[^{3}H]IMIDACLOPRID: SYNTHESIS OF A CANDIDATE RADIOLIGAND FOR THE NICOTINIC ACETYLCHOLINE RECEPTOR

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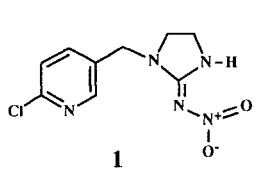
SUMMARY

Imidacloprid is an exceptionally potent insecticide known from physiological studies to act at the nicotinic acetylcholine receptor. To prepare [^{3}H]imidacloprid as a candidate radioligand, 6-chloronicotinoyl chloride was reduced with NaB^2H_4 (in model studies) or NaB^3H_4 in absolute ethanol to 2-chloro-5-pyridinylmethanol which was transformed to 2-chloro-5-chloromethylpyridine on refluxing with thionyl chloride. Coupling with 4,5-dihydro-N-nitro-1H-imidazol-2-amine then gave [$^2H_2$]imidacloprid incorporating about 95% of the deuterium or [$^3H_2$]imidacloprid (25 Ci/mmol) in 80% radiochemical yield. In studies not detailed here [^{3}H]imidacloprid was found to undergo high affinity, specific and saturable binding to a site in insect brain.

Key Words: imidacloprid, insecticide, nicotinic acetylcholine receptor radioligand, tritium labelling

INTRODUCTION

The discovery of 1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine (CAS registry number 105827-78-9) (common name imidacloprid) (1) as a highly potent and effective insecticide[1-3] culminated a long series of developments to increase the potency and photostability of nitromethylene heterocycles such as 2[4,5], 3[6] and 4[7,8]. Electrophysiological studies indicated



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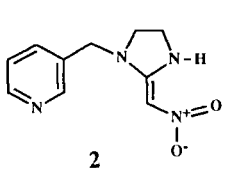

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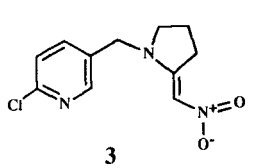

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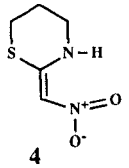

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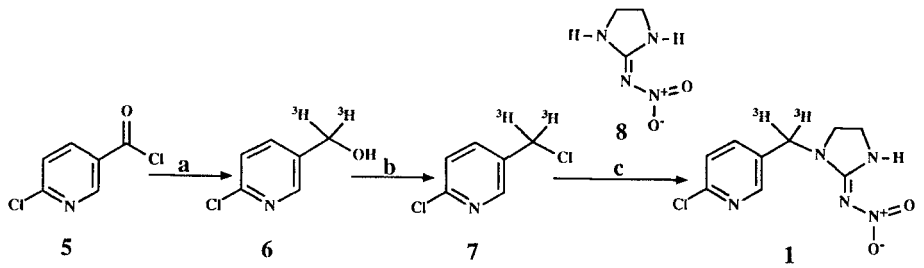
that these compounds act at the nicotinic acetylcholine receptor (nAChR).[4,5,6,8] The exceptional insecticidal potency of 1 suggested that [^{3}H]1 of high specific activity might be a suitable radioligand probe for the insect nAChR.

RESULTS AND DISCUSSION

Consideration was given to labelling in the pyridine ring and in the pyridinylmethyl moiety. Attempts to brominate 2-chloro-5-chloromethylpyridine[9] or its N-oxide[9] using different brominating agents[10-12] in selected solvents were unsuccessful, reflecting the fact that the pyridine ring is highly deactivated by the chloro and chloromethyl substituents. Attention was therefore focused on labelling the pyridinylmethyl substituent. NaB^3H_4 has been used to reduce aldehydes to tritium-labelled alcohols with 25% of the specific activity of NaB^3H_4 (see for example reference 13). A preferred approach from the acyl halide would allow the incorporation of tritium with 50% of the specific activity of NaB^3H_4. The procedure shown below was therefore developed with tritium labelling in the pyridinylmethyl substituent as optimized for [$^2H_2$]1 and applied directly to the synthesis of [$^3H_2$]1 of 25 Ci/mmol.



a NaB^3H_4 (or NaB^2H_4), EtOH; b $SOCl_2$, reflux; c K_2CO_3, CH_3CN, reflux

EXPERIMENTAL

Intermediates. Literature procedures were used to prepare the following compounds in unlabelled form: 5[14], 6[14], 7[9], and 8[2,15].

