SYNTHESIS OF TRITIUM LABELED 2,3,4,5-TETRAHYDRO-1H-3-BENZAZEPINES

Scott W. Landvatter[1], Dale W. Blackburn[1], Anthony J. Villani[1] and Gregory K. Bosch[2]

[1]Smith Kline and French Laboratories
Radiochemistry, F-50
1500 Spring Garden Street
Philadelphia, PA 19101

[2]Chemsyn Science Laboratories
13605 West 96th Terrace
Lenexa, KS 66215

SUMMARY

SKF 82526 (6-chloro-7,8-dihydroxy-1-(4-hydroxyphenyl)-2,3,4,5-tetrahydro-1H-3-benzazepine) has been synthesized in both racemic mono and enantiomerically pure ditritiated form; SKF 38393 (7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine) has also been labeled with tritium. In each case labeling was accomplished via tritiation of the appropriate ring halogenated precursor with tritium gas over Pd catalyst in triethylamine in DMF.

0362-4803/87/020145–11$05.50

Received April 30, 1986
Revised June 16, 1986

Key Words: Tritiation, Benzazepine, Catalytic dehalogenation, Dopamine agonists.

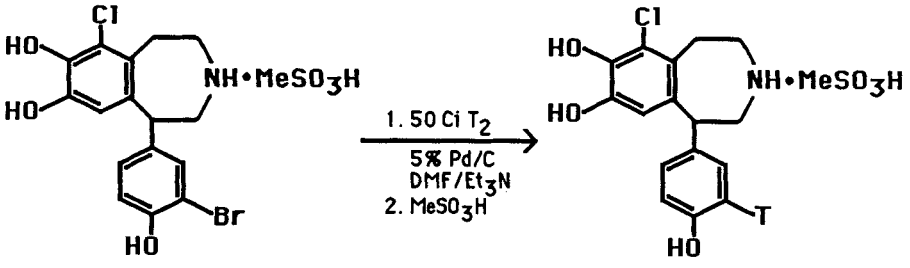
INTRODUCTION

Our study of 3-benzazepine derivatives which interact selectively with the dopamine receptor has focused on 6-chloro-7,8-dihydroxy-1-(4-hydroxyphenyl)-2,3,4,5-tetrahydro-1H-3-benzazepine (SK&F 82526, 1) and 7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SK&F 38393, 2). The central and peripheral dopaminergic activity of SKF 38393 (1) and the peripherally selective agonist activity of SKF 82526 has recently been reported (2). Receptor studies of these two compounds required that they be labeled with tritium at high specific activity (>10 Ci/mmole).

RESULTS AND DISCUSSION

SYNTHESIS OF TRITIATED SK&F 82526. The synthesis of monotritiated SK&F [3-^{3}H]82526 (^{3}H-1) is shown in Scheme I. Bromobenzazepine 3 (SKF 85739, 3) was tritiated with 27 weight percent 5% Pd/C in 1.1% triethylamine/DMF utilizing a 4:1 excess of tritium gas. The crude product was converted to its free base and then its methanesulfonic acid salt; HPLC and TLC analysis of the product showed it to be greater than 98% radiochemically pure. The only discrete radioactive impurity which was detectable by HPLC or TLC (<1%) was product which had also been dechlorinated.

Scheme I



3 ^{3}H-1

Initial synthetic studies using only an equivalent (10 Ci) of tritium gas gave product which was contaminated with a large amount of unreacted bromo precursor as well as having low specfic activity (5.4 Ci/mmole). A potential source of isotope dilution is the four heteroatom hydrogens present in bromobenzazepine 3. A deuterium study on precursor which has been silylated shows only a modest increase in isotope enrichment from 57 to 65% (mass spectral analysis). Thus, the heteroatom hydrogens must contribute only slightly to isotope dilution. However, employing a large excess of tritium gives material which is free from contamination with starting material and which has much higher specific activity (15-23 Ci/mmole).

Tritiated SK&F 82526 (^{3}H-1) appears to be quite sensitive to radiolytic decomposition, especially in methanol (Table 1). A methanol solution at 107 mCi/mL at -78° shows 6-11% decomposition after 11 days (by HPLC and TLC). Storage in methanol at room temperature, as is expected, accelerates this decomposition. The compound appears to be much more stable when stored under argon in 90:10 0.2N aqueous acetic acid/ethanol at a concentration of 1 mCi/mL and -78° where a decomposition rate of only 1-1.5% per week is observed. Other storage

conditions which were studied (90:10 ethanol/0.2N or 0.02N aqueous acetic acid or 90:10 0.02N aqueous acetic acid/ethanol) all show up to 5% of a polar impurity within 10 days. Long term storage should be in liquid nitrogen.

Table 1. Stability of SKF [^{3}H]82526 (^{3}H-1) at a concentration of 107 mCi/mL.

