave the pseudo-tripeptides **29a** and **30a**, which could both be distinguished as distinct 75:25 mixtures of diastereoisomers by NMR and HPLC, so confirming the extent of racemization in the initial reductive amination of **14**. The peptide assembly was completed on these diastereoisomeric mixtures using Boc-protected pentafluorophenol active ester methodology in solution and the diastereoisomers were separated at the final stage to give the enantiomerically pure products **31–34**.

Two further fused-lactam-containing peptides, **35** and **36**, were prepared using solid-phase methodology. The Fmoc-protected, pentafluorophenyl ester fused-lactam derivative **25a** required for this synthesis and the diastereoisomer **26a** were prepared in good overall yield using established methodology (Scheme III).

All final compounds were characterized by FAB mass spectroscopy, reverse-phase HPLC and amino acid analysis (Table I).

Biological Results and Discussion

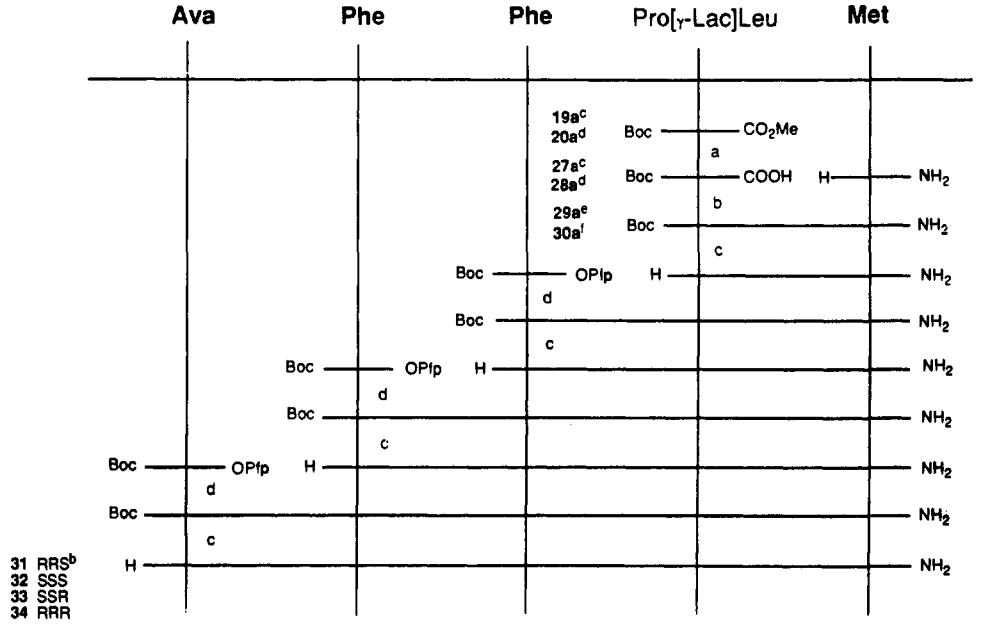
Agonist activity at NK-1 receptors in guinea pig ileum longitudinal smooth muscle (GPI) and at NK-2 receptors in the rat colon muscularis mucosae (RC) was determined from contractile responses recorded under isotonic conditions, at 35 °C, in the presence of atropine (1 μM), mepyramine (1 μM), methysergide (1 μM), and indomethacin (1 μM).[15] Agonist activity at NK-3 receptors was determined from contractile responses of everted rat portal vein (RPV) under isotonic conditions (Krebs–Henseleit solution).[16] Compounds were assayed against substance P (GPI and RC) or neurokinin B (RPV) and equipotent molar ratios calculated. These values have been converted to the EC_{50} values reported in Table II by relating them to mean EC_{50} values for the standard agonists in order to give a clearer impression of the selectivity of each compound.

The N-terminally truncated analogue of substance P, [Ava[6]]-SP(6–11) (**2**), has a similar selectivity for the NK-1

(15) Brown, J. R.; Jordan, C. C.; Ward, P.; Whittington, A. R. Selectivity of Tachykinin Antagonists on Smooth Muscle Preparations. In *Tachykinin Antagonists*; Hakanson, R., Sundler, F., Eds.; Elsevier: Amsterdam, 1985; pp 305–312.

(16) Mastrangelo, D.; Mathison, R.; Huggel, H. J.; Dion, S.; D'Orleans-Juste, P.; Rhaleb, N. E.; Drapeau, G.; Rovero, P.; Regoli, D. The Rat Portal Vein: a Preparation Sensitive to Neurokinins, Particularly Neurokinin B. *Eur. J. Pharmacol.* **1987**, *134*, 321.

Scheme IV. Solution-Phase Synthesis of Fused-Lactam-Containing Peptides[a]



[a] (a) K_2CO_3, MeOH, H_2O. (b) DCC, HOBT, DMF. (c) HCl, AcOH. (d) DMF, 20 °C. [b] First two descriptors for fused lactam ring fusion, third descriptor for adjacent Leu. [c] 75% *RRS*, 25% *SSR*. [d] 75% *SSS*, 25% *RRR*. [e] 75% *RRSS*, 25% *SSRS*. [f] 75% *SSSS*, 25% *RRRS*.

Table I. Characterization of Peptide Analogues[a]

												HPLC[b] t_R, min	mass spec,[c] m/z [MH$^+$]
1				Ava	Phe	Val	Gly		Leu	Met	NH_2	7.85	664
2				Ava	Phe	Phe	Gly		Leu	Met	NH_2	9.21	712
3				Ava	Phe	Phe	Pro		Leu	Met	NH_2	11.95	752
4				Ava	Phe	Phe	D-Pro		Leu	Met	NH_2	ND	752
5		Lys	Asp	Ser	Phe	Val	Gly		Leu	Met	NH_2	ND	895
6		Lys	Asp	Ser	Phe	Phe	Gly		Leu	Met	NH_2	ND	943
11				Ava	Phe	Phe	Gly	[R-γ-Lac]	Leu	Met	NH_2	ND	738
12		Lys	Asp	Ser	Phe	Val	Gly	[R-γ-Lac]	Leu	Met	NH_2	8.38	921
13	Ac	Lys	Asp	Ser	Phe	Val	Gly	[R-γ-Lac]	Leu	Met	NH_2	9.26	963
31				Ava	Phe	Phe	D-Pro	[γ-Lac]	Leu	Met	NH_2	12.18	764
32				Ava	Phe	Phe	Pro	[γ-Lac]	Leu	Met	NH_2	12.37	764
33				Ava	Phe	Phe	Pro	[γ-Lac]	D-Leu	Met	NH_2	13.13	764
34				Ava	Phe	Phe	D-Pro	[γ-Lac]	D-Leu	Met	NH_2	13.67	764
35		Lys	Asp	Ser	Phe	Val	D-Pro	[γ-Lac]	Leu	Met	NH_2	8.75	948
36		Lys	Asp	Ser	Phe	Phe	D-Pro	[γ-Lac]	Leu	Met	NH_2	9.94	996
37[d]				Ava	Phe	Phe	Pro	[R-Spirolac]	Leu	Met	NH_2		

[a] Satifactory amino acid analyses were obtained for all peptides. [b] Retention time on a Brownlee wide-core Aquapore, RP-300(octyl) column using eluants A [H_2O, 0.1% trifluoracetic acid (TFA)] and B (acetonitrile, 0.05% TFA) with gradient elution from 85% A–15% B to 10% A–90% B over 25 min. [c] Found values in agreement with calculated nominal mass + H$^+$. The fragmentation pattern was characteristic of the amino acid sequence claimed. [d] See ref 8b.

receptor to SP with only a 7-fold decrease in potency despite the large reduction in molecular size. [Ava^5]-NKA-(5–10) (1), the corresponding C-terminal fragment of neurokinin A, is only slightly less active than 2 as an agonist at the NK-1 receptor but is markedly more active at the NK-2 receptor. This emphasizes the importance of the Val^7 residue in NKA-related peptides for imparting preferential NK-2 receptor interaction. However, similar NK-2 receptor potency was obtained by replacing the Gly^9 residue (SP nomenclature) in 2 with D-Pro to give 4, which also exhibited reduced activity as an NK-1 receptor agonist. In contrast to this, the corresponding L-Pro^9 analogue 3 had extremely high selectivity for the NK-1 receptor. Introduction of an *R*-γ-lactam constraint between the Gly^9 and Leu^{10} residues in 2 gave 11, which also showed increased NK-2 agonist activity, similar to that of the D-Pro^9 analogue, although without significantly reducing NK-1 activity.

