Synthesis of High-Specific Activity Tritium and Optically Pure [^{14}C]CP-101,606. Enantioselective Crystallization of a Radiochemically Racemic Mixture.

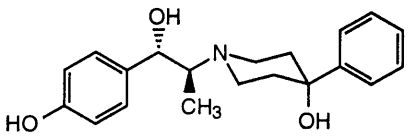
K.E. McCarthy*, S.A. Miller, B.L. Chenard, T.W. Butler, M.L. Dumont, J.Z. Stemple
Departments of Medicinal Chemistry and Analytical Research and Development
Pfizer Inc, Eastern Point Road, Groton, CT 06340

Summary. The syntheses of racemic, high-specific activity tritium and optically pure, carbon-14 labelled CP-101,606 is described. The tritium labelled material was prepared at 35.6 Ci / mmol by hydrogenolysis of the dibromo analog **5**. In the carbon-14 synthesis, the radiolabel was introduced in the final carbon-carbon bond forming step by alkylation of the readily available aminoketone **8** with [^{14}C]methyl iodide. *Threo*-selective sodium borohydride reduction of the ketone and deprotection of the phenol gave racemic [^{14}C]CP-101,606. Enantiomerically pure (+)-[^{14}C]CP-101,606 was obtained by addition of optically pure, unlabelled drug and directly recrystallizing the enantiomerically enriched, but radiochemically racemic, mixture.

Keywords: tritium, carbon-14, CP-101,606, NMDA antagonist, enantioselective crystallization

Introduction

(+)-CP-101,606 (**1**) is a N-methyl-D-aspartate (NMDA) antagonist which interacts with a novel site on the NMDA receptor and lacks α_1 andrenergic activity[1]. This potent new neuroprotective agent exhibits efficacy against glutamate-mediated toxicity as well as a low side-effect profile when compared to other less-selective NMDA antagonists. Potential indications for CP-101,606 include treatment of CNS trauma, ischemic stroke, and Parkinson's diseases. In order to fully elucidate its receptor binding and metabolic profile, both tritium and carbon-14 labelled material were required.



(+)-CP-101,606 (1)

The established route to (+)-CP-101,606 appeared to be directly amenable to both tritium and carbon-14 labelled synthesis except that potentially, an optical resolution of labelled material would be required. Prior to initiating a radiolabelled synthesis however, competitive binding studies

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demonstrated that (+)-CP-101,606, the levorotatory enantiomer, and the racemic mixture possessed equivalent nanomolar IC_{50} activity in the neuroprotective NMDA assay but only micromolar activity in the α_1 assay. Since the pharmacological properties of the racemic mixture were predicted to parallel those of the clinical candidate, we chose to simplify our task and prepare racemic [^{3}H]CP-101,606 in order to evaluate the specific binding properties of this novel antagonist.

Whereas racemic [^{3}H]CP-101,606 was suitable for the *in vitro* assays, optically pure carbon-14 labelled material was required for the full array of drug metabolism studies. Since there was little opportunity to incorporate the label directly into an optically pure intermediate and time constraints prohibited the opportunity to develop an asymmetric synthesis, an efficient optical resolution of the labelled racemate became an important component of this project. At the time the labelled material was requested, racemic CP-101,606 had only been resolved by repeated recrystallizations of its diastereomeric tartrate salts. In light of the potential for limited mass recovery and the knowledge that only low specific activity material was required, we decided to take advantage of an opportunity to resolve the radiolabelled mixture by way of an *enantioselective crystallization.*[2] The plan therefore, was to prepare fully enriched, racemic [^{14}C]CP-101,606, cut with enantiomerically pure unlabelled drug and crystallize to afford low specific activity, optically pure radiolabelled material.

Results and Discussion

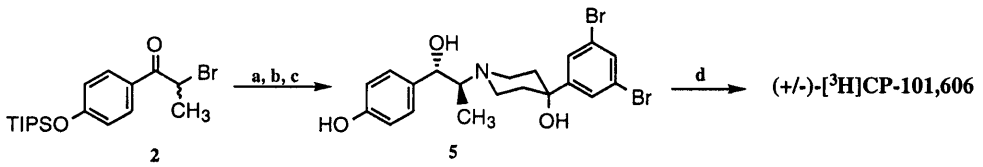
Synthesis of (+/-)-[^{3}H]CP-101,606

As stated above, racemic [^{3}H]CP-101,606 was suitable for receptor binding studies and the straightforward synthesis of this material is depicted in Scheme 1. In order to achieve a specific activity of > 30 Ci / mmol, two halogens were incorporated into the labelling precursor. Thus, coupling of **2** with 4-(3,5-dibromo)phenyl-4-hydroxypiperidine[3] followed by a stereoselective borohydride reduction[1] and deprotection afforded the required substrate for reduction **5**.

