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Research Article

The high specific activity tritium labeling of the ganglion-blocking nicotinic antagonist chlorisondamine

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Summary

Chlorisondamine is a bisquaternary ganglion-blocking nicotinic antagonist that accumulates in dopaminergic, serotonergic, and noradrenergic cell bodies; the mechanism of uptake and time-course of retrograde transport are not known. Chlorisondamine could possibly be taken into monoaminergic neurons when blocked nicotinic receptors on those cells are internalized. In order to more easily study the mechanism of the capture and recycling of chlorisondamine, a ligand is needed that has high specific activity. For that purpose, we now report the preparation of ^{3}H-labeled chlorisondamine (4,5,6,7-tetrachloro-2-[^{3}H]methyl-2-(2-trimethylammoniumethyl)-isoindolinium diiodide) of high specific activity (94 Ci/mmol). The compound was synthesized by quarternization of 4,5,6,7-tetrachloro-2-(2-trimethylammoniumethyl-isoindolinium iodide with pertritiated methyl iodide in dimethylformamide at high temperature in a sealed vessel. Copyright © 2006 John Wiley & Sons, Ltd.

Key Words: chlorisondamine; nicotinic antagonist; tritiation

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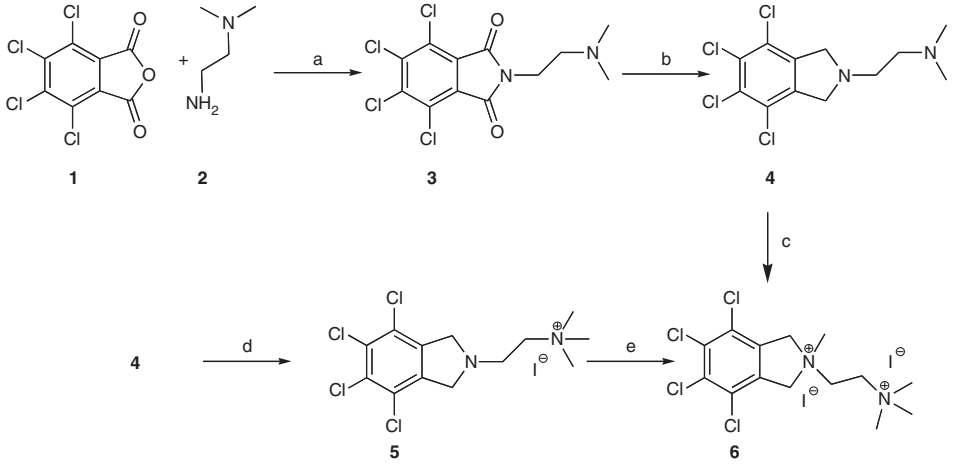
Introduction

Chlorisondamine (**6**, Scheme 1) is a bisquaternary ganglion-blocking nicotinic antagonist with negligible effects at the skeletal muscle endplate.[1,2] While it penetrates the brain poorly when given systemically, a high dose blocks the locomotor stimulating effects[3] and reward-enhancing effects[4] of nicotine. Both the locomotor-stimulating effects[3] and the rewarding effects[5] of nicotine are blocked when the drug is given intracerebro-ventricularly. While effects of chlorisondamine at the autonomic ganglion last only hours or days, blockade of the locomotor-stimulating, rewarding, or reward-enhancing effects of nicotine, effects thought to be mediated by actions of nicotine on the mesocorticolimbic dopamine system, lasts for many weeks.[3–5] The long-lasting central effects of chlorisondamine on nicotine-induced locomotion and reward appear to be due to the intraneuronal accumulation and protection of chlorisondamine by dopaminergic neurons; indeed, radiolabeled chlorisondamine has been found in synaptosome and tissue samples 3 weeks after intracerebroventricular injection.[6,7] Moreover, radiolabelled chlorisondamine injected into the caudate putamen was found, 1 week after injection, not only to label the injection site but also to be concentrated in the zona compacta of the substantia nigra and the dorsal raphe nuclei, nuclei that innervate the caudate with dopaminergic and serotonergic terminals, respectively.[6] Thus chlorisondamine appears to be taken up by monoaminergic nerve terminals expressing the receptors that it blocks, and transported from those terminals to their parent cell bodies.