It was found that replacement of the Ava residue in 1 by the Lys-Asp-Ser peptide sequence, related to the N-terminal region of NKA, gave an NK-2 agonist 5 of equal potency to NKA but with only marginally increased selectivity with respect to the NK-1 receptor. However, introduction of an *R*-γ-lactam constraint into 5, to give analogue 12 (GR64349), markedly reduced the NK-1 agonist activity while maintaining the potent NK-2 agonist activity. GR64349 has proved to be a valuable pharmacological tool since, in addition to its high selectivity (>1000-fold) with respect to NK-1 receptors, it exhibits increased resistance to peptidases in biological preparations when compared with NKA.[17]

We hypothesized that the increase in NK-2 receptor potency of the D-Pro- and *R*-γ-lactam-constrained analogues, 4 and 11, was a result of a more positive entropy of binding, in that these compounds are prearranged by the added constraints into a conformation compatible with

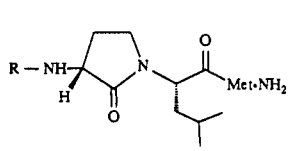
(17) Hagan, R. M.; Ireland, S. J.; Jordan, C. C.; Beresford, I. J. M.; Deal, M. J.; Ward, P. Receptor-Selective, Peptidase-Resistant Agonists at Neurokinin NK-1 and NK-2 Receptors: New Tools for Investigating Neurokinin Function. *Neuropeptides* **1991**, *19*, 127.

Table II. In Vitro Biological Activity

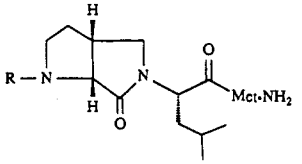
	EC_{50} values, nM		
	GPI (NK-1)	RC (NK-2)	RPV (NK-3)
SP	5.6 ± 0.7	167 ± 20	7500 ± 1200
NKA	13.7 ± 2.1	1.7 ± 0.3	563 ± 99
NKB	68 ± 28	27 ± 12	6.3 ± 1.3
1	86	94	NT
2	38	1190	11250
3	13.4 ± 2.4	13000	>10000
4	830 ± 410	92 ± 24	>30000
5	61	3.0	870
6	45	33	857
11	86	62	10600
12	4237 ± 1295	3.7 ± 0.6	1177 ± 446
13	44 ± 11	1.3 ± 0.3	NT
31	545	15	>18000
32	37700	1190	>8215
33	3732	566	>30000
34	5225	2104	>47000
35	1125	59	2722
36	1030	45	2627
37	307	1390	NT

that required to elicit agonist activity when bound to the NK-2 receptor. This suggestion is supported by the more rigid fused-lactam analogue **31**, which embodies both the D-Pro and *R*-γ-lactam constraints and has further increased NK-2 agonist potency. The torsion angles of the Gly^9 backbone bonds in **2**, which can be accessed by the D-Pro and *R*-γ-lactam constraints in **4** and **11**, are delineated by the broken lines in Figure 2. The fused-lactam constraint in **31** defines a small region of conformational space around $\phi = +75° \pm 20°$, $\psi = +140° \pm 10°$, which, based upon the above argument, approximates the preferred conformation of the Gly^9 residue of neurokinin agonists at the NK-2 receptor. The Pro^9 analogue **3** is consistent with this hypothesis in that it cannot access this conformation (Figure 2) and so is inactive as an NK-2 agonist. Furthermore, three stereoisomers (**32–34**) of the fused-lactam-constrained analogue **31** also exhibited markedly reduced NK-2 agonist activity.

The potent γ-lactam- and fused-lactam-containing analogues **12** and **31** both showed full intrinsic NK-2



12 (R = Lys·Asp·Ser·Phe·Val·)



31 (R = Ava·Phe·Phe·)

agonist activity relative to NKA in the rat colon, despite their pseudopeptide nature and increased structural rigidity in the C-terminal region. In our earlier study,[8b] the NK-1 selective pseudopeptide **37** containing an *R*-spirolactam constraint in positions 9 and 10 was a full agonist at the NK-1 receptor in guinea pig ileum, albeit much less potent than SP. Thus it appears that torsional flexibility around Gly^9 in tachykinin peptides is not a requirement for full intrinsic activity at neurokinin receptors.

Compound **35**, the fused-lactam-containing analogue of **5**, was less potent as an NK-2 agonist than **5** or **31**, indicating that the Lys-Asp-Ser N-terminal sequence is not optimal for this constraint. Interestingly, the replacement of Val^8 in **35** by Phe had no significant effect on potency (compound **36**). This result contrasts with the structure–activity relationship for the nonconstrained peptides **1**, **2**, **5**, and **6** in which Val confers at least 10-fold greater potency than Phe at position 8.

In summary, the γ-lactam-constrained NKA(3–10) analogue **12** (GR64349) is a potent NK-2 agonist with activity comparable to that of NKA, and much greater selectivity with respect to NK-1 (>1000-fold) and NK-3 receptors (>300-fold). These properties, together with the enhanced metabolic stability reported previously, recommend this compound for pharmacological studies in vitro and in vivo. Our subsequent investigation of four stereochemically defined fused-bicyclic constraints provides evidence of a highly stereospecific ligand–receptor interaction for analogue **31**, and further delineates a conformation for full agonist activation of the NK-2 receptor in which Gly^9 occupies the (+,+) region of ϕ,ψ space. The present study therefore extends and complements our earlier work[8b] in which the *R*-spirolactam constraint (see Figure 1) defined a Gly^9 conformation in the (−,+) region of the ϕ,ψ map compatible with full intrinsic agonist activity at the NK-1 receptor.

Experimental Section

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 focusing 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 core RP-300(octyl) aquapore column using eluants A [H_2O, 0.1% trifluoroacetic acid (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/min. Peptide purification was effected by preparative reverse-phase HPLC using a 2-in. diameter Dynamax ODS-2 column or a 1-in. TSK ODS-120T column using the same eluants described for analytical HPLC with an appropriately varied gradient at a flow rate of 45 mL/min (2 in. column) or 15 mL/min. Amino acid analyses were carried out on an LKB 4400 or a Biotronik LC5001 sequencer.

Non-peptide intermediates were characterized by NMR using a Varian XL200, Varian VXR400, or a Brucker AM250 spectrometer. Infrared spectra were recorded on Nicolet 5SXC or Nicolet 20SXB interferometers or Perkin Elmer 580B and 177 dispersive spectrometers. Elemental analyses were obtained for key intermediates on three elements, C, H, and N, and were within ±0.4% of the expected values. High-resolution mass spectra (HRMS) were also recorded for some intermediates. Melting points were recorded on a Riechert heated block apparatus and are uncorrected.

Symbols and abbreviations are in accord with recommendations of the IUPAC–IUB Joint Commission on Biochemical Nomenclature (*Biochem. J.* 1984, *219*, 345). Other abbreviations are as follows: Ac, acetyl; Boc, *tert*-butoxycarbonyl; Ava, δ-aminovaleryl (5-aminopentanoyl); DHBT, 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine; DMF, *N*,*N*-dimethylformamide; Fmoc, (9-fluorenylmethoxy)carbonyl; OPfp, pentafluorophenyl ester; O-*t*-Bu, *tert*-butyl ester; *t*-Bu, *tert*-butyl; TFA, trifluoroacetic acid.

