PREPARATION OF TRITIUM LABELLED BENZAMIDE DOPAMINE-D_2 LIGANDS AT HIGH SPECIFIC ACTIVITY

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SUMMARY

A method for the preparation of tritium labelled potential neuroleptics of the benzamide class possessing highly potent dopamine-D_2 binding properties, is described. The pyrrolidine moiety of the molecule is labelled with tritium by hydrogenation, to a specific activity of up to 58 Ci/mmol and subsequently reacted with an acid chloride. The specificity of the label is confirmed by ^{3}H-NMR.

Key words: Remoxipride, raclopride, dopamine-D_2 ligands, tritiated benzamides, tritium NMR

INTRODUCTION

Brain dopamine receptors mediate a number of biochemical and behavioural effects in experimental animals (1,2,3), and inhibition of dopamine-D2 receptor activity in the brain is suggested to be related to the effects of neuroleptic drugs in schizophrenia (4). However, most classical neuroleptic drugs interact with other receptors in the brain in addition to the dopamine-D2 receptors (5,6). In contrast, the substituted benzamide class of neuroleptics, such as sulpiride (1) and remoxipride (2), are selective, although rather weak, dopamine-D_2 receptor antagonists (7,8). Recently a number of substituted benzamides have been synthesized (9,10,11), which, retaining their selectivity, are very potent dopamine-D_2 antagonists (6,12). Radio-

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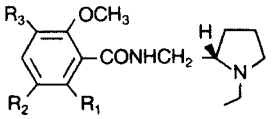


1 $R_1 = R_3 = H, R_2 = SO_2NH_2$ (S(-)-Sulpiride)
2 $R_1 = OCH_3, R_2 = Br, R_3 = H$ (Remoxipride)
3 $R_1 = OH, R_2 = R_3 = Cl$ (Raclopride)

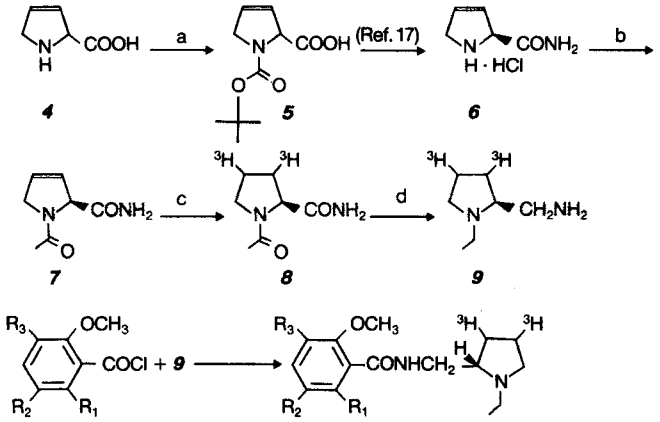
labelling of these new potent substituted benzamides has made it possible to study the dopamine-D_2 receptors in vitro and in vivo (13, 14,15) overcoming the problems associated with either low selectivity ([^{3}H]spiperone, [^{3}H]haloperidol) or low penetration ([^{3}H]sulpiride, [^{3}H]domperidone) into the brain.

The present work describes a general procedure for the synthesis of specifically tritium labelled benzamides and its application to the synthesis of the potential antipsychotic drugs remoxipride (2) and raclopride (3).

RESULTS AND DISCUSSION

A procedure for the preparation of tritium labelled benzamide drugs at high specific activity has been described by Pri-Bar and Buchman (16). In their method the drugs were brominated in the aromatic ring and then hydrodebrominated with tritium in the presence of a catalyst. However, the outcome of this procedure is dependent on the functionality as well as the number of substituents in the aromatic ring. The functionality must be compatible with the bromination reaction as well as the hydrodebromination. In most of our compounds only the 4-position was available for labelling with tritium, limiting the specific activity to 29 Ci/mmol at 100% incorporation. Since in vivo receptor studies and high resolution autoradiography require a very high specific activity, (preferably more than 30 Ci/mmol) depending on the ability of the compound to penetrate the blood-brain barrier, a procedure permitting the introduction of two tritium atoms into the molecule was needed.