Hydrogenolysis of **5** was performed under standard conditions in EtOAc using approximately 800 mCi of carrier-free $^3H_2$ in the presence of 10% Pd/C.[4] Although the uptake of tritium was difficult to quantify at low pressures (175 - 145 mm Hg), the pressure stabilized after 3 hours indicating that the reaction was complete. After stirring at room temperature for an additional hour, ca. 70 mCi of tritium gas was returned to the secondary uranium bed and the reaction was quenched by addition of methanol. The volatile tritium by-products were transferred to a waste vessel under reduced pressure by successive washings with methanol. The reaction flask was then disconnected from the manifold and the contents filtered to remove the catalyst to give 257 mCi of crude product

containing no detectable amount of either the starting material or mono-reduced substrate. Chromatography on SiO_2 gave 212 mCi of >98% radiochemically pure racemic [^{3}H]CP-101,606 having a specific activity of 35.6 Ci / mmol.

Scheme 1



(a) 4-(3,5-dibromo)phenyl-4-hydroxypiperdine, EtOH; (b) $NaBH_4$, EtOH; (c) Bu_4NF,THF; (f) $^3H_2$, Pd/C, EtOAc, Et_3N

Synthesis of racemic [^{14}C]CP-101,606

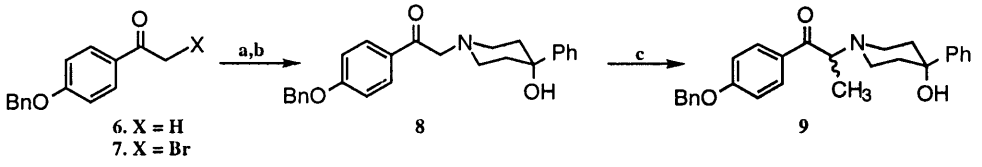
Faced with the prospect that at least 50% of the label would be forfeited as a result of a resolution, we believed it was important to develop an efficient synthesis of racemic material. Of several routes considered, the most straightforward was by alkylation of the amino ketone **8** with $^{14}CH_3I$. The synthesis and chemistry of this key intermediate is shown in Scheme 2.

Bromination[5] of *p*-benzyloxyacetophenone with $CuBr_2$ followed by displacement with 4-hydroxy-4-phenylpiperidine yielded the key intermediate **8**. Isolation of this intermediate by silica gel chromatography resulted in an unexpectedly low yield (39%), even though TLC analysis indicated a much cleaner reaction. Nonetheless, with a sufficient quantity of **8** in hand, a variety of reaction parameters for methylation were screened and the optimal stoichiometry for preparation of **9** was determined to be 1.2 eq. of the ketone and 2.4 eq. NaHMDS in THF. Based on methyl iodide as the limiting reagent, the desired product could be isolated in 55-60% yield. Along with the dimethylated adducts **10** and **11** (13 - 15% each) and a small amount of the trimethylated material **12** was isolated indicating that ~85% of the CH_3I had been consumed. In order to account for the remaining methyl iodide, several reactions were conducted using low specific activity $^{14}CH_3I$. As expected, radio-TLC analysis indicated that along with the same ratio of products observed previously, approximately 15% of the total radioactivity was non-volatile, unidentified material.

Efforts to increase the yield of **9**, particularly by use of a large excess of the enolate, were not fruitful.[6] Surprisingly though, the amino ketone was always completely consumed in these reactions. Upon further investigation, both **13** and **14** could be isolated from the reaction mixture and, in fact, if **8** is treated with base in the absence of methyl iodide, these two compounds are formed in high

yield.[7,8] To avoid this problem, **8** was stored under an argon atmosphere and all reagents and solvents were thoroughly degassed prior to use.[9] We have found that solid NaHMDS affords significantly higher yields than the commercially available solution of NaHMDS in THF, presumably because the solid form is easier to deoxygenate.

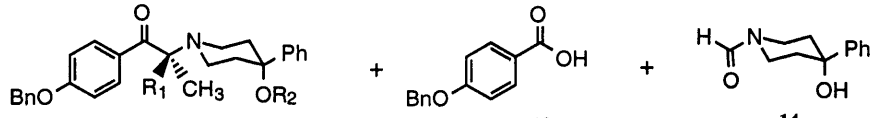
Scheme 2



6. X = H
7. X = Br

8

9



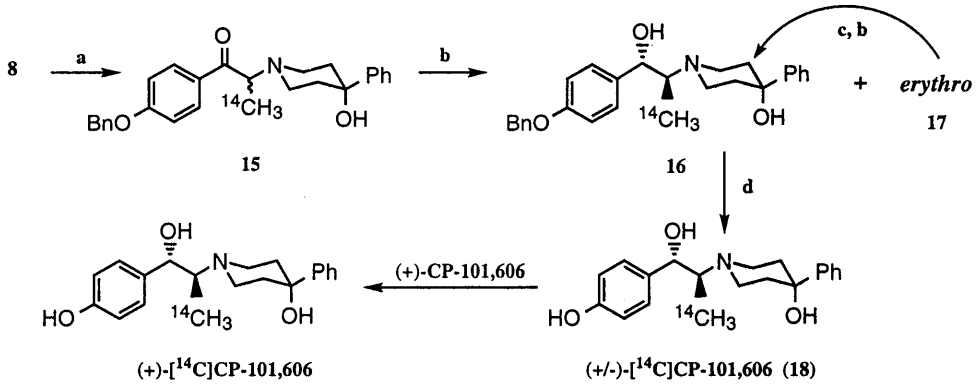
10. $R_1 = CH_3$, $R_2 = H$
11. $R_1 = H$, $R_2 = CH_3$
12. R_1, $R_2 = CH_3$