General Method for Solid-Phase Synthesis of Peptides 1–4 and 11. These analogues were prepared using the Fmoc-polyamide solid-phase method following manual procedures similar to those reported by Atherton et al.[11,18] The dimethylacrylamide–ethylenebisacrylamide–acryloylsarcosine methyl ester copolymer (Cambridge Research Biochemicals; nominal ester functionality 1.0 mmol/g) was derivatized with ethylenediamine and elaborated to the base-labile linked peptide–resin Fmoc-Leu-Met-O-CH_2-*p*-C_6H_4-CO-Nle-resin as described pre-

(18) Atherton, E.; Logan, C. J.; Sheppard, R. C. Peptide Synthesis. Part 2. Procedures for Solid-Phase Synthesis Using N-α-Fluorenylmethoxycarbonylamino Acids on Polyamide Supports. Synthesis of Substance P and of Acyl Carrier Protein 65-74 Decapeptide. *J. Chem. Soc. Perkin Trans. 1* 1981, 538.

viously[18] (hereafter abbreviated as Fmoc-Leu-Met-O-resin). The Nle residue was incorporated as an internal standard for peptide–resin amino acid analysis. Fmoc deprotections were carried out by treatment with a solution of redistilled piperidine (20%) in DMF. Fmoc-protected amino acids were used throughout except for the N-terminal Ava residue, which was Boc-protected. Couplings were performed using preformed symmetrical anhydrides (3 molar equiv) in DMF solution for 1 h and monitored for completion by the ninhydrin test.[19] The Boc-Ava peptide was cleaved from the DMF-washed resin by treatment with a 0 °C solution of ammonia in dry methanol (200 mL/g of resin, saturated at 0 °C) followed by stirring at 20 °C in a stoppered vessel for 24 h. The residue after filtration and evaporation of the methanol extract was Boc-deprotected by treatment with TFA–ethanedithiol (98:2; 100 mL/g of resin) at 0 °C for 30 min. TFA was removed under reduced pressure and the residue partitioned between water and ether. The aqueous layer was freeze-dried to give the crude peptide which was then purified to >95% homogeneity by reverse-phase HPLC and characterized by amino acid analysis and FAB mass spectrometry (Table I).

Ava-Phe-Val-Gly-Leu-Met-NH_2 (1). Fmoc-Leu-Met-O-resin (0.225 g, 0.48 mmol/g) was Fmoc-deprotected and elaborated by successive 1-h acylations with preformed symmetrical anhydrides (0.45 mmol) of the following protected amino acids: Fmoc-Gly-OH, Fmoc-Val-OH, Fmoc-Phe-OH, Boc-Ava-OH. One-half of the assembled peptide–resin was cleaved by ammonolysis, and the product was Boc-deprotected and HPLC-purified to afford 1 (48 mg).

Ava-Phe-Phe-Gly-Leu-Met-NH_2 (2) was prepared by analogous methods to yield 28 mg.

Ava-Phe-Phe-Pro-Leu-Met-NH_2 (3) was similarly prepared from Fmoc-Leu-Met-O-resin (0.42 g, 0.5 mmol/g). One-half of the peptide–resin was processed to afford 71 mg of HPLC-purified peptide.

Ava-Phe-Phe-D-Pro-Leu-Met-NH_2 (4). Synthesis on the same scale as 3 above gave 59 mg of peptide (HPLC purity 98.8%).

Ava-Phe-Phe-Gly-[*R*-γ-Lac]-Leu-Met-NH_2 (11). Boc-Gly-[*R*-γ-Lac]-Leu-OH (9, 294 mg, 0.935 mmol) was treated with *N,N'*-dicyclohexylcarbodiimide (93 mg, 0.43 mmol) in dry dichloromethane (4 mL). After 10 min at 20 °C the resulting suspension was filtered and the filtrate evaporated. The residual symmetrical anhydride was taken up in DMF (3.5 mL) and added to H-Met-O-resin[18] (0.23 g, 0.5 mmol/g). After complete acylation (ninhydrin test, 2 h) the mixture was shaken for a further 1 h. After washing with DMF (×5), *tert*-amyl alcohol (×5), and acetic acid (×5), the Boc-peptide–resin was deprotected with 1.5 M HCl in acetic acid (5-min and 30-min treatments), washed with the reverse sequence of solvents, and neutralized with 10% *N,N*-diisopropylethylamine in DMF. The peptide resin was then washed (DMF) and elaborated by successive acylations with preformed symmetrical anhydrides (0.45 mmol) of the following protected amino acids: Fmoc-Phe-OH, Fmoc-Phe-OH, Boc-Ava-OH. One-half of the final peptide–resin was cleaved by ammonolysis and the product was Boc-deprotected and HPLC-purified to afford the title compound 11 (55 mg).

General Method for Solid-Phase Synthesis of Peptides 5, 6, 12, 13, 35, and 36. These analogues were prepared by the Fmoc-polyamide continuous-flow method[20] using a CRB Pepsynthesizer II semiautomatic synthesizer and following standard protocols supplied by the manufacturer. Couplings were performed using Fmoc-amino acid pentafluorophenyl esters[21] (3–4-fold excess; 50-min reaction) in the presence of DHBT catalyst. Fmoc deprotections were carried out with 20% piperidine in DMF (15-min flow). Kieselguhr-supported dimethylacrylamide–ethylenebisacrylamide–acryloylsarcosine methyl ester copolymer (Pepsyn K, Cambridge Research Biochemicals, sarcosine content 0.25 mmol/g, 16.5 g) was loaded into a 25 cm × 25 mm column and treated by recirculation on the Pepsynthesizer with ethylenediamine for 20 h and then washed with DMF (1.5 h), 10% *N,N*-diisopropylethylamine 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 mol equiv) in DMF (20 mL). After recirculation for 1 h, the reagents were washed out (DMF, 1 h) and the Fmoc group removed. 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 ether and dried in vacuo (17.3 g, 0.23 mmol/g by amino acid analysis). This material, denoted as HO-resin, was used for synthesis of peptides 12 and 13. Peptides 5 and 6 were prepared using an equivalent commercially available functionalized Kieselguhr resin (Pepsyn KB, 0.08 mmol/g). In all preparations, the first residue (Met) was coupled using 2 × 1-h treatments with the preformed symmetrical anhydride (6 mol equiv) from Fmoc-Met-OH in the presence of 4-(dimethylamino)pyridine (1 mol equiv). Ser, Asp, and Lys residues were incorporated using Fmoc-Ser(*t*-Bu)-OPfp, Fmoc-Asp(O-*t*-Bu)-OPfp and Fmoc-Lys(Boc)-OPfp, respectively.

Side-Chain Deprotection and Cleavage from the Solid Support. The fully assembled resin-linked peptide was Fmoc-deprotected, washed (DMF, *tert*-amyl alcohol, acetic acid, *tert*-amyl alcohol, DMF, and ether), and dried in vacuo. Side-chain protecting groups were removed by treatment either with TFA–ethanedithiol (98:2) or N_2-degassed TFA–water (95:5) for 0.5 h at 20 °C, and the peptide–resin was filtered off, washed as above, and dried in vacuo. The peptide was cleaved from the solid support by treatment with acetic acid (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). 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 I).

Lys-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2 (5) was synthesized on Pepsyn KB (1.6 g, 0.08 mmol/g) following the general method. Reverse-phase HPLC purification gave 17 mg of product.

Lys-Asp-Ser-Phe-Phe-Gly-Leu-Met-NH_2 (6) was prepared analogously to 5 on the same scale to obtain 50 mg of pure product.

Lys-Asp-Ser-Phe-Val-Gly-[*R*-γ-Lac]-Leu-Met-NH_2 (12). Fmoc-Met was coupled to the linker-functionalized support (HO-resin, 3.0 g, 0.23 mmol/g; see the general method). Following Fmoc deprotection, a solution of DHBT (0.37 g) and Boc-Gly-[*R*-γ-Lac]-Leu-OPfp (10, 0.994 g) in DMF (5 mL) was applied to the reaction column and recirculated until acylation was complete (ca. 1 h). The reagents were washed out (DMF, *tert*-amyl alcohol, acetic acid, successively) and a saturated solution of HCl in acetic acid was then passed through the column. After a 0.5-h contact time, the column was washed with the above solvents in reverse order and the terminal amino group neutralized by treatment with 10% *N,N*-diisopropylethylamine in DMF. The peptide assembly was completed following the general method and after side-chain deprotection (95:5 TFA–H_2O under N_2), ammonolysis and HPLC purification the title compound 12 (370 mg) was obtained.

Ac-Lys-Asp-Ser-Phe-Val-Gly-[*R*-γ-Lac]-Leu-Met-NH_2 (13) was synthesized on Pepsyn KB (1.0 g, 0.2 mmol/g) following methods analogous to those for 12 above, except that after Fmoc deprotection of the Lys residue, the terminal amino group was acetylated by treatment with a solution of acetic anhydride (0.14 mL) in DMF (1 mL). Preparative HPLC gave pure 13 (98 mg).

