Pergamon

*Bioorganic & Medicinal Chemistry*, Vol. 2, No. 2, pp. 101-113, 1994

0968-0896/94 $6.00 + .00

0968-0896(94)E0003-K

# Rational Design of High Affinity Tachykinin $NK_2$ Receptor Antagonists

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**Abstract**—The rational discovery of a high affinity $NK_2$ receptor antagonist is described utilizing a general strategy for peptoid design. The contribution to $NK_2$ receptor binding affinity for each amino acid of the hexapeptide 'minimum fragment': Leu-Met-Gln-Trp-Phe-Gly$NH_2$ (**8c**), was examined by preparing derivatives where each amino acid in turn was replaced with Ala in an 'alanine scan'. The results from this study indicated the primary importance of the Trp and Phe side-chain for binding and led to the observation that Z-Trp-Phe$NH_2$ (**9a**) is a micromolar affinity $NK_2$ receptor dipeptide lead. Further exploration of structure–affinity via conformationally restricted analogues and N- and C-terminus modifications gave a selective, nanomolar affinity $NK_2$ receptor antagonist, (2,3di-$CH_3$OPh)$CH_2$OCO(*S*)Trp(*S*)α-MePheGly$NH_2$, PD 147714 (**19**) with an $K_i$ = 1.4 nM (hamster urinary bladder membranes and using [$^{125}$I]-iodohistidyl-NKA (0.1 nM) as the radioligand).

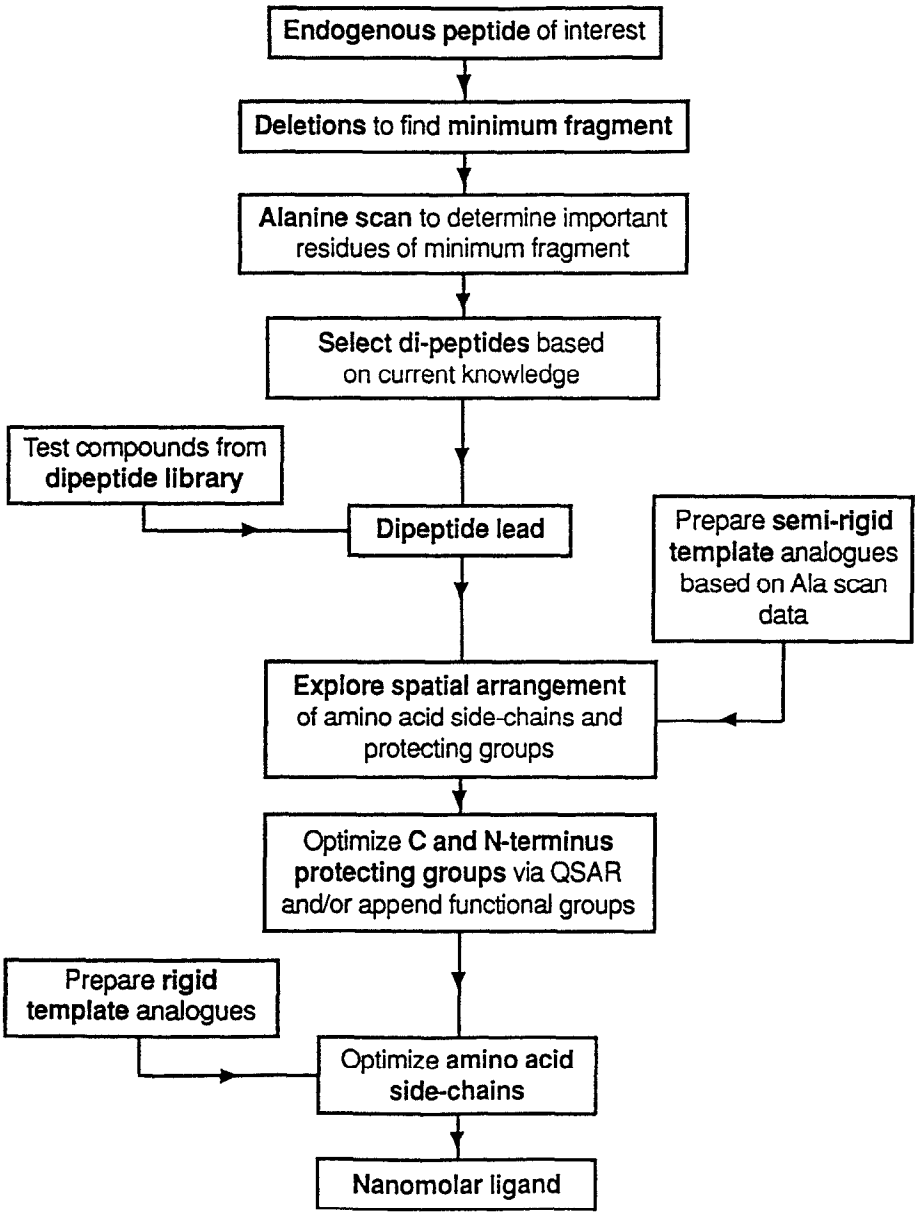
## Introduction

The mammalian tachykinins substance P (**1**), neurokinin A (**2**) and neurokinin B (**3**) interact with at least three types of tachykinin receptor, termed $NK_1$, $NK_2$ and $NK_3$.[1] Neurokinin A and its N-terminally-extended forms, neuropeptide K and neuropeptide γ, bind preferentially to the $NK_2$ receptor and contract smooth muscle of the cardiovascular, gastrointestinal, respiratory and urinary systems.[2] Until recently, a detailed understanding of the physiological and pathophysiological roles of neurokinin A and related tachykinin peptides has been hampered by a lack of selective, high affinity and metabolically stable tachykinin receptor antagonists that possess both good oral bioavailability and CNS penetration. With the exception of the recently described non-peptide $NK_2$ antagonists[3] SR48968 (**4**) and GR 159897, obtained from a random screening/optimization approach, the currently available selective $NK_2$ receptor antagonists are all peptide analogues with limited potency and/or metabolic stability.

Here we describe our strategy and results for the rational design of $NK_2$ receptor antagonists. Our approach was first reported by Horwell[4] and led to the first rationally designed non-peptide antagonist for the cholecystokinin receptor.[5] This strategy (Scheme I) has subsequently been further developed and, in general terms, is as follows. Firstly, to identify a minimum peptide fragment with affinity similar to the endogenous ligand. Then to examine the importance of each amino acid side-chain (via alanine scanning) for binding affinity. From these data, protected dipeptides are selected for evaluation leading to the identification of a micromolar affinity, low molecular weight dipeptide lead. An alternative approach would be to screen a physicochemical information rich library of dipeptides.[6] Once a micromolar dipeptide lead has been identified the next step is to explore the spatial arrangement of the amino acid side-chains and N- and C-terminus groups of the dipeptide. The first part of this step involves further deletions: either moieties within the peptide backbone and amino acid side-chains and/or all or part of the N- and C-terminus groups. Conformationally restricted analogues of the current lead are then prepared. To achieve this, methyl groups are 'appended' along the 'backbone' of the molecule. For a dipeptide, both α-Me and N–Me analogues are synthesized.

Further increases in affinity can then be sought by: optimizing C- and N-terminus groups, via the use QSARs and/or the 'addition' of further binding moieties based on alanine scanning data. Finally, optimization of the amino acid side-chains via QSAR studies and the use of bioisosteres[7] can be carried out, to improve affinity further, and/or alter the physicochemical properties of the compound to produce a more viable 'drug' candidate.

Here we describe the application of the above strategy to the design of a nanomolar affinity, low molecular weight, $NK_2$ receptor antagonist. Studies carried out at Parke-Davis and in other laboratories[8] indicate that the C-terminal hexapeptide of substance P (**5**) is the smallest fragment with measurable affinity for tachykinin receptors. While Folkers *et al.* and others[9] have shown that the systematic introduction of D-amino acid residues (particularly D-Trp) in substance P or C-terminal fragments of it, results in antagonists. Key positions for substitution with D-Trp appear to be $Phe^7$ and $Gly^9$. Further studies using conformationally restricted amino acids led to the cyclic analogue L-659,877 (**6**) and its linear counterpart L-659,874 (**7**). These ligands are high affinity and selective $NK_2$ receptor antagonists.[10] Our interest in $NK_2$ receptors and their physiological relevance together with the desire to explore the general utility of the peptoid strategy led us to select **8c**, a close analogue of **7**, as the minimum peptide fragment for the design of high affinity $NK_2$ receptor antagonists.




**Scheme I.** A strategy for peptoid design.

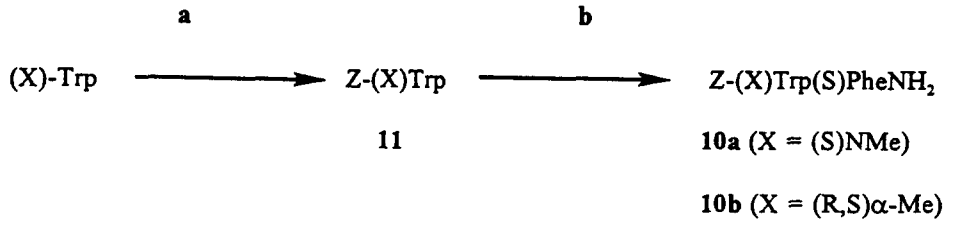
## Chemistry

The standards (**1-7**) were purchased from commercial sources or obtained as gifts.[11] Peptides (**8a–k**) were prepared on an automated continuous flow peptide synthesizer using continuous flow dye monitoring and Fmoc methodology.[12] The N-terminal Boc and 2-Adoc compounds, **8a** and **8b** were prepared by treating the free amino hexapeptide **8c** with $(Boc)_2O$ and 2-adamantyl-chloroformate[13] respectively in DMF with $NEt_3$.

