Synthesis of Tritium Labelled (R) and (S)-3-Aminoquinuclidine: A Ubiquitous Component of Serotonin Receptor Ligands, Part II

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SUMMARY

(S)-3-Aminoquinuclidine-^{3}H **10c-S** having a specific activity of 66 Ci/mmol was prepared in eight steps from isonicotinic acid (**2**). Reduction of **2** with carrier free tritium gas over PtO_2 in DMF gave isonipecotic acid-^{3}H **3c**. Conversion to α-bromo ketone **5c** followed by cyclization afforded 3-quinuclidone-^{3}H **6c**. Racemic 3-amino-quinuclidine-^{3}H **8c** was prepared by conversion of **6c** to oxime **7c** followed by reduction with $NaBH_4/NiCl_2 \cdot 6H_2O$. Racemic **8c** was resolved with (R)-methylbenzyl isocyanate. Hydrolysis of **9c-S,R** afforded (S)-3-aminoquinuclidine-^{3}H **10c-S**. The enantiopurity was >99.5% (S).

Key Word: 3-Aminoquinuclidine-^{3}H, 5-HT_3, Enantiomers.

INTRODUCTION

In our previous manuscript we described[1] the synthesis of (R) and (S)-3-aminoquinuclidine-[^{3}H] enantiomers having a specific activity of 35 Ci/mmole. Various tritiated 5-HT_3 antagonists were prepared from both tritiated enantiomers. These ligands were useful in initial 5-HT_3 receptor studies. However, autoradiographic characterization of receptors which were present in low concentration required higher specific activity ligands. Therefore, we turned our attention to exploring methodology which could furnish 3-aminoquinuclidine-^{3}H having a specific activity significantly greater than 35 Ci/mmole.

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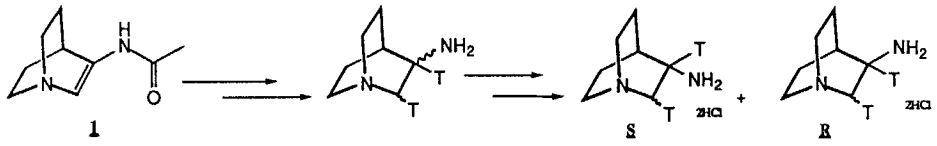
DISCUSSION AND RESULTS

In the preceding paper, we described the synthesis of 3-aminoquinuclidine-^{3}H enantiomers by reduction of an enamide (1) with tritium gas, followed by resolution of the racemic product by chromatographic separation of a diastereomeric derivative ***(Scheme 1)***[1]. However, in order to achieve higher specific activity,