(a) $CuBr_2$, EtOAc, $CHCl_3$; (b) 4-hydroxy-4-phenylpiperidine, EtOH; (c) NaHDMS, CH_3I, THF

Once the conditions for the introduction of radiolabel had been optimized, the synthesis of fully enriched (+/-)-[^{14}C]CP-101,606 was initiated (Scheme 3). Solid NaHMDS (795 mg, 2.4 eq) was degassed and dissolved in freshly distilled THF. After cooling to -78°C, a THF solution of the amino ketone **8** (780 mg, 1.2 eq) was added over six minutes using a gas-tight syringe. To ensure complete enolate formation, the solution was warmed to -20°C for 10 minutes, then cooled to -78°C. At that time, $^{14}CH_3I$ (103 mCi, 57 mCi / mmol, 1.0 eq) was introduced and the reaction flask isolated from the system. The mixture was slowly warmed to room temperature and allowed to stir overnight. Following standard work-up, Radio-TLC and LSC assay indicated complete incorporation of the methyl iodide to give the expected ratio of products. Chromatography afforded 57.7 mCi of radiolabelled **15**. To minimize handling, an ethanol solution of **15** was immediately treated with $NaBH_4$ to give a 3:1 mixture of *threo*-**16** / *erythro*-**17** alcohols. The relatively high percentage of the erythro isomer was a result of the order of addition, since a slow, drop-wise addition of the ketone to a solution of $NaBH_4$ has resulted in up to >19:1 ratio of diastereomers.[1] Nonetheless, the diastereomeric alcohols were easily separated by chromatography and the recovered erythro isomer

recycled to afford additional **16** by a straightforward oxidation[10] and reduction sequence. Hydrogenolysis of **16** afforded racemic [^{14}C]CP-101,606 in 42% overall yield from $^{14}CH_3I$.

Scheme 3



(a) NaHDMS, $^{14}CH_3$-I, THF; (b) $NaBH_4$, EtOH; (c) CrO_3, H_2SO_4, acetone; (d) H_2, Pd/C, EtOH

Enantioselective crystallization to obtain (+)-[^{14}C]CP-101,606

As stated earlier, we chose to take advantage of the low specific activity requirement for (+)-CP-101,606 by directly resolving an enantiomerically enriched mixture. Theoretically, for this process to be chemically feasible a mixture only needs to be enriched above its eutectic point.[11] Whereas this condition could be readily satisfied by addition of the desired, unlabelled enantiomer, this type of artificial enrichment would simultaneously lower the specific activity of that enantiomer. From a radiochemical perspective then, the extent to which a true racemate can be enriched is dictated by both the final specific activity requirements and the initial specific activity of the mixture.[12]

In our case, only low specific activity material of 3.0 mCi / mmol was required for the full metabolic profiling. Since the racemate had been prepared at 57 mCi / mmol, we could dilute the specific activity of the desired isomer 18 fold which would give a 95 : 5 ratio of enantiomers. Using unlabelled material, an ca. 95:5 mixture was prepared by addition of 500 mg of (+)-CP-101,606 to 50 mg of racemic CP-101,606. Upon recrystallization from ethanol, 300 mg (60% theoretical yield) of >99 : 1 optically enriched crystals were isolated. A second recrystallization of this material afforded 200 mg (40% theory) of now optically pure material. Essentially similar results were seen with

mixtures ranging from 90:10 (80% ee, projected specific activity of 5.60 mCi / mmol) up to 95:5 (90% ee). In addition to demonstrating the viability of this approach, we observed that longer crystallization times resulted in higher recovery but lower optical purity. Therefore, we found that it was best to filter the crystals while the solution was still warm. As described below, we are pleased to report that this procedure worked as well with labelled material.

Racemic **18** (53.99 mCi, 0.947 mmol) was dissolved in ethanol and (+)-CP-101,606 was added to give an ca. 93:7 ratio of enantiomers. Note however, that the radiochemical ratio remains unchanged (Figure 1). Crystallization was affected by evaporating the ethanol (with warming) *until the first crystal formed.* The solution was then removed from the heat and closely monitored. When the rate of crystal formation slowed and while the solution was still warm, the mixture was filtered to give 940 mg of a white solid which was enriched both chemically (99:1 ratio) and radiochemically (88:12 ratio) (Figure 2).