Lys-Asp-Ser-Phe-Val-D-Pro-[γ-Lac]-Leu-Met-NH_2 (35) was synthesized on Pepsyn K (2 g, 0.09 mmol/g) following the general method (DHBT catalysis). The fused lactam pseudodipeptide was incorporated using Fmoc-D-Pro-[γ-Lac]-OPfp (**25a**, 0.251 g) in the presence of DHBT (0.063 g). Approximately one-half of the peptide–resin was deprotected and cleaved to give 64 mg of crude product (major HPLC component 72%) which was purified by HPLC to afford the title compound 35 (33 mg).

Lys-Asp-Ser-Phe-Phe-D-Pro-[γ-Lac]-Leu-Met-NH_2 (36) was synthesized analogously to 35 above. The crude product

(19) Kaiser, E. T.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. Color Test for Detection of Free Terminal Amino Groups in the Solid-Phase Synthesis of Peptides. *Anal. Biochem.* **1970**, *34*, 595.

(20) Dryland, A.; Sheppard, R. C. Peptide Synthesis. Part 8. A System for Solid-Phase Synthesis Under Low Pressure Continuous Flow Conditions. *J. Chem. Soc. Perkin Trans. 1* **1986**, 125.

(21) Atherton, E.; Sheppard, R. C. Solid-Phase Peptide Synthesis Using N-α-Fluorenylmethoxycarbonylamino Acid Pentafluorophenyl Esters. *J. Chem. Soc., Chem. Commun.* **1985**, 165.

(major HPLC component 78%) was purified by HPLC to afford the title compound (30 mg, 98% pure by HPLC).

General Methods for Solution-Phase Synthesis of Peptides 31–34 (See Scheme IV). Removal of the *tert*-Butyloxycarbonyl Protecting Group. A saturated solution of hydrogen chloride in acetic acid was prepared by bubbling hydrogen chloride gas through acetic acid (100 mL) for 30 min at room temperature. The Boc-protected peptide (1 mmol) was dissolved in 30 mL of this solution and stirred at room temperature under nitrogen for 2 h before concentrating in vacuo. The residue was redissolved in water (20 mL) and the resulting solution was freeze-dried overnight to give the required amine hydrochloride salt as an amorphous solid which was used without purification in the next coupling step. (Note: it is essential to remove all occluded acetic acid to avoid acetylation in subsequent coupling reactions.)

Peptide Coupling with Boc-Protected Amino Acid Pentafluorophenyl Esters. A mixture of the intermediate peptide amine hydrochloride salt (1 mmol), the appropriate Boc-protected amino acid pentafluorophenyl ester (2 mmol), triethylamine (1.5 mL, 1.1 mmol), and dimethylformamide (3 mL) was stirred at room temperature under nitrogen for 3 h. 4-(2-Aminoethyl)-morpholine (2.62 mL, 2 mmol) was then added and stirring was continued for 1 h before dilution with ethyl acetate (60 mL). The resulting solution was washed with 10% aqueous citric acid (2 × 40 mL), 10% aqueous Na_2CO_3 (2 × 40 mL), and brine (40 mL), and then dried ($MgSO_4$) and concentrated in vacuo to give the required peptide, which was used without purification at intermediate stages of the peptide assembly.

Boc-D-Met-Leu Methyl Ester (7). A mixture of Boc-D-Met-OH (8.6 g, 34.5 mmol), dicyclohexylcarbodiimide (7.83 g, 37.9 mmol), 1-hydroxybenzotriazole (5.81 g, 37.9 mmol), and dimethylformamide (50 mL) was stirred at 20 °C under nitrogen for 10 min. Leu-OMe·HCl (6.3 g, 34.5 mmol) was then added followed by triethylamine (7.0 mL, 50 mmol) and stirring was continued for 16 h. Ethyl acetate (200 mL) was added and stirring continued for 30 min before filtration. The solids were washed with 10% aqueous citric acid (3 × 100 mL), 10% aqueous $NaHCO_3$ (3 × 100 mL), and brine (100 mL) and then dried ($MgSO_4$) and concentrated in vacuo to give an oil (13.0 g, 100%): ^{1}H NMR (DMSO-d_6) δ 0.87 (3 H, d, J = 6 Hz, CH_3CH), 0.92 (3 H, d, J = 6 Hz, CH_3CH), 1.42 (9 H, s, t-Bu), 1.3–1.9 (5 H, m), 2.07 (3 H, s, SCH_3), 2.56 (2 H, t, J = 7 Hz, SCH_2), 3.66 (3 H, s, OCH_3), 4.18 (1 H, m, BocNHCH), 4.34 (1 H, m, CHCO$_2$Me), 6.95 (1 H, d, J = 9 Hz, BocNH), 8.20 (1 H, d, J = 9 Hz, NH); $[\alpha]^{20}_D$ −5.36° (c = 9 mg/mL, MeOH); IR ($CHBr_3$) 1676, 1698, 1737 cm^{-1}. Anal. ($C_{17}H_{32}N_2O_5S$) C, H, N.

(*R*)-2-[3-[(*tert*-Butoxycarbonyl)amino]-2-oxo-1-pyrrolidinyl]-(*S*)-4-methylpentanoic Acid Methyl Ester (8). A solution of Boc-D-Met-Leu-OMe (7) (12.8 g, 34 mmol) in methyl iodide (80 mL; **caution: severe carcinogen**) was allowed to stand for 5 days in the dark before concentrating. The residue was dissolved in CH_2Cl_2 (100 mL) and then reconcentrated; this procedure was repeated four times. The resulting foam was dried in vacuo and then dissolved in dry dimethylformamide (700 mL). This solution was cooled to −40 °C before adding sodium hydride (2.87 g of a 50% dispersion in mineral oil, 60 mmol). The reaction mixture was allowed to warm to −5 ± 3 °C for 45 min before quenching by the careful addition of saturated aqueous NH_4Cl (1200 mL, caution vigorous gas evolution). The reaction mixture was diluted with water (120 mL), 10% aqueous Na_2CO_3 (100 mL) was added, and the resulting mixture was extracted with ethyl acetate (2 × 500 mL). These extracts were washed with water (2 × 300 mL), dried ($MgSO_4$), and concentrated to give an oil (11.0 g). Recrystallization from ethyl acetate–cyclohexane gave 8 (3.9 g, 39%) as a white solid: mp 123–124 °C; ^{1}H NMR ($CDCl_3$) δ 0.92 (3 H, d, CH_3CH), 0.97 (3 H, d, CH_3CH), 1.3–1.6 (1 H, m, (CH$_3$)$_2$CH), 1.45 (9 H, s, t-Bu), 1.6–1.9 (3 H, m), 2.70 (1 H, m), 3.2–3.5 (3 H, m), 3.71 (3 H, s, CO$_2$CH$_3$), 4.22 (1 H, m, NHCH), 4.88 (1 H, m, CHCO$_2$Me), 5.12 (1 H, m, NH); $[\alpha]^{20}_D$ +7.17° (c = 9 mg/mL, MeOH); IR ($CHBr_3$) 1696.6 cm^{-1}. Anal. ($C_{16}H_{28}N_2O_5$) C, H, N.

(R)-2-[3-[(*tert*-Butoxycarbonyl)amino]-2-oxo-1-pyrrolidinyl]-(*S*)-4-methylpentanoic Acid (9). Aqueous NaOH (2 M, 15 mL, 30 mmol) was added to a solution of 8 (3.7 g, 12.5 mmol) in methanol (100 mL) at 0 °C and the reaction mixture was allowed to stand at 0 °C for 18 h before dilution with water (200 mL), washing with ethyl acetate (100 mL), acidification to pH = 2 with 2 M aqueous HCl and extraction with ethyl acetate (3 × 100 mL). These extracts were dried ($MgSO_4$) and concentrated to give 9 (3.12 g, 94%) as a white solid: mp 165–169 °C (lit.[22] 162–165 °C) (from EtOAc–cyclohexane); ^{1}H NMR (DMSO-d_6) δ 0.84 (3 H, m, CH_3CH), 0.88 (3 H, m, CH_3CH), 1.37 (9 H, s, t-Bu), 1.3–1.9 (4 H, m), 2.25 (1 H, m), 3.1–3.4 (2 H, m), 4.09 (1 H, m, NHCH), 4.55 (1 H, m, CHCO$_2$H), 7.17 (1 H, d, J = 9 Hz, NH); $[\alpha]^{20}_D$ +22.65° (lit.[22] $[\alpha]^{24}_D$ +22.1°) (c = 8 mg/mL, MeOH); IR ($CHBr_3$) 1694, 1737 cm^{-1}. Anal. ($C_{15}H_{26}N_2O_5$) C, H, N. The same procedure was carried out on the mother liquors from recrystallization of 8 to give a further identical batch of 9 (2.91 g).

