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

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SUMMARY

(R) and (S)-3-aminoquinuclidines-^{3}H with the specific activity of 35 Ci/mmol were prepared. Reduction of enamide **2** with carrier free tritium gas over RhCODCl dimer, (2S, 3S) Chiraphos in methanol gave racemic amide **9c**. Hydrolysis followed by resolution of the enantiomers with (R)-methyl benzyl isocyanate gave (R) and (S)-3-aminoquinuclidine-^{3}H **10c-S** and **10c-R**. The enantiopurity purity of both isomers was >99.5%.

Key Words: Aminoquinuclidine-^{3}H, 5-HT_3, Zacopride-^{3}H enantiomers

INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter which acts upon various receptors in the body[1]. This family of receptors have been divided into subtypes, i.e., 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, *etc.* and are responsible for numerous pharmacological effects, ranging from emesis to drug addiction[1]. In recent years, agonists and antagonists of 5-HT have been developed to attenuate or enhance those pharmacological effects of interest[1,2]. 5-HT_3 antagonists, in particular, have shown promising results in this regard. Basic research programs at Syntex have identified several 5HT_3 antagonists which contain the (R) or (S) 3-aminoquinuclidine[2] moiety. High specific activity tritiated ligands were required to

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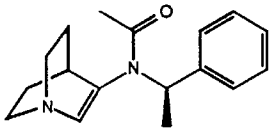
support the ongoing efforts to characterize the pharmacology of the 5-HT_3 receptor. Rather than prepare each ligand, *de novo*, we focussed our efforts on the synthesis of the fragments common to all the ligands of interest, namely, (R) and (S) 3-aminoquinuclidine-^{3}H. This strategy allowed us to prepare a variety of ligands from a single batch of labelled reagent.

RESULTS AND DISCUSSION

At the outset, we envisioned that the desired 3-aminoquinuclidine-^{3}H could be prepared by asymmetric reduction of an enamide with tritium gas in the presence of a chiral homogenous catalyst. Various examples in the literature have shown that reduction of N-acyl enamides can be accomplished with good to excellent stereoselection[3]. Therefore, enamides of type **1** or **2**, which contained an N-acyl group, were expected to be good substrates for asymmetric reduction. Enamide **1** was chosen as our initial target since the presence of the chiral (R)-methylbenzyl group could enhance diastereoselectivity due to double differentiation during the reduction.



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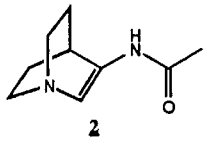
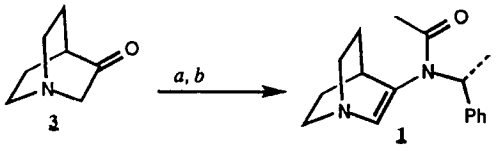


2

Synthesis of **1** is depicted in ***Scheme 1***. 3-Quinuclidone **3** was converted to imine by condensation with (R)-methylbenzyl amine in refluxing benzene. The crude imine was then treated with acetyl chloride followed by pyridine[4] affording a 22% yield of **1** after purification.

Scheme 1



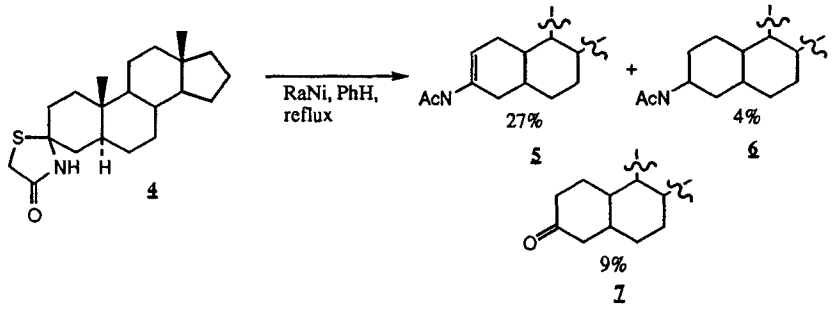
3 **1**

a, (R)-methylbenzyl amine, 4 Å molecular sieves, reflux; *b*, AcCl, 0°, 1h; pyridine 19-24 h, 22% two steps.

