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N-[(*R*,*R*)-(*E*)-1-(4-Chloro-benzyl)-3-(2-oxo-azepan-3-ylcarbamoyl)-allyl]-*N*-methyl-3,5-bis-trifluoromethyl-benzamide: An Orally Active Neurokinin NK_1/NK_2 Antagonist

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Abstract—The stereoselective synthesis of *N*-[(*R*,*R*)-(*E*)-1-(4-chloro-benzyl)-3-(2-oxo-azepan-3-ylcarbamoyl)-allyl]-*N*-methyl-3,5-bis-trifluoromethyl-benzamide (**4**) and its NK_1 and NK_2 receptor binding properties are reported. In addition the potent inhibitory effects in vivo on sar[9]-SP- and β-Ala-NKA-induced airway bronchoconstriction in guinea pigs are demonstrated.

Neurokinins are proposed to be involved in a number of pathological conditions including pain, arthritis, migraine, emesis, cancer, anxiety, depression, schizophrenia, asthma and airway diseases, and NK receptor antagonists have been proposed to have potential clinical benefits.[1] The results obtained from animal and clinical studies in recent years provide an emerging pharmacological evidence that neurokinins play an important role in airway disease induction and progression via the activation of NK_1 and NK_2 receptors.[2] Furthermore, the studies suggest that neurokinin receptor antagonists, especially dual NK_1/NK_2 antagonists, may represent a new treatment option for asthma and other airway diseases, particularly since lung tissue from asthma patients has been shown to over-express NK_1 and NK_2 receptors.[3] As a consequence many pharmaceutical companies have shifted their efforts aiming at selective NK-antagonists towards the discovery of dual NK_1/NK_2 antagonists.

Our efforts aiming at the discovery of dual NK_1/NK_2 antagonists as potential anti-asthma agents led to the discovery of a series of *N*-[(*E*)-3-carbamoyl-1-(4-chloro-benzyl)-allyl]-*N*-methyl-3,5-bis-trifluoro-methyl-benzamides, as dual NK_1/NK_2 antagonists, derived originally from the selective NK_1 antagonist **CGP49823** (Fig. 1).[4,5] Formal elimination of a CH_2-group from the piperidine ring led to a new series of ''open chain'' neurokinin receptor antagonists. In general, compounds from this structural class exhibited highly potent affinity to the NK_1 receptor (inhibition of ^{3}H-sar[9]-substance *P* binding to bovine retinal membranes) and a number of compounds exhibited an additional affinity to the NK_2 receptor (inhibition of ^{125}I-NKA binding to human NK_2-CHO-cells). In these binding assays, **1** and **2** exhibited IC_{50} values of 0.7 nM (NK_1) and 55 nM (NK_2), and 10 nM (NK_1) and 49 nM (NK_2) respectively.[5]

Compound **1** which exhibited highly potent affinity to the NK_1 receptor and a promising affinity to the NK_2 receptor was prepared and tested as a mixture of 4 stereoisomers. In order to find out which isomers (and to what degree) are responsible for the affinity of **1** to the neurokinin receptors, all 4 isomers have been prepared as outlined in Scheme 1. Coupling of the racemic acid **3** with either enantiomerically pure D(*R*)- or L(*S*)-α-amino-ε-caprolactam [6] followed by deprotection and acylation led in both cases to a mixture of two diastereoisomers which could easily be separated on a standard flash-chromatography column.

In order to assign the absolute stereochemistry of the chiral center in the carbon chain of the four isomers, we

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chose to work out a stereoselective synthesis to prepare specifically one isomer as an enantiomerically pure compound. In the 4-amino-piperidine series of selective NK_1 antagonists (e.g., **CGP49823**) it was shown that the compounds possessing the *S*-configuration at C-2 exhibited higher affinity to the NK_1 receptor in comparison to the corresponding *R*-enantiomers. Therefore, we focused our efforts on the stereoselective preparation of an isomer that possessed the same stereochemistry at the corresponding chiral center.