The amides studied are all, in principle, products of the coupling of (S)-2-(aminomethyl)-1-ethylpyrrolidine with a substituted benzoic acid and their structures differ only in the aromatic part of the molecule. Consequently, for the synthesis of the labelled compounds, an inviting strategy was to insert the label into the pyrrolidine beforehand. This would allow the incorporation of tritium into all the structures, independent of the substituents on the aromatic ring, simply through coupling of the labelled amine with the appropriate acid derivative.



R_1 = OCH_3,	R_2 = Br,	R_3 = H **10**
R_1 = OH,	R_2 = R_3 = Cl	**11**
R_1 = OH,	R_2 = R_3 = CH_2CH_3	**12**
R_1 = OH,	R_2 = CH_2CH_3,	R_3 = Br **13**
R_1 = OH,	R_2 = R_3 = Br	**14**
R_1 = OH,	R_2 = CH_2CH_3,	R_3 = H **15**
R_1 = OH,	R_2 = CH_2CH_3,	R_3 = Cl **16**

Scheme 1.

Reagents: a) $[(CH_3)_3COCO]_2O$, b) $(CH_3CO)_2O$, CH_3COOH

c) $^3H_2$/PdO, DMF d) $LiAlH_4$, diethyl ether

Following the method of Felix et al. (17) 3,4-dehydroproline 4 (Scheme 1) was converted into the optically active (S)-3,4-dehydroprolinamide hydrochloride 6. The demonstrated (18) lability of the free base of 6 suggested the use of non-basic reaction conditions for the subsequent acylation step in order to minimize racemization. Therefore, 6 was treated with acetic anhydride in acetic acid at room temperature in the presence of sodium acetate to afford the diamide 7. To attain specificity in the reductive tritiation of 7 the choice of catalyst and solvent might be critical. For example, carrying out the reduction of N-acetyl-3,4-dehydroproline with PtO_2 in 2M HCl showed an extensive randomization of label while PdO in CH_3OH worked successfully (17). Thus, by employing PdO in DMF, compound 8 was obtained, specifically labelled in positions 3 and 4 of the pyrrolidine ring as demonstrated by ^3H NMR (vide infra). Reduction of the amide functions of 8 with $LiAlH_4$ in ether was performed on the crude reaction products of the tritiation step. Refluxing for three days provided after work-up an etheral solution of [3,4-$^3H_2$]diamine 9. This solution served as a "pool" of tritium labelled precursor and in order to facilitate the handling of high activity material in the synthesis, the diamine was kept at a high radioactive concentration (100-250 mCi/ml). Consequently, this called for a prompt usage of the compound as it suffered from rapid decomposition (19). Divided in up to four parts, each pool should yield the same number of benzamides when reacted with the appropriate acid chlorides. In this manner compounds 10 to 16 were synthesized with a specific activity range of 19 to 58 Ci/mmol.

The integrity of the assymmetric centre at position 2 of the derivatives 6 through 9 was demonstrated when diamine 9 from a cold run was checked for enantiomeric purity. GLC analysis of the (S)-N-trifluoroacetyl prolylamide of 9 showed a 94% enantiomeric purity indicating highly stereoconservative (20) reaction conditions. The localization