Attempted reduction of **1** with D_2, in the presence of $(RhCODCl)_2$, (2S, 3S)-Chiraphos in MeOH led only to recovered starting material. This was attributed to the steric bulk which existed around the reduction site. In fact, attempts to reduce **1** with H_2 and Pd/C under neutral or acid conditions were also ineffective, leading to either recovered starting material **1** or decomposition products. We turned our attention, therefore, to the less hindered substrate **2**.

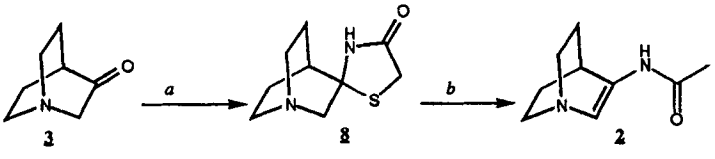
Crossly and Djerassi [5] have shown that various steroids containing a keto group can be converted into an enamide in modest yield. For example treatment of 5-α-androstan-3-one with thioglycolic acid and ammonium carbonate in refluxing benzene led to a thioindazolidanone **4** which upon reduction with Raney-Nickel (RaNi) in benzene afforded three products: the desired enamide **5**, amide **6** and ketone **7**. The yield of the reaction as well as product distribution was highly dependent upon the age of RaNi and the solvent employed ***(Scheme 2)***.

Scheme 2



Applying this methodology to the problem at hand ***(Scheme 3)***, 3-quinuclidone **3** was converted to thioindazolidanone **8** with thioglycolic acid and $(NH_4)_2CO_3$, in refluxing benzene in 65% yield. Reduction of **8** with RaNi gave the desired enamide **2** in a 55-69% yield . A trial reduction of **2** with D_2 in the presence of (2S, 3S)-Chiraphos in EtOH ***(Scheme 4)*** proceeded smoothly to amide **9b**. Hydrolysis of **9b** in aq HCl afforded deuterated 3-aminoquinuclidine dihydrochloride **10b**. Surprisingly, HPLC analysis of the glucose isothiocyanate

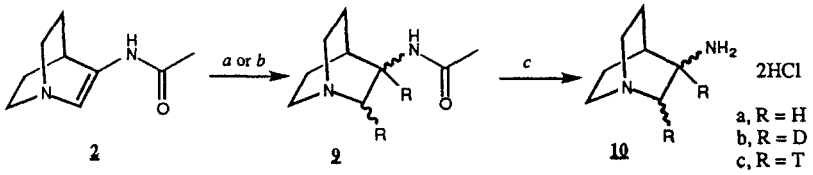
Scheme 3



a, Thioglycolic acid, $(NH_4)_2CO_3$, PhH, 65%; *b*, RaNi, PhH, reflux, 19 h, 55-65%.

(GITC)[6] derivative of **10b** revealed only a 2% diastereomeric excess (de). The nearly complete lack of asymmetric induction required the development of a method to effectively separate a diastereomeric derivative of racemic **10**. Racemic secondary amines have been resolved by separation of their diastereomeric ureas derived from (R)-methylbenzylisocyanate[7]. The pure enantiomers were then obtained by removing the chiral auxiliary in refluxing

Scheme 4

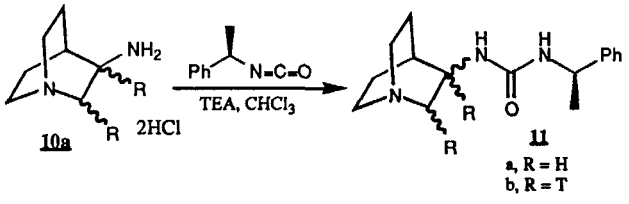


2 9 10

a, $(RhCODCl)_2$, D_2, (2S, 3S)-Chiraphos, MeOH, 4 h; *b*, H_2, 5% Pd/C, 10% MeOH:EtOAc, 48 h; *c*, 6 N aq HCl, 100°, 3 h.