Figure 1. After addition of (+)-CP-101,606

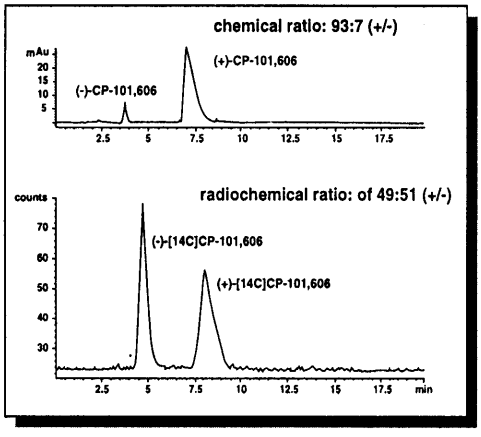
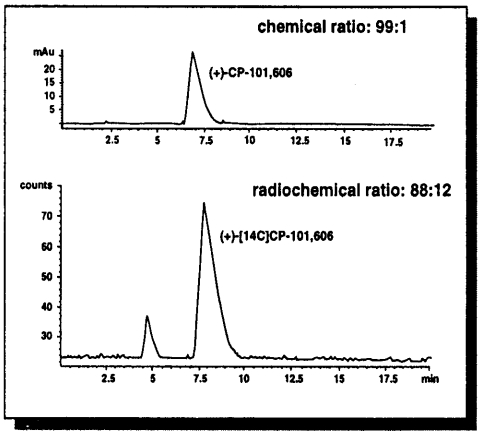


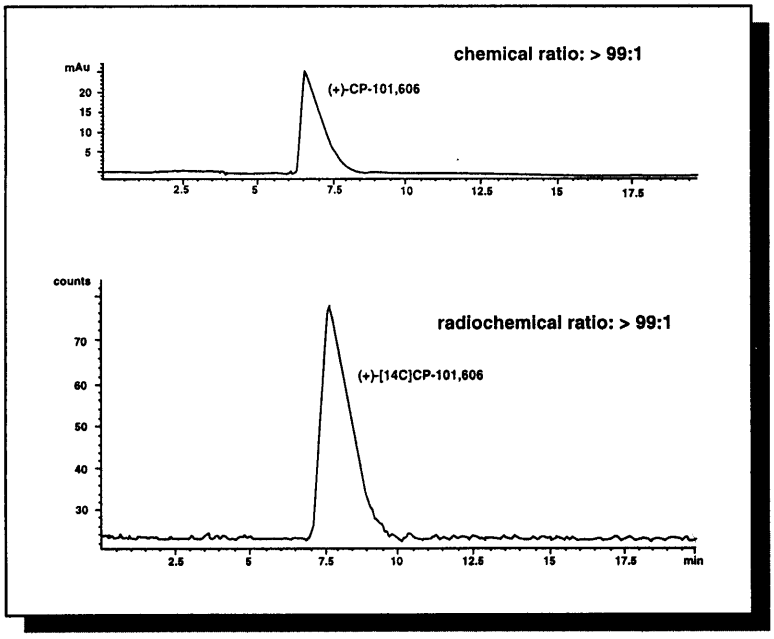
Figure 2. After the first recrystallization



This solid was recrystallized from ethanol to yield 500 mg of a >99:1 (chemical) and 98:2 (radiochemical) enantiomeric mixture. Subsequent recrystallizations yielded a total of 450 mg (7.32 mCi, 5.33 mCi/mmol) of chemically, radiochemically and enantiomerically pure (+)-[^{14}C]CP-101,606 as determined by both UV and radioisotope detection (Figure 3). In order to increase the radiochemical efficiency of this process, the mother liquors were combined, cut with 3.67 mmols of

(+)-CP-101,606 and processed as above to yield an additional 2.71 mCi (2.38 mCi/mmol) of enantiomerically pure (+)-[^{14}C]CP-101,606.

Figure 3. (+)-[^{14}C]CP-101,606 after the final recrystallization.



Conclusion

The syntheses of racemic [^{3}H]CP-101,606 at 35.6 Ci /mmol and optically pure [^{14}C]CP-101,606 have been described. In carbon-14 synthesis, racemic drug was prepared in three steps in 42% overall yield from [^{14}C]methyl iodide. By taking advantage of the low specific activity requirements for the carbon-14 labelled material and the propensity for non-racemic mixtures to crystallize in enantiomerically pure form, optically pure [^{14}C]CP-101,606 was obtained by an enantioselective crystallization. This protocol effectively circumvented the need for chemical derivatization, chiral prep-HPLC separation or the development of an enantioselective synthesis. Thus under the right circumstances, an enantioselective crystallization of a radiochemically racemic mixture may be a viable process for the preparation of low specific activity, enantiomerically pure materials.

Experimental

All reagents were purchased from Aldrich Chemical Company and were used without purification unless indicated. ^{1}HNMR spectra were recorded on a Bruker 250 MHz NMR spectrophotometer. Thin Layer Chromatography analysis were performed on Baker plates and Radio-TLC scans were recorded on a Bioscan Linear 2000 analyzer. Radioassays were obtained on a Wallac 1409 liquid scintillation counter. HPLC analysis was carried out on the Hewlett-Packard HP 1050 Automated LC system. This system is comprised of the HP 1050 quaternary pump, HP 1050 series programmable auto sampler, 35900E A/D converter and the HP 1050 diode array detector which was connected, in series, to a β-RAM flow-through radioisotope detector (IN/US Systems, Inc.) The flow through radioactivity detector was operated in the solid scintillation counting mode using a 250μL YtSi cell.

