Stereoselective Preparation of *N*-[(*R,R*)-(*E*)-1-(3,4-dichlorobenzyl)-3-(2-oxoazepan-3-yl)carbamoyl]allyl-*N*-methyl-3,5-bis(trifluoromethyl)benzamide, a Potent and Orally Active Dual Neurokinin NK_1/NK_2 Receptor Antagonist

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In a program aimed at the development of neurokinin antagonists, *N*-[(*R*,*R*)-(*E*)-1-(3,4-dichlorobenzyl)-3-(2-oxoazepan-3-yl)carbamoyl]allyl-*N*-methyl-3,5-bis(trifluoromethyl)benzamide (**1**, DNK333) has been discovered as a potent and balanced neurokinin (tachykinin) NK_1/NK_2 receptor antagonist. Enantiomerically pure (>99.5% ee) **1** can be prepared in 6 + 1 synthetic steps starting from commercially available optically active BOC-D-3,4-dichlorophenylalanine in an overall yield of ca. 25−30%. **1** showed potent affinities to cloned human NK_1 ($pK_i = 8.38$) and NK_2 ($pK_i = 8.02$) receptors. When **1** was compared to the other possible three diastereoisomers, it could be demonstrated that only the *R*,*R*-isomer (**1**) exhibits potent and balanced affinity for the cloned human NK_1 and NK_2 receptors. **1** exhibited favorable pharmacokinetic properties in guinea pigs following oral administration and demonstrated in vivo activity in pharmacological models of substance P- and neurokinin A (NKA)-induced bronchoconstriction in guinea pigs after intravenous and in squirrel monkeys after oral application.

Introduction

Neurokinins are members of a family of small peptides known as tachykinins that are widely distributed throughout the central and peripheral nervous systems.[1] The most prominent mammalian neurokinins are substance P (SP), neurokinin A (NKA), neurokinin B (NKB), and the recently discovered hemokinin 1.[2,3] Neurokinins exhibit their effects via specific G-protein-coupled receptors (NK_1, NK_2, and NK_3 receptors). Substance P and hemokinin A display the highest affinity for the NK_1 receptor, NKA displays the highest affinity for the NK_2 receptor, and NKB displays the highest affinity for the NK_3 receptor.[4] Neurokinins and the neurokinin receptors have been proposed to be involved in a number of pathological conditions including pain, arthritis, migraine, emesis, cancer, anxiety, depression, schizophrenia, asthma and airways diseases, inflammatory bowel disease, and incontinence, and NK receptor antagonists have been proposed to have potential clinical benefits.[5]

Over the past few years, many pharmaceutical companies have successfully shifted research efforts aimed at selective NK_1 or NK_2 receptor antagonists toward the identification of non-peptide dual NK_1/NK_2 receptor antagonists,[6] since increasing evidence was found for the contribution of both the NK_1 and the NK_2 receptors on a variety of diseases.[7,8]

We have recently described *N*-[1-(arylmethyl)-3-carbamoyl]allyl-*N*-methylbenzamides as a series of compounds capable of delivering potent, orally active dual

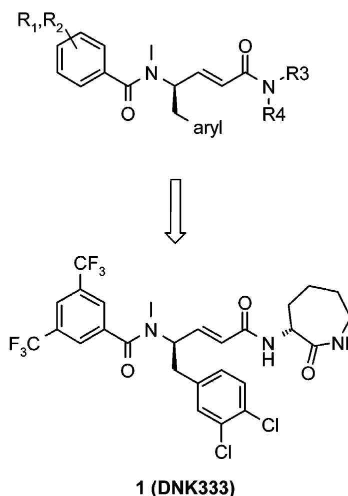

Figure 1. *N*-[1-(Arylmethyl)-3-carbamoyl]allyl-*N*-methylbenzamides and the structure of **1**.

NK_1/NK_2 receptor antagonists.[9] From this series **1**, (DNK333, (*N*-[(*R*,*R*)-(*E*)-1-(3,4-dichlorobenzyl)-3-(2-oxoazepan-3-yl)carbamoyl]allyl-*N*-methyl-3,5-bis(trifluoromethyl)benzamide) was found to exhibit potent and balanced affinity at NK_1 and NK_2 receptors[9] (Figure 1). We here describe the stereospecific synthesis of **1** and the preparation of its stereoisomers, together with NK_1 and NK_2 affinity data. We also provide results of in vivo studies with **1** in bronchoconstriction studies as well as the results from pharmacokinetic studies.