Scheme 1. Synthesis of starting materials and standards. Reaction conditions: (a) Toluene/Δ (93%), (b) $LiAlH_4$/THF/Δ (43%), (c) MeI (2.1–3.0 eq.)/90% EtOH or DMF/100°C (65–68%), (d) MeI (1.1 eq.)/absolute EtOH/20°C (58–71%), (e) MeI (1.05 eq.)/DMF/100°C (58%)

While it is known to accumulate in dopaminergic, serotonergic, and noradrenergic[8] cell bodies, the mechanism of uptake and time-course of retrograde transport are not known. One possibility is that chlorisondamine is taken into monoaminergic neurons when blocked nicotinic receptors on those cells are internalized. The mechanism of this capture and recycling of chlorisondamine is of considerable interest and could be more easily studied with a ligand that has high specific activity.

The tritiated chlorisondamine used for these studies[6] was prepared, as previously reported, by tritium exchange[9] between chlorisondamine and tritium gas; a method that provided the material with a specific activity of 3.0 Ci/mmol and 98% purity. We thought that it would be more convenient to use tritiated methyl iodide as a source of radioactivity since the direct precursor of chlorisondamine has two nucleophilic sites that can be alkylated stepwise with methyl halides. This strategy would yield labeled chlorisondamine with very high specific activity and in good yield.

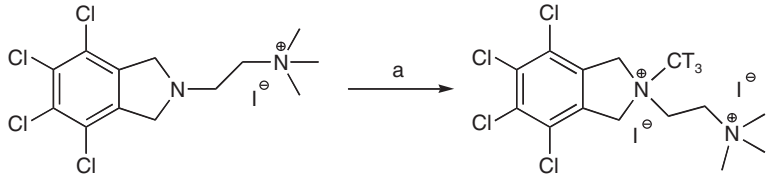
Results and discussion

The starting material, 4,5,6,7-tetrachloro-2-(2-dimethylaminoethyl)-isoindoline (**4**, Scheme 1), was prepared from commercially available tetrachlorophthalanhydride (**1**) by a condensation[10] with *N,N*-dimethylethylenediamine (**2**), followed by reduction[11] of the resulting phthalimide (**3**) with lithium aluminum hydride in two steps (40% overall yield) utilizing published procedures (Scheme 1). Two previously reported procedures for bisquaternization to obtain **6** with methyl iodide used refluxing in 90% ethanol [12] or dimethylformamide.[11] Both procedures call for an excess of methyl iodide (*ca.* 5–6 eq.). With the goal of minimizing the amount of methyl iodide used, the procedures above were modified using sealed vessels (heated to 90–100°C). Heating of diamine **4** in 90% ethanol in the presence of 3.0 eq. of methyl iodide for 5 days gave a 68% yield of chlorisondamine (**6**) after recrystallization. Lowering the amount of methyl iodide to 2.1 eq. and shortening the reaction time (1 day) also gave a satisfactory yield (65%) of **6**. However, in that case the presence of a trace amount of the mono salt **5** was observed in the ^{1}H-NMR spectrum. When DMF was used as a solvent[11] a similar results were obtained.

In 1957, Rosen *et al.*,[11] reported that the nitrogen atoms of 4,5,6,7-tetrachloro-2-(2-dimethylaminoethyl)-isoindoline (**4**) displayed remarkably different reactivity towards various alkylating reagents. The terminal nitrogen was easily alkylated under mild conditions, while the alkylation of the isoindoline nitrogen required increased temperatures and higher pressures. Rosen *et al.*,[11] rationalized this in terms of the negative inductive effect of the four chlorine substituents on the aromatic ring significantly decreasing the basicity of the isoindoline nitrogen. According to Rosen *et al.*,[11] this basicity is

further decreased in the presence of the β-ammonio cation-bearing side-chain group in **5**. When both of those influences are present, the basicity of isoindoline nitrogen was said to be decreased by a factor of about 50 000–65 000 (p*K*a from 7.7 to 2.9–3.0). They further reported the monomethochloride, monomethobromide, and monomethiodide salts of **4** in 1957.[11,13] We used this differential reactivity to selectively prepare the monomethiodide **5** by the reaction of **4** with 1.1 eq. of methyl iodide in absolute ethanol at ambient temperature. The desired product **5** precipitated out of the reaction mixture and was obtained in good yield (58%) after two recrystallizations. A second quarternization, using a slight excess of methyl iodide (1.05 eq., in DMF in a sealed tube) proceeded smoothly to give the bismethiodide (chlorisondamine, **6**) in good yield (58%, recrystallized). A similar procedure using 90% ethanol gave a lower yield (35%) of bismethiodide, and it was contaminated with starting material.