Solvent	decomposition at 10-11 days	temp
MeOH	6-11%	-78°
90:10 0.2N HOAc/EtOH	1.4-2.1%	-78°
90:10 EtOH/0.2N HOAc	5%	-78°
90:10 EtOH/0.02N HOAc	5%	-78°

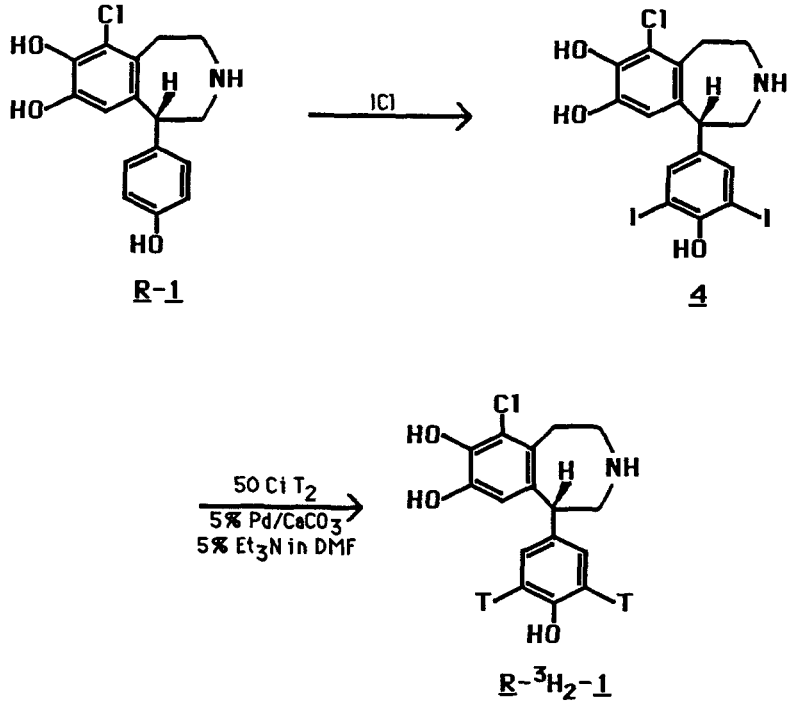
Though the monotritiated material has a specific activity of up to 23.0 Ci/mmole, the receptor activity is necessarily much less since only the R isomer is biologically active. In order to maximize both specific activity and biological activity we chose to tritiate an enantiomerically pure dihalogenated precursor.

Ditritio SK&F R-82526 ($^3H_2$-1) was synthesized as shown in Scheme II. SK&F R-82526 (R-1, 4) was iodinated with excess iodine monochloride giving diiodobenzazepine 4 in good yield. This was then tritiated with tritium gas over 200 weight percent 5% Pd/$CaCO_3$ (added in 2 portions) in 5% triethylamine in DMF at a concentration of 2 mg/mL. SK&F [$^3H_2$]R-82526 ([$^3H_2$-1) was thus obtained at a specific activity of 19.4 Ci/mmole and a radiochemical purity of 97.5% after HPLC purification.

Model studies conducted on the diiodobenzazepine 4 with

deuterium indicate that increasing the reactant concentration from 2 to 20 mg/mL slows the reaction by nearly a factor of 30. Increasing the amount of base or the weight percent catalyst, on the other hand, enhances the reaction rate, presumably by minimizing catalyst poisoning. Similarly, $Pd/CaCO_3$ gives optimum results when compared to Pd/C.

Scheme II



R-1 4 R-$^3H_2$-1

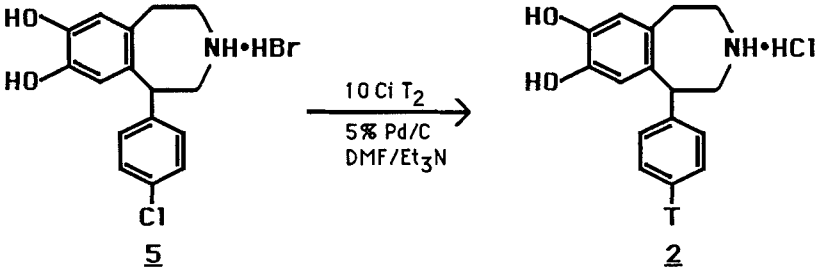
Tritiation itself proceeds smoothly provided that the catalyst has been predried, otherwise product having a low specific activity (ca. 10 Ci/mmol) is obtained. However, even with dry catalyst, the product obtained from tritiation has a specific activity of only 19.4 Ci/mmole, or 33% of the theoretical activity. A possible, but unconfirmed, source of

isotope dilution may be residual hydrogen on the catalyst.