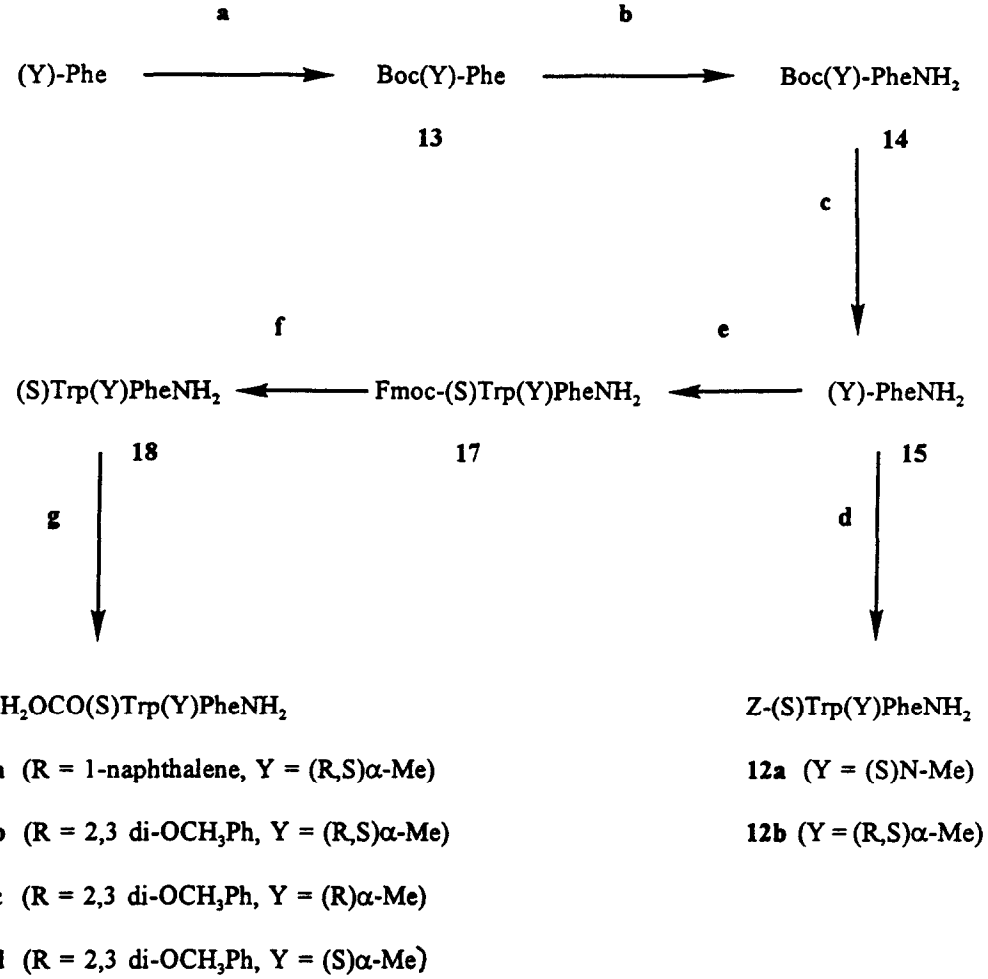
The dipeptide lead **9a** was prepared by reacting N-[(phenylmethoxy)carbonyl]-(*S*)-tryptophan, activated with ethylchloroformate, with (*S*)-phenylalaninamide. The conformationally restricted analogues **9b** and **9c** were obtained from (*S*)-tryptophyl-(*S*)-phenyl alaninamide and (*R*) or (*S*)-1-phenethyloxy chloroformate respectively.

The synthesis of the N-MeTrp and α-MeTrp analogues (**10a** and **b**) is outlined in Scheme II. The appropriate Trp derivative was reacted with benzylchloroformate to give the *Z*-protected compound (**11**). Subsequently, acid (**11**) was activated and reacted with (*S*)-phenylalaninamide to give **10a** or **b**.

The N-MePhe derivative (**12a**) was synthesized by the method shown in Scheme III. N-(*t*-Butyloxycarbonyl)-(Y)-phenylalanine (**13**, Y = (*S*)-N-Me) was activated, in this case with ethylchloroformate, to give the mixed anhydride which was then converted to the primary amide (**14**, (*S*)-N-Me). Deprotection of the Boc group furnished the amine (**15**, (*S*)-N-Me), and reaction of this with a mixed anhydride of N-[(phenylmethoxy)carbonyl]-(*S*)-tryptophan gave the desired product **12a**. The α-MePhe analogue (**12b**) was prepared in a similar manner (Scheme III).




**Scheme II.** Synthetic scheme for compound **10a–b**. **a**: $PhCH_2OCOCl$, $K_2CO_3$ (aq), dioxane; **b**: i. DCCI/Pfp, EtOAc, ii. (*S*)$PheNH_2$, $NEt_3$.



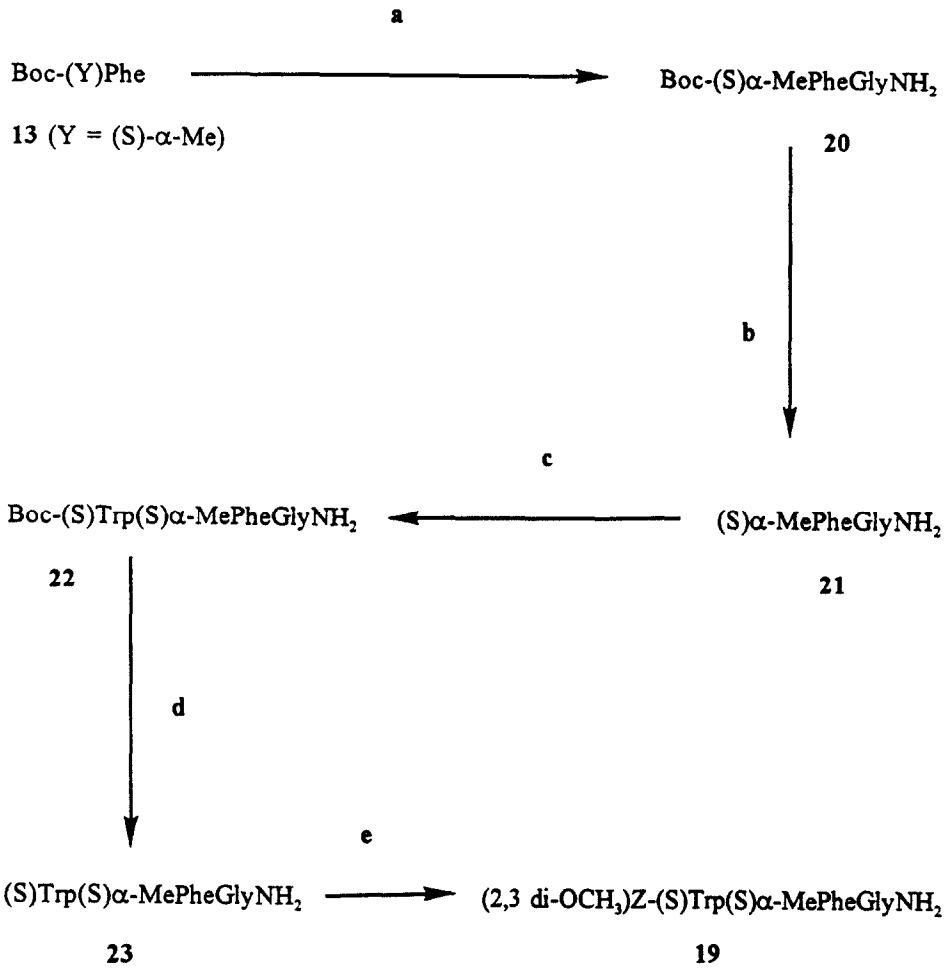

**Scheme III.** Synthetic scheme for compound **12a–b**, **16a–d**. **a**: $(Boc)_2O$, $Na_2CO_3$(aq), dioxane; **b**: i. DCCI/HOBt, $CH_2Cl_2$, ii. $NH_3$(g), DMF; **c**: TFA; **d**: i. Z-(*S*)Trp, EtOCOCl, $NEt_3$, $CH_2Cl_2$, ii. **15**, $CH_2Cl_2$/THF; **e**: Fmoc-(*S*)TrpOPfp, DMF; **f**: piperidine/ DMF; **g**: i. $RCH_2OH$, pyridine, triphosgene, EtOAc, ii. **18**, $NEt_3$, THF.

The α-Me$PheNH_2$ analogues **16a–d** where the N-terminus moiety is varied, were prepared as shown in Scheme III. Briefly this was as follows: (Y)-phenylalaninamide (**15**, Y = α-Me) was treated with *N*-[(9*H*-fluoren-9-ylmethoxy)-carbonyl]-(*S*)-tryptophan-pentafluorophenyl ester to give the dipeptide (**17**, Y = α-Me). This was subsequently deprotected to give the amine (**18**, Y = α-Me) which was reacted with the appropriate chloroformate to give the desired compounds **16a–d**.

Finally the nanomolar affinity ligand **19** was obtained by the method outlined in Scheme IV. **13** (Y = (*S*)α-Me) was activated and reacted with glycinamide to give the 2° amide (**20**). The Boc group was then removed with TFA and the amine (**21**) reacted with N-(*t*-butyloxycarbonyl)-(*S*)-tryptophan to give the tripeptide (**22**). Deprotection of this followed by reaction with the mixed 4-nitrophenolcarbonate of 2,3-dimethoxybenzylalcohol gave the desired ligand **19**.

## Results

Compounds were tested for affinity at $NK_2$ tachykinin receptor binding sites using [$^{125}$I]-iodohistidyl-NKA (0.1 nM) binding to hamster urinary bladder membranes.[14] The results and structures are collated in Table 1.




**Scheme IV.** Synthetic scheme for compound **19**. **a**: i DCCI/HODhbt, THF, ii. $GlyNH_2$·HCl, $NEt_3$, DMF; **b**: TFA, $CH_2Cl_2$; **c**: i. Boc-(*S*)Trp, DCCI/HOBt, DMF, ii. **21**, DMF; **d**: TFA, $CH_2Cl_2$, **e**: (2,3 di-$OCH_3$)$PhCH_2OCO_2$(4-$NO_2$Ph).

*Minimum fragment*

Initially we examined the effect of varying the Ac moiety of L-659,874 (**7**) replacing it with bulkier groups such as Boc (**8a**) or 2-Adoc (**8b**) or removing it altogether (**8c**). This study showed that the bulkier groups have no significant effects on affinity for $NK_2$ receptors.

Interestingly, the free amino analogue (**8c**) displayed the highest affinity for the $NK_2$ receptor ($K_i$ = 11.7 nM). As a consequence this compound was selected as the minimum fragment.

*Alanine scan and related studies*

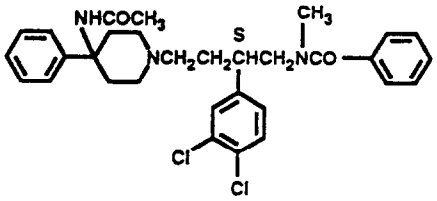
The contribution to binding affinity of each amino acid side-chain was examined by preparing analogues of **8c** where each amino acid in the peptide in turn was replaced with L-Alanine (derivatives **8d–i**). The compounds where the Trp (**8g**) or Phe (**8h**) were replaced showed very low affinity for the $NK_2$ receptor ($K_i$ = >10,000 nM and 8,300 nM respectively), indicating the primary importance of these amino acid side-chains for binding. A smaller, but significant decrease in $NK_2$ receptor binding was observed for the analogues where the Met (**8e**, $K_i$ = 138 nM) and Gln (**8f**, $K_i$ = 156 nM) were replaced with Alanine.