4-(3,5-Dibromo)phenyl-4-hydroxypiperidine hydrochloride. A solution of 1,3,5-tribromobenzene (15.75 g, 50.0 mmol) in diethyl ether (500 mL) was chilled to -78°C and butyllithium (20.8 mL, 50.0 mmol, 2.4 M in hexane) was added drop-wise over 30 min. The reaction was stirred 30 minutes, then 1-*tert*-butyloxycarbonylpiperidin-4-one (5.0 g, 25 mmol in 100 mL of diethyl ether) was added drop-wise over 30 minutes. The reaction was stirred 2 hours at -78°C, then quenched with water and allowed to warm to ambient temperature. The phases were separated and the organic layer was washed with brine, dried over calcium sulfate and concentrated. The residue was flash chromatographed on silica gel with an elution gradient of 1 - 20% ethyl acetate / hexane to give 6.76 g (62%) of 4-(3,5-dibromophenyl)-4-hydroxy-1-*tert*-butyloxycarbonylpiperidine as a light yellow foam. ^{1}HNMR ($CDCl_3$) δ 7.56 (m, 3H), 4.06 (br d, 2H, J = 13 Hz), 3.21 (t, 2H, J = 13 Hz), 1.93 (dt, 2H, J = 4.5, 13 Hz), 1.80 (s, 1H), 1.68 (d, 2H, J = 13 Hz), 1.48 (s, 9H). The product was estimated to be 88% pure and contaminated by 12% of 1-*tert*-butyloxycarbonylpiperidin-4-one (NMR triplets at δ 3.71 and 2.44). This material was used without further purification.

The product of the above reaction (6.76 g, 15.5 mmol) was dissolved in diethyl ether (150 mL) and dioxane saturated with HCl (15 mL) was added. The mixture was stirred 30 minutes at ambient temperature, then chilled to 0°C and HCl gas was bubbled into the solution for 3 minutes. The reaction was allowed to warm to ambient temperature and stir overnight. Nitrogen gas was bubbled through the mixture to remove HCl gas and the precipitate was filtered to afford 3.27 g of a cream colored solid. The filtrate was again saturated with HCl gas and stirred 6 h. Again the mixture was purged with nitrogen gas and the precipitate collected (1.63 g). The HCl hydrolysis was repeated a third time to yield 0.45 g more product. In this fashion, 5.45 g (94%) of 4-(3,5-dibromo)phenyl-4-hydroxypiperidine hydrochloride was obtained as a cream colored solid. This material was used without purification.

1-(4-Triisopropylsilyloxyphenyl)-2-[4-(3,5-dibromophenyl)-4-hydroxy]-piperidin-1-yl-propan-1-one (3). A mixture of 4-triisopropylsilyloxy-α-bromopropiophenone (3.0 g, 7.79 mmol)[1], 4-(3,5-dibromophenyl)-4-hydroxypiperidine hydrochloride (2.89 g, 7.79 mmol) and triethylamine (3.26 mL, 23.4 mmol) in ethanol (200 mL) was refluxed overnight. The solvent was removed at reduced pressure and the residue was partitioned between ethyl acetate and water. The phases were separated and the organic layer was washed with brine, dried over calcium sulfate and concentrated. The residue was chromatographed on silica gel with 1 - 15% ethyl acetate / hexane to give 3.55 g (71%) of 1-(4-triisopropylsilyloxyphenyl)-2-[4-(3,5-dibromophenyl)-4-hydroxy]-piperidin-1-yl-propan-1-one as a crunchy off-white foam. ^{1}HNMR ($CDCl_3$) δ 8.03 (d, 2H, J = 9.0 Hz), 7.57-7.53 (m, 3H), 6.92 (d, 2H, J = 8.5 Hz), 4.14 (q, 1H, J = 7.0 Hz), 2.85 (dd, 2H, J = 2.0, 9.5 Hz), 2.77-2.70 (m, 1H), 2.60 (dt, 1H, J = 2.5, 11.5 Hz), 2.13-1.92 (m, 2H), 1.74-1.56 (m, 3H), 1.32 (d, 3H, J = 7 Hz), 1.36-1.18 (m, 3H), 1.12 (d, 18H, J = 7 Hz).