Chemistry

1 (*R*,*R*-isomer) was prepared starting from commercially available BOC-D-3,4-dichlorophenylalanine (**2**), which, after N-methylation and esterification with

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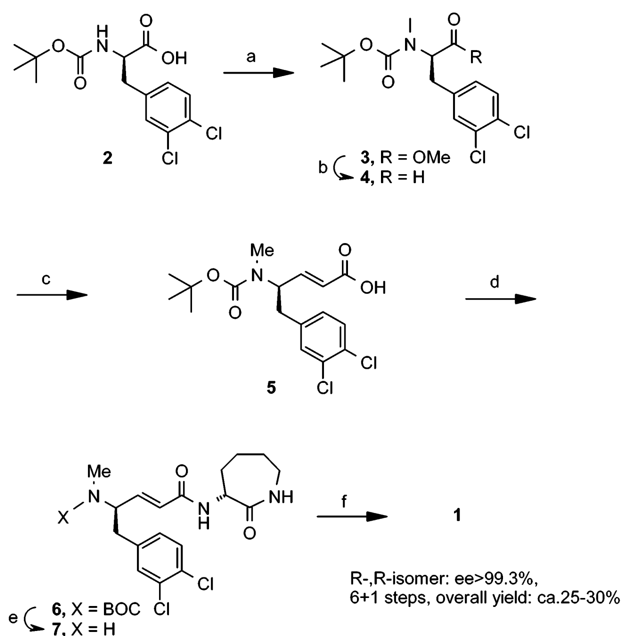
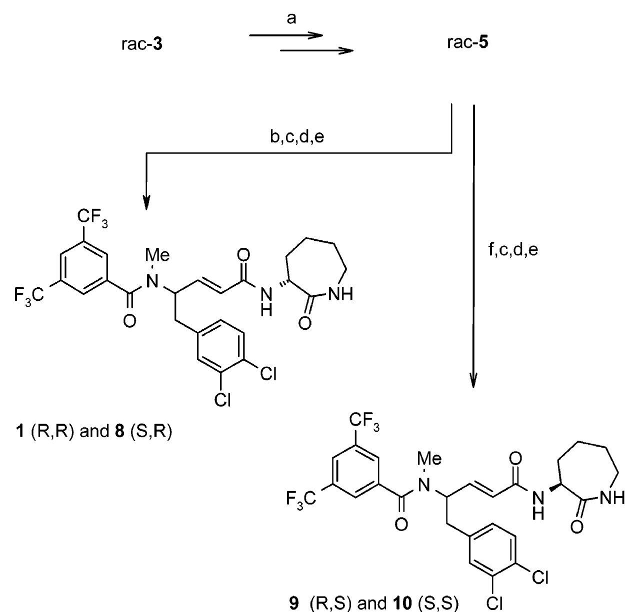
Scheme 1. Preparation of **1** (*R,R*-Isomer)[a]



[a] Reagents and conditions: (a) MeI, Ag_2CO_3, DMF; (b) DIBAH, toluene, −78 °C; (c) n-BuLi, trimethylsilyl P,P-diethylphosphonoacetate, then dilute HCl; (d) D-α-amino-ϵ-caprolactam, EDC, DMAP, CH_2Cl_2; (e) TFA, CH_2Cl_2; (f) 3,5-bis(trifluoromethyl)benzoyl chloride, Et_3N, CH_2Cl_2, crystallization from CH_2Cl_2/*n*-pentane.

Scheme 2. Preparation of Diastereoisomers[a]



[a] Reagents and conditions: (a) reaction conditions, see Scheme 1; (b) D-α-amino-ϵ-caprolactam, EDC, DMAP, CH_2Cl_2; (c) TFA, CH_2Cl_2; (d) 3,5-bis(trifluoromethyl)benzoyl chloride, Et_3N, CH_2Cl_2; (e) chromatography on silica gel; (f) L-α-amino-ϵ-caprolactam, EDC, DMAP, CH_2Cl_2.

excess methyl iodide in the presence of silver oxide in DMF, was converted to the ester **3**. The ester group of this compound was reduced with diisobutylaluminum hydride (DIBAH) in toluene at −78 °C to afford the corresponding aldehyde **4**. Chain elongation under Wittig−Horner conditions with trimethylsilyl P,P-diethylphosphonoacetate[10] in THF followed by hydrolysis of the trimethylsilyl ester group with diluted hydrochloric acid during the workup procedure yielded *R*-4-(*tert*-butoxycarbonylmethylamino)-5-(3,4-dichlorophenyl)pent-2-enoic acid **5**. Condensation of **5** with D-α-amino-ϵ-caprolactam[11] in the presence of *N*-ethyl-*N'*,*N'*-dimethylaminopropylcarbodiimide-HCl (EDC) and 4-(dimethylamino)-pyridine (DMAP) afforded *N*-[(*R*,*R*)-(*E*)-1-(3,4-dichlorobenzyl)-3-(2-oxoazepan-3-yl)carbamoyl]allyl-*N*-methylcarbamic acid *tert*-butyl ester (**6**). Removal of the BOC protecting group with trifluoroacetic acid in dichloromethane, subsequent acylation of the nitrogen with 3,5-bistrifluoromethylbenzoyl chloride in the presence of triethylamine in dichloromethane, and purification on silica gel led to the final product in an overall yield of ca. 25−30%. Crystallization of the product from CH_2Cl_2/*n*-pentane yielded **1** as a crystalline compound with a melting point of 127−129 °C. An enantiomeric excess (ee) value of >99.5% could be determined by HPLC analysis using a Chiralcel OJ column (Scheme 1).