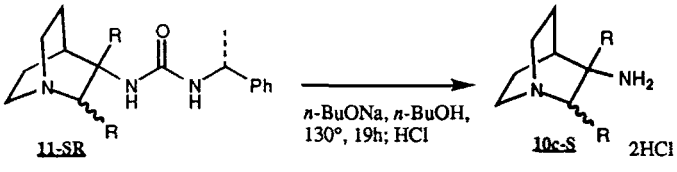
ethanol or butanol. This method was reported to be specific for secondary amines only. However, we found that the urea of a primary amine could be resolved using (R)-methylbenzylisocyanate, then hydrolyzed, in very good yield, by heating in sodium butoxide-butanol. Thus, treatment **10a** with (R)-methylbenzylisocyanate gave a mixture of diastereomeric ureas **11a** which was separable by radial chromatography on silica gel ***(Scheme 5)***. Hydrolysis of the chiral auxiliary from the (S,R) diastereomer using *n*-BuONa in *n*-BuOH at 130° furnished (S)-3-aminoquinuclidine **10a-S** with >99.5% enantiopurity ***(Scheme 6)***.

Scheme 5



10a 11

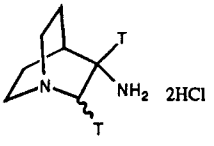
Scheme 6



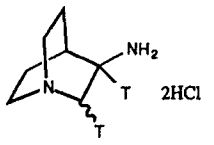
11-SR 10c-S

Applying the above process for the synthesis of the labelled enantiomers **10c-S** and **10c-R**, **2** was reduced with carrier free tritium gas, $(RhCODCl)_2$, (2S, 3S)-Chiraphos in EtOH for 24 h. The homogenous chiral catalyst system was used in place of Pd/C, despite the lack of chiral induction. This choice was made because, in our experience, the former affords cleaner reaction mixtures and, in general,

higher specific activity products. Tritiated amide **9c** was obtained in 46% radiochemical yield after purification. Acidic hydrolysis of **9c** gave racemic 3-aminoquinuclidine-^{3}H dihydrochloride **10c** which was converted to a mixture of diastereomers **11b** with (R)-methybenzylisocyanate [>99.5% (R)]. Upon separation of the diastereomers by radial chromatography, pure (R,R) and (S,R) diastereomers were obtained in a 3:5 ratio along with a significant amount of mixed fractions. The specific activity of the (S,R) diastereomer was determined by mass spectrometry to be 33-35 Ci/mmol. The pure (R,R) and (S,R) diastereomers were hydrolyzed to their corresponding enantiomers with *n*-BuONa/*n*-BuOH at 130° affording enantiomerically pure >99.5 (R) and (S)-3-aminoquinuclidine-^{3}H, **10c-R** and **10c-S**, respectively.



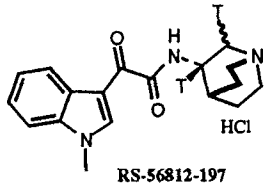
10c-S



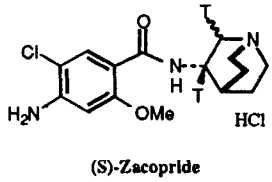
10c-R

Both enantiomers were used to prepare various 5-HT$_3$ antagonists ***(Scheme 7)***. Thus, coupling of (S)-3-aminoquinuclidine-^{3}H **10c-S** with methylindole glyoxayl chloride in EtOAc gave RS-56812-197-^{3}H in 85% yield. The same fragment when condensed with 4-amino-3-chloronaphthalic anhydride in refluxing MeOH furnished RS-56532-197-^{3}H in 66% yield. Coupling of **10c-S** with 6-chloro-5-amino-2-methoxy benzoic acid (DCC, HOBT, TEA, in THF) afforded (S)-Zacopride-^{3}H in 88%. The same procedure with (R)-3-aminoquinuclidine-^{3}H **10c-R** furnished (R)-Zacopride-^{3}H in 98% yield.

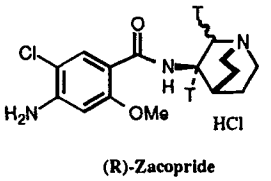
Scheme 7



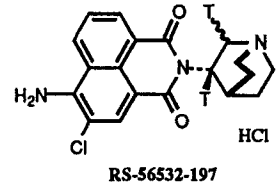
RS-56812-197



(S)-Zacopride



(R)-Zacopride



RS-56532-197

The specific activity of each compound was determined by HPLC (external standard method) or by mass spectrometry to be between 32-36 Ci/mmol.

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 DuPont/New England Nuclear Corp. (10 Ci, 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. Specific activity determination was done using the HPLC external standard method unless otherwise stated. "Dried" refers to drying the organic layer over anhydrous $MgSO_4$ unless otherwise stated.