Finally, the effect on $NK_2$ receptor affinity for analogues of **8c** where an amino acid was removed from the N- and C-terminus was examined. The similar affinity of **8j** ($K_i$ = 43.1 nM) and **8d** ($K_i$ = 40.1 nM) confirmed the results of the Alanine scan indicating that the Leu side-chain is not important. Whereas the decrease in binding found for compound **8k** ($K_i$ =200 nM) compared to **8c** ($K_i$ = 11.7 nM), where the C-terminal $GlyNH_2$ has been removed, suggests that the C-terminal amide itself binds or affects the overall conformation.

*Dipeptide lead*

The above study indicated that the key amino acid side-chains for binding of the hexapeptide (**8c**) are the continuous fragment containing Trp and Phe residues. Evaluation of a number of N-terminal protected TrpPhe derivatives in the binding assay led to the identification of Z-$TrpPheNH_2$ (**9a**, $K_i$ = 2700 nM) as a micromolar affinity $NK_2$ receptor dipeptide lead.

**Table 1.** $NK_2$ tachykinin receptor binding affinities

| Step in Strategy | Compound No | Structure | $NK_2$ receptor binding affinity, $K_i$ (nM) |
|---|---|---|---|
| Standards etc | 1 | ArgProLysProGlnGlnPhePheLeuMetNH$_2$ | 295 (–33,+37)[a] |
| | 2 | HisLysThrAspSerPheValGlyLeuMetNH$_2$ | 1.62 (–0.14,+0.15) |
| | 3 | AspMetHisAspPhePheValGlyLeuMetNH$_2$ | 3.13 (–0.45,+0.53) |
| | 4 (SR48968) |  | 5.56 (–1.25,+1.62) |
| | 5 | GlnPhePheGlyLeuMetNH$_2$ | 1200 (–70,+70) |
| | 6 (L-659,877) | Cyclo (GlnTrpPheGlyLeuMet) | 8.13 (–0.72,+0.78) |
| | 7 (L-659,874) | Ac-LeuMetGlnTrpPheGlyNH$_2$ | 19.8 (–3.0,+3.5) |
| | 8a | Boc-LeuMetGlnTrpPheGlyNH$_2$ | 14.2 (–2.1,+2.6) |
| | 8b | 2-Adoc-LeuMetGlnTrpPheGlyNH$_2$ | 25.5 |
| Minimum | 8c | LeuMetGlnTrpPheGlyNH$_2$ | 11.7 (–1.3,+1.6) |
| fragment | 8d | AlaMetGlnTrpPheGlyNH$_2$ | 40.1 |
| Alanine scan | 8e | LeuAlaGlnTrpPheGlyNH$_2$ | 138 |
| | 8f | LeuMetAlaTrpPheGlyNH$_2$ | 156 (–49,+72) |
| | 8g | LeuMetGlnAlaPheGlyNH$_2$ | >10,000 |
| | 8h | LeuMetGlnTrpAlaGlyNH$_2$ | 8,300 |
| | 8i | LeuMetGlnTrpPheAlaNH$_2$ | 28.1 |
| | 8j | MetGlnTrpPheGlyNH$_2$ | 43.1 |
| | 8k | LeuMetGlnTrpPheNH$_2$ | 200 (–55,+75) |
| Dipeptide lead | 9a | Z-TrpPheNH$_2$[b] | 2700 (–270,+290) |
| Conformational | 9b | Ph(R)CH(CH$_3$)OCOTrpPheNH$_2$[c] | 6300 (–1000,+1100) |
| restricted | 9c | Ph(S)CH(CH$_3$)OCOTrpPheNH$_2$ | >10,000 |
| analogues | 10a | Z-(NCH$_3$) TrpPheH$_2$ | 7500 |
| | 10b | Z-(R,S) α-MeTrpPheNH$_2$ | >10,000 |
| | 12a | Z-Trp (NCH$_3$) PheNH$_2$ | 6700 |
| | 12b | Z-Trp (R,S) α-MePheNH$_2$ | 327 (–69,+86) |
| Optimize | 16a | 1-NaphthylCH$_2$OCOTrp (R,S) αMePheNH$_2$ | 62.8 (–3.2,+3.4) |
| N-terminus | 16b | (2,3diOCH$_3$Ph) CH$_2$OCOTrp (R,S) α-MePheNH$_2$ | 37.6 (–5.5,+6.4) |
| | 16c | (2,3diOCH$_3$Ph) CH$_2$OCOTrp (R) α-MePheNH$_2$ | ~10,000 |
| | 16d | (2,3diOCH$_3$Ph) CH$_2$OCOTrp (S) α-MePheNH$_2$ | 17.2 |
| Optimize C-terminus, Nanomolar ligand | 19 | (2,3diOCH$_3$Ph) CH$_2$OCOTrp (S) α-MePheGlyNH$_2$ | 1.4 (–0.2,+0.4) |

[a] $K_i$ values are given as geometric means -, + sem from at least 3 separate experiments.
[b] Z = Benzyloxycarbonyl.
[c] All amino acids have (*S*)-stereochemistry unless indicated.

*Explore spatial arrangement*

Having identified Z-TrpPheNH$_2$ (**9a**) as a micromolar lead the next step in the strategy is to explore the spatial arrangement of the amino acid side-chains and N- and C-terminus groups; the first part of this step involves further deletions. Preparation and evaluation of analogues where the C- and N-terminal groups were deleted (data and compounds not shown[15]) led to a significant loss of affinity in all cases. The second part involves the preparation of conformationally restricted analogues of **9a**, in particular the incorporation of methyl groups along the peptide backbone to give compounds **9b–c**, **10a–b**, **12a–b**.

All of these have lower, some significantly lower, binding affinities for the $NK_2$ receptor than **9a** apart from **12b**. The (*R,S*)α-MePhe derivative (**12b**, $K_i$ = 327 nM) has an affinity eight times greater than **9a** ($K_i$ = 2,700 nM).

*Optimize N-terminus group*

Compound **12b** was subsequently progressed to the next step in the strategy which involved optimization of the N-terminus group. Earlier work at Parke-Davis indicated a positive, though shallow, correlation between $NK_2$ receptor affinity and the MR[16] of the group *R* in *R*-OCO–TrpPheNH$_2$. This observation led to the preparation of the 1-naphthylmethoxy analogue of **12b**, **16a** ($K_i$ = 62.8 nM) and a further significant increase (~6-fold) in affinity. **16a** is a very lipophilic compound (*clogP* = 4.7) in order to reduce this the 1-naphthyl group was replaced by the 2,3-dimethoxyphenyl moiety to give **16b**. The 2,3-dimethoxyphenyl group has a similar cMR to the 1-naphthyl (cMR = 3.9 cf to 4.4) whilst its *clogP* is significantly less (*clogP* = 2.2 cf to 3.5). Interestingly, **16b** ($K_i$ = 37.6 nM) has a higher affinity for the $NK_2$ receptor as well as a lower *clogP* (*clogP* = 3.4) than **16a**. As a consequence, the [(2,3-dimethoxyphenyl)-methoxy]carbonyl moiety was selected as the N-terminus group. Compound **16b** consists of a mixture of two epimers, enzymatic resolution of (*R,S*)α-MePhe by the method of Turk *et al.*[17] allowed us to explore the stereochemical preference further through the preparation of the individual isomers of **16b** (the *S,R* stereoisomer (**16c**) and the *S,S* stereoisomer (**16d**)). The *S,S* isomer ($K_i$ = 17.2 nM) has an affinity approximately twice that of the mixture **16b**, while the *R,S* isomer ($K_i$ = 10,000 nM) is virtually inactive.

*Optimize C-terminus group*

Next we examined the effect of 'appending' certain functional groups to the C-terminus. The Alanine scan and related studies for LeuMetGlnTrpPheGlyNH$_2$ (**8c**) indicated the secondary, although still significant, importance of the GlyNH$_2$ for binding (cf **8k** $K_i$ = 200 nM and **8c**, $K_i$ = 11.7 nM). Thus a GlyNH$_2$ was appended to the C-terminus of **16d** ($K_i$ = 17.2 nM) to give compound **19** ($K_i$ = 1.4 nM) resulting in a 10-fold improvement in binding.

*Nanomolar ligand*

The SAR of the semi-rigid compounds was additive at the N- and C-terminal when orientated by the preferred conformational constraint. This then gave compound **19** as a selective nanomolar $NK_2$ receptor ligand, with a $K_i$ = 14 nM for the $NK_2$ receptor (hamster urinary bladder) and $K_i$ values > 2000 nM versus seventeen other receptors, including $NK_1$, $NK_3$, $CCK_B$ receptors and L-type calcium channels. Further work has shown it is an $NK_2$ receptor antagonist *in vitro* with a $pK_B$ = 7.9 vs [β-Ala$^8$]NKA(4-10)-induced contraction of the rat colon.

## Conclusion

This study exemplifies the utility of the peptoid design strategy outlined in Scheme I. An Alanine scan has shown that the key amino acid side-chains for $NK_2$ receptor binding affinity of the hexapeptide (**8c**) are Trp and Phe. Evaluation of a number of N-terminal protected TrpPhe derivatives led to the identification of *Z*-TrpPheNH$_2$ (**9a**) as a micromolar affinity $NK_2$ receptor dipeptide lead. From **9a** we have designed a selective nanomolar affinity, $NK_2$ receptor antagonist (**19**). This was achieved by the 'addition' of two methoxy groups to the N-terminus phenyl ring, a methyl group to the α-position of the Phe and a GlyNH$_2$ to the C-terminus. Further studies will be reported shortly describing modifications to **19** leading to small molecule 'peptoid' drug candidates containing no peptide bonds.

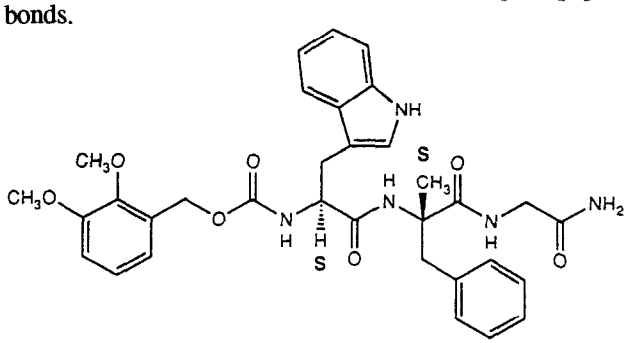



**Figure 1.** Chemical structure of $NK_2$ receptor nanomolar affinity ligand **19**

## Experimental Section

*Biological assays*

(1) $NK_2$ receptor binding assay. These were performed as previously described.[14]
(2) Rat isolated colon *in vitro* assay.