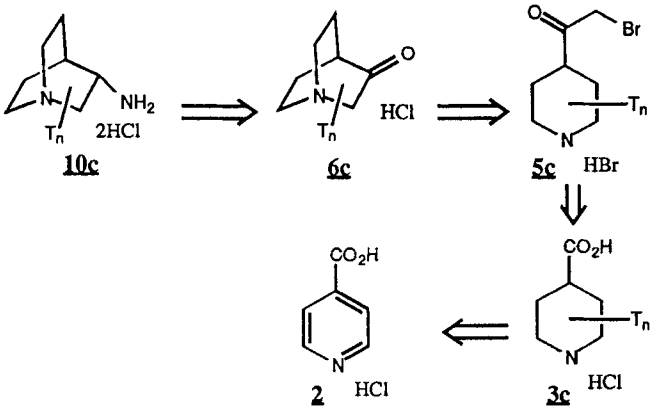
Scheme 1



1 S R

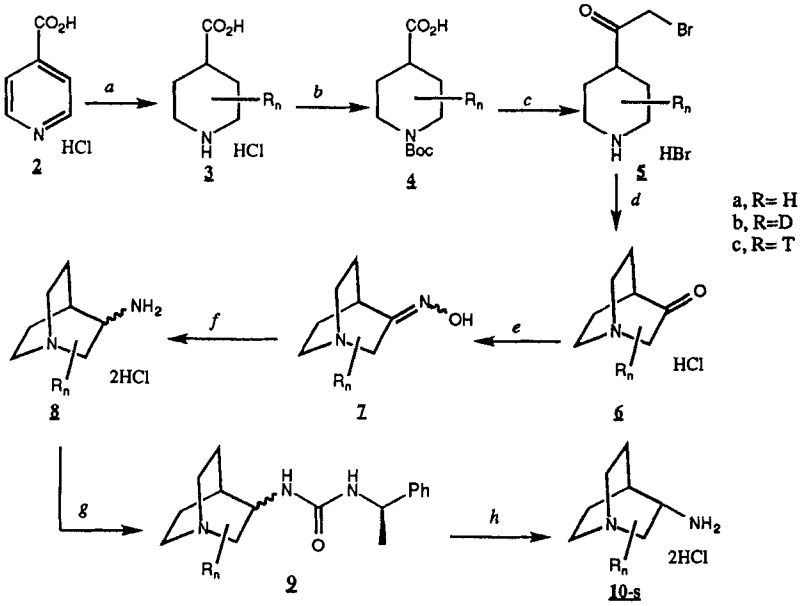
a substrate containing multiple sites of unsaturation was required. Isonicotinic acid is a readily available substrate which meets this requirement. In fact, we used this very material in our recent synthesis of 3-aminoquinuclidine-^{14}C[2]. That synthesis was based on the intramolecular cyclization of 4-(α-bromoacetyl)-piperidine which was derived from isonipecotic acid *via* the intermediate diazoketone. Adaptation of this approach to the problem at hand would afford an opportunity to achieve a high degree of tritium incorporation by the simple expedient of substituting tritium for hydrogen in the first step. This strategy was particularly attractive because reductions of pyridines to piperidines with tritium gas have been used in our laboratory to furnish high specific activity (60-180 Ci/mmole) products[3].
The retrosynthetic scheme ***(Scheme 2)*** summarizes our intended approach. Since tritiation is the first step in this carrier free sequence, implementation of such a multistep, microscale synthesis was expected to be an a challenging operational problem.

Scheme 2



Reduction conditions and subsequent reactions were developed and optimized using deuterium ***(Scheme 3)***. Thus, isonicotinic acid hydrochloride **2** was reduced with D_2 over PtO_2 in DMF. When the reduction was allowed to proceed for 3 d, deuterated **3b** was obtained with an average incorporation of 3.98 D atoms as determined by mass spectroscopy. Increasing the reaction time to 7 d changed the

Scheme 3



a, $^3H_2$ or $^2H_2$, PtO_2, DMF, 3d; *b*,(Boc)$_2$O, Na_2CO_3, dioxane, H_2O; *c*, 1.$(COCl)_2$, CH_2Cl_2. 2. CH_2N_2, Et_2O. 3. HBr, HOAc; *d*, K_2CO_3, CH_3CN, 90°; *e*, $HONH_2$.HCl, NaOAc, MeOH; *f*, $NaBH_4$, $NiCl_2.6H_2O$, MeOH; HCl; *g*, R-methylbenzyl isocyanate, TEA, $CHCl_3$; *h*, *n*-BuONa, *n*-BuOH, 130°.

average D incorporation only modestly to 4.3 D atoms. In order to elaborate the carboxylic acid, the piperidine nitrogen was protected as the N-Boc derivative. Conversion of **4b** to the acid chloride, followed by treatment with diazomethane afforded the α-diazoketone. Addition of HBr accomplished three transformations simultaneously; the diazo function was displaced to give the desired α-bromoketone, the Boc group was removed, and the liberated piperidine nitrogen was protonated, thereby preventing premature reaction with the α-bromoketone. Nitrogen deprotection and simultaneous neutralization was critical because carefully controlled intramolecular cyclization conditions were essential in order to achieve a good yield of quinuclidone **6b**. Formation of 3-quinuclidone-D_n **6b** by intramolecular displacement of the α-bromoketone **5b** by the piperidine nitrogen was accomplished in 61% yield by slow addition to a suspension of K_2CO_3 in CH_3CN. Mass spectral analysis of **6b** showed no loss of deuterium (3.95 D per molecule).