3-[N-(R)-Methylbenzyl]-3-acetamido-1-azabicyclo[2.2.2]oct-2-ene (1). To a solution of 3-quinuclidone (1.0 g, 8 mmol) in benzene (20 mL, 0.4 M) was added (R)-methylbenzyl amine (1.02 g, 8.4 mmol) and 4 Å molecular sieves. The mixture was heated at reflux for 24 hr, cooled to 0°, and treated with acetyl chloride (1.25 mL). The resulting solution was stirred at 0° for 1 h then treated with 8 mL of pyridine. The mixture was stirred for 24 h at room temp, poured into saturated aq $NaHCO_3$, and extracted with EtOAc. The combined organic layers were washed with brine, dried, and concentrated. Chromatography on silica gel with 30% (2%NH_4OH /MeOH)/ 70% EtOAc afforded 546 mg of **1** (22%).

TLC: 30%(2% NH_4OH/MeOH) /EtOAc R_f 0.3. **IR** (KBr) 3031, 2450, 2874, 1653, 1495, 1385, 1344, 1315, 1223, 1203, 1126, 1080, 1060, 1035, 797, 760, 702, 679, 603 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 0.80 (m, 1H), 1.25-1.35 (m, 1H), 1.36-2.06 (m, 5H), 2.15-2.39 (m, 4H), 2.42-2.44 (m, 2H), 2.80-2.90 (m, 2H), 5.9-6.1 (m, 1H), 6.2 (d, 1H, *J*=2.3 Hz), 7.26-7.33 (m, 5H). **MS** (EI) m/z (rel. inten.) 270 (M+, 50), 255 (20), 227 (70), 165 (35), 123 (70), 105 (100).

3-(Thioindazolidinone)-1-azabicyclo[2.2.2]octane (8). To a mixture of **3** (0.5 g, 4 mmol) and $(NH_4)_2CO_3$ (4.49 g, 45.8 mmol) in benzene (25 mL) was added thioglycolic acid (3.21 g, 2.4 mL) dropwise *via* syringe. Gas evolution was apparent. Once the effervescence stopped, the mixture was heated at reflux for 30 h while water was removed from the reaction mixture using a Dean-Stark trap. The mixture was concentrated, dissolved in EtOAc, and washed with satd aq $NaHCO_3$. The aq layer was extracted with EtOAc. The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated. Chromatography on silica gel with 30%(2% NH_4OH/MeOH)/EtOAc afforded 0.5 g of **2** (63.5%).

MP 187.6-188.6 °C. **TLC**: silica gel: 30%(2% NH_4OH/MeOH)/CH_2Cl_2; R_f 0.2. **IR** (KBr): 3445, 3165, 3061, 2959, 2878, 1693, 1448, 1371, 1321, 1215, 1074, 1053,

970, 854, 823, 788, 630 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 1.26-1.72 (m, 2H), 1.93-1.99 (m, 2H), 2.0-2.09 (m. 1H), 2.80-2.99 (m, 4H), 3.28 (d, 2 H, *J*=16Hz), 3.59 (d, 2H, *J*=16 Hz), 9.0 (S, 1H). **MS** (EI) m/z (rel. inten.) 198 (M+, 15), 181 (5) 169 (5) 91 (100), 70 (24), 48 (95).

3-Acetamido-1-azabicyclo[2.2.2]oct-2-ene (2). A suspension of RaNi (8 g) was washed with EtOH five times then benzene five times. Benzene (50 mL) was added and 15 mL was removed by distillation to remove residual moisture. Compound **8** (0.723 g, 3.7 mmol) was dissolved in 20 mL of benzene and half the volume was removed by distillation. This solution was added to the RaNi and the mixture was heated at reflux for 42 h. The reaction was cooled, filtered through celite, and concentrated. Chromatography on silica gel with a 15% - 30% gradient of (2%NH_4OH/MeOH)/CH_2Cl_2 afforded 400 mg of **2** (70% based on recovered **8**).

TLC: silica gel, 30%(2% NH_4OH/MeOH)/ CH_2Cl_2,; R_f 0.53. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 1.44-1.64 (m, 4 H), 1.99 (s, 3H), 2.44-2.50 (m, 2H), 2.64-2.79 (m, 1H), 2.81-2.88 (m, 2H), 6.69 (s, 1H), 9.35 (S, 1H). **MS** (EI) m/z (rel. inten.) 166 (M+, 100), 123 (77), 95 (100), 82 (50), 43 (58).