*Tissue preparation.* Segments of descending colon from 200–300 g male Sprague Dawley rats were cleared of their contents and mounted on a glass pipette of maximum outer diameter 5 mm. The outer muscle layers of the colon were carefully stripped off using moist cotton wool and discarded; the remaining tissue (*muscularis mucosæ* and epithelium) was retained. Preparations of the *muscularis mucosæ* from the rat colon, approximately 25 mm in length, were set up under a resting tension of 1 gf (9.8 mN) for isometric recording of smooth muscle contraction (Grass FT.03C force–displacement transducer, Graphtec MKVII Linearcorder) in 3 mL silanized-glass organ baths, containing a modified Krebs–Henseleit solution of the following composition (mM): NaCl, 118; KCl, 5.9; $MgSO_4$, 1.2; $CaCl_2$, 2.5; $NaH_2PO_4$, 1.2; $NaHCO_3$, 25.5; glucose, 11; indomethacin, 0.005; atropine, 0.01; mepyramine, 0.01; methysergide, 0.01. The solution was maintained at 37 °C and was continuously gassed with a mixture of oxygen/carbon dioxide: 95/5.

*Experimental procedure.* Non-cumulative concentration–response curves to the selective $NK_2$ agonist [β-Ala$^8$]NKA(4-10) were constructed in the colon by addition of increasing doses (≤10 μL) to the organ bath. To assess the effect of putative antagonists, concentration–response curves to the agonist were obtained in the absence, and then the presence of known concentrations of the presumptive antagonist. Contractile responses to [β-Ala$^8$]NKA(4-10) were expressed as a percentage of the maximum response for the one preparation, and concentration–response data fitted by a least-squares iterative method in Inplot (Graphpad Software Inc.) to the logistic function $R = 100 \cdot x^n/(EC_{50}{}^n + x^n)$, where $R$ is the response, $x$ is the agonist concentration, $EC_{50}$ is the 'location parameter' for the curve (approximates to the value for $EC_{50}$, the concentration of agonist producing 50% of the maximum response), and $n$ is the 'slope factor' of the curve. The effect of the antagonist was observed as a rightward shift of the log(concentration)–response curve, and generally this was quantified in terms of the 'dose ratio' (DR) between $[EC_{50}]_A$ and $[EC_{50}]_C$, these parameters being the measures made in the presence and absence of antagonist respectively. By repeating estimates for DR using at least three separate observations at any one concentration of antagonist, and not less than three concentrations of antagonist, the affinity of the antagonist was derived by Schild analysis from the plot of log(DR-1) against–log[antagonist].[18] The plots were analyzed by regression analysis, and if the slope of the line of best fit was not significantly different from unity, the intercept on the abscissa from the regression analysis with the slope constrained to unity was taken as the negative logarithm of $K_B$ ($pK_B$), the estimate of the antagonist dissociation constant.

*Chemistry*

Melting points were determined with a Mettler FP80 or a Reichert Thermovar hotstage apparatus. Proton NMR spectra were recorded on a Bruker AM300 spectrometer; chemical shifts were recorded in parts per million (ppm) downfield from tetramethylsilane. IR spectra were recorded with the compound either neat (oils and liquids) or as a Nujol mull on a sodium chloride disc on a Perkin-Elmer 1750 Fourier transform spectrophotometer. Optical rotations were determined with a Perkin-Elmer 241 polarimeter. Mass spectra were recorded with a Finnegan 4500 or VG Analytical ZAB-E. Elemental analyses indicated by the symbols of the elements were within ± 0.4% of theoretical values and were determined by Medac Ltd, Uxbridge, U.K. Normal phase silica gel used for chromatography was Merck No. 9385 (230–400 mesh); reverse-phase silica gel used was Lichroprep RP-18 (230–400 mesh); both were supplied by E. Merck, A.G., Darmstadt, Germany. Anhydrous solvents were purchased in septum-capped bottles from Fluka Chemicals Ltd, Glossop, U.K. and dispensed by syringe.

*Synthesis of peptides 8a–k*

The peptides were synthesized on an automated continuous flow peptide synthesizer (LKB/Pharmacia 4170) utilizing dye monitoring at 600 nm (acid violet dye). Fmoc amino acid pentafluorophenyl esters with HOBt catalysis in DMF solution was used in the acylation steps, the amino acid components being used in a five-fold excess. 20% Piperidine in DMF was used as the deprotecting reagent while the peptides were cleaved from the resins using TFA/thioanisole/ethanedithiol (95:4:1) at room temperature for 2–3 h. Fmoc amino acid pentafluorophenol esters and HOBt were purchased from Pharmacia. Syntheses employed 0.1 mmol/g Novasyn KR resin (Nova-Biochem) to yield the peptides as C-terminal amides on cleavage.

The peptides were purified by HPLC chromatography using a Beckman Instruments 'System Gold' equipped with a Microsorb C18 column (5 μ, 22 x 250 mm). The mobile phase consists of A (0.1% aq TFA) and B (0.1% TFA in MeCN). Peptide purity was determined by analytical HPLC: Beckman System Gold equipped with a Microsorb C18 column (5 μ, 4.6 x 250 mm).

The identity of peptides were determined by amino acid analysis and FAB-MS spectroscopy. The hydrolysis of the peptides was carried out using 6 N aqueous HCl in the presence of phenol at 110 °C for 24 h and the subsequent analysis on a Pharmacia 4151 amino acid analyzer.

*Boc-LeuMetGlnTrpPheGlyNH$_2$ 8a*

**8c** was prepared by standard solid phase procedures and cleaved by TFA/thioanisole/ethanedithiol (95:4:1) as outlined above. The crude peptide was triturated under $Et_2O$, taken up in MeOH, treated with activated charcoal, filtered and the solvent removed *in vacuo*. To a stirred solution of **8c** (50 mg, 0.056 mmol) in DMF (3 mL) was added di-*t*-butyldicarbonate (13.5 mg, 0.062 mmol) and subsequently triethylamine (12 mg, 0.123 mmol) in DMF (2 mL) dropwise. Stirring was continued at room temperature overnight, the solvent was reduced to 0.5 mL *in vacuo* and $H_2O$ (10 mL) was added. The precipitated product was then filtered, washed with $H_2O$, dried, dissolved in MeOH, treated with activated charcoal, filtered again and then evaporated to dryness to yield the pure acylated peptide **8a** (28 mg, 57%); purity >98%. HPLC anal.: $t_R$ = 16.8 min, gradient 20–80% B in A, $t$ = 20 min, flow = 1 mL/min; Amino acid analysis: Gln (0.97), Gly (0.97), Met (1.01), Leu (0.97), Phe (1.00), Trp (ND); MS m/e (FAB) 880.5 (M + H).

*2-Adoc-LeuMetGlnTrpPheGlyNH$_2$ 8b*

To a stirred solution of **8c** (50 mg, 0.056 mmol) in DMF (3 mL) was added 2-adamantylchloroformate[13] (13 mg, 0.06 mmol) in DMF (2 mL) followed by triethylamine (12 mg, 0.123 mmol) in DMF (2 mL) dropwise. Stirring was continued at room temperature for 60 min after which time $CH_2Cl_2$ (30 mL) was added; the resulting white precipitate was filtered, washed with $CH_2Cl_2$ and dried. The crude product was purified by HPLC (gradient 60–100% B in A; v = 10 mL/min, 20 min) to yield the pure acylated peptide **8b** (12 mg, 22%); purity >98% HPLC anal.: $t_R$ = 9.2 min, 60–100% B in A, $t$ = 20 min, flow= 1 mL/min;

Amino acid analysis: Gln (1.02), Gly (1.05), Met (1.02), Leu (1.02), Phe (1.00), Trp (ND); MS m/e (FAB) 981 (M + Na).

*H-LeuMetGlnTrpPheGlyNH$_2$·TFA 8c*

The peptide was prepared by standard solid-phase procedures and cleaved by TFA/thioanisole/EDT (95:4:1) as outlined above. The crude peptide was triturated under $Et_2O$, taken up in MeOH, treated with activated charcoal, filtered and the solvent removed *in vacuo*. The semi-crude product was purified by HPLC (gradient 30–70% B in A; v = 10 mL/min, 20 min) to obtain the pure product **8c** (21 mg, 70%) purity >98%. HPLC anal.: $t_R$ = 10.8 min, gradient 20–80% B in A; *t* = 20 min, flow = 1 mL/min. Amino acid analysis: Gln (0.99), Gly (1.08), Met (1.12), Leu (1.10), Phe (1.00), Trp (ND); MS m/e (FAB) 780 (M + H), 802 (M + Na).

*H-AlaMetGlnTrpPheGlyNH$_2$·TFA 8d*

The semi-crude product was purified by HPLC (gradient 30–70% B in A; v = 10 mL/min, 20 min) to obtain the pure peptide **8d** (22 mg, 26%); purity >98%. HPLC anal.: $t_R$ = 9.7 min, gradient 20–80% B in A, *t* = 20 min, flow = 1 mL/min; Amino acid analysis: Gln (1.00), Gly (0.97), Ala (0.97), Met (0.98), Phe (1.00), Trp (ND); MS m/e (FAB) 738 (M + H), 760 (M + Na).

*H-LeuAlaGlnTrpPheGlyNH$_2$·TFA 8e*

The semi-crude product was purified by HPLC (gradient 30–70% B in A; v = 10 mL/min, 20 min) to yield the pure peptide **8e** (5 mg, 6%); purity >98%. HPLC anal.: $t_R$ = 9.5 min, 20–80% B in A, t = 20 min, flow = 1 mL/min; Amino acid analysis: Gln (1.01), Gly (0.99), Ala (0.99), Leu (1.01), Phe (1.00), Trp (ND); MS m/e (FAB) 720 (M + H), 742 (M + Na).

*H-LeuMetAlaTrpPheGlyNH$_2$·TFA 8f*

The semi-crude product was purified by HPLC (gradient 30–70% B in A; v = 10 mL/min, 20 min) to yield the pure peptide **8f** (23 mg, 28%), purity >98%. HPLC anal.: $t_R$ = 11.3 min, 20–80% B in A, *t* = 20 min, flow = 1 mL/min; Amino acid analysis: Gly (1 .01), Met (1.03), Leu (1.02), Phe (0.95), Trp (ND); MS m/e (FAB) 723 (M + H).