The *R,R*-isomer was prepared starting from commercially available D-4-chlorophenyl-alanin-methylester (**8**) which, after BOC-protection followed by *N*-methylation, was reduced to the aldehyde. Chain elongation using trimethylsilyl-*P,P*-diethyl-phosphonoacetate and an acidic work up procedure led to the carboxylic acid **11**. Introduction of D-α-amino-ε-caprolactam (**D-13**) in the presence of EDC, removal of the BOC protecting group and subsequent acylation of the nitrogen with 3,5-bistrifluoro-methylbenzoyl-chloride led to the final product in an overall yield of 20% after recrystallisation from CH_2Cl_2/*n*-pentane (ee = 95% before and ee = 98.5% after recryst.) (Scheme 2). During the synthesis starting with D-4-chlorophenyl-alanin-methylester (**8**, ee >97%) no significant epimerisation of the chiral center could be observed.[7] After checking all intermediates for optical purity, we showed that the very minor loss of optical purity had occurred during the *N*-methylation of *N*-BOC-D-4-chlorophenyl-alanin-methylester in the presence of silver oxide and DMF at the beginning of the synthesis (Scheme 2).

On the basis of the chromatographical behaviour of **4** (*R,R*-isomer), the absolute configuration of the stereocenters of all the isomers produced starting from **3** could be assigned and all four compounds were tested for their binding affinities in the NK-receptor assays.[8,9] As can be seen from the NK_1 and NK_2-binding values in Table 1, the *R,R*-isomer (**4**) clearly exhibits the highest affinity for both the NK_1 and the NK_2 receptor. Changing the stereochemistry of the backbone chiral center from R to S (resulting in the *S,R*-isomer, (**5**) leads to a decrease in binding affinities to the NK_1 and NK_2 receptors by a factor of 30 and 5 respectively. Inversion of the stereochemistry at the chiral center of the caprolactam moiety (leading to the *R,S*-isomer, (**7**) results in reduced binding

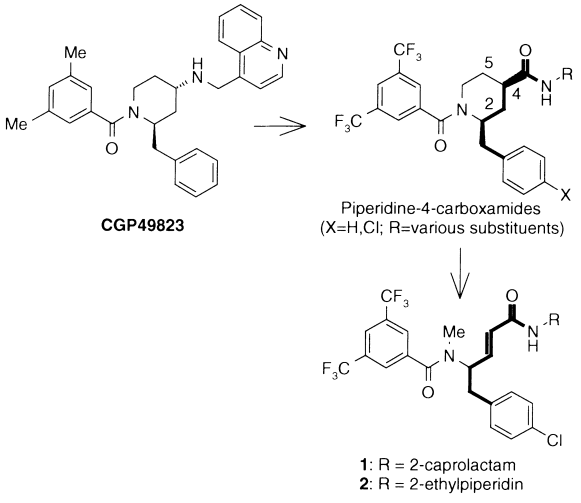
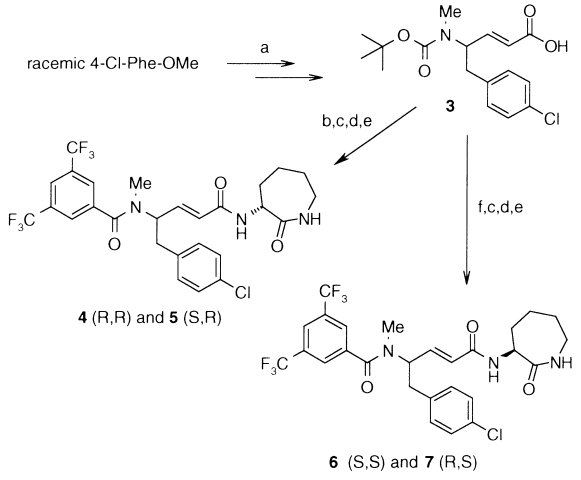
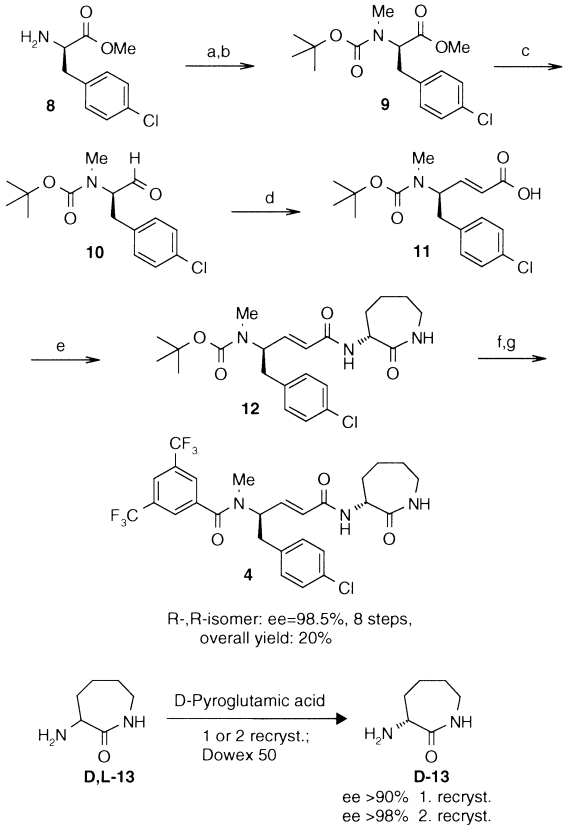


Figure 1. The discovery of 5-aryl-4-benzoyl-amino-pent-2-ene-carboxamides.