(*R*)-2-[3-[(*tert*-Butoxycarbonyl)amino]-2-oxo-1-pyrrolidinyl]-(*S*)-4-methylpentanoic Acid Pentafluorophenyl Ester (10). Dicyclohexylcarbodiimide (1.81 g, 8.8 mmol) was added to a stirred solution of 9 (2.53 g, 8 mmol) and pentafluorophenol (1.62 g, 8.8 mmol) in DMF (25 mL) and the mixture was stirred at 20 °C under nitrogen for 3 h before concentration in vacuo. The residue was suspended in ethyl acetate (150 mL) and filtered, and the filtrate washed with 10% aqueous citric acid (2 × 50 mL), saturated aqueous $NaHCO_3$ (2 × 50 mL), and water (50 mL) and then dried and concentrated. The residual oil was purified by flash chromatography (70 g SiO_2) eluted with EtOAc–petroleum ether (1:4) to give 10 (2.2 g, 57%) as a pale yellow oil which crystallized on standing: mp 58–61 °C; ^{1}H NMR ($CDCl_3$) δ 0.98 (3 H, d, J = 6 Hz, CH_3CH), 1.02 (3 H, d, J = 6 Hz, CH_3CH), 1.4–1.7 (1 H, m, CH(CH$_3$)$_2$), 1.7–2.0 (3 H, m), 2.72 (1 H, m), 3.3–3.6 (2 H, m), 4.26 (1 H, m, NHCH), 5.14 (1 H, m, NHCH$_2$), 5.28 (1 H, dd, J = 9, 7 Hz, CHCO$_2$); $[\alpha]^{20}_D$ −9.45° (c = 10 mg/mL, MeOH); IR ($CHBr_3$) 1697 cm^{-1}.

(1′*S*)-3-[[[1-(Methoxycarbonyl)-3-methylbutyl]amino]-methyl]-1*H*-pyrrole-2-carboxylic Acid Ethyl Ester, with 25% *R* Isomer (15a). To a solution of L-leucine methyl ester hydrochloride (50 g, 0.275 mmol) in water (60 mL) was added Raney nickel catalyst (~1 g). A solution of the cyanopyrrole 14[13] (9.5 g, 0.050 mmol) in ethanol (60 mL) was added and the mixture hydrogenated at 100 psi in a Parr hydrogenator for 48 h. The catalyst was removed by filtration through Celite and washed with 50% aqueous ethanol (3 × 50 mL) and water (50 mL). The filtrate and washings were evaporated to dryness and after adjustment of the pH to 8 with 1 M NaOH solution, the mixture was extracted with ether (3 × 70 mL) and the extract washed with water (80 mL), dried over sodium sulfate, and evaporated to leave an oil which was chromatographed on silica (400 g) eluted with hexane–ethyl acetate (70:30) to give the product 15a (12.2 g, 71%): ^{1}H NMR ($CDCl_3$) δ 0.83 (3 H, d, J = 7 Hz), 0.88 (3 H, d, J = 7 Hz), 1.33 (3 H, t, J = 7 Hz, OCH$_2$CH_3), 1.46 (2 H, t, J = 7 Hz), 1.68 (1 H, m), 2.02 (1 H, s, NH), 3.33 (1 H, t, J = 7 Hz, NHCH), 3.65 (3 H, s, CO$_2$CH_3), 3.82 and 3.94 (2 H, AB q, J = 14 Hz, CH_2NH), 4.30 (2 H, q, J = 7 Hz, OCH_2), 6.22 (1 H, t, J = 3 Hz, H$_1$), 6.82 (1 H, t, J = 3 Hz, H$_5$), 9.24 (1 H, broad s, NH); a chiral shift reagent study using tris[3-[(heptafluoropropyl)hydroxymethylene]-(+)-camphorato]europium(III) revealed a 3:1 mixture of enantiomers—see the text; IR ($CHBr_3$) 1688, 1730, 3450 cm^{-1}. Anal. ($C_{20}H_{32}N_2O_6$) C, H, N.

(1′*R*)-3-[[[1-(Methoxycarbonyl)-3-methylbutyl]amino]-methyl]-1*H*-pyrrole-2-carboxylic Acid Ethyl Ester, with 10% *S* Isomer (15b). Method A. D-Leucine methyl ester hydrochloride and cyanopyrrole 14, as described for 15a but with more vigorous stirring of the hydrogenation reaction, gave 15b (61%) as a colorless oil: ^{1}H NMR identical to that of 15a; a chiral shift reagent study showed a 9:1 mixture of enantiomers with the major enantiomer the same as the minor enantiomer of 15a.

Method B. Raney nickel (~1 g slurry in water) was added to a solution of D-leucine methyl ester hydrochloride (20 g, 110 mmol) in pH 7 buffer (50 mL). A solution of 14 (3.6 g, 22 mmol) in ethanol (40 mL) was added and the mixture was hydrogenated at 100 psi in a Parr pressure vessel with vigorous stirring for 24 h. The catalyst was removed by filtration under nitrogen, washed with ethanol and water, and the filtrate was evaporated to dryness. The crude product was purified by chromatography on silica

(22) Merck & Co. Inc. Analogs of Substance P and Eledoisin. European Patent Application 176436 A2, 1986.

(250 g) eluting with ethyl acetate–cyclohexane (2:3) to give **15b** (3.8 g, 59%) as a colorless oil. A chiral shift reagent investigation as for **15a** showed a ca. 12:1 mixture of enantiomers.

(1′*S*)-1-[(1,1-Dimethylethoxy)carbonyl]-3-[[[1-(methoxycarbonyl)-3-methylbutyl]amino]methyl]-1*H*-pyrrole-2-carboxylic Acid Ethyl Ester, with 25% *R* Isomer (16a). A solution of the pyrrole **15a** (12.2g, 0.041 mol) was dissolved in acetonitrile (370 mL), and di-*tert*-butyl dicarbonate (9.92 g, 0.045 mol) and 4-(dimethylamino)pyridine (0.5 g, 4 mmol) were added. After stirring for 24 h, the mixture was evaporated to leave an orange oil which was chromatographed on silica to yield a pale yellow oil, **16a** (13.0 g, 80%): ^{1}H NMR ($CDCl_3$) δ 0.82 (3 H, d, J = 7 Hz), 0.89 (3 H, d, J = 7 Hz), 1.31 (3 H, t, J = 7 Hz, OCH_2CH_3), 1.43 (2 H, t, J = 7 Hz), 1.52 (9 H, s, O-*t*-Bu), 1.70 (1 H, m), 1.87 (1 H, s, NH), 3.26 (1 H, t, J = 7 Hz, NHC*H*), 3.67 (3 H, s, CO_2CH_3), 3.57 and 3.73 (2 H, AB q, J = 14 Hz, CH_2NH), 4.29 (2 H, q, J = 7 Hz, OCH_2CH_3), 6.18 (1 H, d, J = 3 Hz, H_4), 7.17 (1 H, d, J = 3 Hz, H_5); IR ($CHBr_3$) 1734 (broad), 3330 cm^{-1}. Anal. ($C_{20}H_{32}N_2O_6$) C, H, N.

(1′*R*)-1-[(1,1-Dimethylethoxy)carbonyl]-3-[[[1-(methoxycarbonyl)-3-methylbutyl]amino]methyl]-1*H*-pyrrole-2-carboxylic acid ethyl ester (16b), with 10% *S* isomer, was prepared from **15b** as described above (68%): ^{1}H NMR and IR identical to those of **16a**. Anal. ($C_{20}H_{32}N_2O_6$) C, H, N.