*H-LeuMetGlnAlaPheGlyNH$_2$·TFA 8g*

The semi-crude product was purified by HPLC (gradient 30–70% B in A; v = 10 mL/min, 20 min) to yield the pure peptide **8g** (45 mg, 58%); purity >98%. HPLC anal.: $t_R$ = 14.3 min, 20–80% B in A, *t* = 20 min, flow = 1 mL/min; Amino acid analysis: Gln (1.05), Gly (0.96), Ala (0.98), Met (1.00), Leu (1.03), Phe (1.04; MS *m/e* (FAB) 665 (M + H).

*H-LeuMetGlnTrpAlaGlyNH$_2$·TFA 8h*

The semi-crude product was purified by HPLC (gradient 30–70% B in A; v = 10 mL/min, *t* = 20 min) to yield the pure peptide **8h** (30 mg, 37%); purity >98%. HPLC anal.: $t_R$ = 7.8 min, 20–80% B in A, *t* = 20 min, flow = 1 mL/min; Amino acid analysis: Gln (1.02), Gly (0.97), Ala (1.00), Met (1.03), Leu (1.03), Trp (ND); MS m/e (FAB) 704 (M + H).

*H-LeuMetGlnTrpPheAlaNH$_2$·TFA 8i*

The semi-crude product was purified by HPLC (gradient 30–70% B in A; v = 10 mL/min, *t* = 20 min) to yield the pure peptide **8i** (22 mg, 24%); purity >98%. HPLC anal.: $t_R$ = 10.7 min, 20–80% B in A, *t* = 20 min, flow = 1 mL/min; Amino acid analysis: Gln (1.00), Ala (0.96), Met (1.00), Leu (1.01), Phe (1.00), Trp (ND); MS m/e (FAB) 794 (M + H).

*H-MetGlnTrpPheGlyNH$_2$·TFA 8j*

The semi-crude product was purified by HPLC (gradient 30–70% B in A; v = 10 mL/min, *t* = 20 min) to yield the pure peptide **8j** (33 mg, 42%); purity >98%. HPLC anal.: $t_R$ = 15.8 min, 20–80% B in A; *t* = 20 min, flow = 1 mL/min; amino acid analysis: Gln (1.04), Gly (1.00), Met (1.02), Phe (0.96), Trp (ND); MS m/e (FAB) 667 (M + H).

*H-LeuMetGlnTrpPhe-NH$_2$·TFA 8k*

The semi-crude product was purified by HPLC (gradient 30–70% B in A; v = 10 mL/min, *t* = 20 min, to yield the pure peptide **8k** (22 mg, 30%); purity >98%. HPLC anal.: $t_R$ = 10.9 min, 20–80% B in A; v = 10 mL/min, *t* = 20 min, to yield the pure peptide **8k** (22 mg, 24%); purity >98%. HPLC anal.: $t_R$ = 10.9 min, 20–80% B in A, *t* = 20 min, flow = 1 mL/min; amino acid analysis: Gln (0.98), Met (0.99), Leu (0.96), Phe (1.00), Trp (ND); MS m/e (FAB) 723 (M + H).

*N-[(Phenylmethoxy)carbonyl)-(S)-tryptophyl-(S)-phenylalaninamide 9a*

A solution of N-[(phenylmethoxy)carbonyl]-(*S*)-tryptophan (0.34 g, 1 mmol) in $CH_2Cl_2$ (10 mL) was cooled to -10 °C and stirred whilst triethylamine (0.14 mL, 1 mmol) and ethylchloroformate (0.085 ml, 1 mmol) were added. The mixture was stirred for a further 10 min and a cold solution of (*S*)-phenylalaninamide (0.16 g, 1 mmol) in THF/$CH_2Cl_2$ (1 mL/5 mL) added. The mixture was stirred and allowed to warm to room temperature for a further 2 h. The solvent was removed *in vacuo* and the solid washed with $H_2O$ and acetone to give **9a** (0.35 g, 75%); m.p. 212.5–213.5 °C; $^1$H NMR (DMSO-$d_6$) δ 2.5–2.84, 2.88–2.99 (2 x 2H, 2 x m, 2 x $CH_2$), 4.26 (1H, m, α-CH), 4.48

(1H, m, α-CH), 4.94 (2H, s, $CH_2O$), 6.97 (1H, t, 7 Hz, indole C(5)H), 6.94–7.43 (16H, m, $CONH_2$, OCONH, ArH), 7.59 (1H, d, 8 Hz, indole C(2)H), 8.02 (1H, d, 8 Hz, CONH), 10.81 (1H, s, indole NH); IR 3296, 1658 $cm^{-1}$; MS m/e (FAB) 485 (M + H); Anal. $C_{28}H_{28}N_4O_4$ (C, H, N).

*N-[((R)-1-phenylethyloxy)carbonyl]-(S)-tryptophyl-(S)-phenylalaninamide* 9b

N-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-(*S*)-tryptophan pentafluorophenyl ester (18.8 g, 31.7 mmol) and (*S*)-phenylalaninamide (4.6 g, 31.7 mmol) were stirred together overnight in DMF (200 mL) at room temperature. Then the solution was reduced to half its volume *in vacuo* and $H_2O$ (750 mL) added to precipitate a white solid which was filtered and collected. The dried solid was dissolved in 20% solution of piperidine in DMF (100 mL) and stirred for 20 min. Then a white solid was removed and discarded. The filtrate was reduced *in vacuo* and the residue partitioned between EtOAc and 10% citric acid solution. The aqueous layer was adjusted to pH 10 with $Na_2CO_3$. The aqueous layer was then extracted with EtOAc, dried ($MgSO_4$) and the solvent removed *in vacuo* to give a white solid, (*S*)-tryptophyl-(*S*)-phenylalaninamide (7.16 g, 65%). m.p. 160–168 °C; $^1H$ NMR (DMSO-$d_6$) δ 1.72 (2H, bs, $NH_2$), 2.59–2.99 (4H, m, 2 x β-$CH_2$), 3.44 (1H, m, α-CH), 4.49 (1H, m, α-CH), 6.95–7.58 (12H, m, ArH, $CONH_2$), 7.95 (1H, d, 8Hz, CONH), 10.83 (1H, s, indoleNH); IR 3583, 3309, 1659 $cm^{-1}$; MS m/e (EI) 351 (M + H); Anal. $C_{20}H_{22}N_4O$ (C, H, N). This was used in the next step without further purification.

Pyridine (0.16 mL, 2 mmol) in $CH_2Cl_2$ (1 mL) was added dropwise to a solution of triphosgene (0.22 g, 0.75 mmol) and (*R*)-1-phenethylalcohol (0.24 g, 2 mmol) in $CH_2Cl_2$ (5 mL) at 0 °C. The mixture was stirred at this temperature for 1 h. The solvent was removed *in vacuo* and residue taken up in EtOAc (10 mL) and filtered. (*S*)-Tryptophyl-(*S*)-phenylalaninamide (0.7 g, 2 mmol) and triethylamine (0.2 g, 2 mmol) were added to the filtrate. The mixture was stirred at room temperature for 48 h and the white precipitate that formed was then filtered off. The filtrate was collected and the solvent removed *in vacuo* to give a white solid. This was purified by flash chromatography on silica eluting with a mixture of $CH_2Cl_2$ /MeOH (95:5) to give **9b** (0.11 g, 22%) m.p. 228–232 °C; $^1H$ NMR (DMSO-$d_6$) δ 1.33 (3H, d, 6.5 Hz, $CH_3$), 2.78–3.08 (4H, m, 2 x β-$CH_2$), 4.21 (1H, m, α-H), 4.46 (1H, m, α-H), 5.56 (1H, q, 6.5 Hz, PhC($CH_3$)H), 6.95–7.94 (19H, m, ArH, $CONH_2$, CONH, OCONH), 10.78 (1H, S, indole NH); IR 3583, 3303, 1651 $cm^{-1}$; MS m/e (FAB) 499 (M + H); Anal. $C_{29}H_{30}N_4O_4 \cdot 0.5H_2O$ (C, H, N).

*N-[((S)-1-Phenylethyloxy)carbonyl]-(S)-tryptophyl-(S)-phenylalaninamide* 9c

Prepared as described for **9b** from (*S*)-1-phenethylalcohol and (*S*)-tryptophyl-(*S*)-phenylalaninamide (0.26 g, 36%), m.p. 137–144 °C; $^1H$-NMR (DMSO-$d_6$) δ 1.40 (3H, d, 6.5 Hz, $CH_3$), 2.78–3.08 (4H, m, 2 x β-$CH_2$), 4.17 (1H, m, α-H), 4.50 (1 H, m, α-H), 5.57 (1H, q, 6.6 Hz, PhC($CH_3$H)), 6.91–7.92 (19H, m, ArH, $CONH_2$, CONH, OCONH), 10.74 (1H, s, indoleNH); IR 3583, 3300, 1667 $cm^{-1}$; MS m/e (FAB) 499 (M + H); Anal. $C_{29}H_{30}N_4O_4 \cdot 0.4H_2O$ (C, H, N)

*N-Methyl-N-[(phenylmethoxy)carbonyl]-(S)-tryptophyl-(S)-phenylalaninamide* 10a

N-Methyl-(*S*)-tryptophan (1.0 g, 5 mmol), benzylchloroformate (0.85 mL, 5 mmol) and $K_2CO_3$ (1.2 g, 9 mmol) were suspended in dioxan (1.5 mL) and $H_2O$ (1 mL) and stirred for 2 h. The solvents were then removed *in vacuo* and the residue taken up in EtOAc. This was washed with 2 M HCl, $H_2O$, brine and dried ($MgSO_4$) and the solvent removed *in vacuo* to give **11** (X = (*S*)-N–Me) as a dark straw coloured liquid (1.25 g, 7.7%). IR 3345, 1740, 1684 $cm^{-1}$; this was used in the next step without further purification.

**11** (X = (*S*)-N–Me) (0.85 g, 2.4 mmol), 1,3-dicyclohexylcarbodiimide (0.56 g, 2.4 mmol) and pentafluorophenol (0.44 g, 2.7 mmol) were dissolved in EtOAc (20 mL) and the mixture stirred at room temperature for 1 h. The mixture was filtered to remove the white solid (dicyclohexylurea) and the filtrate treated with (*S*)-phenylalaninamide hydrochloride (0.5 g, 2.5 mmol) and triethylamine (0.35 mL, 2.5 mmol). The mixture was then stirred at room temperature for 6 h. The white solid was removed by filtration and washed with EtOAc. The filtrate was washed with 10% aqueous citric acid, 10% aqueous $Na_2CO_3$ and $H_2O$, dried ($MgSO_4$) and the solvent removed *in vacuo*. The residue was purified by reverse phase column chromatography eluting with mixtures of MeOH: $H_2O$ (4:6 to 8:1) to give **10a** as a white solid (0.7 g, 86%) m.p. 65–77 °C. $^1H$ NMR (DMSO-$d_6$) δ 2.51 (3H, s, N–$CH_3$), 2.80–3.25 (4H, m, 2 x β-$CH_2$), 4.49 (1H, m, α-H), 4.74 (1H, m, α-H), 5.00 (2H, m, Ph$CH_2$O), 6.88–7.60 (18H, m, ArH, $CONH_2$ CONH), 10.76 (1H, s, indole NH). IR 3323, 1669 $cm^{-1}$; MS m/e (FAB) 500 (M + H); Anal. $C_{29}H_{30}N_4O_4 \cdot 0.25H_2O$ (C, H, N).