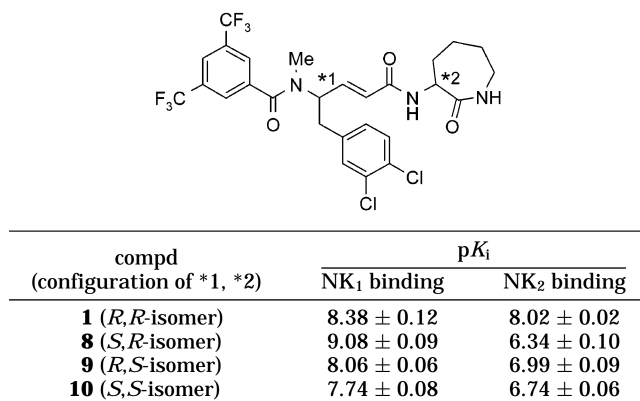
The remaining three stereoisomers (and **1**) can be prepared as outlined in Scheme 2 starting from racemic **3**[12] under exactly the same reaction sequence used for the stereospecific preparation of **1** (see Scheme 1). Coupling of the racemic carboxylic acid derivative rac-**5** with either enantiomerically pure D(*R*)- or L(*S*)-α-amino-ϵ-caprolactam[11] in the presence of "EDC and DMAP, followed by cleavage of the BOC group and acylation of the nitrogen with 3,5-bistrifluoromethylbenzoyl chloride, led in both cases to a mixture of two diastereoisomers that could easily be separated by standard flash chromatography on silica gel to afford the diastereoisomers **8**−**10** as pure compounds (Scheme 2).

Results and Discussion

By comparison of the chromatographical behavior of compounds **8**−**10** with **1** (*R,R*-isomer), the absolute configuration of the stereocenters of the three remaining isomers could be assigned, and all four compounds were tested for their binding affinities to NK_1 and NK_2 receptors. In comparison to the other three possible isomers, **1** (*R,R*-isomer) clearly exhibits the most potent NK_2 binding affinity showing at least 10-fold higher affinity for this receptor in comparison to the other isomers. With respect to affinity for human NK_1 receptors however, the *S,R*-isomer (**8)** is some 5 times more potent than **1** but exhibits the lowest affinity to the NK_2 receptor. Of the four stereoisomers, the *S,S*-isomer **10** (i.e., enantiomer of **1)** exhibits the lowest affinity to the NK_1 receptor. Only the *R,R*-isomer (**1**) exhibits potent and balanced affinity for the cloned human NK_1 and NK_2 receptors (Table 1).

Following confirmation of activity in in vitro functional tests, where the compound demonstrated blockade of bronchoconstrictor responses induced by selective NK_1 (pA_2 = 7.93) and selective NK_2 (pA_2 = 7.27) agonists in the guinea pig isolated trachea model,[13] in in vivo experiments the ability of **1** to antagonize either Sar^9 substance P (a selective and stable NK_1 receptor agonist) or (Ala^5,β-Ala^8)-α-NKA (fragments 4−10) (a selective and stable NK_2 receptor agonist) induced bronchoconstriction in anesthetised guinea pigs was tested. The results are presented in Figure 2. **1**, 1 mg/kg, dosed iv at 5 min or 1 h prior to administration of the NK_1 agonist Sar^9-SP induced 15.8- or 15.7-fold shifts in the dose−response curves to the right, respectively. **1**, 10 mg/kg, dosed iv at 5 min or 1 h prior to adminis-

Table 1. In Vitro Binding Affinities (pK_i Values) of **1** and Compounds **8**–**10** to Human NK_1 and NK_2 receptors[a]



compd (configuration of *1, *2)	pK_i	
	NK_1 binding	NK_2 binding
1 (*R,R*-isomer)	8.38 ± 0.12	8.02 ± 0.02
8 (*S,R*-isomer)	9.08 ± 0.09	6.34 ± 0.10
9 (*R,S*-isomer)	8.06 ± 0.06	6.99 ± 0.09
10 (*S,S*-isomer)	7.74 ± 0.08	6.74 ± 0.06

[a] $n = 3$ for all compounds.

tration of the NK_2 agonist (Ala^5,β-Ala^8)-α-NKA (fragments 4–10) demonstrated 2.3- or 2.6-fold shifts in the dose–response curves to the right, respectively.

There is no loss of activity up to 1 h after iv administration of **1**, and the relative potency was ca. 6-fold in favor of NK_1 blockade, a finding that is broadly consistent with the in vitro relative antagonist potency at guinea pig NK_1 ($pA_2 = 7.93$) and NK_2 ($pA_2 = 7.27$) receptors (guinea pig isolated trachea[13]).