The potential for isomerization of the enantiomerically pure product to the racemic compound during tritiation was checked by exposing a portion of pure enantiomer to hydrogen gas under identical conditions as those used during tritiation. The isolated product was checked for optical purity (0.2% solution in methanol); no change in specific rotation was observed. Thus, isomerization during tritiation should not occur.

SYNTHESIS OF SK&F 38393 (2). SK&F 38393 (2) was tritiated as shown in Scheme III. Chlorobenzazepine 5 (5) was treated with tritium

Scheme III



5 2

gas over dry 31 weight percent Pd/C in 1% triethylamine in DMF. The product was obtained with a radiochemical purity of greater than 98% after HPLC purification and a specfific activity of 9.95 Ci/mmole. The source of isotope dilution in this synthesis is not clear since the catalyst itself was extensively dried.

CONCLUSION

Three benzazepine derivatives have been synthesized in tritium labeled form via tritium-halogen replacement. Key features of these syntheses include the careful control of reactant concentration, catalyst used, triethylamine concentration in DMF, drying of catalyst and

the use of excess tritium gas to both drive the reaction to completion and to maximize specific activity. The success of the tritiation of SKF R-82526 in particular indicates the feasibility using simple tritiation techniques to produce enantiomerically pure labeled products even when they have potential isomerization sites such as the doubly benzylic proton in SKF 82526.

EXPERIMENTAL

Tritiation was performed by Chemsyn Science Labs (formerly Midwest Research Institute) in Kansas City, MO. Instrumental and analytical parameters are given in each synthesis. All solvents were of analytical reagent grade or better; HPLC solvents were filtered (0.45 μm nylon filter) and degassed prior to use. HPLC radioactivity was monitored with a Radiomatic Flo-1 radioactivity detector or with scintillation counting using Biofluor liquid scintillation cocktail (New England Nuclear). TLC radioactivity was monitored with a Bioscan BID 100 or a Berthold Linear Analyzer.

R,S-6-Chloro-7,8-dihydroxy-1-(4-hydroxy-[3-^{3}H]phenyl)-2,3,4,5-tetrahydro-1H-3-benzazapine (SK&F R,S-82526, ^{3}H-1). Bromobenzazepine 3 (83.1 mg, 0.18 mmol, 3) was dissolved in 4.5 mL DMF and was added to 22.1 mg 5% Pd/C (dried at 120° 0.5 h) in a dry Erlenmeyer with side arm. The Erlenmeyer was attached to a Toepler pump and the mixture degassed by 3 freeze/thaw cycles. Tritium gas (50 Ci, 0.86 mmol) was transfered to the frozen (liq N_2) reaction mixture and the mixture warmed to room temperature and stirred 9 min. The system was vented and progress of the reaction monitored by TLC (silica gel, 60:20:10:10 acetone/$CHCl_3$ /H_2O/HOAc, R_f =0.36). The assay showed 9.9 Ci tritium of uptake and no starting material left. The solvent was removed in

vacuo and labile tritium removed with MeOH. The residue was taken up in MeOH containing 12 mL $MeSO_3H$ and the catalyst removed via filtration through celite. The filtrate was evaporated to dryness and 3 mL H_2O added. The aqueous solution was treated with 2 mL Ar-purged 5% $NaHCO_3$ containing a little ascorbic acid and the precipitate collected by centrifugation. The precipitate was washed (2 x, 5% $NaHCO_3$) and the residue dissolved in 5 mL MeOH with 12 mL $MeSO_3H$.

The product was purified by replacement of MeOH with EtOAc via repeated evaporation. The EtOAc supernate (ca. 10 mL) was removed via pipet and centrifugation and the residue (2.5 Ci, 65.1 mg) stored in 10 mL MeOH at -80° : Specific activity: 15.2 Ci/mmol (determined by UV at 282.4 nm, ε=3650); Radiochemical purity : >98% (determined by HPLC: DuPont Zorbax C-8, 4.6mm x 25cm, 55:45 MeOH/1.3 g/L aqueous camphorsulfonic acid, 0.5 mL/min, UV at 254 and 280 nm, retention time = 8.00 min and TLC radioscanning: silica gel, 60:20:10:10 acetone/$CHCl_3$/H_2O/HOAc).