*N-[(Phenylmethoxy)carbonyl]-α-methyl-(R,S)-tryptophyl-(S)-phenylalaninamide* 10b

Prepared in a similar manner to **10a** from α-methyl-(*R,S*)-tryptophan to give **10b** as a white solid (0.15 g, 57%) m.p. 78–83 °C, $^1H$ NMR (DMSO-$d_6$) δ 1.20 (1.5H, s, 0.5 x $CH_3$), 1.60 (1.5H, s, 0.5 x $CH_3$), 2.37 (0.5H, dd, 6, 13.5 Hz, 0.5 x $CH_2$Ph), 2.85–3.42 (3.5H, m, 1.5 x $CH_2$Ph, $CH_2$ indole), 4.45–5.35 (3H, m, α-H Phe, $CH_2O$), 6.00–7.55 (19H, m, ArH, $CONH_2$, CONH, OCONH), 8.12 and 8.27 (1H, 2 x s, indoleNH); IR 3308, 1667 $cm^{-1}$; MS m/e (CI) 499.6 (M + H); Anal. $C_{29}H_{30}N_4O_4 \cdot 0.5H_2O$ (C, H, N).

*N-[(Phenylmethoxy)carbonyl]-(S)-tryptophyl-α-methyl-(R,S)-phenylalaninamide* 12b

*N-[(1,1-Dimethylethoxy)carbonyl]-α-methyl-(R,S)-phenylalaninamide* 14 *(Y =(R,S)-α-Me)*

α-Methyl-(*R,S*)-phenylalanine (5.0 g, 28 mmol) was dissolved in warm 10% $Na_2CO_3$ solution (60 mL) and then

cooled to 0 °C. *t*-Butyloxycarbonyl anhydride (6.39 g, 29.3 mmol) in dioxan (50 mL) was added dropwise and the mixture stirred to 0 °C for 1 h. The mixture was then allowed to warm to room temperature and stirred for a further 24 h. The solvents were then distilled off *in vacuo* and the residue taken up in $H_2O$. This was then washed with $CH_2Cl_2$ (3x), acidified with citric acid, and extracted with $CH_2Cl_2$ (3x). The organic extracts were combined, dried ($MgSO_4$), and the solvent distilled off *in vacuo* to give **13** (Y = (*R,S*)α-Me) as a white solid (6.2 g, 90%): $^1$H NMR (DMSO-$d_6$) δ 1.18 (3H, s, $CH_3$), 1.41 (9H, s, $(CH_3)_3C$), 2.91 (1H, d, 13 Hz, CHH), 3.31 (1H, d, 13 Hz, CHH), 6.71 (1H, bs, OCONH), 7.09 (2H, d, ArH), 7.25 (3H, m, ArH), 12.50 (1H, bs, $CO_2H$). This was used in the next step without further purification.

**13** (Y = (*R,S*)α-Me) (6.7 g, 22 mmol) was dissolved in $CH_2Cl_2$ (80 mL) and 1,3-dicyclohexylcarbodiimide (4.9 g, 24 mmol) followed by 1-hydroxybenzotriazole monohydrate (3.9 g, 18.8 mmol) added and the mixture stirred for 0.5 h at room temperature. DMF (15 mL) was then added, the solution cooled to -10 °C and a slow stream of $NH_3$ (g) bubbled through. The mixture thickened almost immediately so a further amount of DMF (50 mL) was added. After 0.5 h, the addition of $NH_3$ (g) was stopped and the white precipitate removed by filtration and washed with EtOAc. The washings were combined with the filtrate and the solvent distilled off *in vacuo*. The residue was partitioned between $H_2O$ (500 mL) and $CH_2Cl_2$ (300 mL). The aqueous layer was washed with $CH_2Cl_2$ (3x), the organic extracts combined, washed with a saturated $NaHCO_3$ solution, dried ($MgSO_4$), and the solvent distilled off *in vacuo*. The white solid obtained was further purified by flash column chromatography on silica, eluting with a mixture of $CHCl_3$:MeOH (95:5) to give **14** (Y = (*R,S*)α-Me) as a white solid (4.4 g, 66%): $^1$H NMR (DMSO-$d_6$) δ 1.33 (3H, s, $CH_3$), 1.41 (9H, s, $(CH_3)_3C$), 3.31 (2H, bs, β-$CH_2$), 6.25 (1H, bs, OCONH), 7.15 (6H, m, Ar, CONH), 7.41 (1H, bs, *N*-CONH); IR 3313, 1687, 1657 $cm^{-1}$.

*N-[(Phenylmethoxy)carbonyl]-(S)-tryphophyl-α-methyl-(R,S)-phenylalaninamide* ***12b***

**14** (Y = (*R,S*)α-Me) (4.4 g, 15 mmol) was stirred in TFA (15 mL) at room temperature for 15 min. The TFA was distilled off *in vacuo* to give α-methyl-(*R,S*)-phenylalaninamide (**15**) as its TFA salt . $^1$H NMR (DMSO-$d_4$) δ 1.48 (3H, s, $CH_3$), 3.03 (1H, d, 14 Hz, CHH), 3.16 (1H, d, 14 Hz, CHH), 7.21–7.36 (5H, m, ArH), 7.72 and 7.95 (2 x H, 2 x s, $CONH_2$), 8.06 (3H, bs, $NH_3$); IR 3186, 1674 $cm^{-1}$. The salt was taken up in EtOAc and the organic solution carefully washed with saturated $NaHCO_3$ solution, dried ($MgSO_4$) and the solvent removed *in vacuo* to give **15** (Y = (*R,S*)-α-Me) as the free base (1.9 g, 70%).

N-[(Phenylmethoxy)carbonyl]-(*S*)-tryptophan (0.39 g, 1.2 mmol) was dissolved in $CH_2Cl_2$ (20 mL) and then cooled to -10 °C. Triethylamine (0.16 ml, 1.2 mmol) followed by ethylchloroformate (0.2 g, 1.2 mmol) were added and the mixture stirred for 20 min. Then a cold solution (-10 °C) of **15** (Y = (*R,S*)α-Me) (0.34 g, 1.2 mmol) in $CH_2Cl_2$/THF (5/5 mL) was added. The solution was allowed to warm to room temperature and stirred overnight. The solvents were then distilled off *in vacuo* and the residue taken up in $CH_2Cl_2$ (30 mL). This was then washed with citric acid (2 x 30 mL) and $H_2O$ (30 mL) dried ($MgSO_4$) and the solvent distilled off *in vacuo* to give **12b**. This was purified by flash chromatography on silica eluting with a mixture of EtOAc: Hexane (2:1) to give **12b** as a white amorphous solid (98 mg, 17%), m.p. 76–77 °C. $^1$H NMR ($CDCl_3$) δ 1.37 (0.5 x 3H, s, 0.5 x $CH_3$), 1.44 (0.5 x 3H, s, 0.5 x $CH_3$), 2.65–3.35 (4H, m, $CH_2$Indole, $CH_2$Ph), 4.30 (1H, m, α-H Trp), 5.06 (m, $CH_2O$), 5.20–8.30 (19H, m, ArH, Indole NH, OCONH, CONH, $CONH_2$); IR 3309, 1668 $cm^{-1}$; MS m/e (CI) 499.5 (M + H); Anal. $C_{29}H_{30}N_4O_4 \cdot 0.5H_2O$ (C, H, N).

*N-[(Phenylmethoxy)carbonyl]-(S)-tryptophyl-N-methyl-(S)-phenylalaninamide* ***12a***

Prepared by a similar method to **12b** from N-methyl-N-[(1,1-dimethylethoxy)carbonyl]-(*S*)-phenylalaninamide to give **12a** as a white amorphous solid. m.p. 75–100 °C; $^1$H NMR ($CDCl_3$) δ 2.21–3.31 (6H, m, 2 x CH, 2 x $CH_2$), 2.97 (3H, s, $CH_3$), 4.41–8.32 (21H, m, 2 x CH, $CH_2O$, ArH, $CONH_2$, OCONH, indoleNH); IR 3315, 3063, 1683 $cm^{-1}$; MS m/e (CI) 499 (M + H); Anal $C_{29}H_{30}N_4O_4 \cdot 0.5H_2O$ (C, H, N).

*N-[(1-Naphthalenylmethoxy)carbonyl]-(S)-tryptophyl-α-methyl-(R,S)-phenylalaninamide* ***16a***

*(S)-Tryptophyl-α-methyl-(R,S)-alaninamide* ***18*** *(Y = (R,S)α-Me)*

**15** (Y = (*R,S*)α-Me) (1.8 g, 10.1 mmol) and *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-(*S*)-tryptophan pentafluorophenylester (6.0 g, 10.1 mmol) were stirred in DMF (50 mL) at room temperature for 18 h. The solution was concentrated *in vacuo* and $H_2O$ (400 mL) added. The aqueous suspension was extracted with EtOAc (3x) and the organic extracts combined, dried ($MgSO_4$) and the solvent removed *in vacuo* to give crude **17** (Y = (*R,S*)α-Me). This was used in the next step without further purification.

Crude **17** (Y = (*R,S*)α-Me) (3.65 g, 6.2 mmol) was dissolved in a 20% solution of piperidine in DMF (20 mL) and stirred at room temperature for 20 min. The mixture was then concentrated *in vacuo* and $H_2O$ (400 mL) added; the white precipitate formed was then removed by filtration. Citric acid was added to the filtrate to pH 3 and washed with EtOAc (3x). The aqueous layer was made alkaline (pH 9) with solid $Na_2CO_3$ and extracted with EtOAc (3x). The organic extracts were combined, dried ($MgSO_4$) and the solvent removed *in vacuo* to give **18** as a pale yellow solid (2.2 g). Purification by flash chromatography on silica, eluting with $CH_2Cl_2$:MeOH (10:1) gave the product **18** (Y = (*R,S*)α-Me) as a white foam (1.8 g, 80%): m.p. 79–89 °C; $^1$H NMR (DMSO-$d_6$) δ 1.45 (0.5 x 3H, s, 0.5$CH_3$), 1.51 (0.5 x 3H, s, 0.5$CH_3$),

1.81 (2H, bs, $NH_2$), 2.59 (0.5 x H, dd, 10, 14 Hz, CHH), 2.76 (0.5 x H, dd, 9, 14 Hz, CHH), 3.15, 3.45 (4H, 2 x m, $CH_2$, $0.5CH_2$, CH), 6.95–7.60 (12H, m, ArH, $CONH_2$), 8.16 (0.5 x H, s, 0.5 CONH), 8.28 (0.5 x H, s, 0.5CONH), 10.85 (1H, s, indoleNH); IR 3310, 1660 $cm^{-1}$.