Monomethiodide salt **5** was easily prepared and it seemed likely that the second alkylation with labeled methyl iodide could be accomplished using the above-mentioned procedure (sealed tube/CT_3I/DMF). The labeling was successfully carried out by Amersham Biosciences using an excess of CT_3I in DMF in a sealed vessel at 100°C to give crude product in >60% radiochemical purity (Scheme 2). Purification on a reverse phase support gave pure product (**7**) with the desired high specific activity (94 Ci/mmol by MS). Since the addition of three tritium atoms would give a maximum specific activity of 82 Ci/mmol, there was probably some nonspecific exchange as well. Mass spectroscopic analysis (MALDI-TOF) of unlabeled material showed weak molecular peaks (355.12, 357.13, 359.12) and strong base peaks (296.09, 298.09, 299.09) corresponding to the loss of the $(CH_3)_3N$ moiety. The spectrum of labeled material showed an analogous pattern, with the corresponding peaks having a larger mass of 6 amu, confirming the successful labeling. The labeling on the much less reactive site was very important since the strongest peak in the MS spectra corresponded to the loss of the alkylated side-chain nitrogen. The labeled moiety was preserved for easier detection. In summary, we prepared labeled chlorisondamine **7** with very high specific



5 **7**

Scheme 2. Quaternization with labeled methyl iodide. Reaction conditions: (a) CT_3I/DMF/sealed tube/100°C

activity and in good yield, and this material should be suitable for the contemplated biological experiments.

Experimental

Tetrachlorophthalic anhydride (96%), *N,N*-dimethylethylenediamine (95%), and methyl iodide (CH_3I, 99.5%, stabilized over copper) were purchased from Sigma Aldrich and used without further purification. Diethyl ether (Et_2O) was freshly distilled from $LiAlH_4$ before use. The melting points were taken on a Thomas Hoover apparatus and are corrected. The Ace Glass threaded thick-walled tubes (of *ca.* 24 ml volume) were used for the reactions at elevated temperatures, with proper safety shielding. The actual labeling was performed by Tritium Custom Preparations Group, Amersham Biosciences, The Maynard Centre, Whitchurch, Cardiff, CF147YT. The NMR spectra were recorded on a Varian Gemini 300 spectrometer in d_6-DMSO (2.50 ppm). The mass spectra were measured at NIDDK/NIH, at Amersham Biosciences facilities, and at NIDA/IRP (MALDI-TOF), the latter using a PE-Biosystems DE-PRO mass spectrometer (Farmingham, MA), equipped with a nitrogen laser (337 nm) firing at 3 Hz using an α-cyano-4-hydroxycinnamic acid matrix, prepared as a saturated solution in 50% v/v EtOH/H_2O).

*4,5,6,7 -Tetrachloro-2-(2-dimethylamino-ethyl)-isoindole-1,3-dione (**3**)*[10]

A solution of *N,N*-dimethylethylenediamine (**2**, 13.2 mL, 0.12 mol, 1.20 eq.) in dry toluene (100 ml) was added dropwise to a solution of tetrachlorophthalic anhydride (**1**, 28.6 g, 0.10 mol) in toluene (500 ml) at ambient temperature. The resulting white suspension was stirred at room temperature for 1 h. A Dean-Stark trap was attached and the reaction mixture was heated to reflux for 3 h, by which time the theoretical amount of H_2O was collected. After cooling to room temperature, the reaction mixture was washed with H_2O (2×100 ml), 5% $NaHCO_3$ (2×100 ml) and H_2O again (2×100 ml). The organic layer was dried over anhydrous Na_2SO_4, filtered, evaporated and recrystallized from $CHCl_3$ (*ca.* 150 ml) to give the first crop of **3** as pale-yellow crystals (20.96 g, 59%), m.p. 180–183°C (Lit.[11] m.p. 183–185°C). Evaporation of mother liquors and trituration of the residue with toluene (200 ml) gave an additional amount of **3** as yellow crystals (11.75 g, 33%). HRMS $[M\text{-}H]^+$ calculated for $C_{12}H_{11}N_2O_2Cl_4$: 354.9575, found 354.9579.

