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Preparation and Radiolabelling of CP-96,345, the First Non-Peptide Substance P Antagonist

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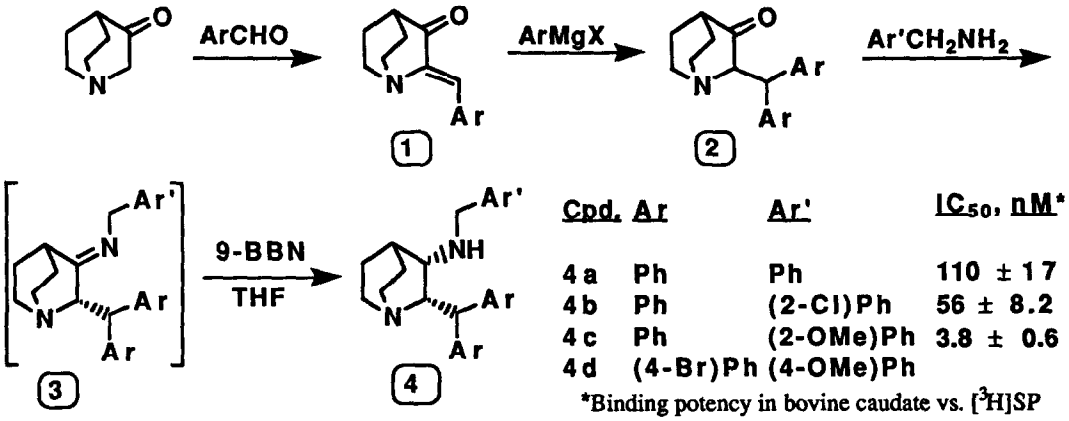
Abstract: The preparation of CP-96,345, a potent, non-peptide Substance P antagonist, in both enantiomerically pure and radiolabelled forms is described. In addition, the absolute configuration of CP-96,345 was determined to be 2S,3S.

We recently described the discovery of CP-96,345, the first non-peptide Substance P antagonist[1]. Substance P (SP), a peptide neurotransmitter first described in the 1930's[2] and isolated and characterized more recently[3], has been the focus of an intense research effort for many years[4]. As a potent, specific, and enantioselective SP receptor antagonist, CP-96,345 offers a unique opportunity to study the biochemistry and pharmacology of SP and to evaluate the therapeutic potential of SP antagonists. To facilitate laboratory studies, we prepared radiolabelled CP-96,345 and determined its absolute configuration.

CP-96,345 and its congeners were prepared by the route shown in Scheme 1[5]. In order to generate the desired *cis* relationship at positions 2 and 3 of the quinuclidine nucleus, imine 3 was reduced stereoselectively with 9-borabicyclo[3.3.1]nonane:



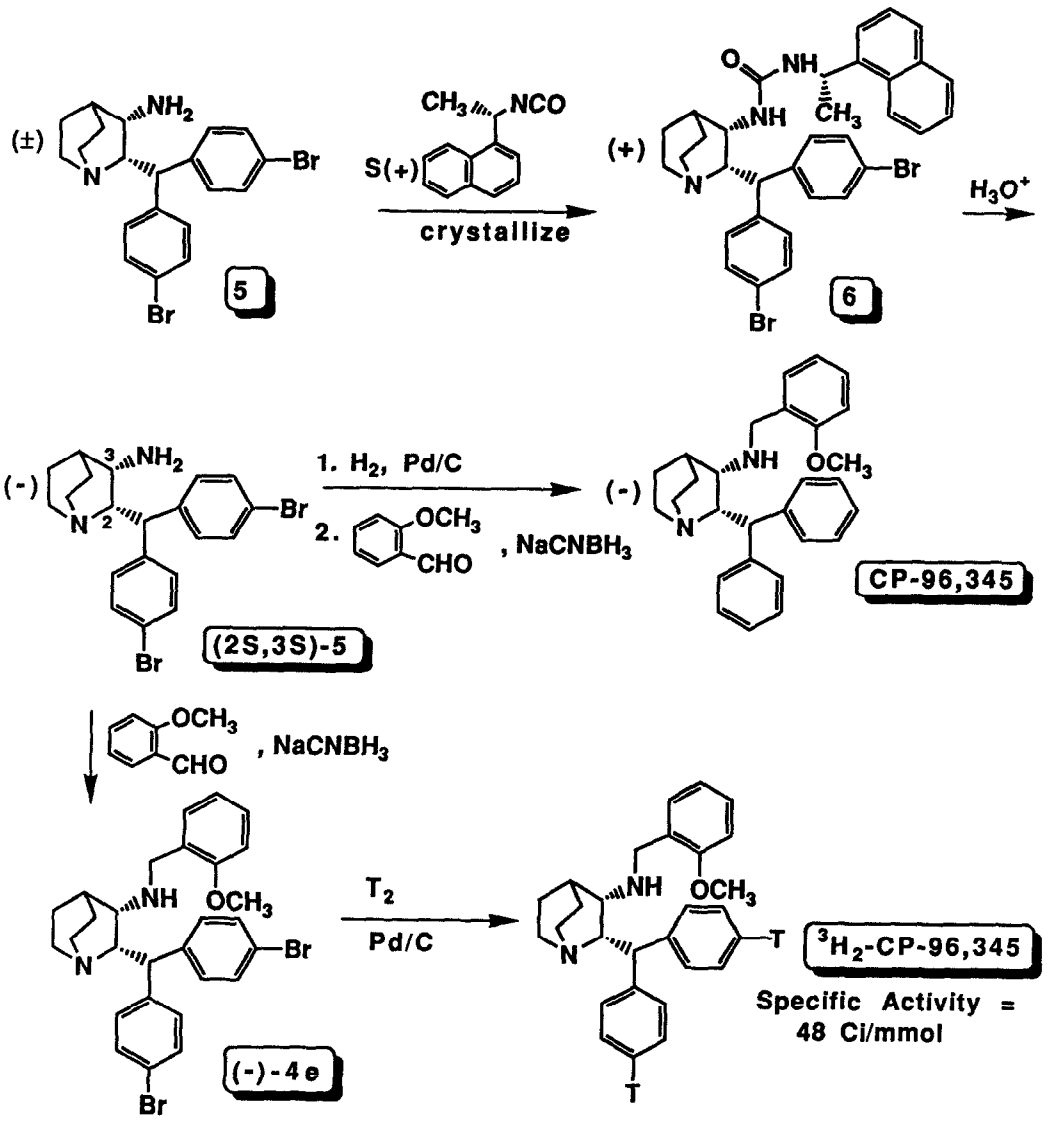
Cpd.	Ar	Ar'	IC_{50}, nM*
4a	Ph	Ph	110 ± 17
4b	Ph	(2-Cl)Ph	56 ± 8.2
4c	Ph	(2-OMe)Ph	3.8 ± 0.6
4d	(4-Br)Ph	(4-OMe)Ph	

*Binding potency in bovine caudate vs. [^{3}H]SP

Scheme 1. Synthesis and SAR of Quinuclidine SP Antagonists

During SAR evaluation, compound **4c** emerged as the most potent member of this series of SP receptor antagonists[6]. Accordingly, **4c** was selected for radiolabelling. Its synthesis began with compound **5**, which

Scheme 2. Preparation of CP-96,345 and $^3H_2$-CP-96,345



was conveniently prepared by removal of the p-methoxybenzyl group from **4d** in refluxing 48% hydrobromic acid (Scheme 2). Removal of alternative protecting groups was less successful, with the benzyl group (compound **4a**) requiring prolonged heating and the 3,4-dimethoxybenzyl group leading to extensive decomposition. Since direct resolution of **4c** via crystallization of its diastereomeric salts with chiral acids proved unsuccessful, resolution of **5** was carried out by conversion to a mixture of diastereomeric urea derivatives, using commercially available chiral (S)-(+)-1-(1-naphthyl)ethyl isocyanate[7]. Compound **6** crystallized directly from the reaction and could be purified to constant optical rotation by a single recrystallization (in contrast, separation of the corresponding diastereomeric ureas lacking the bromine atoms required a tedious column chromatography). Heating **6** in 70% sulfuric acid conveniently afforded (-)-**5** by way of the unsubstituted urea intermediate in which the naphthylethyl moiety had been excised *via* solvolysis. The other enantiomer, (+)-**5**, was obtained by using the R-(-) isomer of the chiral isocyanate.