1 was tested in a second in vivo study, the bronchoconstrictor response to the selective NK_2 receptor agonist β-Ala^8-NKA, in the anesthetised squirrel monkey. When administered orally 2 h prior to the agonist, **1** inhibited β-Ala^8-NKA induced bronchoconstriction dose-dependently with ED_{50} values of 1 mg/kg (airways resistance) and 2.8 mg/kg (dynamic compliance) (Figure 3).

The pharmacokinetic properties of **1** were investigated in guinea pigs (3 mg/kg, iv; 10 mg/kg, po) following administration of **1** as a cremophor formulation (iv) and as microemulsion or cremophor oral formulations. A terminal elimination half-life of ca. 4.2 h after intravenous administration and apparent termination half-lives of 3.8 h (microemulsion formulation) and 5.4 h (cremophor formulation) after oral administration of the compound could be observed. After oral administration of 10 mg/kg **1**, an AUC_{0-24h} of 6378 ng h/mL and a bioavailability of approximately 41% can be observed when the compound was applied as a microemulsion. A prolonged half-life but lower AUC of 2712 ng h/ml and a bioavailability of just 17.4% were observed after oral application of **1** as a cremophor formulation (Table 2).

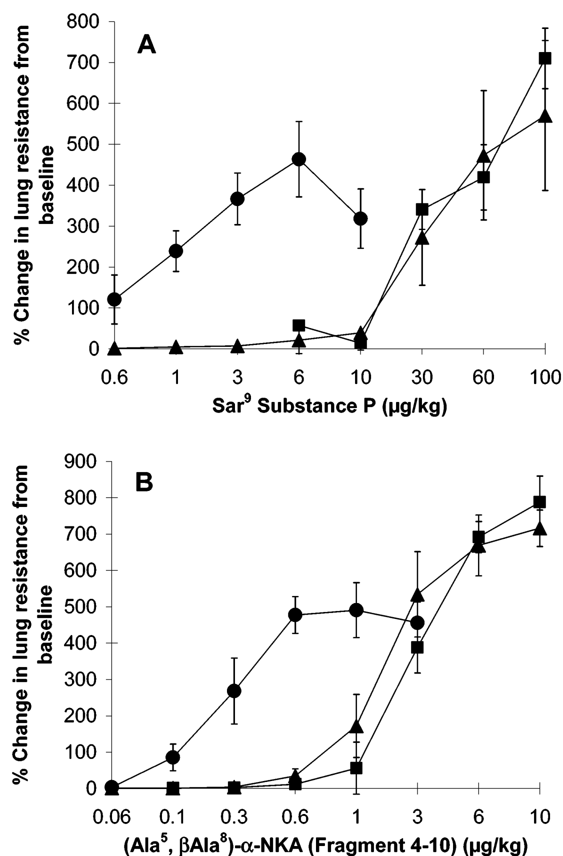


Figure 2. Inhibition of NK_1 (A) and NK_2 (B) agonist induced bronchoconstriction in guinea pigs. Inhibitory effects on airway resistance of 1 mg/kg (upper graph) or 10 mg/kg (lower graph) **1** dosed iv: 5 min (▲, $n = 9$ or 5) or 1 h (■, $n = 4$ or 5) prior to establishing a dose–response curve to Sar^9SP, a selective NK_1 receptor agonist (A), or to (Ala^5,β-Ala^8)-α-NKA (fragments 4–10), a selective NK_2 receptor agonist (B) compared with vehicle dosed iv (●, $n = 4$, both).

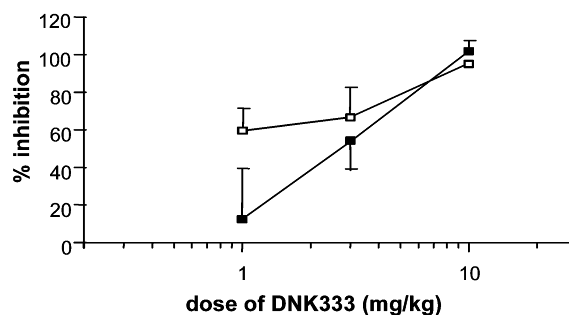


Figure 3. Inhibition of changes in airway resistance (□) and dynamic compliance (■) induced by β-Ala^8-NKA (1 mM aerosol) by **1**. Dose–response curves for **1** in the anesthetized squirrel monkey, $n = 12$.