Preparation of [$^2H_2$]1 via [$^2H_2$]6 and [$^2H_2$]7. 6-Chloronicotinoyl chloride (5) (210 mg, 1.2 mmol) was added to a stirred solution of NaB^2H_4 (40 mg, 0.96 mmol) and aqueous NaOH (0.01 N, 0.1 mL) in absolute ethanol (10 mL) at 0 °C and stirring was continued for 40 min at 0 °C and 40 min at 25 °C. Ethyl acetate

(5 mL) and aqueous NaH_2PO_4 (1.0 M, 1 mL) were added and the organic extract was separated, dried ($MgSO_4$) and concentrated in vacuo to give 200 mg of crude [$^2H_2$]6 which was used in the next step without purification. ^1H NMR ($CDCl_3$) δ: 8.3 (d, J=2.2 Hz, 1H), 7.7 (dd, J=2.2, 8.0 Hz, 1H), 7.3 (d, J=8.0 Hz, 1H), 4.5 (s, 0.1H corresponding to 95% ^2H incorporation). ^{13}C NMR ($CDCl_3$) δ: 151.2, 147.9, 139.9, 137.9, 124.0, 41.5 (m).

A solution of crude [$^2H_2$]6 (180 mg, 1.24 mmol) and excess thionyl chloride (0.3 mL) in chloroform (5 mL) was refluxed for 3 h then the solvent and thionyl chloride were removed under reduced pressure to give 200 mg of crude [$^2H_2$]7. ^1H NMR ($CDCl_3$) δ: 8.3 (d, J=2.1 Hz, 1H), 7.7 (dd, J=2.1, 8.1 Hz, 1H), 7.3 (d, J=8.1 Hz, 1H), 4.5 (s, 0.1 H corresponding to 95% ^2H incorporation). ^{13}C NMR ($CDCl_3$) δ: 150.3, 148.4, 139.5, 132.3, 124.5, 41.2 (m).

A portion of the [$^2H_2$]7 (180 mg, 1.1 mmol) was mixed with 8 (146 mg, 1.1 mmol) and K_2CO_3 (600 mg) in acetonitrile (10 mL). This mixture was refluxed overnight, cooled to room temperature, water (2 mL) was added, and the reaction products were recovered by extraction into ethyl acetate (30 mL) which was dried ($MgSO_4$) and concentrated in vacuo. The crude product was fractionated by radial TLC (1 mm silica gel plate, 5% methanol in chloroform) to give pure [$^2H_2$]1 (250 mg, 80% yield). ^1H NMR ($CDCl_3$) δ: 8.3 (d, J=2.2 Hz, 1H), 8.2 (s, 1H), 7.7 (dd, J=2.2, 8.1 Hz, 1H), 7.4 (d, J=8.1 Hz, 1H), 4.5 (s, 0.1H corresponding to 95% ^2H incorporation), 3.8 (t, J=8.9 Hz, 2H), 3.5 (t, J=8.9 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ: 161.1, 151.4, 149.2, 139.0, 129.6, 124.6, 45.0, 41.5. ^2H NMR ($CDCl_3$) δ: 4.5 (s). MS NCI, m/z (rel. abund.): M^- (2.3), 241 (25), 239 (67), 211 (40), 209 (100), 195 (7), 173 (12), 111 (17) and 68 (22).

Preparation of [$^3H_2$]1. A solution of NaB^3H_4 (1 Ci, 50.9 Ci/mmol, ~0.020 mmol, from Amersham) and aqueous NaOH (0.01 N, 50 μL) in absolute ethanol (1.0 mL) was stirred at room temperature while 5 (10 mg, 0.056 mmol) was added. The mixture was stirred for 90 min, diluted with ethyl acetate (3 mL) followed by aqueous NaH_2PO_4 (1.0 M, 0.3 mL), dried ($MgSO_4$), and then filtered through silica gel in a Pasteur pipet and lyophilized. To the solid residue was added chloroform (5 mL) and thionyl chloride (0.2 mL) and the solution was refluxed for 3 h. Following lyophilization and dissolving the residue in acetonitrile, K_2CO_3 (40 mg) and 8 (12 mg) were added and the mixture was refluxed overnight

then cooled to room temperature. Water (0.5 mL) was added and the organic layer was extracted with ethyl acetate (5 mL), dried ($MgSO_4$), filtered and concentrated by lyophilization. The crude residue was purified by radial TLC as above to give 800 mCi of [$^3H_2$]1 in 80% radiochemical yield. The specific activity (25 Ci/mmol) was calculated by measuring the UV absorption of 1.1 mCi of [^{3}H]1 in absolute ethanol [λ_{max} (270 nm), ϵ=22,000]. ^{3}H NMR (CD_3OD) δ: 4.5 (d, J=9.0 Hz).

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