Of many methods available for conversion of a ketone to primary amine, the most suitable was formation of an oxime **7b** with hydroxylamine hydrochloride and NaOAc in MeOH Followed by reduction with $NaBH_4$, $NiCl_2.6H_2O$ in MeOH at -30°[5]. The resulting crude racemic 3- aminoquinuclidine was then converted to a mixture of diastereomeric ureas **9b** with (R)-methylbenzyl isocyanate. These diastereomers were separated chromatographically as previously reported[1]. The above process was applied to the synthesis of tritiated 3-aminoquinuclidine. Thus, **2** was reduced with carrier-free tritium gas over PtO_2 in DMF for 3 d affording 4,550 mCi of tritiated isonipecotic acid hydrochloride **3c**. A portion of this product was converted to N-Boc protected isonipecotic acid with $(Boc)_2O/Na_2CO_3$ to give **4c** in 63% yield. The protected amine was then converted to the key labelled intermediate 4-(α-bromoacetyl)-piperidine-^{3}H, **5c**, in 76% yield by reaction of **4c** with $(COCl)_2$ followed by CH_2N_2 and HBr/HOAc. Slow addition, *via* addition funnel, of a dilute CH_3CN solution of **5c** to K_2CO_3 in a large volume of CH_3CN at 90° furnished 3-quinuclidone-^{3}H hydrochloride **6c** in 81% yield. Treatment of **6c** with hydroxylamine hydrochloride followed by reduction of the oxime **7c** with $NaBH_4$, $NiCl_2.6H_2O$ in MeOH gave a 51% yield of racemic 3-aminoquinuclidine-^{3}H (**8c**). Following our previous synthesis, **8c** was converted to a mixture of diastereomeric ureas with (R)-methylbenzyl isocyanate[1]. Chromatography on silica gel followed by preparative HPLC separation of the diastereomers, 23 mCi of **9c-S,R** isomer and 80 mCi of **9c-R,R**[6] isomer were obtained. The specific activity was determined for the (S,R) urea by the HPLC external standard method, to be 66 Ci/mmol, approximately twice the enrichment obtained by reduction of enamide (**1**). The **9c-S,R** diastereomer was hydrolyzed with *n*-BuONa in *n*-BuOH at 130° to give (S)-3-aminoquinuclidine-^{3}H (**11-S**). The enantiopurity of (**11-S**) was determined to be >99.5% by HPLC analysis of its glucose isothiocyanate derivative[1]. The (R,R) diastereomer, (**9c-R,R**) was not hydrolysed to (R)-3-aminoquinuclidine-^{3}H since there was no immediate need for that enantiomer.

EXPERIMENTAL

Unlabelled reagents were purchased from Aldrich Chemical Co. and were used without purification. Solvents were HPLC grade. Carrier free tritium gas was purchased from New England Nuclear Corp. (10 Ci ampules, 58 Ci/mmol). Radiochromatography was performed on a BioScan 200 Scanner. Radioassays were obtained using a Packard 4000 liquid scintillation counter. UV spectra were obtained using a Hitachi UV-265 spectrometer. NMR spectra were recorded using a Varian EM 390 spectrometer. IR spectra were recorded using a Nicolet 5PC FT-IR spectrometer. MS spectra were obtained on a Finnigan-MAT 8230 spectrometer.

Isonipecotic acid hydrochloride-^{3}H, 3c. A mixture of **2** (8 mg, 0.05 mmol) and PtO_2 (8 mg) was dried under vacuum in a 5 mL septum side-arm flask; DMF (2 mL) was injected in the flask. The resulting mixture was degassed. Carrier free tritium gas (10 Ci, 58 Ci/mmol, 0.17 mmol) was transferred by a Toepler pump into the liquid nitrogen cooled flask. The mixture was warmed to room temperature and stirred for 4 d. The bulk of volatile activity was removed by vacuum transfer to a waste bulb. The crude mixture was filtered through a 0.45 μm nylon filter, acidified with 10% aq HCl and concentrated twice from EtOH:H_2O (1:1 v/v). TLC (8:2: 0.1 CH_2Cl_2:MeOH:HOAc) indicated 60% of the activity (4.55 Ci) was the desired compound. No further purification was done.
Total Activity: 4.55 Ci. **TLC**: silica gel; ; 8:2: 0.1 CH_2Cl_2:MeOH:HOAc; R_f 0.25.

N-(*t*-Butoxycarbonyl)isonipecotic acid-^{3}H, 4c. To a solution of **3c** (2 Ci) in dioxane/H_2O (5 mL, 1:1 v/v) was added Na_2CO_3 (18 mg). The mixture was stirred for 5 min and $(Boc)_2O$ (18 mg) was added. The mixture was stirred at room temperature for 12 h, concentrated and reconstituted in H_2O. The aq layer was extracted with EtOAc to remove the neutral components, then acidified to pH 3 and extracted with EtOAc. Combined organic layers were dried over Na_2SO_4 and concentrated. The mixture was reconstituted in EtOAc. TLC (2:1:0.1 hexane:EtOAc:HOAc) indicated that 75% of the activity (1.26 Ci) was due to the desired compound. No further purification was done.
Total Activity: 1.26 Ci. **TLC**: silica gel, 8:2:0.1 hexane:EtOAc:HOAc; R_f 0.63. **^{1}H NMR** **4a** (300 MHz, $CDCl_3$) δ 4.1 (broad m, 2H), 2.8 (broad m, 2H), 2.5 (m, 1H), 1.9 (broad m, 2H), 1.7 (broad m, 2H), 1.45 (s, 9H). **MS** **4a** (EI) m/z (rel. inten.) 229 (M+, 60), 191 (80), 174 (22), 130 (100). **MS** **4b** (LSIMS) m/z (rel. distribution) 229-235 (d_0= 2%, d_1=5%, d_2=10%, d_3=15%, d_4=21%, d_5=22%, d_6=17%, d_7=8%).