Conclusions

With the availability of potent and orally bioavailable dual NK_1/NK_2 neurokinin receptor antagonists, it is now possible to further elucidate the role of NK_1 and NK_2 receptors in contributing to pathophysiological processes, in particular in respiratory diseases, and to define their utility as drugs. *N*-[(*R*,*R*)-(*E*)-1-(3,4-dichlorobenzyl)-3-(2-oxoazepan-3-yl)carbamoyl]allyl-*N*-methyl-3,5-bis(trifluoromethyl)benzamide (**1**) has been discovered as a potent, balanced (human receptors), orally active dual NK_1/NK_2 receptor antagonist. The compound can be prepared as an enantiomerically pure product (>99.5% ee) from commercially available optically active starting material using a short synthesis comprising 6 + 1 chemical steps in an overall yield of ca. 30%. We have also shown that **1** exhibits potent and balanced affinity to cloned human NK_1 and NK_2 receptors. NK_1 and NK_2 receptor blocking activity could be demonstrated in bronchoconstriction studies in guinea pigs (after intravenous administration) and in squirrel monkeys (after oral administration). Favorable pharmacokinetic properties after oral administration of the compound formulated as a microemulsion to guinea pigs have been observed.

Table 2. Pharmacokinetic Parameters of **1** in Guinea Pigs

route of administration	$t_{1/2}$ (h)	C_{max} (ng/mL)	AUC_{0-24h} (ng h/mL)	bioavailability (%)
guinea pig, iv, $n = 6$, 3 mg/kg	4.2		4687	
guinea pig, po, $n = 7$, 10 mg/kg	3.8	600	6378 (microemulsion)	41
	5.4	415	2712 (cremophor)	17.4

Experimental Section

General. Melting points were determined on a Reichert melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 781 spectrometer. Proton NMR (^{1}H NMR) were recorded at 300 MHz on a Varian Mercury spectrometer and at 400 MHz on Bruker AMX 400 and Varian VXR-400 S spectrometers. Deutero-DMSO or $CDCl_3$ were used as solvents. In some cases NMR spectra were recorded at 120 or 150 °C to ease NMR spectral interpretation. Chemical shifts are given in δ units (ppm) and relative to deuterated solvent. NMR multiplicity are denoted by s (singlet), d (doublet), m (multiplet), and bs (broad singlet). HPLC experiments were carried out on Chiralcel OJ or AD columns (25 cm × 0.46 cm), with hexane/2-propanol = 85:15 + 0.1% TFA gradients at a flow rate of 1 mL/min and detection at 210 nM. Optical rotations were measured in absolute ethanol on a Perkin-Elmer 241 polarimeter at 20 °C. Elemental analyses were performed in the labs of Solvias AG Elemente-Analytik, Basel (CH), Switzerland, and are within ±0.4% of theoretical values unless otherwise noted. Reagents and starting materials were obtained from commercial sources unless otherwise stated. Reactions were in general carried out under an argon atmosphere. Solvents used were distilled or dried by standard techniques prior to use. After aqueous workup, magnesium sulfate was used as drying agent for the organic phase. Chromatography on silica gel was carried out on Merck silica gel 60 (230−400 mesh) under flash conditions.

(*R*)-2-(*tert*-Butoxycarbonylmethylamino)-3-(3,4-dichlorophenyl)propionic Acid Methyl Ester (3). To a solution of (*R*)-2-*tert*-butoxycarbonylamino-3-(3,4-dichlorophenyl)propionic acid (2.0 g, 5.7 mmol) in DMF (30 mL) was added silver oxide (8 g), methyl iodide (20 g), and acetic acid (0.8 mL). This mixture was stirred at room temperature for 20 h. Then Et_2O (200 mL) was added and the resulting suspension was filtered. The filtrate was washed with water (three times) and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash chromatography (hexane/EtOAc = 5:1) to give **3** (2.0 g, 93%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$, room temp): δ 7.36 (d, 1H), 7.25 (s, 1H), 7.02 (m, 1H), 4.86 and 4.52 (2m, 1H), 3.76 (bs, 3H), 3.28 (m, 1H), 3.00 (m, 1H), 2.74 and 2.70 (2s, 3H), 1.40 and 1.35 (2s, 9H). Colorless oil. Anal. ($C_{16}H_{21}NO_4Cl_2$) C, H, N, Cl.

[(*R*)-1-(3,4-Dichlorobenzyl)-2-oxoethyl]methylcarbamic Acid *tert*-Butyl Ester (4). A solution of (*R*)-2-(*tert*-butoxycarbonylmethylamino)-3-(3,4-dichlorophenyl)propionic acid methyl ester (**3**) (2.0 g, 5.52 mmol) in toluene (50 mL) was cooled to −78 °C. Then diisobutylaluminum hydride (11.5 mL of 20% solution in toluene) was added dropwise over a period of 0.5 h. After an additional stirring period of 1 h, methanol (2 mL) was added and the reaction mixture warmed to 0 °C and poured into 50 mL of an ice-cold citric acid solution (18 g/50 mL). This mixture was stirred at 0 °C for 2 h. Then the layers were separated and the aqueous layer was extracted twice with Et_2O. The combined organic layers were washed with water and brine, dried, and concentrated under vacuum to give **4** (1.76 g, 96%) as a pale-yellow oil. This material was used without further purification in the next step. ^{1}H NMR (300 MHz, $CDCl_3$, room temp): δ 9.60 (s, 1H), 7.36 (d, 1H), 7.25 (s, 1H), 7.02 (m, 1H), 4.14 and 4.02 (2m, 1H), 3.24 (m, 1H), 3.02−2.80 (m, 1H), 2.76 and 2.68 (2s, 3H), 1.44 and 1.37 (2s, 9H).