*4,5,6,7-Tetrachloro-2-(2-dimethylaminoethyl)-isoindoline (**4**)*[11]

Tetrachlorophthalimide derivative **3** (10.68 g, 0.03 mol) was added portion-wise to a stirred suspension of $LiAlH_4$ (4.50 g, 0.12 mol, 4.0 eq.) in dry Et_2O (600 ml) over *ca.* 20 min. The resulting yellow–green suspension was heated to

reflux under an argon atmosphere for 3 h, after which the heating was turned off and the reaction mixture was allowed to stir overnight at ambient temperature. After cooling in an ice bath to 0°C the excess hydride was carefully quenched with 10% aqueous Na_2SO_4 (24 ml) and the resulting slurry was stirred 1 h at room temperature. A fine granular precipitate was filtered and washed with Et_2O (5×75 ml). The pale-yellow filtrate was dried over anhydrous $MgSO_4$ (*ca.* 2 h), filtered and evaporated to give crude **4**. Recrystallization from acetone (*ca.* 20 ml) gave **4** as fine needles (4.24 g, 43%), m.p. 68–69.5°C (Lit.[11] m.p. 71.5–73°C). HRMS $[M\text{-}H]^+$ calculated for $C_{12}H_{15}N_2Cl_4$: 326.9989, found 326.9986.

*4,5,6,7-Tetrachloro-2-methyl-2-(2-trimethylammoniumethyl)-isoindolinium diiodide (**6**): procedure A*[12]

In a thick-walled tube, CH_3I (114 µL, 1.83 mmol, 3.0 eq.) was added to a stirred solution of 4,5,6,7-tetrachloro-2-(2-dimethylaminoethyl)-isoindoline (**4**, 0.200 g, 0.61 mmol) dissolved in 90% EtOH (1 ml) The monomethiodide salt **5** rapidly precipitated. The tube was briefly purged with argon, tightly capped and heated in an oil bath at 90°C for 5 days. After cooling to room temperature, the reaction mixture was cooled in an ice bath and the precipitate filtered and washed with absolute EtOH (2×3 ml) to give 0.355 g of crude **6**, which was recrystallized from 95% EtOH (50 ml + *ca.* 7 ml of H_2O) to furnish 0.255 g of chlorisondamine (**6**) as white needle-like crystals (68%, m.p. 239–241°C, Lit.[11] m.p. 239–241°C). ^{1}H-NMR (d_6-DMSO) δ 5.23 (ABq, $J = 15.0$ Hz, 4 H), 4.25 (m, 2 H), 4.01 (m, 2 H), 3.40 (s, 1 H), 3.19 (s, 9 H). ^{13}C-NMR (d_6-DMSO) δ 132.8, 132.1, 126.7, 70.0, 57.4, 56.7, 53.2 (3 Me), 52.0 (Me). Analytically calculated for $C_{14}H_{20}Cl_4I_2N_2$: C 27.48%, H 3.29%, N 4.58%; found: C 27.71%, H 3.34%, N 4.58%.

*Procedure B for 4,5,6,7-Tetrachloro-2-methyl-2-(2-trimethylammoniumethyl)-isoindolinium diiodide (**6**)*

In a thick-walled tube (*ca.* 24 ml volume), CH_3I (78 µL, 1.28 mmol, 2.1 eq.) was added to a stirred solution of 4,5,6,7-tetrachloro-2-(2-dimethylaminoethyl)-isoindoline (**4**, 0.200 g, 0.00061 mol) dissolved in 90% EtOH (1 ml) The monomethiodide salt **5** quickly precipitated. The tube was briefly purged with argon, tightly capped and heated in an oil bath at 94°C for 1 day. After cooling to room temperature, the reaction mixture was cooled further in an ice bath and the precipitate was filtered off and washed with abs. EtOH (2×3 ml) to give 0.318 g of crude product. The ^{1}H-NMR spectrum (d_6-DMSO) revealed the presence of a small amount of monomethiodide **5** (*ca.* 6% by integration of ^{1}H-NMR spectra). Recrystallization from 90% EtOH (*ca.* 15 ml) gave 0.242 g of chlorisondamine (**6**) as white crystals (65%, m.p. 239–241°C).

