# Synthesis of a Tritium Labelled Azaspirane: SK&F 105685

Scott W. Landvatter, Stephen G. Senderoff and J. Richard Heys

Department of Synthetic Chemistry
Radiochemistry Section
SmithKline Beecham Pharmaceuticals
P.O.Box 1539
King of Prussia, Pennsylvania 19406-0939

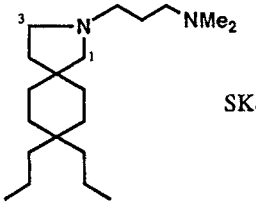
## Summary

SK&F 105685 (**1**) was prepared in tritium labelled form by two different methods. Moderate specific activity material was obtained via sodium borotritide reduction on an iminium perchlorate precursor which itself had been derived from **1**. High specific activity material was obtained via catalytic reduction of a terminal double bond.

**Key Words:** Iminium perchlorate, mercuric acetate oxidation, sodium borotritide, catalytic reduction

## Introduction

SK&F 105685 (**1**) is a novel immunomodulatory azaspirane being investigated for the treatment of rheumatoid arthritis. Azaspiranes, such as SK&F 105685, induce suppressor cells and this induction appears to be responsible for the therapeutic effect (1). Synthesis of the molecule in both moderate and high specific activity tritium labelled form was required for both pharmacologic and radioligand binding studies in order to further investigate the mechanism of action of SK&F 105685. In particular, binding to stromal cells and stromal cell membranes, which may lead to enhanced suppressor cell production, was to be investigated with the labelled compound.




SK&F 105685 (**1**)

## Discussion

There are several valuable strategies for the synthesis of tritium labelled compounds in which no aromatic position is available for tritium-halogen exchange labelling. One

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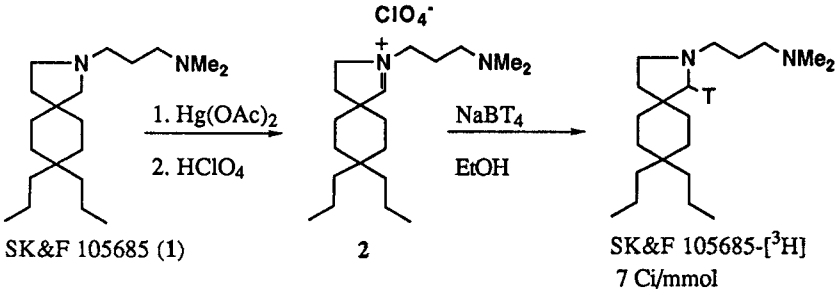
strategy is the total synthesis of an unsaturated precursor which can then be reduced catalytically to the tritiated product. A more direct strategy is to directly convert the unlabelled product into an unsaturated precursor which can then be reduced to reform the product in tritiated form. In the synthesis of tritium labelled **1**, we have utilized both of these strategies.

Dehydrogenation of cyclic teritary amines can be accomplished by reaction with mercuric acetate. The resulting oxidized compound may be isolated either as an enamine or as an iminium salt (2-4). Mercuric acetate dehydrogenation of **1** could thus provide rapid access to tritiated **1** via catalytic reduction of the isolated enamine or borotritide reduction (5) of the iminium salt.

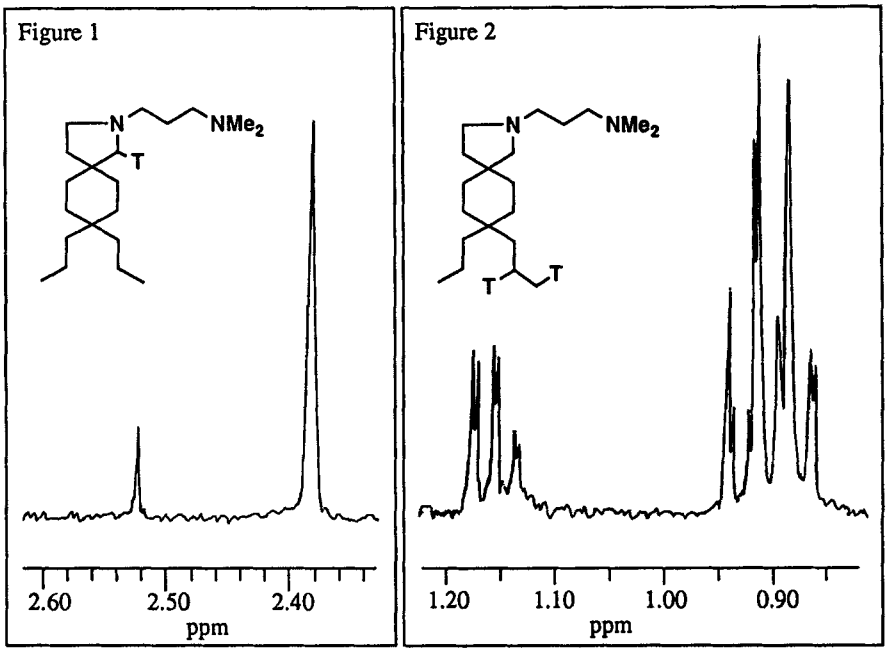
SK&F 105685 was directly converted to iminium perchlorate **2** by mercuric acetate oxidation of **1** (Scheme I). Although several regioisomeric iminium perchlorates are possible from this oxidation, proton NMR analysis of the product isolated after recrystallization suggested that it consisted mainly of the regioisomer shown in the Scheme. The proton NMR spectrum showed a downfield singlet at δ8.91 corresponding to the olefin proton and two triplets at δ3.86 and δ4.18 corresponding to the two methylene groups alpha to the iminium nitrogen.