4-(Bromoacetyl)piperidine hydrobromide-^{3}H, 5c. To **4c** (1.26 mCi) in benzene/ CH_2Cl_2 (5 mL, 1:1 v/v) at 0° was added $(COCl)_2$ (0.1 mL) followed by one drop of DMF. Gas evolution was apparent. After 1 h, the mixture was concentrated in vacuo and reconstituted in benzene. This process was repeated three times. Then, the mixture was dissolved in Et_2O and was added to excess freshly prepared and dried CH_2N_2 at 0°. The mixture was stirred at 0° for 3 h and excess CH_2N_2 was quenched with glacial HOAc. 33% HBr/HOAc (3 mL)[4] was added and the mixture was allowed to reach room temperature slowly and was stirred overnight. Upon dilution with MeOH and concentration *in vacuo* (three times), TLC analysis (2:1:0.1 hexane:EtOAc:HOAc) indicated that 76% of the activity (965 mCi) was the desired compound. No further purification was done.
Total Activity: 965 mCi. **TLC**: silica gel, 2:1:0.1 hexane:EtOAc:HOAc; R_f 0.34. **^{1}H NMR** **5a** (300 MHz, DMSO-d_6) δ (8.6, broad s, 1H), 4.5 (s, 2H), 3.4 (m, 2H), 2.9 (3, 3H), 2.0 (m, 2H), 1.7 (m, 2H). **MS** **5a** (CI, NH_3) m/z (rel. inten.) 205 (M+ + 1, 30),

126 (100). **MS 5b** (LSIMS) m/z (rel. distribution) 205-212 (d_0= 7.7%, d_1=6.4%, d_2=11.1%, d_3=15.9%, d_4=18.9%, d_5=19.6%, d_6=13.4%, d_7=5.2%, d_8=1.8%).

3-Keto-1-azabicyclo[2.2.2]octane dihydrochloride-^{3}H, 6c. To a suspension of K_2CO_3 (9 mg) in dry CH_3CN (50 mL) at 90° (bath temp) was added **5c** (965 mCi) dissolved in 3% DMF/CH_3CN (75 mL) dropwise *via* an addition funnel over 5 h. The mixture was stirred for an additional 3 h, cooled to room temperature and stirred over night. The mixture was filtered through a sintered glass funnel, and the supernatant was acidified to pH 3 with conc. HCl. The mixture was concentrated and reconstituted in MeOH. TLC analysis [20%(5%NH_4OH/ MeOH)/CH_2Cl_2] indicated that 61% of the activity (782.8 mCi) was due to the desired compound. No further purification was done.
Total Activity: 783 mCi. **TLC**: silica gel, 20%(5%NH_4OH/ MeOH)/CH_2Cl_2; R_f 0.74

1-Azabicyclo[2.2.2]octane-3-oxime-^{3}H, 7c. To a solution of **6c** (663.5 mCi) in MeOH (10 mL) was added $HONH_2$.HCl (20 mg) and NaOAc (35 mg). The mixture was stirred at room temperature. After 1 h, the mixture was concentrated and purified on silica gel with 10%(5%NH_4OH/MeOH)/CH_2Cl_2 with increasing polarity to 30%(5%NH_4OH/ MeOH) /CH_2Cl_2. After chromatography, the residual NaOAc was removed by dissolving the product in CH_2Cl_2 and filtering it through a 0.45 μm nylon filter. TLC 10%(5%NH_4OH/ MeOH) / CH_2Cl_2 analysis indicated that 96% of the activity (475.35) was due to the desired product.
Total Activity: 475.35 mCi. **TLC**: silica gel, 10%(5%NH_4OH/MeOH) /CH_2Cl_2; R_f 0.25. **^{1}H NMR 7a** (300 MHz, $CDCl_3$) δ 3.85 (s, 2H), 3.6 (overlapping s and m, 2H), 3.1 (m, 2H), 2.75 (m, 1H), 1.9 (m, 2H). **MS 7a** (EI) m/z (rel. inten.) 140 (M+, 40), 123 (100). **MS 7b** (EI) m/z (rel. distribution) 229-235 (d_0= 2%, d_1=5%, d_2=11%, d_3=18%, d_4=23%, d_5=22%, d_6=14%, d_7=4%).