3-Acetamido-1-azabicyclo[2.2.2]octane-^{3}H (9c). A mixture of **2** (8.3 mg, 0.05 mmol), (S,S)-Chirophos (3 mg), and $(RhCODCl)_2$ (3 mg) was dried under vacuum in a 5 mL septum side-arm flask. EtOH (3 mL) was injected into the flask and the resulting yellow solution was degassed. Carrier free tritium gas (10 Ci, 58 Ci/mmol, 0.17 mmol) was transferred by means of a Toepler pump into the liquid nitrogen cooled flask. The mixture was warmed to room temperature and stirred for 44 h. The bulk of the volatile activity was removed by distilling half of solvent into a waste flask connected to the vacuum line. The crude mixture was filtered through a 0.45 μm nylon filter, then concentrated from EtOH three times to remove exchangeable tritium. Radio-TLC [30%(5% NH_4OH/MeOH)/CH_2Cl_2)] of the crude mixture indicated 83% of the activity was due to the desired compound (1.6 Ci). Purification on silica gel column with a gradient of 20-40%(5%NH_4OH/MeOH)/ CH_2Cl_2 furnished 1.35 Ci of **9c** (46% radiochemical yield).

TLC: silica gel, 30%(5%NH_4OH/ MeOH)/CH_2Cl_2; Rf 0.25. **^{1}H NMR** **9a** (300 MHz, $CDCl_3$) δ 1.75-199 (m, 1H), 2.08 (s, 3H), 2.37-2.40 (m, 2H), 3.14-3.34 (m, 3H), 3.37-3.42 (m, 1H), 3.72-3.86 (m, 2H), 4.36 (m, 1H), 8.44 (d, 1H, J=7 Hz). **MS** **9a** (EI) m/z (rel. inten.)168 (M+, 19), 109 (42), 70 (60), 59 (100), 44 (70). **MS** **9b** (EI) m/z (rel. distribution) 168-173 (d_0=15%, d_1=39%, d_2=34%, d_3=12%).

3-Amino-1-azabicyclo[2.2.2]octane-^{3}H-dihydrochloride (10c). To **9c** (1.35 Ci) was added 6 M aq HCl (15 mL). The mixture was warmed to 100° and stirred for 3.5 h. The mixture was cooled to room temperature and assayed to

afford 1.34 Ci of product. An aliquot was removed and a GITC derivative was prepared with glucose isothiocyanate. HPLC analysis against cold standard showed a 50:50 mixture of enantiomers of **10c**.

TLC: silica, 50%(5%NH_4OH/MeOH)/CH_2Cl_2; R_f 0.01. **MS (10b)** (EI) m/z (rel. distribution) 126-129 (d_0=13%, d_1=36%, d_2=38%, d_3=13%). **HPLC** (GITC derivative): Beckman Ultrasphere, ODS C18, 5 µm , 4.5 x 250, 23% CH_3CN/) 0.03 M TEAP pH 3 buffer, 1 mL/min, 214 nm.

SR and RR-3-(Methylbenzyl urea)-1-azabicyclo[2.2.2]octane-^{3}H (11b). To **10c** (1.35 Ci) in 2% TEA/$CHCl_3$ (15 mL) was added (R)-methylbenzyl isocyanate [Fluka, 0.1 mL, >99.5 % (R)] at room temperature. The mixture was stirred for 2.5 h and concentrated. Partial separation of the diastereomers was achieved by radial chromatography (2 mm rotor, 3 mL/min) using 40%(5%NH_4OH /MeOH)/CH_2Cl_2 as the eluting solvent to afford 265 mCi of (S,R) isomer, 773.2 mCi of mixed (S,R), (R,R) isomer, 158 mCi (R,R) isomer.