***threo*-1-(4-Triisopropylsilyloxyphenyl-2-[4-(3,5-dibromo)phenyl-4-hydroxy]-piperidin-1-yl-propan-1-ol (4).** An ice cold mixture of sodium borohydride (0.21 g, 5.56 mmol) and ethanol (50 mL) was stirred 10 min. and then 1-(4-triisopropylsilyloxyphenyl)-2-[4-(3,5-dibromophenyl)-4-hydroxy]-piperidin-1-yl-propan-1-one (3.55 g, 5.56 mmol in 50 mL of ethanol) was added drop-wise over 15 min. The reaction was allowed to warm to ambient temperature and stir overnight. Additional sodium borohydride (0.10 g) was added and the reaction was stirred 6 h more. The white precipitate was collected and rinsed with ethanol and weighed 0.84 g. The filtrate was treated with sodium borohydride (0.10 g) and stirred overnight. The white precipitate was collected and rinsed with ethanol and weighed 2.56 g. The combined precipitate (3.40 g) was recrystallized from ethanol to afford 3.00 g (84%) of 1-(4-triisopropylsilyloxyphenyl)-2-[4-(3,5-dibromophenyl)-4-hydroxy]-piperidin-1-yl-propan-1-ol as fluffy white needles (mp 235-236.5°C). Analysis calculated for $C_{29}H_{43}Br_2NO_3Si$: C, 54.29; H, 6.76; N, 2.18. Found: C, 54.17; H, 6.50; N, 2.35.

***threo*-1-(4-Hydroxy)phenyl-2-[4-(3,5-dibromo)phenyl-4-hydroxy]-piperidin-1-yl-propan-1-ol (5).** The product of the above reaction (0.53 g, 0.827 mmol) was dissolved in tetrahydrofuran (20 mL) and tetrabutylammonium fluoride (1.25 mL, 1.25 mmol, 1 M tetrahydrofuran solution) was added. The reaction was stirred for 1 hour at ambient temperature and then concentrated. The residue was chromatographed on silica gel with an elution gradient of 25 - 50% ethyl acetate / hexane to give 0.20 g (50%) of 1-(4-hydroxyphenyl)-2-[4-(3,5-dibromophenyl)-4-hydroxy]-piperidin-1-yl-propan-1-ol as a white solid (mp 232-234°C). Analysis calculated for $C_{20}H_{23}Br_2NO_3$: C, 49.51; H, 4.78; N, 2.89. Found: C, 49.77; H, 4.58; N, 2.76.

***threo*-1-(4-Hydroxyphenyl)-2-[4-[3,5-^{3}H]phenyl)-4-hydroxy]-piperidin-1-yl-propan-1-ol (+/-)-[^{3}H]CP-101,606.** A solution of 1-(4-hydroxyphenyl)-2-[4-(3,5-dibromophenyl)-4-hydroxy]-piperidin-1-yl-propan-1-ol (2.2 mg, 0.0045 mmol) in THF (0.7 mL) containing 10 μL Et_3N was added 10% palladium on carbon (4.5 mg) under an helium atmosphere.

The reaction mixture was freeze / thaw degassed three times and then exposed to tritium gas (800 mCi, 58 Ci / mmol) and allowed to stir for 4 h at ambient temperature. The reaction was then cooled to -78°C and the unreacted tritium gas (ca. 70 mCi) re-adsorbed onto a secondary uranium bed. The volatile by-products were then vacuum transferred to a receiving flask with methanol (3 x 1mL) and the mixture diluted with methanol and passed through a 0.45 micron filter to remove the catalyst. The entire lot was purified by chromatography on silica gel eluting with ethyl acetate / hexane to afford 212 mCi of 1-(4-hydroxyphenyl)-2-[4-[3,5-^{3}H]phenyl)-4-hydroxy]-piperidin-1-yl-propan-1-ol which had a radiochemical purity of > 98% and a specific activity of 36.51 Ci / mmol as determined by mass spectral analysis.

1-(4-Benzyloxy)phenyl-2-bromoethan-1-one (7). $CuBr_2$ (18.4 g, 82.54 mmol) was added to a solution of 4-benzyloxyacetophenone (9.2 g, 40.65 mmol) in EtOAc (360 mL) and $CHCl_3$ (360 mL) and heated to the reflux temperature for 5 hours, then cooled to room temperature and filtered to remove the inorganic salts. The organics were twice washed with sat. $NaHCO_3$, brine then dried over $MgSO_4$ and concentrated to give 11.03 grams of a tan solid consisting of 85:15 mixture of 7 : 6 as determined by NMR spectroscopy. This mixture was used without further purification. An analytical sample of pure 7 was isolated by crystallization from EtOAc / hexane. ^{1}HNMR ($CDCl_3$) δ 7.91 (d, 2H, J = 6.5 Hz), 7.25 (m, 5H), 7.05 (d, 2H, J = 6.5 Hz), 5.10 (s, 2H), 4.36 (s, 2H); Mass spec (CI) 305, 307 (M^+ + 1); R_f (3 : 1 hexane / EtOAc) 0.28.