Analysis[8] of single crystal X-ray data for compound (-)-**5**, facilitated by the bromine atoms, indicated that it possesses the 2S,3S absolute configuration. Hydrogenolytic removal of the bromines followed by attachment of the side chain *via* reductive amination with 2-methoxybenzaldehyde[9] provided (-)-**4c** and, starting with (+)-**5**, compound (+)-**4c**. The success of the resolution was demonstrated by receptor binding studies, which showed the (-) enantiomer to have an IC_{50} value of 3.4 ± 0.8 nM, while the (+) enantiomer had an IC_{50} value of $81{,}000 \pm 12{,}000$ nM[1]. The activity of the (+) enantiomer is presumably due to the presence of residual (-)-**4c**.

Finally, the 2-methoxybenzyl side chain was attached to compound (-)-**5** to provide (-)-**4e**, the precursor for radiolabelling. Exchange of the bromines for tritium was carried out at atmospheric pressure for one hour in the presence of triethylamine; purification was effected by HPLC, and the final product, $^3H_2$-CP-96,345, with a radiochemical purity of 97.2%, had a specific activity of 48.1 curies/mmole. As will be reported elsewhere[10], tritiated CP-96,345 has proven especially useful in autoradiographic studies characterizing SP binding sites in brain; further studies will be reported in due course.

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5. The route to compound **4a** shown in Scheme 1 is based on chemistry reported by Warawa, *et. al.*, see: Warawa, E.J.; Mueller, N.J.; Jules, R.; *J. Med. Chem.* **1974**, *17*, 497.

6. The procedure for [^{3}H]SP binding to bovine caudate membranes which was used for SAR evaluation was based on the literature protocol of Perrone, M.H.; Diehl, R.E.; Haubrich, D.R.; *Eur. J. Pharmacol.* **1983**, *95*, 131. Caudates were removed from bovine brains and stored at -80°C until use. Tissue was thawed, weighed, and homogenized in 50 volumes (w/v) ice-cold 50 mM TRIS buffer (pH 7.7), then centrifuged twice at 30,000 x g for 20 min at 2-4°C. The pellet was suspended in assay buffer (50 mM TRIS-HCl (pH 7.7), 1 mM $MnCl_2$, 0.02% BSA, 40 μg/mL bacitracin, 4 μg/mL leupeptin, 2 μg/mL chymotrypsin, 30 μg/mL phosphoramidon), and the assay conducted in 5 mL polystyrene tubes with 100 μL of test compound, 100 μL of ligand (0.5 nM final concentration, 36-55 Ci/mmol), and 800 μL tissue preparation (20 mg original wet weight/tube). After incubation in the dark at room temperature for 20 min, the assay was terminated by filtration onto GF/B filters which had been presoaked in 0.2% polyethyleneimine for 1-2 hr. The filters were washed (5 x 1 sec) with ice-cold 50 mM TRIS-HCl buffer (pH 7.7) using a Brandell Harvesting System, and the filters quantified for radioactivity by liquid scintillation counting. Standard errors are indicated following the IC_{50} values.

7. Aldrich Chemical Co., 29,595-7.

8. A representative crystal, grown from isopropanol/methylene chloride, was surveyed and a 1 Å data set (maximum sin θ/λ=0.5) was collected on a Nicolet R3m/μ diffractometer. Atomic scattering factors were taken from the International Tables for X-ray Crystallography (Birmingham: Kynoch Press, 1974, Vol IV, pp. 55, 99, 149). All crystallographic calculations were facilitated by the SHELXTL system (Sheldick, G.M. *SHELXTL. User Manual*, Nicolet Instrument Co., **1981**.) All diffractometer data were collected at room temperature. A trial structure was obtained by direct methods. This trial structure refined routinely. *A difference map revealed a disordered dichloromethane molecule of crystallization, which was best fit using four molecules of CH_2Cl_2 each with an occupancy of 0.25.* Hydrogen positions were calculated wherever possible. The hydrogens on nitrogen were located by difference Fourier techniques. The hydrogen parameters were added to the structure factor calculations but were not refined. The shifts calculated in the final cycle of least squares refinement were less than 0.1 of their corresponding deviations. The final R-index was 0.077. A final difference Fourier revealed no missing or misplaced electron density. *The absolute configuration was determined by the method of Ibers and Hamilton* (Hamilton, W.C. *Acta Cryst.* **1965**, *18*, 502. Ibers, J.; Hamilton, W.C. *Acta Cryst.* **1964**, *17*, 781). Pertinent crystal, data collection, and refinement parameters, as well as coordinates, anisotropic temperature factors, distances, and angles are available from the authors.

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