Scheme 1. Preparation of diastereoisomers. (a) Reaction conditions: see Scheme 2. (b) D-α-amino-ε-caprolactam, EDC, DMAP, CH_2Cl_2; (c) TFA, CH_2Cl_2; (d) 3,5-Bistrifluoromethyl-benzoyl-chloride; Et_3N, CH_2Cl_2; (e) Chromatography on silica gel; (f) L-α-amino-ε-caprolactam, EDC, DMAP, CH_2Cl_2.



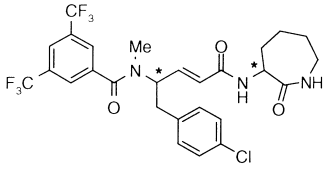
Scheme 2. Stereospecific preparation of **4**. (a) $(BOC)_2O$, Et_3N; b) MeI, Ag_2CO_3, DMF; (c) DIBAH, toluene, −78 °C; (d) *n*-BuLi, trimethylsilyl-*P,P*-diethylphosphonoacetate, then citric acid; (e) D-α-amino-ε-caprolactam, EDC, DMAP, CH_2Cl_2; (f) TFA, CH_2Cl_2; (g) 3,5-Bistrifluoromethylbenzoylchloride; Et_3N, CH_2Cl_2, chromatography, recrystallisation.

affinities to the NK_1 and NK_2 receptors by a factor of 7 and 5 respectively. In comparison to the *R,R*-isomer (**4**), it's enantiomer the *S,S*-isomer (**6**) exhibits a 170 times lower binding affinity to the NK_1 and a 12 times lower affinity to the NK_2 receptor.

Following confirmation of activity in in vitro functional tests (Ca^{2+} influx measurements and IL6 release in cultured cell lines), the ability of **4** to antagonise either sar^9-substance *P* (selective and stable NK_1 agonist)- or β-Ala^8-NKA (selective and stable NK_2 agonist)-induced bronchoconstriction (airway pressure measurement) in anaesthetised guinea pigs was also tested.[10] As can be seen from the results shown in Table 2, after oral application 2 h prior to the challenge with the agonists, **4** is able to inhibit sar^9-substance *P*-induced bronchoconstriction (NK_1) with an ED_{50} of 0.036 mg/kg and the β-Ala^8-NKA-induced bronchoconstriction (NK_2) with an ED_{50} of 0.9 mg/kg. An inhibition of 85% after a 1 mg/kg oral dose 12 h prior to the challenge with the NK_1 agonist could also be observed indicating a long duration of action (Table 2).

From a series of *N*-[(*E*)-3-carbamoyl-1-(4-chloro-benzyl)-allyl]-*N*-methyl-3,5-bis-trifluoro-methyl-benzamides, **1** (a mixture of 4 isomers) has been identified as a dual NK_1/NK_2 antagonist exhibiting highly potent affinity to the NK_1 receptor and good affinity to the NK_2 receptor. Consequently, all four isomers were prepared and tested as enantiomerically pure compounds in NK_1 and NK_2 binding assays. In addition, a stereoselective synthesis (8 steps) starting from a commercially available D-amino acid derivative has been worked out, which allows the preparation of the *R,R*-isomer (**4**) in an overall yield of 20% and with an ee >98%. **4** was also shown to exhibit a protective effect against sar^9-substance *P*- or β-Ala^8-NKA-induced bronchoconstriction in guinea pigs after oral application.

Table 1. In vitro binding affinities of compounds **1** and **4**–**7** to NK_1- and NK_2-receptors



Compound	NK_1 binding IC_{50}, nM[a]	NK_2 binding IC_{50}, nM[a]
1 (Mixture of 4 stereoisomers)	0.76	55
4 (*R,R*-isomer)	0.5	24
5 (*S,R*-isomer)	16	123
6 (*S,S*-isomer)	86	300
7 (R,S-isomer)	3.6	120

[a]Values are means of three experiments.