TLC: silica gel, 40%(5%NH_4OH/MeOH)/ CH_2Cl_2; R_f 0.5. **^{1}H NMR 11a-SR** isomer (300 MHz, $CDCl_3$) δ 1.25 (m, 2H), 1.46 (d, 3H, *J*=15 Hz), 1.56 (m, 2H), 1.76 (q, 1H, *J*=3 Hz), 2.20 (s, 1H), 2.32 (broad dd, 1H, *J*=4.7), 2.58-2.76 (m, 5H), 3.18-3.27 (m, 1H), 3.70 (m, 1H), 4.60-4.75 (m, 2H), 4.91 (d, 1H, J=6.3 Hz), 7.3-7.4 (m, 5H). **MS 11a-SR** isomer (EI) m/z (rel. inten.) 273 (M^+, 45), 153, (10), 125, (20), 109 (100), 70 (60). **MS 11b-SR** (LSIMS) m/z (rel. distribution) 274-280 (T_0=22%, T_1=41%, T_2=29%, T_3=8%). **Specific activity:** 35.6 Ci/mmol, by MS.

(S)-3-Amino-1-azabicyclo[2.2.2]octane dihydrochloride (10c-S). Freshly prepared *n*-BuONa in *n*-BuOH (14 mL) was added to **11b-SR** (43.6 mCi). The mixture was warmed to 130° for 6 d, then cooled to room temperature. The pH of the solution was adjusted to two with aq HCl in EtOH. The mixture was concentrated, dissolved in H_2O, and extracted with EtOAc to remove the neutral components[8]. The aq layer was concentrated, reconstituted in 30% H_2O/EtOH, and assayed for radioactivity and enantiopurity[9].

Total Activity: 39.6 mCi[10]. **Specific Activity**: 35 Ci/mmol. This was determined after conversion of the 3-aminoquinuclidine to 5-HT_3 ligands. **Enantiopurity**: >99.5% (S), determined by making the GITC derivative followed by HPLC analysis (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).

(R)-3-Amino-1-azabicyclo[2.2.2]octane dihydrochloride (10c-R). The same procedure as described for the preparation of **10c-S** was followed but this time 36.56 mCi of **11b-RR** was used instead.

Total Activity: 27.28 mCi[10]. **Specific Activity**: 36 Ci/mmol. This was determined after conversion of the 3-aminoquinuclidine to 5-HT_3 ligands. **Enantiopurity**: >99.5% (R), determined by making the GITC derivative followed by HPLC analysis (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).

(S)-3-(1-Azabicyclo[2.2.2]-octane-3-yl)-oxoacetamido-1-methylindole hydrochloride-3H, RS-56812-197-^{3}H. (S)-3-Aminoquinuclidine **10c-S** dihydrochloride (20 mCi) was neutralized with 5%NH_4OH/MeOH. The mixture was concentrated and reconstituted in EtOAc. Freshly prepared methylglyoxayl chloride (13 mg) was added and the mixture was stirred at room temperature. After 20 min, TLC analysis indicated that the product was the major component of the reaction mixture. Stirring was continued overnight. The mixture was concentrated, redissolved in 5%NH_4OH/MeOH to liberate the free base, and concentrated again. Chromatography on silica gel with 10%(5%NH_4OH/MeOH)/CH_2Cl_2 afforded 11.59 mCi of product. The product was converted to the HCl salt with 0.12 M HCl in EtOH.

Total Activity: 11.59 mCi. **Specific Activity**: 35 Ci/mmol. **HPLC:** Beckman Ultrasphere ODS C18, 5 μm, 4.5 x 250, 20%CH_3CN/0.03 M TEAP pH 3 buffer, 1 mL/min, 214 nm. **TLC**: silica gel, 10%(5%NH_4OH/MeOH)/CH_2Cl_2; R_f 0.4, RP-C_{18}, 70%CH_3CN/30% 0.03 M TEAP pH 3 buffer, R_f 0.38.