(*E*)-(*R*)-4-(*tert*-Butoxycarbonylmethylamino)-5-(3,4-dichlorophenyl)pent-2-enoic Acid (5). To a solution of trimethyl P,P-diethylphosphonoacetate (2.74 g, 10.2 mmol) in THF (27 mL), n-BuLi (6.6 mL, 1.6 M in hexane) was added at 0 °C. After the mixture was stirred for 1 h, a solution of **4** (2.15 g, 6.48 mmol in 8 mL of THF) was added. Then the cooling bath was removed and the reaction mixture was stirred for 1 h. To the reaction mixture, 0.1 N HCl (100 mL) was added. After being stirred for 20 min, the reaction mixture was extracted three times with EtOAc. The combined organic layers were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc) to give **5** (1.83 g, 76%) as a colorless oil. ^{1}H NMR (400 MHz, DMSO, +150 °C): δ 7.49 (d, 1H), 7.48 (s, 1H), 6.78 (dd, 1H), 5.85 (d, 1H), 3.00 (m, 2H), 2.68 (s, 3H), 1.36 (s, 9H). Anal. ($C_{17}H_{21}Cl_2NO_4$) H, N. C: calcd, 54.56; found, 54.15.

[(*E*)-(*R*)-1-(3,4-Dichlorobenzyl)-3-((*R*)-2-oxoazepan-3-ylcarbamoyl)allyl]methylcarbamic Acid *tert*-Butyl Ester (6). A solution of **5** (0.5 g, 1.34 mmol), D-α-amino-ε-caprolactam (0.172 g, 1.34 mmol), *N*-ethyl-*N*′-(3-dimethylaminopropyl)-carbodiimid hydrochloride (0.405 g, 2.01 mmol), and 4-(dimethylamino)pyridine (0.16 g, 1.34 mmol) in methylene chloride (15 mL) was stirred at room temperature for 20 h. The reaction mixture was concentrated in vacuo and the residue was purified by flash chromatography to yield a colorless oil (0.46 g, 76%). The oil was recrystallized from Et_2O to give **6** as white crystals. Mp 153−154 °C. $[\alpha]^{20}_D$ +1.6° (*c* 2.50, EtOH). ^{1}H NMR (400 MHz, DMSO, +120 °C): δ 7.62 (bs, 1H), 7.49 (d, 1H), 7.47 (s, 1H), 7.37 (bs, 1H), 7.22 (d, 1H), 6.62 (dd, 1H), 6.18 (d, 1H), 4.80 (bs, 1H), 4.51 (m, 1H), 3.25−3.12 (m, 2H), 2.95 (m, 2H), 2.68 (s, 3H), 1.90 (m, 2H), 1.80−1.73 (m, 2H), 1.46 (m, 1H), 1.30 (s, 9H), 1.29 (m, 1H). Anal. ($C_{23}H_{31}N_3O_4Cl_2$) C, H, N, Cl.

(*E*)-(*R*)-5-(3,4-Dichlorophenyl)-4-methylaminopent-2-enoic Acid (*R*)-2-Oxoazepan-3-yl)amide (7). A solution of **6** (0.230 g, 0.478 mmol) and trifluoroacetic acid (1.5 mL) in methylene chloride (10 mL) was stirred for 4 h at room temperature. The reaction mixture was concentrated in vacuo, and the residue was disolved in 50 mL of EtOAc. This solution was washed twice with 0.1 N NaOH, water, and brine, dried, and concentrated in vacuo to give **7** as a crude product that was directly used in the next step.