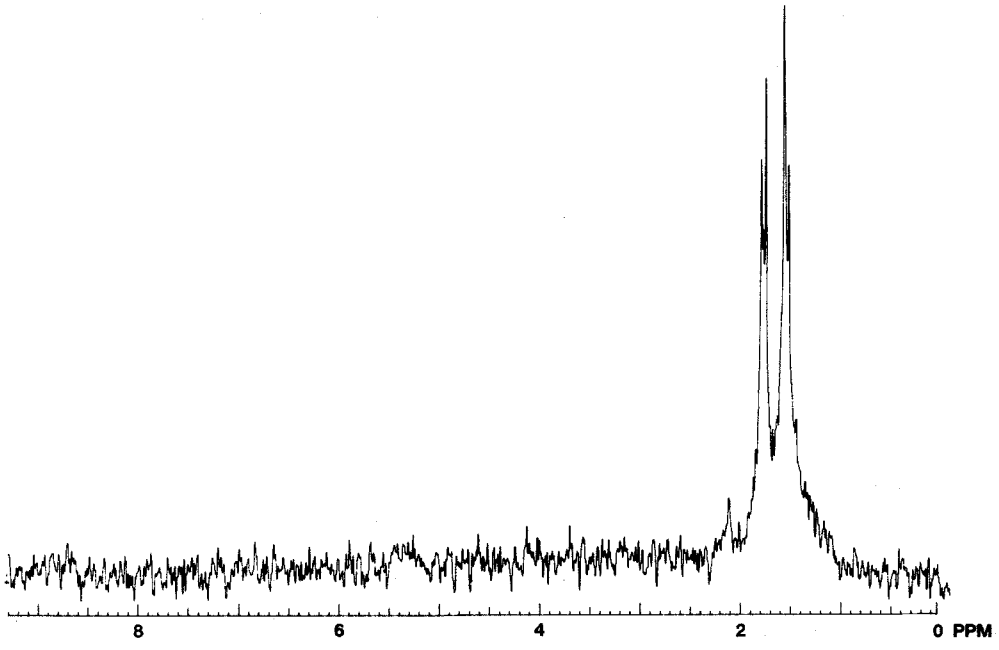


Fig. 1. Proton decoupled ^{3}H NMR spectrum of 10 (50 mCi) obtained after 3000 scans at 212.8 MHz.

of tritium atoms to positions 3 and 4 was confirmed by the ^{3}H NMR of [^{3}H] remoxipride (10) (Fig. 1). The proton decoupled spectrum exhibits essentially only peaks from two doublets of equal intensities and with a coupling constant, J (^{3}H^{3}H) = 10.3 Hz, consistent with a doubly-labelled cis- [3,4-$^3H_2$]pyrrolidine (21).

EXPERIMENTAL

Melting points were obtained on a Mettler FP 61 apparatus and are uncorrected. ^{1}H and ^{3}H NMR spectra were obtained on a JEOL FX 200 spectrometer with CD_3OD as solvent using Me_4Si as internal standard and ghost- reference (22). The ^{3}H NMR sample was prepared as described in ref. 22. Mass spectrum (EI, 70 eV) was recorded on a LKB 2091 mass

spectrometer. Elemental analysis were performed by Analytische Laboratorien, Elbach, W. Germany. Tritium gas (98%) was purchased from Amersham International plc, Amersham. Buchs, England. Radiochemical purity was determined from TLC using a Berthold LB 283 TLC Linear Analyzer. TLC analyses were done on silica gel 60 F_{254} (Merck) glass plates developed in $CHCl_3/CH_3OH$/conc. NH_3 (9:1:0.05). HPLC was performed on a 4.6 mm x 25 cm Whatman Partisil PXS 10/25 ODS column eluted with n-hexane/isopropanol/conc. NH_3 (85:15:1). Radioactivity was determined in a Packard Tri-Carb 460 C liquid scintillation spectrometer using Bioflour (New England Nuclear) as the counting medium.

N-tert-butyloxycarbonyl-3,4-dehydro-(R,S)-proline (5).

To a solution of 4, (1.58 g, 14 mmol) in 40 ml of dioxane-water (25:15), was added 14 ml of 1M NaOH at 0°C followed by di-tert-butyldicarbonate (3.30 g, 15 mmol). The cooling bath was removed and the reaction mixture was stirred at room temperature until it became clear (~1 h). After concentration by means of a rotary evaporator, ethyl acetate (100 ml) was added. While cooled in an ice-water bath, the pH was adjusted to pH 2 with 0.5 M $KHSO_4$ and the organic phase separated. The aqueous solution was extracted with ethyl acetate (2 x 75 ml) and the organic extracts were combined and dried (Na_2SO_4). Evaporation to dryness and recrystallization of the residue from ethyl acetate gave 2.58 g (87%) of 5, m.p. 113-115°C. (Lit. (17) m.p. 113-115.5°C.

(S)-3,4-Dehydroprolinamide hydrochloride (6).

Following the published procedure (17), 5 was resolved, amidated and deprotected to give 6, m.p. 202-205°C (decomp.), $[\alpha]_D^{25}$ - 285.7 (c. 0.77, CH_3OH) (Lit. (17) m.p. 192.5 - 196°C $[\alpha]_D^{25}$ - 276.6, c. 0.95, CH_3OH) Calcd. for $C_5H_8N_2O$ x HCl: C 40.38; H 6.06; N 18.84; O 10.77; Cl 23.86. Found: 40.57; H 6.02; N 18.78; O 10.82; Cl 23.74.