[2*R*-[2α,3α(*S)]]-1-[(1,1-Dimethylethoxy)carbonyl]-3-[[[1-(methoxycarbonyl)-3-methylbutyl]amino]methyl]-2-pyrrolidinecarboxylic Acid Ethyl Ester, with 25% 2*S*[2α,3α(*S**)] Isomer (17a).** The Boc-protected pyrrole **16a** (7.5g, 0.019 mol) was dissolved in methanol (50 mL) and the solution added to 5% rhodium on alumina (~1 g) in a Parr hydrogenator. The mixture was hydrogenated at 300 psi with vigorous stirring for 24 h, and then the catalyst was removed by filtration and washed with methanol. The combined filtrate and washings were evaporated to leave an oil which was purified by column chromatography on silica eluted with hexane–ethyl acetate (80:20) to yield **17a** (1.76 g, 23%): ^{1}H NMR ($CDCl_3$) 3:1 rotameric mixture, δ 0.91 (3 H, d, J = 7 Hz), 0.94 (3 H, d, J = 7 Hz), 1.28 and 1.30 (3 H, 2 × t, J = 7 Hz, OCH_2CH_3), 1.41 and 1.46 (9 h, 2 × s, O-*t*-Bu), 1.46–1.95 (6 H, m), 2.32 (1 H, m, NHC*H*), 2.60 (2 H, m, CH_2NH), 3.2–3.4 (2 H, m), 3.65 (1 H, m, NHC*H*), 3.70 (3 H, s, CO_2CH_3), 4.20 (2 H, m, $CO_2CH_2CH_3$), 4.28 and 4.35 (1 H, 2 × d, J = 7 Hz, $CHCO_2$-Et); IR ($CHBr_3$) 1688, 1730, 3350 cm^{-1}. Anal. ($C_{20}H_{36}N_2O_6$) C, H, N.

[2*S*-[2α,3α(*R)]]-1-[(1,1-Dimethylethoxy)carbonyl]-3-[[[1-(methoxycarbonyl)-3-methylbutyl]amino]methyl]-2-pyrrolidinecarboxylic Acid Ethyl Ester, with 25% 2*R*[2α,3α(*R**)] Isomer (18a).** After isolation of **17a**, further elution of the column gave isomer **18a** (2.02 g, 27%) along with a fraction containing a mixture of isomers (1.80 g, 24%): ^{1}H NMR ($CDCl_3$) 3:1 rotameric mixture, δ 1.25_{minor} and 1.28_{major} (3 H, 2 × t, J = 7 Hz, $CO_2CH_2CH_3$), 1.40_{major} and 1.46_{minor} (9 H, 2 × s, *t*-Bu), 1.4–2.1 (6 H, m), 2.50 (3 H, m), 3.2–3.4 (2 H, m), 3.65 (1 H, m, NHC*H*), 3.70 (3 H, s, CO_2CH_3), 4.15 (2 H, m, $CO_2CH_2CH_3$), 4.28_{major} and 4.36_{minor} (1 H, 2 × d, J = 7 Hz, $CHCO_2Et$); IR ($CHBr_3$) 1688, 1730, 3300 cm^{-1}. Anal. ($C_{20}H_{36}N_2O_6$) C, H, N.

[2*S*-[2α,3α(*S)]]-1-[(1,1-Dimethylethoxy)carbonyl]-3-[[[1-(methoxycarbonyl)-3-methylbutyl]amino]methyl]-2-pyrrolidinecarboxylic Acid Ethyl Ester, with 10% 2*R*[2α,3α(*S**)] Isomer (17b), and [2*R*-[2α,3α(*R**)]]-1-[(1,1-dimethylethoxy)carbonyl]-3-[[[1-(methoxycarbonyl)-3-methylbutyl]amino]methyl]-2-pyrrolidinecarboxylic Acid Ethyl Ester, with 10% 2*S*[2α,3α(*R**)] Isomer (18b).** Isomers **17b** and **18b** were prepared in a similar manner as the above from **16b**. Isomer **17b**: ^{1}H NMR ($CDCl_3$) identical to that of **17a**. Anal. ($C_{20}H_{36}N_2O_6$) C, H, N. Isomer **18b**: ^{1}H NMR ($CDCl_3$) identical to that of **18a**. Anal. ($C_{20}H_{36}N_2O_6$) C, H, N.

[3a*R*(3aα,6aα,2′*S)]-1-[(1,1-Dimethylethoxy)carbonyl]octahydro-α-(2-methylpropyl)-6-oxopyrrolo[3,4-*b*]pyrrole-5-acetic Acid Methyl Ester, with 25% 3a*S*(3aα,6aα,2′*S**) Isomer (19a). Method A.** A solution of the amino diester (**17a**, 1.9 g, 4.8 mmol) in triethylamine (20 mL) was refluxed for 48 h, after which time the solvent was removed and the residue taken up in ethyl acetate, washed with 1 M HCl, water, brine then dried and concentrated to yield a pale yellow oil (1.6 g). Trituration with ether–hexane followed by filtration gave **19a** (0.75 g, 47%) as a white solid; ^{1}H NMR (DMSO-d_6, 100 °C) δ 0.87 (3 H, d, J = 7 Hz), 0.92 (3 H, d, J = 7 Hz), 1.41 (9 H, s, O-*t*-Bu), 1.47 (1 H, m, $CH(CH_3)_2$), 1.5–1.8 (3 H, m, $CH_2CH(CH_3)_2$ + H_{3A}), 2.14 (1 H, m, H_3), 2.93 (1 H, m, H_{3a}), 3.08 (1 H, dd, J = 10, 2 Hz, H_{4A}), 3.14 (1 H, m, H_{2A}), 3.41 (1 H, d, J = 10, 7 Hz, H_{2B}), 3.53 (1 H, dd, J = 10, 6 Hz, H_{4B}), 3.66 (3 H, s, CO_2CH_3), 4.43 (1 H, d, J = 8 Hz, H_{6a}), 4.59 (1 H, dd, J = 10, 6 Hz, $CHCO_2Me$); IR ($CHBr_3$) 1690, 1740 cm^{-1}; HRMS calcd for $C_{18}H_{30}N_2O_3$ 355.2233, found 355.2237.

Method B. A solution of the amino diester (**17a**, 1.7 g, 4.24 mmol) in methanol (10 mL) and pH 4.7 buffer (10 mL) was refluxed for 18 h then diluted with water and extracted into ether. The organic layer was washed with 2 M HCl, water, and brine and then dried and concentrated to leave a brown oil. This was purified by column chromatography on silica eluted with hexane–ethyl acetate (70:30) to yield **19a** (1.05 g, 70%).

Crystal Structure Analysis of 19a. A sample of **19a** prepared as above (method A) was crystallized from ether–hexane. A crystal of dimensions 0.10 × 0.10 × 0.15 mm was used.

Crystal data: $C_{18}H_{30}N_2O_5$, M = 354.4, orthorhombic, a = 12.305 (2) Å, b = 16.932 (4) Å, c = 19.315 (3) Å, V = 4024 Å^3, space group *Pnab*, Z = 8, D_c = 1.17 g cm^{-3}, Cu radiation, λ = 1.54178 Å, μ(Cu-Kα) = 7 cm^{-1}, F(000) = 1536. Data were measured on a Nicolet R3m diffractometer with Cu-Kα radiation (graphite monochromator) using ω-scans. A total of 2053 independent reflections ($2\theta \leq 100°$) were measured, of which 1145, had $|F_o| > 3\sigma(|F_o|)$, and were considered to be observed. The data were corrected for Lorentz and polarization factors; no absorption correction was applied. The structure was solved by direct methods. The non-hydrogen atoms were refined anisotropically. The positions of the hydrogen atoms were idealized, C–H = 0.96 Å; assigned isotropic thermal parameters, U(H) = 1.2U_{eq}(C); and allowed to ride on their parent carbon atoms. The methyl groups were refined as rigid bodies. Refinement was by block-cascade full-matrix least-squares to R = 0.098, R_ω = 0.095 [$\omega^{-1} = \sigma^2(F) + 0.00180F^2$]. The maximum and minimum residual electron densities in the final ΔF map were 0.35 and −0.34 eÅ^{-3}, respectively. The mean and maximum shift/error in the final refinement were 0.028 and 0.124, respectively. Computations were carried out on an Eclipse S140 computer using the SHELXTL program system. Tables of atom coordinates, bond lengths, bond angles, anisotropic temperature factors and hydrogen coordinates are available as supplementary material.