*N-[(1-Naphthalenylmethoxy)carbonyl]-(S)-tryptophyl-α-methyl-(R,S)-phenylalaninamide 16a*

Pyridine (0.11 mL, 1.4 mmol) was added dropwise to a stirred solution of 1-naphthalene methanol (0.22 g, 1.4 mmol) and triphosgene (0.15 g, 0.51 mmol) in $CH_2Cl_2$ (5 mL) at 0 °C and stirred for 5 min. The solvent was removed *in vacuo* and the resulting oil triturated with $Et_2O$ and filtered. The filtrate was added to a solution of **18** (Y = (*R,S*)α-Me) (0.26 g, 0.71 mmol) and triethylamine (0.10 mL, 0.71 mmol) in THF (25 mL) and stirred overnight. The solvent was removed *in vacuo* to give a white solid which was partitioned between EtOAc and 10% citric acid solution. The organic layer was washed with saturated $NaHCO_3$ solution and $H_2O$ and dried ($MgSO_4$). Further purification by flash column chromatography on silica eluting with a mixture of $CH_2Cl_2$:MeOH (95:5) gave **16a** as a white foam, m.p. 102–113°C; $^1H$ NMR (DMSO-$d_6$) δ 1.40 (3H, s, $CH_3$), 2.87 (1H, m, β-$CH_2$), 3.05–3.45 (3H, m, β-$CH_2$), 4.15–4.40 (1H, m, α-CH), 5.30–5.50 (2H, m, $CH_2O$), 6.90–8.00 (21H, m, ArH, OCONH, CONH, $CONH_2$), 10.70 (1H, s, indoleNH); MS m/e (FAB) 549 (M + H); Anal. $C_{33}H_{32}N_4O_4 \cdot 0.4H_2O$ (C, H, N).

*N-[[(2,3-Dimethoxyphenyl)methoxy]carbonyl]-(S)-tryptophyl-α-methyl-(R,S)-phenylalaninamide 16b*

Pyridine (0.048 mL, 0.6 mmol) was added dropwise to a solution of 2,3-dimethoxybenzylalcohol (0.1 g, 0.6 mmol) and triphosgene (0.065 g, 0.21 mmol) in EtOAc (5 mL) at 0 °C. The mixture was stirred at this temperature for 10 min and the precipitate removed by filtration. The filtrate was added dropwise at room temperature to a solution of **18** (Y = (*R,S*)α-Me) (0.1 g, 0.27 mmol) in EtOAc (5 mL) and stirred for 48 h. The solution was then washed with 10% citric acid solution, saturated aqueous $NaHCO_3$ and $H_2O$, dried ($MgSO_4$) and the solvent removed *in vacuo* to give a residue. This was purified by flash chromatography on silica, eluting with a mixture of $CH_2Cl_2$/MeOH (95:5) to give **16b** as a white solid (0.08g, 53%) m.p. 87–102 °C. $^1H$ NMR (DMSO-$d_6$) δ 1.39 (3H, s, α-$CH_3$), 2.83–3.37 (4H, m, 2 x β-$CH_2$), 3.62 (0.5 x 3H, s, 0.5 x $OCH_3$), 3.64 (0.5 x 3H, s, 0.5 x $OCH_3$), 3.78 (3H, s, $OCH_3$), 4.15–4.32 (1H, m, α-H Trp), 4.93 (2H, s, $CH_2OCO$), 6.78–7.78 (17H, m, Ar, $CONH_2$, CONH, OCONH), 10.78 (1H, s, indoleNH). IR 3307, 1666 $cm^{-1}$; MS m/e (FAB) 559 (M + H); Anal. $C_{31}H_{34}N_4O_6 \cdot 0.5H_2O$ (C, H, N).

**16c** and **16d** were prepared in a similar manner to that described above for **16b** starting from *R*- and *S*-α-methylphenylalanine respectively. These were obtained by the method of Turk *et al.*[17] from racemic α-methyl-phenylalanine.

*N-[[(2,3-Dimethoxyphenyl)methoxy]carbonyl]-(S)-tryptophyl-α-methyl-(R)-phenylalaninamide 16c*

White solid; m.p. 78–88 °C. $^1H$ NMR (DMSO-$d_6$) δ 1.39 (3H, s, α-$CH_3$), 2.84–3.32 (4H, m, 2 x β-$CH_2$), 3.62 (3H, s, $OCH_3$), 3.78 (3H, s, $OCH_3$), 4.24 (1H, m, α-H Trp), 4.92 (2H, s, $CH_2OCO$), 6.78–7.70 (17H, m, ArH, $CONH_2$, CONH, OCONH), 10.77 (1H, s, indoleNH); IR 3335, 1669 $cm^{-1}$; MS m/e (FAB) 559 (M + H); Anal. $C_{31}H_{34}N_4O_6$ (C, H, N).

*N-[[(2,3-Dimethoxyphenyl)methoxy]carbonyl]-(S)-tryptophyl-α-methyl-(S)-phenylalaninamide 16d*

White solid; m.p. 83–86 °C. $^1H$ NMR (DMSO-$d_6$) δ 1.38 (3H, s, α-$CH_3$), 2.85–3.40 (4H, m, 2 x β-$CH_2$), 3.64 (3H, s, $OCH_3$), 3.78 (3H, s, $OCH_3$), 4.16 (1H, m, α-H Trp), 4.92 (2H, s, $CH_2O$), 6.80–7.77 (17H, m, ArH, $CONH_2$, CONH, OCONH), 10.78 (1H, s, indole NH). IR 3324, 1664 $cm^{-1}$; MS m/e (FAB) 559 (M + H); Anal $C_{31}H_{34}N_4O_6$ (C, H, N).

*N-[[(2,3-Dimethoxyphenyl)methoxy]carbonyl]-(S)-tryptophyl-α-methyl-(S)-phenylalanylglycinamide 19*

*N-[(1,1-dimethylethoxy)carbonyl]-α-methyl-(S)-phenylalanyl-glycinamide 20*

1,3-Dicyclohexylcarbodiimide (4.95 g, 24 mmol) was added to a solution of **13** (Y = (*S*)α-Me)[17] (6.70 g, 24 mmol) in THF (100 mL) at -15 °C and stirred for 5 min. 3-Hydroxy-1,2,3-benzotriazine-4-(3*H*)-one (3.91 g, 24 mmol) and an additional volume of THF (20 mL) were added to the mixture at -10 °C for 1 h and at 0 °C for 4 h. After leaving overnight at 5 °C a white solid precipitated. This was filtered and dried to give the Dhbt ester of **13** (Y = (*S*)α-Me). The crude Dhbt ester (4.2 g, 10 mmol) and triethylamine (1.4 mL, 11 mmol) were dissolved in DMF (50 mL) and glycinamide hydrochloride (1.1 g, 11 mmol) added. The mixture was stirred at room temperature for 72 h. The mixture was evaporated to dryness *in vacuo* and the residue taken up in EtOAc. This was washed with saturated aqueous $NaHCO_3$, 1N HCl solution and $H_2O$, dried ($MgSO_4$) and the solvent removed *in vacuo* to give **20** as a white solid (2.1 g, 62%). m.p. 78–82 °C; $^1H$ NMR ($CDCl_3$) δ 1.38 (3H, s, $CH_3$), 1.46 (9H, s, $(CH_3)_3$), 3.09 (1H, d, 13.8 Hz CHHPh), 3.36 (1H, d, 13.8 Hz CHHPh), 3.81 (1H, dd, 17.3, 5.7 Hz, CHH), 4.11 (1H, dd, 17.4, 7.0 Hz, CHH), 4.73 (1H, s, OCONH), 5.32 (1H, bs, CONHH), 6.62 (1H, bt, CONH), 7.10–7.25 (3H, m, ArH, CONHH), 7.25–7.35 (3H, m, Ar). IR 3319, 1669, 1521 $cm^{-1}$; MS (CI) 336 (M + H); Anal. $C_{17}H_{25}N_3O_4 \cdot 0.25H_2O$ (C, H, N).

*α-Methyl-(S)-phenylalanyl-glycinamide 21*

Trifluoroacetic acid (30 ml) was added dropwise to a stirred solution of **20** (3.7 g, 11 mmol) in $CH_2Cl_2$ (100 mL). The mixture was stirred at room temperature for 4 h, the solvent removed *in vacuo* and the residue triturated with $Et_2O$ to give **21** as its TFA salt (3.7 g, 95%). $^1H$ NMR

(DMSO) δ 1.52 (3H, S, $CH_3$), 3.05 (1H, d, 14.1 Hz, CHHPh), 3.20 (1H, d, 14.0 Hz, CHHPh), 3.75 (2H, d, 5.6 Hz, $CH_2CO$), 7.10 (1H, s, CONHH), 7.20–7.35 (6H, m, ArH, CONHH), 8.06 (3H, bs, $NH_3$), 8.60 (1H, bt, CONH). MS m/e (CI) 236 (100%) (M + H); Anal. $C_{12}H_{17}N_3O_2 \cdot C_2HF_3O_2$ (C, H, N).