Stepwise monoquarternizations

*4,5,6,7-Tetrachloro-2-(2-trimethylammoniumethyl)-isoindolinium iodide (**5**).* CH_3I (190 µl, 0.433 g, 0.00305 mol, 1.1 eq.) was added to a stirred solution of 4,5,6,7-tetrachloro-2-(2-dimethylaminoethyl)-isoindoline **4** (0.910 g, 0.00277 mol) dissolved in absolute EtOH (10 mL). The reaction mixture was stirred at ambient temperature under an argon atmosphere for 2 days. After cooling in an ice bath, the precipitate was filtered off and washed with ice-cold absolute EtOH (2 × 5 ml), dried and recrystallized from 95% EtOH (*ca.* 20 ml) to give 0.990 g (76%) of **5** as a pink/yellow solid (m.p. 241–243°C). The compound was dissolved in 95% EtOH, boiled with charcoal, filtered hot, and cooled to give 0.756 g (58%) of **5** as off-white crystals (m.p. 241.5–243°C). ^{1}H-NMR (d_6-DMSO) δ 4.17 (s, 4 H), 3.52 (t, J = 5.4 Hz, 2 H), 3.18 (m, 2 H), 3.14 (s, 9 H), ^{13}C-NMR (d_6-DMSO) δ 139.6, 129.8, 125.9, 62.4, 58.9, 52.8, 48.5. Analytically calculated for $C_{13}H_{17}Cl_4IN_2$: C 33.22%, H 3.65%, N 5.96%; found: C 32.97%, H 3.64%, N 5.80%. HRMS: $[M\text{-}H]^+$ calculated for $C_{13}H_{17}Cl_4N_2$: 341.0146, found 341.0144.

*4,5,6,7-Tetrachloro-2-methyl-2-(2-trimethylammoniumethyl)-isoindolinium diiodide (**6**)*[11]. In a thick-walled tube (inner volume *ca.* 25 ml), CH_3I was added (7 µL, 0.112 mmol, 1.05 eq.) to 4,5,6,7-tetrachloro-2-(2-trimethylammoniumethyl)-isoindolinium iodide **2** (0.050 g, 0.106 mmol) dissolved in dry *N,N*-dimethylformamide (1 ml). The tube was briefly purged with argon, tightly capped and heated in an oil bath at 100°C for 1 day. After cooling to room temperature, the reaction mixture (dark-brown solution) was diluted with acetone (3 ml) and cooled to 0°C. The precipitate was filtered and recrystallized from 95% EtOH (7 ml plus *ca.* five drops of H_2O) to give **6** as white crystals (0.038 g, 58%), m.p. 241–242.5°C). Analytically calculated for $C_{14}H_{20}Cl_4I_2N_2$: C 27.48%, H 3.29%, N 4.58%; found: C 27.63%, H 3.25%, N 4.37%.

*4,5,6,7-Tetrachloro-2-CT$_3$-2-(2-trimethylammoniumethyl)-isoindolinium diiodide (**7**).* [^{3}H]chlorisondamine was prepared by combining monomethiodide **5** (10.1 mg, 0.02 mmol) in a borosilicate glass tube with *N,N*-dimethylformamide (1 ml) and [^{3}H]methyl iodide (10 Ci, total activity) and heating the mixture at 100°C for 16 h. The volatile materials were pumped off at high vacuum, labile tritium removed by repeated evaporation of EtOH (3 × 1 ml), and the orange residue was dissolved in EtOH: H_2O mixture (3:1; 40 ml). Yield: 839 mCi. Crude radiochemical purity: > 60%, specific activity: 94 Ci/mmol. Purified (~100 mCi) by column chromatography (SepPak C18; 3 cm^3; 500 mg) eluting with MeOH: H_2O: 55% hydriodic acid (80:20:0.2). Desired fractions were collected and analyzed. Yield: ~40 mCi. Radiochemical purity was ascertained by thin layer chromatography on Whatman LKC18F in: (i) MeOH: H_2O: trifluoroacetic acid (90:10:0.2): 97%, (ii) MeOH: H_2O: 55% hydriodic

acid (80:20:0.2): 96% and also on cellulose (Merck 5718) in 1-butanol saturated with 0.1 M ammonia to be 97%. Labeled material co-eluted with the non-labeled material and the mass spectrum was consistent with the proposed structure and non-labeled reference. TOF MS ES^+ (Amersham): $C_{14}H_{20}Cl_4N_2^{2+}$: *m/z*: 178.0285, 179.0253, 180.0248. Labeled material: $C_{14}H_{17}T_3Cl_4N_2^{2+}$: 181.0381, 182.0377, 183.0345.

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