[3a*S*(3aα,6aα,2′*R)]-1-[(1,1-Dimethylethoxy)carbonyl]octahydro-α-(2-methylpropyl)-6-oxopyrrolo[3,4-*b*]pyrrole-5-acetic acid methyl ester, with 25% 3a*R* (3aα,6aα,2′R*) isomer (20a),** was prepared in a manner similar to that of **19a** (method B) starting from **18a** (73%); ^{1}H NMR (DMSO-d_6, 100 °C) δ 0.88 (3 H, d, J = 6 Hz), 0.92 (3 H, d, J = 6 Hz), 1.42 (9 H, s, O-*t*-Bu), 1.52 (1 H, m, $CH(CH_3)_2$), 1.6–1.8 (3 H, m, $CH_2CH(CH_3)_2$ + H_{3A}), 2.11 (1 H, m, H_{3B}), 2.90 (1 H, m, H_{3a}), 3.11 (1 H, dd, J = 10, 2 Hz, H_{4A}), 3.17 (1 H, m, H_{2A}), 3.39 (1 H, dt, J = 10, 7 Hz, H_{2B}), 3.48 (1 H, dd, J = 10, 6 Hz, H_{4B}), 3.65 (3 H, s, CO_2CH_3), 4.47 (1 H, d, J = 8 Hz, H_{6a}), 4.59 (1 H, dd, J = 9, 6 Hz, $CHCO_2Me$); IR ($CHBr_3$) 1700, 1735 cm^{-1}.

[3a*S*(3aα,6aα,2′*S)]-1-[(1,1-Dimethylethoxy)carbonyl]octahydro-α-(2-methylpropyl)-6-oxopyrrolo[3,4-*b*]pyrrole-5-acetic acid methyl ester, with 10% 3a*R*(3aα,6aα,2′*S**) isomer (19b), and [3a*R*(3aα,6aα,2′*R**)]-1-[(1,1-dimethylethoxy)carbonyl]octahydro-α-(2-methylpropyl)-6-oxopyrrolo[3,4-*b*]pyrrole-5-acetic acid methyl ester, with 10% 3a*S*(3aα,6aα,2′*R**) isomer (20b),** were prepared in a manner similar to that of **19a** (method B) and **20a** starting from **17b** and **18b**. The corresponding diastereoisomers were identical by ^{1}H NMR and HRMS.

[3a*R*(3aα,6aα,2′*S)]-Octahydro-α-(2-methylpropyl)-6-oxopyrrolo[3,4-*b*]pyrrole-5-acetic Acid Methyl Ester, with 25% 3a*S*(3aα,6aα,2′*S**) Isomer (21a).** The Boc-protected pyrrolidine **19a** (1 g) was stirred in HCl/AcOH (10 mL) at room temperature for 1 h, whereupon the solvents were removed and on trituration with ether yielded a solid which was filtered off and washed with ether to leave the hydrochloride salt as a white solid (0.8 g, 100%); ^{1}H NMR ($CDCl_3$) δ 0.90 (6 H, d, J = 7 Hz, $(CH_3)_2CH$), 1.44 (1 H, m, $(CH_3)_2CH$), 1.70 (2 H, t, J = 7 Hz), 1.78 (1 H, m, H_{3A}), 2.48 (1 H, m, H_{3B}), ca. 3.20 (1 H, m, H_{3a}), 3.3–3.6 (2 H, m, H_{2A} and H_{2B}), 3.17 (1 H, d, J = 10 Hz, H_{4A}), 3.68 (3 H,

s, CO_2CH_3), 3.78 (1 H, dd, J = 10, 7 Hz, H_{4B}), 4.72 (2 H, m, H_{6a} + $CHCO_2Me$), 9.20 (2 H, broad s, NH_2^+).

[3a*S*(3aα,6aα,2'*R)]-Octahydro-α-(2-methylpropyl)-6-oxopyrrolo[3,4-*b*]pyrrole-5-acetic acid methyl ester, with 25% 3a*R*(3aα,6aα,2'*R**) isomer (22a),** was prepared in a similar manner from isomer **20a** (71%): ^1H NMR (D_2O) δ 0.88 (3 H, d, J = 7 Hz), 0.93 (3 H, d, J = 7 Hz), 1.50 (1 H, m, $CH(CH_3)_2$), 1.80 (2 H, m, $CH_2CH(CH_3)_2$), 2.00 (1 H, m, H_{3A}), 2.44 (1 H, m, H_{3B}), 3.2–3.4 (3 H, m, H_{3a} + H_{2A} + H_{2B}), 3.45 (1 H, d, J = 10 Hz, H_{4A}), 3.76 (3 H, s, CO_2CH_3), 3.81 (1 H, dd, J = 10, 7 Hz, H_{4B}), 4.72 (2 H, m, H_{6a} + $CHCO_2Me$).

[3a*R*(3aα,6aα,2'*S)]-1-[(9-Fluorenylmethoxy)carbonyl]-octahydro-α-(2-methylpropyl)-6-oxopyrrolo[3,4-*b*]pyrrole-5-acetic Acid Pentafluorophenyl Ester, with 25% 3a*S*(3aα,-6aα,2'*S*) Isomer (25a).** The amino ester **21a** (821 mg, 2.82 mmol) in methanol (15 mL) was cooled in ice and then 2 M NaOH solution (4.25 mL, 8.5 mmol) was added and the mixture brought up to room temperature overnight. The solution was evaporated to low bulk to remove methanol and then sodium carbonate (647 mg, 6.1 mmol) was added followed by a partial suspension of Fmoc-OSu (1.22 g, 4.2 mmol) in acetone (10 mL). The mixture was left at room temperature for 4 h and then brought to pH 2 with 10% citric acid solution and extracted with ethyl acetate. The extract was washed with water and brine and then dried and evaporated to dryness to yield a solid which on further treatment with ethyl acetate and filtering gave a white solid, **23a** (1.2 g, 91%).

To a solution of the Fmoc-protected acid **23a** (1.19 g, 2.56 mmol) in dichloromethane (20 mL) and DMF (0.5 mL) were added pentafluorophenol (489 mg, 2.65 mmol) and DCC (547 mg, 2.65 mmol), and the mixture was left at room temperature for 2 h. The mixture was then filtered to remove DCU and, the filtrate and washings were evaporated to leave a solid which was dissolved in ethyl acetate (50 mL) and left for 1 h. The solid was filtered off and washed with more ethyl acetate, and then the combined filtrate and washings were evaporated to dryness. This solid was subjected to column chromatography on silica eluting with EtOAc–hexane (1:2) to yield an oil which was crystallized from EtOAc–hexane to yield a white solid, **25a** (661 mg, 41%): mp 135–145 °C; ^1H NMR ($CDCl_3$) δ 1.02 (6 H, d, J = 7 Hz), 1.5–2.0 (5 H, m), 3.00 (1 H, m, H_{3a}), 3.20 (1 H, d, J = 10 Hz, H_{4A}), 3.4–3.7 (2 H, m, H_{2A} and H_{2B}), 3.76 (1 H, m, H_{4B}), 4.0–4.7 (3 H, m), 4.74 (1 H, d, J = 9 Hz, H_{6a}), 5.28 (1 H, m, $CHCO_2Pfp$), 7.34 (4 H, m), 7.61 (1 H, m), 7.75 (3 H, m); IR (Nujol) 1700, 1790 cm^{-1}.

[3a*S*(3aα,6aα,2'*R)]-1-[(9-Fluorenylmethoxy)carbonyl]-octahydro-α-(2-methylpropyl)-6-oxopyrrolo[3,4-*b*]pyrrole-5-acetic acid pentafluorophenyl ester, with 25% 3a*R*(3aα,-6aα,2'*R**) isomer (26a),** was prepared in a similar manner from isomer **22a** (66%) mp 157–158 °C; ^1H NMR (DMSO-d_6) rotameric mixture, δ 0.96 (6 H, m), 1.5–2.0 (4 H, m), 2.18 (1 H, m, H_{3A}) 3.03 and 2.93 (1 H, m, H_{3a}), 3.12 and 3.18 (1 H, 2 × d, J = 10 Hz, H_{4A}), 3.2–3.5 (2 H, m, H_{2A} + H_{2B}), 3.60 (1 H, m, H_{4B}), 4.30 (3 H, m), 4.66 and 4.78 (1 H, 2 × d, J = 8 Hz, H_{6a}), 5.05 and 5.16 (1 H, 2 × m, $CHCO_2Pfp$), 7.2–7.5 (4 H, m), 7.66 (1 H, d, J = 8 Hz), 7.83 (1 H, d, J = 8 Hz), 7.90 (2 H, d, J = 8 Hz); IR ($CHBr_3$) 1705, 1790 cm^{-1}; $[\alpha]^{20}_D$ −133° (c = 1%, $CHCl_3$).