(S)-N-acetyl-3,4-dehydroprolinamide (7).

Compound 6 (77 mg, 0.52 mmol) in acetic acid (600 µl) was treated with NaOAc (50 mg, 0.61 mmol) for 4 hours. Acetic anhydride (70 µl, 0.74 mmol) was added and the mixture was stirred for 48 hours. Filtration and evaporation to dryness left a glassy residue which was crystallized from ethanol. Recrystallization from the same solvent gave 35 mg (44%) of 7, m.p. 165-167°C (decomp.) $[\alpha]_D^{25}$ - 394° (c. 0.52, CH_3OH) (Lit. (23) m.p. 167.5-171.5°C, $[\alpha]_D^{25}$ - 395.09 (c. 1.07, CH_3OH)) ^{1}H NMR (200 MHz) : δ 2.01 and 2.11 (two s, 3H), 4.35 (m, 2H), 5.08 (m, 1H), 5.85 (m, 1H), 6.04 (m, 1H). MS m/z (rel. int) : 111 (8), 110 (43), 69 (9), 68 (100), 67 (11), 43 (20). Calcd. for $C_7H_{10}N_2O_2$ C 54.53; H 6.54; N 18.17; O 20.76. Found; C 54.37; H 6.44; N 18.12; O 20.60.

(S)-2-(Aminomethyl)-1-ethyl[3,4-$^3H_2$]pyrrolidine (9).

A solution of 7 (5.5 mg, 36 µmol) in DMF (250 µl) was stirred at room temperature under carrier-free tritium gas (10 Ci) in the presence of PdO (2.4 mg). After 20 hours the reaction mixture was freeze-degassed and the solvent distilled in vacuo. Labile tritium was removed by repeated lyophilization with ethanol. The residue was dissolved in ethanol, filtered to remove the catalyst and evaporated to dryness. Ether (2 ml) and $LiAlH_4$ (70 mg) were added and the mixture heated under reflux for 3 days. To the reaction mixture was successively added, with cooling, H_2O (70 µl), 15% NaOH (70 µl) and H_2O (210 µl). Separation of the liquid phase gave an etheral solution (~1.5 ml) of 9 which was dried (Na_2SO_4) and used directly in the preparation of 10 and 11.

Preparation of benzamides 10-16.

The benzamides were prepared by the following general procedure, exemplified by the synthesis of compounds 10 and 11.

(S)-2-[(3-Bromo-2,6-dimethoxybenzamido)methyl]-1-ethyl[3,4-$^3H_2$]-pyrrolidine ([^{3}H]Remoxipride) (10).

The etheral solution from the preparation of 9 (750 µl) was treated with 3-bromo-2,6-dimethoxybenzoyl chloride (24) (approximately 2 eq.) in methylene chloride (100 µl). After 15 min TLC showed almost complete conversion of 9 and 2M NH_3 (1 ml) was added. The organic phase was separated and dried (Na_2SO_4). The total amount of activity in the solution was ~200 mCi. Concentration and purification by HPLC furnished 105 mCi of 10.

(S)-2-[(3,5-Dichloro-2-hydroxy-6-methoxybenzamido)methyl]-1-ethyl [3,4-$^3H_2$]pyrrolidine. ([^{3}H] Raclopride) (11).

To a slurry of 3,5-dichloro-2-hydroxy-6-methoxybenzoic acid (25) (60 mg, 0.48 mmol) in toluene (2 ml), thionyl chloride (100 µl) was added followed by one drop of DMF. The reaction mixture was stirred at room temperature until the gas evolution had stopped and the solution had become clear. Excess thionyl chloride was removed with toluene by repeated evaporation to dryness with toluene on a rotary evaporator. The residue was dissolved in methylene chloride (5 ml). Two 50 µl portions of this solution were added to the remaining part of the etheral solution of 9 and the mixture left for 48 hours. TLC showed ~80% conversion and 2M NH_3 (1 ml) was added. The organic layer was separated, dried (Na_2SO_4) and concentrated. Purification by HPLC gave 64 mCi of 11.

The specific activity of 10 and 11 was 58 Ci/mmol as measured by quantitative HPLC analysis. The radiochemical purity of the two compounds was greater than 94% and 98% respectively as determined by TLC.

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