1-(4-Benzyloxy)phenyl-2-(4-hydroxy-4-phenyl)piperdin-1-yl-ethan-1-one (8). To 6.0 grams (17.7 mmol) of 7 in 300 mL ethanol was added 4-hydroxy-4-phenylpiperdine (3.2 g, 18.0 mmol) and triethylamine (5 mL, 35.7 mmol). The reaction mixture was heated to 80°C for 1.5 hours, cooled to room temperature and concentrated. The residue was taken up in EtOAc and washed with successively with water (2x) and brine, then dried ($MgSO_4$) and concentrated to give 7.4 grams of a reddish oil. Flash chromatography on SiO_2 (55% EtOAc / hexane with 1% Et_3N) gave 2.8 grams (39%) of pale yellow solid which was stored under an argon atmosphere. ^{1}HNMR ($CDCl_3$) 7.81 (d, 2H, J = 6 Hz), 7.45 - 7.15 (m, 10H), 6.90 (d, 2H, J = 6 Hz), 5.04 (s, 2H), 3.68 (s, 2H), 2.75 (d, 2H); 2.50 (app t, 2H), 2.18 (app t, 2H), 1.62 (d, 2H), 1.55 (s, 1H); Mass spec. (CI) 403 (M^+ + 1).

1-(4-Benzyloxy)phenyl-2-(4-hydroxy-4-phenyl)piperdin-1-yl-[3-^{14}C]propan-1-one (15). To a to -78°C suspension of NaHMDS (795 mg, 4.34 mmol) in dry THF (40 mL) was added a solution of amino-ketone 8 (870 mg, 2.17 mmol) in dry THF (15 mL) over 6 minutes. This mixture was stirred at -78°C for 10 minutes, warmed to -20°C for 10 minutes and then re-cooled to -78°C and evacuated. [^{14}C]Methyl iodide (103 mCi, 57 mCi / mmol) was vacuum transferred into the reaction. After stirring at -78°C for 1 hour, the reaction mixture was warmed to room temperature and stirred overnight. The reaction was quenched with methanol (3 mL), and the volatiles removed *in vacuo*, and the residue partitioned between EtOAc/H_2O. The organic layer was dried over Na_2SO_4, filtered, and concentrated to a yellow oil (crude yield ~105 mCi) which was dissolved in 2 mL CH_2Cl_2 and

loaded onto a silica gel column. Elution with 20% EtOAc/hexanes gave 57.74 mCi (56.1%) of >99% radiochemically pure **15** which co-eluted with unlabelled standard **9**. Analytical data for **9**: ^{1}HNMR ($CDCl_3$) 8.12 (d, 2H, J = 8.4 Hz), 7.48 - 7.23 (m, 10H), 6.99 (d, 2H, J = 8.4 Hz), 5.12 (s, 2H), 4.10 (q, 2H, J = 6.7 Hz), 2.87 - 2.62 (m, 4H); 2.09 (m, 2H), 1.72 (m, 2H), 1.30 (d, 2H, J = 6.7 Hz); Mass spec. (CI) 416 (M^+ + 1); Radio TLC: (SiO_2) R_f: (30% EtOAc/Hexanes) 0.34.

***threo*-1-(4-Benzyloxy)phenyl-2-(4-hydroxy-4-phenyl)piperdin-1-yl-[3-^{14}C]propan-1-ol (16).** The ketone **15** (72.3 mCi, 1.27 mmol,) was suspended in EtOH (11 mL), cooled to 0°C and treated with solid $NaBH_4$ (96 mg, 2.53 mmol). After stirring at 0°C for 1 hour, the slurry was warmed to room temperature and stirred overnight (ca. 18 hours). The reaction was quenched with H_2O (0.5 mL) to precipitate any dissolved **16**, and filtered through a cotton plug. The retained solid was washed with EtOH (2 mL) and then dissolved in EtOAc / MeOH / dichloromethane to give 43 mCi of 9:1 mixture of diasteriomeric alcohols. The filtrate (mostly EtOH) contained 25 mCi of a 1:1 mixture of alcohols. The two product lots were combined, concentrated and suspended in H_2O (15 mL). After stirring for 3 hours (to dissolve any remaining borate salts), the slurry was filtered to give 64.7 mCi of an off-white solid.[13] This material was chromatographed (50:50 EtOAc/hexanes, SiO_2) to give 45.4 mCi desired alcohol **16**, and 15.84 mCi of the erythro diastereomer **17**. Radio-TLC (silica gel): R_f (50% hexanes/ethyl acetate) threo alcohol 0.43; erythro 0.13. Analytical data for unlabelled **16**. ^{1}HNMR ($CDCl_3$) 7.52 (app d, 2H, J = 8.6 Hz), 7.48 - 7.23 (m, 10H), 6.94 (app d, 2H, J = 8.6 Hz), 5.25 (broad s, 2H), 5.05 (s, 2H), 4.23 (d, 2H, J = 9.9 Hz), 3.06 (m, 1H), 2.72 - 2.57 (m, 4H), 2.15 (m, 2H), 1.82 (m, 2H), 0.81 (d, 3H, J = 6.5 Hz); Mass spec. (CI) 416 (M^++1).

Conversion of 17 to 16. To 15.8 mCi (0.28 mmol) of **17** in 30 mL acetone was added 20 µL of concentrated H_2SO_4 followed by 200 µL of a 2.1 molar solution of freshly prepared Jones reagent.[10] The reaction mixture was stirred for 10 minutes then quenched by addition of water (10 mL) and sat. $NaHCO_3$ (15 mL). EtOAc was added (ca. 50 mL) and the mixture stirred for one hour, then the layers were separated and the aqueous washed with ethyl acetate and CH_2Cl_2. The combined organics were dried (Na_2SO_4) and concentrated and the crude ketone (14.8 mCi) reduced as described above to give an additional 9.5 mCi of chemically and radiochemically pure **16**.