[3a*R*(3aα,6aα,2'*S)]-1-[(1,1-Dimethylethoxy)carbonyl]octahydro-α-(2-methylpropyl)-6-oxopyrrolo[3,4-*b*]pyrrole-5-acetic Acid, with 25% 3a*S*(3aα,6aα,2'*S**) Isomer (27a).** The amine diester **19a** (247 mg, 0.62 mmol) was dissolved in methanol (5 mL) and water (0.5 mL) and then potassium hydroxide (48 mg, 1.36 mmol) added. After stirring at room temperature overnight, the organic solvents were removed under vacuum, and the aqueous brine solution was washed with ether to remove any nonacidic impurities. Acidification of the aqueous layer and extraction with dichloromethane followed by drying of the organic layer and concentration yielded a white solid **27a** (190 mg, 90%): ^1H NMR ($CDCl_3$) δ 0.93 (3 H, d, J = 6 Hz), 0.97 (3 H, d, J = 6 Hz), 1.45 (1 H, m, $CH(CH_3)_2$), 1.48 (9 H, s, O-t-Bu), 1.70 (3 H, m, H_{3A} + $CH_2CH(CH_3)_2$), 2.20 (1 H, m, H_{3B}), 2.92 (1 H, m, H_{3a}), 3.07 (1 H, d, J = 10 Hz, H_{4A}), 3.40 and 3.50 (1 H, 2 × broad m, H_{2A} + H_{2B}), 3.70 (1 H, dd, J = 10, 7 Hz, H_{4B}), 4.58 and 4.80 (2 H, 2 × broad m, H_{6a} + $CHCO_2H$), 5.90 (broad s, CO_2H + H_2O). Anal. ($C_{17}H_{28}N_2O_5$) C, H, N.

[3a*S*(3aα,6aα,2'*R)]-1-[(1,1-Dimethylethoxy)carbonyl]octahydro-α-(2-methylpropyl)-6-oxopyrrolo[3,4-*b*]pyrrole-5-acetic acid, with 25% [3a*R*(3aα,6aα,2'*R**)] isomer (28a),** was prepared in a similar manner from the isomer **20a** (96%): ^1H NMR (DMSO-d_6) rotameric mixture, δ 0.84 (3 H, d, J = 7 Hz), 0.90 (3 H, d, J = 7 Hz), 1.40 (9 H, s, O-t-Bu), 1.4–1.8 (4 H, m), 2.10 (1 H, m, H_{3B}), 2.90 (1 H, m, H_{3a}), 3.11 (1 H, d, J = 10 Hz, H_{4A}), 3.20 (1 H, m, H_{2A}), 3.50 (1 H, m, H_{2B}), 3.42 (1 H, dd, J = 10, 7 Hz, H_{4B}), 4.48 (2 H, m, H_{6a} + $CHCO_2H$).

[3a*R*(3aα,6aα,2'*S,1''*S**)]-*N*-[1-(Aminocarbonyl)-3-(methylthio)propyl]-1-[(1,1-dimethylethoxy)carbonyl]octahydro-α-(2-methylpropyl)-6-oxopyrrolo[3,4-*b*]pyrrole-5-acetamide, with 25% 3a*S*(3aα,6aα,2'*S**,1''*S**) Isomer (29a).** A solution of the acid **27a** (133 mg, 0.39 mmol) in dichloromethane (10 mL) was treated with Met-NH_2·HCl (87 mg, 0.47 mmol) and triethylamine (190 μL, 1.17 mmol) at ice-bath temperature followed by BOP-Cl (110 mg, 0.43 mmol). After standing for 48 h at 0 °C, ethyl acetate was added and after washing with 10% citric acid, water, 10% sodium bicarbonate, and then brine, the solution was dried and concentrated to yield the product **29a** (78 mg, 68%); HPLC indicated a mixture of two isomers in a ~2:1 ratio.

[3a*S*(3aα,6aα,2'*R,1''*R**)]-*N*-[1-(Aminocarbonyl)-3-(methylthio)propyl]-1-[(1,1-dimethylethoxy)carbonyl]octahydro-α-(2-methylpropyl)-6-oxopyrrolo[3,4-*b*]pyrrole-5-acetamide, with 25% 3a*R*(3aα,6aα,2'*R**,1''*R**) Isomer (30a).** This was prepared in an identical manner to the above from **28a** (67%). Again, HPLC indicated the presence of two isomers in a ~2:1 ratio.

Pharmacological Methods. Animals. Male AH/A rats (250–350 g; Glaxo) and male Dunkin-Hartley guinea pigs (300–500 g; Porcellus) were used in these studies.

Guinea Pig Ileum Longitudinal Muscle (GPI). The longitudinal smooth muscle myenteric plexus (GPI) was prepared from excised guinea pig ileum.[23] GPI preparations were mounted in 3-mL organ baths (37 °C) filled with Tyrode's medium containing atropine, indomethacin, mepyramine, methysergide, and ondansetron (all at 1 μM). Mechanical activity was recorded isometrically via Grass FT.03 transducers and using a resting tension of 0.3 g.

Rat Colon Muscularis Mucosae (RC). Rat colon muscularis mucosae were prepared as described by Bailey and Jordan.[24] RC preparations were mounted in 3-mL organ baths under a resting tension of 0.5 g and bathed as for GPI preparations. Mechanical activity was recorded isotonically using Chemlab CS-IT100 transducers.

Rat Everted Portal Vein (RPV). Segments of rat isolated portal vein were everted and suspended in 3-mL organ baths (37 °C) under a resting tension of 0.5 g and bathed in modified Krebs–Henseleit medium. Mechanical activity was recorded as for GPI preparations.

Drugs and Solutions. Tyrode's solution had the following composition (mM): NaCl (137), KCl (2.8), $NaHCO_3$ (11.9), $MgCl_2$ (2.1), NaH_2PO_4 (0.32), $CaCl_2$ (1.8), and glucose (5.6). Modified Krebs–Henseleit medium contained (mM) NaCl (118), KCl (4.7), $NaHCO_3$ (25), KH_2PO_4 (1.2), $MgSO_4$ (0.6), $CaCl_2$ (2.5), and glucose (11). Media were prepared using glass-distilled water and A.R.-grade reagents (BDH).

SP and NKB were purchased from Cambridge Research Biochemicals, Peninsula, or Bachem. Stock solutions of peptides (5 mM) were prepared in acetic acid (0.01 M) or dimethyl sulfoxide and stored in aliquots under nitrogen at −20 °C.

Experimental Design. Substance P (SP) was used as the reference agonist with GPI and RC preparations; neurokinin B (NKB) was used with RPV preparations. Concentration–contraction response curves to agonist were constructed non-cumulatively using serially-increasing concentrations applied in 7 min (GPI) or 15 min (RC and RPV) time cycles. To ensure reproducibility, tissues were used in the study only if two consecutive control curves to the reference agonist were found to have EC_{50} values consistent to within a factor of 2 and maxima reproducible to within ±15%. The activity of test compounds

(23) Rang, H. P. Stimulant Actions of Volatile Anaesthetics on Smooth Muscle. *Br. J. Pharmacol.* **1964**, *22*, 356–365.

(24) Bailey, S. J.; Jordan, C. C. A Study of [D-Pro^2,D-Phe^7,D-Trp^9]-Substance P and [D-Trp^{7,9}]-Substance P as Tachykinin Partial Agonists in the Rat Colon. *Br. J. Pharmacol.* **1984**, *82*, 441–451.

was assayed using 3 + 3 (GPI) or 2 + 2 (RC) randomized latin square designs. Equipotent molar ratios were estimated from the assays and converted to EC_{50} values by relating them to the mean EC_{50} value for the reference agonist. Estimates of $EC_{50} \pm$ SE mean were obtained by nonlinear curve-fitting analysis[25] of concentration–response data from at least four separate tissue preparations.

(25) DeLean, A.; Munson, P. J.; Rodbard, D. Simultaneous Analysis of Families of Sigmoidal Curves: Application to Bioassay, Radioligand Assay, and Physiological Dose-Response Curves. *Am. J. Physiol.* 1977, *235*, E97–E102.

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Supplementary Material Available: Further information concerning the X-ray analysis of compound **19a** (atom coordinates, bond lengths, bond angles, anisotropic temperature factors and hydrogen coordinates) (5 pages). Ordering information is given on any current masthead page.