*N-[(1,1-Dimethylethoxy)carbonyl]-(S)-tryptophyl-α-methyl-(S)-phenylalanyl-glycinamide* ***22***

N-[(1,1-Dimethylethoxy)carbonyl]-(*S*)-tryptophan (1.0 g, 3.3 mmol) was dissolved in DMF (20 mL) and 1-hydroxybenzotriazole hydrate (0.5 g, 3.7 mmol) added, followed by 1,3-dicyclohexylcarbodiimide (0.68 g, 3.3 mmol). The mixture was stirred at room temperature for 1 h and then **21** (0.8 g, 3.4 mmol) in DMF (5 mL) was added. The mixture was stirred for a further 48 h and the solvent removed *in vacuo*. The residue was taken up in EtOAc and filtered. Washed with saturated aqueous $NaHCO_3$, 1N HCl and $H_2O$, dried ($MgSO_4$) and the solvent removed *in vacuo* to give a foam. This was purified by flash chromatography on silica eluting with EtOAc to give **22** as a white solid (1.25 g, 73%); m.p. 108–114 °C. $^1H$ NMR ($CDCl_3$) δ 1.35 (9H, s, $C(CH_3)_3$), 1.45 (3H, s, $CH_3$), 3.00 (1H, d, 13.7 Hz, CHHPh). 3.10–3.35 (3H, m, 3 x CHH), 3.77 (2H, d, 6.2Hz, $CH_2$), 4.15–4.25 (1H, m, CH), 5.13 (1H, s, NH), 5.48 (1H, s, NH), 6.29 (1H, s, NH), 6.95–7.00 (2H, m), 7.06 (1H, 2.2 Hz, ArH), 7.10–7.30 (7H, m), 7.41 (1H, d, 8.0 Hz, ArH), 7.56 (1H, d, 7.8 Hz, ArH), 8.38 (1H, s, indoleNH); IR 3310, 2980, 1653 $cm^{-1}$; MS m/e (FAB) 522 (M + H); Anal. $C_{28}H_{35}N_5O_5 \cdot 0.5H_2O$ (C, H, N).

*(S)-Tryptophyl-α-methyl-(S)-phenylalanyl-glycinamide* ***23***

**22** (1.6 g, 3 mmol) was dissolved in $CH_2Cl_2$ (100 mL) and trifluoroacetic acid (50 mL) added. The mixture was stirred at room temperature for 2 h and then the solvents removed *in vacuo*. The residue was triturated with $Et_2O$ to give a solid. This was partitioned between 10% aqueous $NH_3$ solution and $CH_2Cl_2$. The aqueous layer was separated and extracted with $CH_2Cl_2$ (2x). The organic extracts were combined, dried ($MgSO_4$) and removed *in vacuo*. The solid was purified by flash chromatography on silica eluting with $CH_2Cl_2$: MeOH (10:1) to give **23** as a white solid (1.2 g, 93%); m.p. 105–108 °C. $^1H$ NMR ($CDCl_3$) δ (3H, s, $CH_3$), 3.02–3.12 (2H, m, CHH, CHHPh), 3.21 (1H, dd, 14.4 Hz, CHH), 3.32 (1H, d, 13.7 Hz, CHHPh), 3.57 (1H, dd, 17.1 5.9 Hz, CHH), 3.75–3.80 (1H, m, CH), 3.86 (1H, dd, 17.2, 6.7 Hz, CHH), 5.42 (1H, s, NHC), 6.14 (1H, bt, $NHCH_2$), 7.05–7.10 (3H, m), 7.14 (1H, t, 7.0 Hz, ArH), 7.20–7.30 (5H, m, $+CHCl_3$), 7.39 (1H, d, 8.0 Hz, ArH), 7.55 (1H, s, indoleNH, CONHH), 7.64 (1H, d, 7.8 Hz, Ar), 8.40 (1H, s, indoleNH); IR 3307, 1661 $cm^{-1}$; MS m/e (CI) 422 (M + H); Anal. $C_{23}H_{27}N_5O_3 \cdot 0.25H_2$ (C, H, N).

*N-[[(2,3-Dimethoxyphenyl)methoxy]carbonyl]-(S)-tryptophyl-α-methyl-(S)-phenylalanyl-glycinamide* ***19***

2,3-Dimethoxybenzylalcohol (3.36 g, 20 mmol) and pyridine (1.7 mL, 20 mmol) were dissolved in $CH_2Cl_2$ and cooled to 0 °C. 4-Nitrophenylchloroformate (4.8 g, 24 mmol) was dissolved in $CH_2Cl_2$ (50 mL) and added dropwise to the above over 15 min. The mixture was stirred at room temperature overnight, washed with $H_2O$, dried ($MgSO_4$) and the solvent removed *in vacuo*. The residue was triturated with $Et_2O$ to give the mixed carbonate as a crystalline solid (4.5 g, 68%). m.p. 87–89 °C; $^1H$ NMR ($CDCl_3$) δ 3.89 (3H, s, $OCH_3$), 3.91 (3H, s, $OCH_3$), 5.37 (2H, s, $CH_2$), 6.95–7.12 (3H, m, ArH), 7.39 (2H, d, 9.1 Hz, ArH), 8.27 (2H, d, 9.1 Hz, ArH); IR 2940, 1766, 1525, 1486 $cm^{-1}$; MS m/e (CI) 333 (M); Anal. $C_{23}H_{27}N_5O_3 \cdot 0.25H_2O$ (C, H, N).

The mixed carbonate (0.4 g, 1.2 mmol) described above, **23** (0.5 g, 1.2 mmol), and triethylamine (0.17 mL, 2.3 mmol) were dissolved in DMF (25 mL) and stirred at room temperature overnight. The solvent was removed *in vacuo* and the residue taken up in EtOAc. This was washed with 10% aqueous $Na_2CO_3$ solution, 1N HCl, 10% aqueous $Na_2CO_3$, dried ($MgSO_4$) and the solvent removed *in vacuo*. The residue was purified by flash chromatography on silica eluting with a mixture of $CH_2Cl_2$: MeOH (9:1) to give **19** as a white solid (0.4 g, 55%); m.p. 106–110 °C, $^1H$ NMR ($d_6$-DMSO) δ 1–25 (3H, s, $CH_3$), 2.85–2.95 (1H, m, CHH). 3.00–3.10 (2H, m, 2 x CHH), 3.30–3.40 (1H, obscured by water, CHH), 3.50 (1H, dd, 16.8, 5.4 Hz, CHH), 3.66 (3H, s, $OCH_3$), 3.60–3.75 (1H, m, CHH), 3.80 (3H, s, $OCH_3$), 4.25–4.35 (1H, m, CH), 4.88 (1H, d, 12.4 Hz, CHH), 4.99 (1H, d, 12.4 Hz, CHH), 6.80–6.85 (1H, m, ArH), 6.90–7.20 (12H, m, $CONH_2$ indole (5C)H, (6C)H, (2C)H, and ArH), 7.35 (1H, d, 8.0 Hz, indole(7C)H), 7.58 (1H, d, 6.3 Hz, CONHCH), 7.63 (1H, d, 7.5 Hz, indole(4C)H), 7.87 (1H, bt, $CONHCH_2$), 8.31 (1H, s, CONHC), 10.82 (1H, s, indoleNH). MS m/e ($FAB^+$) 616 (70%) $(M + H)^+$ 638 (100%) $(M + Na)^+$. Anal. $C_{33}H_{37}N_5O_7 \cdot 0.5H_2O$ (C, H, N)

## Acknowledgements

The authors thank Dr G Ratcliffe, Mrs L Terry and Mrs R Franks for their technical assistance.

## References and Notes

1. Guard, S.; Watson, S. P. *Neurosci. Int.* **1991**, *18*, 149.

2. Maggi, C. A.; Quartara, L.; Giuliari, S.; Patacchini, R. *Drugs of the Future* **1993**, *18*, 155.

3. (a) Advenier, C.; Emonds-Alt, X.; Vilain, P.; Goulaouie, P.; Proietto, V.; Van Broeck, D.; Naline, E.; Nelia, G.; Le Fur, G.; Breliere, J. C. *Br. J. Pharmacol.* **1992**, 105, 78P; (b) Cooper, A. W. J.; Hagan, R. M. W09314084, 1993.

4. Horwell, D. C. In *Topics in Medicinal Chemistry, 4th SCI-RSC Medicinal Chem. Symp.*, p. 62, Leeming, P. R., Ed.; Royal Society of Chemistry; Letchworth, 1988.

5. Horwell, D. C.; Hughes, J.; Hunter, J. C.; Pritchard, M. C.; Richardson, R. S.; Roberts, E.; Woodruff, G. N. *J. Med. Chem.* **1991**, *34*, 404.

6. Horwell, D. C.; Howson, W.; Ratcliffe, G. S.; Rees, D. C. *BioMed. Chem. Lett.* **1993**, *3*, 799.

7. Thornber, C. W. *Chem. Soc. Rev.* **1979**, *8*, 563.

8. Lavelle, S.; Beaujouan, J. C.; Torrens, Y.; Saffroy, M.; Viger, A.; Chassaing, G.; Marquet, A.; Glowinski, J. In *Substance P*, p. 85, Jordan, C.; Oehme, P., Eds.; Taylor and Francis; London, 1985.

9. (a) Folkers, K.; Hong, J.; Rosell, S.; Bjorkroth, U. *Acta Physiol. Scand.* **1981**, *111*, 505; (b) Regoli, D.; Escher, D.; Mizrahi, J. *J. Pharmacol.* **1984**, *28*, 301; (c) Dutta, A. S.; Gormley, J. J.; Graham, A. S.; Briggs, I.; Growcott, J. W.; Jamieson, A. *J. Med. Chem.* **1986**, *29*, 1163; (d) Regoli, D.; Mizrahi, J.; D'Orleans-Juste, P.; Dion, S.; Drapeau, G.; Escher, E. *Eur. J. Pharm.* **1985**, *109*, 212.

10. McKnight, A. T.; Maguire, J. J.; Williams, B. J.; Foster, A. C.; Trigett, R.; Iversen, L. L. *Regul. Pep.* **1988**, *22*, 127.

11. Compounds **1–3** and **6** were obtained from Bachem U.K. Ltd, Saffron Walden, U.K. Compound **5** was obtained from Peninsula Labs, CA, U.S.A. Compound **7** was obtained from CRB, Cambridge, U.K. We thank Dr X. Emonds-Alt, Sanofie Recherche, Montpellier, France for the kind gift of SR 48968 (compound **4**).

12. Atherton, E.; Sheppard, R. C. In *Solid Phase Peptide Synthesis, A Practical Approach*, Oxford University Press; Oxford, 1989.

13. Eden, J. M.; Higginbottom, M.; Hill, D. R.; Horwell, D. C.; Hunter, J. C.; Martin, K.; Pritchard, M. C.; Raham, S. S.; Richardson, R. S.; Roberts, E. *Eur. J. Med. Chem.* **1993**, *28*, 37.

14. Buck, S. J.; Shatzer, S. A. *Life Sci.* **1988**, *42*, 2701.

15. Boyle, S. *et al.*, personal communication.

16. MR = Molar refractivity, see Taylor, P. J. In *Comprehensive Medicinal Chemistry*, Vol. 4, p. 250, Ramsden, C. A., Ed., Pergamon Press; Oxford, 1990.

17. Turk, J.; Panse, G. T.; Marshall, G. R. *J. Org. Chem.* **1975**, *40*, 953.

18. Arunlakshana, O.; Schild, H. O. *Br. J. Pharmacol.* **1954**, *14*, 48.

(*Received* 13 *December* 1993; *accepted* 6 *January* 1994)