Table 2. In vivo activity of **4** against NK_1- and NK_2-agonist-induced bronchoconstriction in guinea pigs

Inhibition of sar^{9}SP-induced bronchoconstriction ED_{50}, mg/kg	Inhibition of β-Ala^8-NKA-induced bronchoconstriction ED_{50}, mg/kg
0.036 (−2 h)	0.9 (−2 h)
0.03 (−4 h)	0.73 (−4 h)
85% (1 mg/kg, −12 h)	n.d. (−12 h)

Acknowledgements

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7. Analytical data, **4**: mp 110–112 °C. $[\alpha]_D^{20} = +42°$ ($c = 0.895$, EtOH). ^{1}H NMR (400 MHz, δ, DMSO, +120 °C) 8.02 (s, 1H); 7.67 (b, 1H); 7.55 (s,b, 2H); 7.41 (b, 1H); 7.31 (d, 2H); 7.23 (b, 2H); 6.69 (dd, 1H); 6.34 (dd, 1H); 4.98 (b, 1H); 4.51 (m, 1H); 3.17 (m, 1H); 3.06 (d, 2H); 2.82 (s,b, 3H); 1.96–1.22 (m, 6H). Anal. calcd for $C_{27}H_{26}ClF_6N_3O_3$: C: 54.97, H:4.44, N:7.12. Found: C: 54.94, H:4.73, N:7.13. Raman (pulv., cm^{-1}): 1643 (-CH=CH-C=O), 1095 (Ph-Cl), 1003 (1,3,5-trisubst. phenyl). ee-values determined using HPLC on Chiracel OJ (hexane:ethanol = 9:1 + 0.1% TFA).

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9. Inhibition of [^{125}I]-NKA binding to transfected Chinese hamster ovary cells (CHO cells) expressing recombinant human neurokinin 2 receptors: The assay was performed in 96 well plates (Nunclon) containing 200 μL 20 mM HEPES buffer, pH 7.4 containing 2 mM $MnSO_4$ and 6 mM $MgCl_2$, 3×10^5 h NK_2 CHO cells, 0.05 nM [^{125}I]-NKA (2200 Ci $mmol^{-1}$) and various drug concentrations. Nonspecific binding was estimated

in the presence of 50 nM NKA. The mixture was incubated for 20 min at room temperature after which the unbound ligand was removed by rapid filtration and washed 4 times with ice cold Tricine buffer. Filter bound radioactivity was counted in Microscint 20 in a scintillation counter. All samples were measured in triplicate. Culture conditions and cell isolation for h NK_2 CHO cells: Subramanian, N.; Ruesch, C.; Bertrand, C. *Biochem. Biophys. Res. Comm.* **1994**, *200*, 1512.

10. Dunkin–Hartley guinea-pigs (500–700g) were anaesthetised with ip urethane (1.5 g kg^{-1}), tracheotomised and ventilated with a constant-volume ventilator (Model 683; Harvard apparatus Co., S. Natick, MA) at a frequency of 60 breaths min^{-1}. Pavulon (pancuronium bromide, Organon, 1 mg kg^{-1}) and atropine (Fluka, 1mg kg^{-1}) were administered (iv) to prevent spontaneous breathing and cholinergic reflexes, respectively. The tidal volume was adjusted to about 1 mL 100 g^{-1} body weight so as to maintain normal arterial blood gases. Intratracheal pressure was measured with a differential pressure transducer (Model DP 45-28, Validyne Engineering Corp., Northridge, CA). Polyethylene catheters (250 I.U. ml^{-1} heparin in 0.9% NaCl) were inserted into the right jugular vein for drug injection and the left carotid for blood pressure measurements (Statham transducer P23XL). All signals were recorded using a computer data-acquisition system (Mi^2 Bio Report software, Modular Instruments). The timing of anaesthesia and animal preparation were such that after a baseline period was obtained, Sar^9-SP (3 μg kg^{-1}; ED_{80} dose for increase in intratracheal pressure) or β-Ala^8-NKA (0.8 μg kg^{-1} ED_{80} dose) was injected, corresponding to a time of 2, 4 or 12 h since the oral dosing of vehicle or drug. The antagonists were given in doses ranging from 0.01 to 1 mg kg^{-1} in a vehicle consisting of 0.0067–0.67% DMSO in 0.5% methylcellulose, in a volume of 10 mL kg^{-1}. Five to six animals per dose were studied. The percent inhibition for each animal was calculated by dividing the elicited change in intratracheal pressure for the antagonist-treated animals by the mean value obtained for the vehicle-treated group. A linear regression analysis was then performed of the logarithmically transformed dose data and the ED_{50} value interpolated.