***N*-[(*E*)-(*R*)-1-(3,4-Dichlorobenzyl)-3-(*R*)-2-oxoazepan-3-ylcarbamoyl)allyl]-*N*-methyl-3,5-bis(trifluoromethyl)-benzamide (1).** To a solution of **7** (ca. 0.144 g, ca. 0.375 mmol), triethylamine (0.27 mL), and 4-(dimethylamino)pyridine (0.01 g) in dichloromethane (3.5 mL), 3,5-bis(trifluoromethyl)benzoyl chloride (0.081 mL dissolved in 1 mL of methylenchloride) was added dropwise at 0 °C. The reaction mixture was stirred for 0.5 h at 0 °C and 2 h at room temperature. Then EtOAc (30 mL) was added, and the resulting mixture was washed with 0.1 N HCl, water, and brine. The organic layer was dried and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc) followed by crystallization from methylenchloride/*n*-pentane to yield **1** as white crystals (0.142 g, 61%). Mp 127−129 °C. $[\alpha]^{20}_D$ +39.9° (*c* 0.94, EtOH). ee ≥ 99.5 (t_R = 21.64 min, Chiralcel OJ, hexane/2-propanol = 85:15 + 0.1% TFA, flow rate 1 mL/min). ^{1}H NMR (400 MHz, DMSO, +150 °C): δ 8.02 (s, 1H), 7.65 (s, 2H), 7.55 (bs, 1H), 7.44 (m, 2H), 7.28 (bs, 1H), 7.21 (bs, 1H), 6.71 (dd, 1H), 6.32 (dd, 1H), 5.03 (bs, 1H), 4.51 (m, 1H), 3.18 (m, 2H), 3.05 (m, 2H), 2.82 (s, 3H), 1.96 (m, 2H), 1.75 (m, 2H), 1.45 (m, 1H), 1.3 (m, 1H). Anal. ($C_{27}H_{25}Cl_2F_6N_3O_3$) C, H, N, Cl, F. IR ($CH_2Cl_2$, cm^{-1}): 1668 (−CH=CH−C=O), 1643 (Ph−C=O), 1286 (Ph−CF_3), 975 (C−H, trans disubstituted C=C).

***N*-[(*E*)-(*S*)-1-(3,4-Dichlorobenzyl)-3-(*R*)-2-oxoazepan-3-ylcarbamoyl)allyl]-*N*-methyl-3,5-bis(trifluoromethyl)-benzamide (8).** **8** is a white crystalline powder (from methylene chloride/*n*-pentane). Mp 114−122 °C. $[\alpha]^{20}_D$ −59.6° (*c* 0.55, EtOH). ^{1}H NMR (400 MHz, DMSO, +150 °C). ^{1}H NMR (400 MHz, DMSO, +120 °C): δ 8.02 (s, 1H), 7.65 (s, 2H), 7.55 (bs, 1H), 7.44 (m, 2H), 7.28 (bs, 1H), 7.23 (bs, 1H), 6.70 (dd,

1H), 6.315 (dd, 1H), 5.03 (bs, 1H), 4.50 (m, 1H), 3.18 (m, 2H), 3.05 (m, 2H), 2.82 (s, 3H), 1.96 (m, 2H), 1.75 (m, 2H), 1.45 (m, 1H), 1.3 (m, 1H). Anal. ($C_{27}H_{25}Cl_2F_6N_3O_3$) C, H, N.

***N*-[(*E*)-(*R*)-1-(3,4-Dichlorobenzyl)-3-(*S*)-2-oxoazepan-3-ylcarbamoyl)allyl]-*N*-methyl-3,5-bis(trifluoromethyl)-benzamide (9).** **9** is a white crystalline powder (from methylenchloride/*n*-pentane). Mp 115−120 °C. $[\alpha]^{20}_D$ +58.7° (*c* 0.65, EtOH). ^{1}H NMR (400 MHz, DMSO, +120 °C): δ 8.03 (s, 1H), 7.67 (d, 1H), 7.60 (s, 2H), 7.44 (m, 2H), 7.40 (bs, 1H), 7.20 (bs, 1H), 6.68 (dd, 1H), 6.37 (dd, 1H), 5.06 (bs, 1H), 4.52 (m, 1H), 3.15 (m, 2H), 3.07 (m, 2H), 2.81 (s, 3H), 1.90 (m, 2H), 1.80−1.63 (m, 2H), 1.45 (m, 1H), 1.3 (m, 1H). Anal. ($C_{27}H_{25}Cl_2F_6N_3O_3$) C, H, N, Cl.

***N*-[(*E*)-(*S*)-1-(3,4-Dichlorobenzyl)-3-(*S*)-2-oxoazepan-3-ylcarbamoyl)allyl]-*N*-methyl-3,5-bis(trifluoromethyl-benzamide (10).** **10** is a white crystalline powder (from methylene chloride/*n*-pentane). Mp 122−127 °C. $[\alpha]_D^{20}$ −39.25° (*c* 0.53, EtOH). ^{1}H NMR (400 MHz, DMSO, +120 °C): δ 8.03 (s, 1H), 7.67 (d, 1H), 7.60 (s, 2H), 7.44 (m, 2H), 7.40 (bs, 1H), 7.20 (bs, 1H), 6.70 (dd, 1H), 6.35 (dd, 1H), 5.06 (bs, 1H), 4.51 (m, 1H), 3.15 (m, 2H), 3.07 (m, 2H), 2.81 (s, 3H), 1.90 (m, 2H), 1.80−1.63 (m, 2H), 1.45 (m, 1H), 1.3 (m, 1H). Anal. ($C_{27}H_{25}Cl_2F_6N_3O_3$) C, H, N, Cl.