***threo*-1-(4-hydroxy)phenyl-2-(4-hydroxy-4-phenyl)piperdin-1-yl-[3-^{14}C]propan-1-ol (+/-)-[^{14}C]CP-101,606 (18).** The alcohol **16** (54 mCi, ~0.95 mmol) was dissolved in 10 mL THF (10 mL) and added treated with Pd/C (40 mg). The mixture was degassed and opened to an atmosphere of H_2. After stirring for a total of 39 hours (H_2 replaced 3 times), the reaction was filtered through celite (50% EtOAc/MeOH) then SiO_2. The filtrate was concentrated *in vacuo* to give 53 mCi of a white powder (98% radiochemically pure) which was used without further purification. See reference 1 for analytical data. Radio-TLC: (silica gel) R_f (EtOAc/1% NH_4OH) 0.39.

(+)-(1S,2S)-1-(4-Hydroxy)phenyl-2-(4-hydroxy-4-phenyl)piperdin-1-yl-[3-^{14}C]propan-1-ol. (+)-[^{14}C]CP-101,606. Racemic [^{14}C]CP-101,606 (53.99 mCi, 309.7 mg, 0.947 mmol), carrier (+)-CP-101,606 (1.5 g, 4.58 mmol) and ethanol (40 mL) were combined and the mixture heated until complete dissolution. This solution was filtered and an aliquot analyzed by chiral HPLC (Figure 1). The ethanol solution was slowly concentrated until the first crystal formed (20-25 mL left). The flask was removed from the heat source and watched carefully. When the rate of crystal formation slowed (the solution was still warm), the mixture was filtered to give 940 mg white crystals. HPLC analysis indicated a 99 : 1 chemical ratio and an 88 : 12 radiochemical ratio (Figure 2). This crystallization procedure was repeated twice on successive solid lots to yield 294 mg (5.33 mCi) of white crystals 100% ee by UV detection, >99.4 % ee by radioisotope detection. The filtrate from the third recrystallization was subjected to the warming/filtration process to yield an additional 155 mg (2.53 mCi) of white crystals; 100% ee by UV detection and > 99.3% ee by radio-isotope detection. The specific activity of the combined lots was determined to be 16.32 uCi / mg by microgravimetric assay.

HPLC Conditions: Chiral: ULTRON ES-OVM[14] 4.6 x 150 mm; Mobile phase: 10% acetonitrile/90% 0.01 M KH_2PO_4 (pH=6.5); Flow rate: 1.5 mL/minute; Retention time: $UV_{(226\ nm)}$, 6.53 minutes; radiochemical, 7.67 minutes. Achiral: Rainin Microsorb C-18 (5*u*), 4.6 x 250 mm column; Mobile phase: 85% 0.05 M KH_2PO_4 (pH 7.10 with KOH)-15% methanol; 1.0 mL/minute; Retention time: $UV_{(226\ nm)}$, 21.17 minutes; radiochemical, 21.64 minutes.

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4. The hydrogenolysis with $^3H_2$ was performed on the IN/US Trisorber manifold purchased from IN/US Systems Inc., 5809 North 50th Street, Tampa, Florida.

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6. Protection of the tertiary hydroxyl was considered, however since yield of desired product was reasonable and both **10** and **12** result from bis-methylation of the enolate, it was felt that any effort invested screening protecting groups would offset any potential increase in the overall yield.

7. The auto-oxidation of α-aminoketones under basic conditions has been previously reported. See Schirmann, P.J., Matthews, R.S., Dittmer, D.C. *J. Org. Chem.*, 48, 4426, (1983).

8. Compounds **13** and **14** were formed in ca. 80% yield based on NMR and mass spectral data of the crude reaction mixture.

9. The instability of this intermediate was unexpected since both the desired product **9** and the related intermediate **5** were known to be stable. Apparently, the additional substitution at the alpha position of the aminoketone leads to enhanced stability. For example, over 20 aminoketones of type **9** were prepared in reference 1 and no mention of their instability was made.

10. The Jones reagent can be prepared by dissolving CrO_3 (267 mg) in 0.23 mL H_2SO_4 and 0.7 mL water.

11. See references 2a, p.p. 88 - 92 and 2b, p.p. 381 - 389.

12. The relationship between the chemical and radiochemical requirements which must be satisfied can be described by the equation:

eutectic point x specific activity required < specific activity of the racemate.

13. In retrospect, a better work-up would have been to quench with 15-20 mL H_2O, and remove the EtOH *in vacuo*. After stirring the residue in water for ~ 3 hours to remove any borate salts, the product can be isolated by extraction with either methylene chloride or ethyl acetate.

14. The ULTRON-ES OVM column was purchased from Mac-Mod Analytical Inc., 127 Commons Court, Chadds Ford, Pennsylvania 19317.