(S)-4-Amino-N-(1-azabicyclo[2.2.2]-oct-yl)-5-chloro-2-methoxy-1-benzamide hydrochloride-^{3}H, (S)-Zacopride-^{3}H. DCC (78 mg, 0.379 mmol), HOBT (93 mg, 0.025 mmol), and 4-amino-5-chloro-2-methoxybenzoic acid (50 mg, 0.253 mmol) were dissolved in THF (3 mL) and the mixture was stirred at room temperature. **10c-S** (10.83 mCi) was concentrated out of EtOH/H_2O. EtOH was added and the mixture was concentrated once more to remove residual water. Then, 2 mL of THF and 0.15 mL of TEA were added to liberate the free base. The mixture was sonicated and stirred at room temperature. After 6 h, the contents of the flask containing DCC, HOBT and benzoic acid derivative were added to (S)-3-aminoquinuclidine-^{3}H. The reaction was stirred over night, diluted with EtOH and filtered through a nylon filter. Chromatography on silica gel[11] with 10-15% gradient (5%NH_4OH/ MeOH)/CH_2Cl_2 afforded 9.51 mCi (88%) of (S)-Zacopride-^{3}H. HPLC analysis of the product indicated presence of some unlabelled impurities. Therefore, the product was further purified by preparative HPLC (Beckman ultrasphere ODS C18, 5 μm, 4.5 x 250, 15%CH_3CN/85% 0.01 M NH_4OAc pH 5 buffer, 1 mL/min, 214 nm). In this way, 5.09 mCi of pure (S)-Zacopride-^{3}H was obtained. The product was converted to HCl salt with 0.12 M HCl in EtOH.

Total Activity: 5.09 mCi. **Specific Activity**: 34.5 Ci/mmol. **TLC**: silica gel, 20%(5%NH_4OH/MeOH)/CH_2Cl_2; R_f 0.4, RP-C18, 50% CH_3CN/0.03 M TEAP pH 3

buffer; R_f 0.36. **HPLC**: Vydac C18, 9% CH_3CN/0.03 M TEAP pH 3 buffer, 1 mL/min, 214 nm.

(R)-4-Amino-N-(1-azabicyclo[2.2.2]-oct-yl)-5-chloro-2-methoxy-1-benzamide hydrochloride-^{3}H, (R)-Zacopride-^{3}H. The same procedure as described for the preparation of (S)-Zacopride was followed but this time 8.8 mCi of **10c-R** was used instead.

Total Activity: 4.03 mCi. **Specific Activity**: 36 Ci/mmol. **TLC**: silica gel, 15%(5%NH_4OH/MeOH)/CH_2Cl_2; R_f 0.44, RP-C18, 50% CH_3CN/0.03 M TEAP pH 3 buffer; R_f 0.28. **HPLC**: Beckman Ultrasphere ODS C18, 5 µm, 4.5 x 250, 15% CH_3CN/NH_4OAc pH 5 buffer, 1 mL/min, 214 nm.

(S)-6-Amino-2-(1-azabicyclo[2.2.2]-oct-yl)-5-chloro-2,3-dihydro-1,3-dioxo-1H-benzo[de]isoquinoline hydrochloride-^{3}H, RS-56532-197-^{3}H. (S)-3-Aminoquinuclidine-^{3}H **10c-S** (16.8 mCi) was concentrated from 30% H_2O/EtOH and reconstituted in 5%NH_4OH/MeOH to liberate the free amine. After 1 h, the mixture was concentrated, 10 mL of MeOH and 8 mg of the 4-amino-3-chloronaphthalic anhydride were added (yellow soln). The mixture was warmed to 60° for 6 d. Dilution with EtOH followed by filtration gave 14.25 mCi of crude product. Purification on silica gel using a 10-20% gradient of (5%NH_4OH/MeOH) /CH_2Cl_2 gave 2.05 mCi of RS-56532-^{3}H. The remaining activity was unreacted starting material. RS-56532-^{3}H was further purified on silica gel with 40% MeOH/EtOAc and 10%(5%NH_4OH/MeOH)/CH_2Cl_2 to afford 0.989 mCi of pure compound.

Total Activity: 0.989 mCi. **Specific Activity**: 31.9 Ci/mmol. **TLC**: 15%(5% NH_4OH /MeOH)/CH_2Cl_2; R_f 0.58, RP-C18, 40%CH_3CN/0.03 M TEAP pH 3 Buffer, R_f 0.15. **HPLC**: Beckman ultrasphere ODS C18, 5 µm, 4.5 x 25, 25 % CH_3CN/0.03 M TEAP pH 3 buffer, 1 mL/min, 220 nm

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8. The free amine was very water soluble, therefore, no aq extraction was possible.
9. The compound contained NaCl and methylbenzyl amine as impurities since chromatographic purification (silica gel) was not possible.
10. The total activity obtained after the hydrolysis was always lower than the expected value even though no formal work-up was involved!
11. In order to get a high yield, it is crucial to avoid a water workup and to transfer the crude mixture onto the chromatography column with EtOH.