NK_1, NK_2 Receptor Binding Assays. Cloned human receptors, expressed in CHO cells, were purchased from NEN Life Sciences. Ninety-six-deep-well plates were used, with each well containing 375 μL of a membrane receptor suspension (1:50 dilution of the original stock), 125 μL of ^{3}H-[Sar^{9}Met-$(O_2)^{11}$]SP (final concentration of 0.5 nM) for NK_1 or ^{125}I-neurokinin A (final concentration of 1.4 nM) for NK_2 recetors, 25 μL of **1**, **8**, **9**, or **10**, all prepared and diluted in HEPES buffer composition: 20 mM HEPES, 1 mM $MnCl_2$, pH 7.4. Nonspecific binding was determined in the presence of 0.3 μL of Sar^{9}SP or 10 μL of NKA for the NK_1 receptor or the NK_2 receptor, respectively. The mixture was incubated for 60 min at 28 °C, after which the unbound ligand was removed by inverse flash filtration into 96-well filter plates followed by three washes with ice-cold Tris buffer (50 mM, pH 7.4). The filter plates were dried for 15 min at 56 °C, followed by the addition of 50 μL of scintillation, and sealed on top with an adhesive sheet. The radioactivity was counted in a Packard 96-well plate counter.

Neurokinin-Induced Bronchoconstriction in the Guinea Pig. Male Dunkin−Hartley guinea pigs (400−550 g) were anesthetized and mechanically ventilated. The jugular vein and carotid artery were cannulated for injection of drug and blood pressure measurements, respectively. All signals were recorded using a computer data acquisition system (Lfr, Mumed Systems). After establishment of a stable baseline, dose−response curves were constructed for Sar^9 substance P (0.6−10 μg/kg) or (Ala^5,β-Ala^8)-α-neurokinin (fragments 4−10) (0.06−3 μg/kg). The agonists were dissolved in 0.9% NaCl and injected iv every 15 min as increasing quarter-log increments. Increases in lung resistance were determined. The timing of anesthesia and animal preparation were such that the dose−response curves to Sar^9 substance P or (Ala^5,β-Ala^8)-α-neurokinin (fragments 4−10) were obtained 5 min and 1 h after the iv injection of vehicle or **1**. **1** was given at doses of 1 or 10 mg/kg in a vehicle of 2% DMSO/17% cremophor EL/ 81% NaCl. Results were analyzed by calculating the percent change from baseline of each agonist response. Best-fit dose−response graphs were constructed, and values corresponding to a 300% increase in resistance to agonist were calculated to quantify the inhibitory effects of the antagonists. Student's *t*-test (for normally distributed data) and Mann−Whitney rank sum test (for nonparametric data) were used to evaluate the significance of the changes in resistance values at 300%. Results are quoted as the mean ± SEM. Significance was determined as $P < 0.05$.

Neurokinin-Induced Bronchoconstriction in the Squirrel Monkey. Squirrel monkeys known to respond with bronchoconstriction to an aerosol of 1 mM β-Ala^8-NKA were studied every 2 weeks in a randomized fashion. Conscious monkeys were dosed orally with either vehicle (3% DMSO/8.8% cremophor/88.2% saline) or **1** (1, 3, and 10 mg/kg). After 70 min, the animals were anesthetized and airway parameters were monitored. The monkeys were exposed 2 h after administration of **1** to an aerosol of a 1 mM solution of β-Ala^8-NKA for 5 min.

Pharmacokinetic Testing. Male Dunkin−Hartley guinea pigs (350 g) were anesthetized with 4% halothane (Rhone Merieux, U.K.) and 1:1 oxygen/nitrous oxide (2 L each per min) and maintained at a reduced level for surgery (1.5% halothane). Analgesia was initiated using 0.06 mg/kg intramuscular buprenorphine (Vestergesic, Reckitt and Coleman, U.K.). The carotid artery was cannulated with pp50 polythene tubing (Portex, U.K.) attached to a 1 mL syringe containing 500 IU/mL heparin (Multiparin, CP Pharmaceuticals Ltd, U.K.) in 0.9% w/v saline. For animals receiving an intravenous dose, the jugular vein was also cannulated. All cannulae were exteriorized between the shoulders and tied in place. Animals were allowed to recover, and up to 6 h postcannulation, each cannula was flushed with 50 μL of 500 IU/mL heparinized 0.9% w/v saline. Animals to be dosed orally had food withdrawn up to 18 h predose. The following morning, cannula patency was checked using 101 IU/mL heparinized 0.9% w/v saline. **1** was administered to guinea pigs at 3 mg/kg via the jugular vein (iv, $n = 6$) or at 10 mg/kg orally (po, $n = 7$) as a microemulsion (2% DMSO/20% Sandimmun placebo/78% saline) or cremophor/2% DMSO formulation. Blood samples were taken at 5, 30, 60, 120, 240, 360, 720, and 1440 min (iv) and at 60, 120, 240, 360, 480, 720, and 1440 min (oral). Blood samples were analyzed for **1** by HPLC mass spectrometry (LC−MS).

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