R-6-Chloro-7,8-dihydroxy-1-(3,5-diiodo-4-hydroxyphenyl)-2,3,4,5-tetrahydro-1H-3-benzazepine (SK&F R-101793, 4). SK&F R-82526 (1, 5) was dissolved (300 mg, 0.75 mmol) in 20 mL 50% aqueous HOAc. To this, at room temperature, was added 0.60 mL ICl (12 mmol, Aldrich) and the mixture stirred 45 min. The precipitate which formed was filtered and washed successively with with H_2O and ether until the precipitate was an ochre color. The precipitate was transferred to a round bottom flask and stirred vigorously with 100 mL saturated sodium hydrosulfite until the solid was an off white (ca. 4 h). The precipitate was filtered, washed (H_2O, ether) and triturated (MeOH) giving benzazepine 4 as an off white powder (392 mg, 85%). HPLC analysis (LiChrosorb RP-18, 4.6mm

x 25cm, eluted with 70:29:1 H_2O/EtOH/HOAc from 0-5 min then a gradient to 50:49:1 from 5-15 min and then isocratic at 1 mL/min, UV at 278 nm, retention time = 20.80 min) showed only a single peak: mp 245° (d); mass spectrum (CI, CH_4) m/z (relative intensity) 558 (38, M + 1), 432(25), 283(40), 281(36), 78(100), 58(61); ^{1}H-NMR (270 MHz, DMSO-d_6) 3.1-3.7 (m, 6, CH_2), 4.33-4.42 (m, 1, ArCH), 6.11 (s, 1, ArH), 7.53 (s, 2, ArH ortho to I).

R-6-Chloro-7,8-dihydroxy-1-([3,5-$^3H_2$]-4-hydroxyphenyl)-2,3,4,5-tetrahydro-1H-3-benzazepine (SK&F R-[$^3H_2$]82526 ,R-1). The hydrogenation catalyst (5% Pd/$CaCO_3$, 18 mg) and a 12 mL side arm flask were dried at 130° for 1 h. Diiodobenzazepine 4 (18.4 mg, 0.0331 mmol) was dissolved in 9.5 mL 5% Et_3N in DMF (both distilled from CaH_2) and combined with catalyst. The reaction mixture was degassed by three freeze/thaw cycles. Tritium gas (50 Ci, 0.86 mmol) was introduced via a Toepler pump into a calibrated gas buret connected to the reaction flask. The substrate was completely dissolved after being stirred 45 min. After an additional 1 h, 18.4 mg catalyst in 0.5 mL DMF was added and the mixture stirred overnight. The total uptake of tritium was 2.6 mL (ca. 0.1 mmol). The solvents were removed in vacuo and the residue stirred with 2 x 2 mL portions of EtOH followed by evaporation to remove labile tritium. The catalyst was removed via filtration through celite giving 479 mCi of crude product. The EtOH was removed in vacuo and the solid dissolved in 0.7 mL 90:10:1 H_2O/EtOH/HOAc, filtered through a 0.45 μm MSI nylon 66 filter and the filter rinsed giving ca. 1.5 mL of solution. Four 0.1 mL samples were purified by HPLC (Whatman Partisil 5 ODS-3, 10mm x 50cm, eluted with 90:10:1 H_2O/EtOH/HOAc at 4 mL/min from 0 to 5 min, then a gradient over 15 min to 75:25:1, UV at 280 nm, retention

time = 13.9 min) giving 9.46 mCi of pure R-1: Specific activity: 19.4 Ci/mmol (determined by UV at 282.4nm, ε=3650); Radiochemical purity: >97.5% (determined by HPLC with a Radiomatic Flo-1 detector).

R,S-7,8-Dihydroxy-1-([4-^{3}H]phenyl)-2,3,4,5-tetrahydro-1H-3-benzazepine (SK&F 38393, 2). Chlorobenzazapine 5 (14.6 mg, 0.036 mmol, 4) was dissolved in 1 mL DMF and 0.01 mL Et_3N. This was added to 4.5 mg dry 5% Pd/C (dried at 130° for 1h in the reaction flask). The flask was attached to a Toepler pump and the reaction mixture degassed in vacuo via 2 freeze/thaw cycles. Tritium gas (10 Ci, 0.17 mmol) was introduced and the mixture stirred 18 h. Solvents were removed in vacuo and labile tritium removed with 2 x 5 mL portions 0.012N HCl in 2-propanol. The residue was dissolved in MeOH and filtered giving 330 mCi of crude product. A portion of the crude product was purified by HPLC (Zorbax C-8, 4.6mm x 25cm, eluted with 55:45 MeOH/1.3 g/L aqueous camphorsulfonic acid at 1 mL/min, UV at 254, 280nm, retention time = 4.40 min) giving 8.5 mCi pure 2: Specific activity: 9.95 Ci/mmol (determined by UV at 283nm, ε=3330); Radiochemical purity: >98% (determined by HPLC and TLC, 50:20:10:10 EtOAc/acetone/H_2O/HOAc, R_f =0.43).

ACKNOWLEDGEMENTS

We would like to thank Dr. Robert Roth, of Chemsyn Science Labs, for his assistance in the preparation of these compounds and Drs. Tikam Jain and Joseph Weinstock of Smith Kline and French Laboratories for their suggestions on the synthesis of the diiodobenzazepine and SKF 82526 respectively.

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