3-Amino-1-azabicyclo[2.2.2]octane dihydrochloride-^{3}H, 8c. To a solution of **7c** (475.35 mCi) in MeOH (10 mL) at -30° was added $NiCl_2.6H_2O$ (70 mg) and $NaBH_4$ (50 mg). Gas evolution was apparent. After 3 h, TLC analysis [20%(5%NH_4OH/ MeOH) / CH_2Cl_2] indicated presence of starting material. Therefore, more $NaBH_4$ (10 mg)[7] was added. After 0.5 h, TLC showed drastically different pattern as had been seen earlier. This may have been due to N-B bond formation. To test this idea, an aliquot was removed and heated to 70° in 10% aq HCl/EtOH (1:1 v/v) for 1 h. TLC analysis indicated complete conversion to aminoquinuclidine. Consequently, the remaining activity was subjected to hydrolysis. TLC analysis indicated (5%NH_4OH/MeOH) that 95.6% of the activity (396 mCi) was due to the desired compound. No further purification was done.

Total Activity: 396.27 mCi[8]. **TLC**: silica gel, 5%NH_4OH/MeOH; R_f 0.12.

(S,R) and (R,R)-3-(R-Methylbenzyl urea)-1-azabicyclo[2.2.2]octane-^{3}H, 9c. To a solution of **8c** (396 mCi) in 5% TEA/$CHCl_3$ (5 mL) was added (R)-methylbenzyl isocyanate (0.2 mL). The mixture was stirred at room temperature and monitored by TLC. After 2 h, the mixture was filtered through 0.45 μm nylon filter, concentrated and purified on silica gel with 40%(5%NH_4OH/MeOH)/CH_2Cl_2 to afford 317 mCi of product. Chromatography on silica gel separated the impurities and afforded 199 mCi mixture of (S,R) and (R,R) urea. Preparative HPLC on a Biotage PBD column with 8%CH_3CN/ (0.2%NH_4OH-0.1%Et_2NH/H_2O), 1 mL/min, 220 nm, achieved the separation of diastereomers.
Total Activity: (S,R) isomer **9c-S,R** 23 mCi, (R,R) isomer **9c-R,R**, 80 mCi[6].
Specific Activity: (S,R) isomer **9c-S,R** 66 Ci/mmol, determined based on HPLC external standard method. **TLC**: silica gel, 40%(5%NH_4OH/MeOH)/CH_2Cl_2; R_f 0.5.
HPLC: Beckman ultrasphere ODS C18, 5 μm, 15%CH_3CN/0.03 M TEAP pH 3 buffer, 1 mL/min, 220 nm.

(S)-3-Amino-1-azabicyclo[2.2.2]octane dihydrochloride-^{3}H, 10c.
Freshly prepared *n*-BuONa in *n*-BuOH (14 mL) was added to **9c-S,R** (17 mCi). The mixture was warmed to 130° and monitored by TLC. After 9 d, the mixture was cooled to room temperature, EtOH and 10% aq HCl were added to pH 2. The mixture was concentrated, dissolved in H_2O and extracted with EtOAc to remove the neutral components[9]. The aqueous layer was concentrated and reconstituted in 30% H_2O/EtOH and assayed for activity and enantiopurity by HPLC[10].
Total Activity: 13.29 mCi[8]. **Specific Activity**: 66 Ci/mmol, determined by HPLC external standard and was based on specific activity of (S,R) urea.
Enantiopurity: >99.5% (S), determined by making the GITC derivative and analysis on HPLC using Beckman Ultrasphere ODS C18, 5 μm, 4.5 x 250, 18% CH_3CN/0.03 M TEAP pH 3 buffer, 1 mL/min, 214 nm.

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4. 48% HBr was used.

5. *a.* Ipaktschi, J. *Chem. Ber.*117, 856, (1984). *b.* Nose, A.; Kudo, T. *Chem .Pharm. Bull.* 29, 1159, (1981).

6. The (R,R) diastereomer still contained 6% of the (S,R) isomer.

7. Most likely, extra $NaBH_4$ was not needed.

8. The total activity obtained after the hydrolysis was always lower than the expected value. This did not represent the actual activity present in the flask.

9. The free based amine was very water soluble, therefore, no aqueous extraction was possible.

10. The compound contained NaCl and methylbenzyl amine as impurity since no chromatography (silica gel) was possible on the compound.