Scheme I




Radiolabelled **1** at moderate specific activity (7 Ci/mmol) was obtained via sodium borotritide (75 Ci/mmol) reduction of **2** in absolute ethanol. Tritium NMR (benzene-$d_6$) analysis (Figure 1) of the labeled product indicated the presence of two labeled species. The major labeled species (88%) had the triton in the expected C-1 position based on the structure of **2**. A small amount (12%) of the species tritiated at C-3 was also observed. Thus, a small amount of the corresponding regioisomeric iminium perchlorate (with olefin at C-3 rather than C-1) was probably contaminating singly recrystallized **2**. This two step sequence thus provided rapid access to tritiated **1** at moderate specific activity.

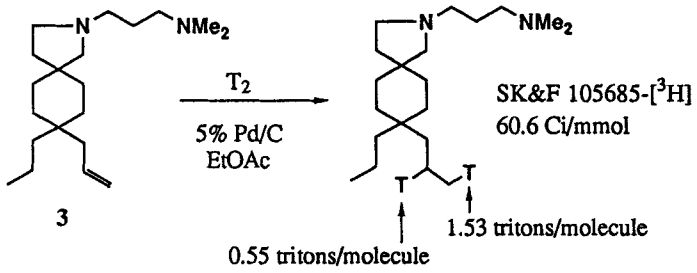
High specific activity **1** (>30 Ci/mmol) was unavailable by this route. Attempts to oxidize **1** with mercuric acetate and isolate the enamine after base treatment failed due to product decomposition upon concentration. This is known to occur in compounds which contain a reactive olefinic bond, and dimers (6) or intractable mixtures (7) may result. Labeling was therefore accomplished by using an olefinic precursor (**3**) which had been prepared by total synthesis. This olefin was subjected to catalytic reduction with 10




Figures 1 and 2. Figure 1 shows the proton decoupled $^3$H-NMR of azaspirane **1** with tritium at C-1 (δ2.39, 88%) and C-3 (δ2.53, 12%). Figure 2 shows the proton decoupled $^3$H-NMR of high specific activity **1** with uneven tritium distribution in the propyl group.

Ci of tritium gas over 5% palladium on carbon in ethyl acetate and afforded tritiated **1** at a specific activity of 60.6 Ci/mmol. It is interesting to note that chemical ionization (ammonia) mass spectrometry indicated a $t_0$:$t_1$:$t_2$:$t_3$:$t_4$ distribution of 11:20:29:29:11 in high specific activity **1**-[$^3$H]. Tritium NMR (benzene-$d_6$) analysis (Figure 2) showed the label to be located, as expected, entirely in the propyl group. However, the distribution of label is uneven. The ratio of tritium in the terminal methyl group to tritium in the adjacent methylene group is 2.78:1.

Scheme II




## Conclusion

SK&F 105685 has been prepared in tritium labelled form by two different methods. Moderate specific activity material was obtained by a two-step process from

unlabeled product. Mercuric acetate oxidation provided an iminium perchlorate precursor which, after borotritide reduction, gave tritium labelled **1** at 7 Ci/mmol. High specific activity material (60.6 Ci/mmol) was obtained via catalytic reduction of a terminal double bond.

## Experimental

**2-(3-Dimethylamino)propyl-8,8-dipropyl-2-azoniaspiro[4.5]dec-1-ene perchlorate hydroperchlorate (2)** 2-(3-Dimethylamino)propyl-8,8-dipropyl-2-azaspiro-[4.5]decane (SK&F 105685, 132 mg, 0.43 mmol) was dissolved in 2 mL of 5% aqueous acetic acid. This solution was added to a solution of 573 mg of mercuric acetate (1.80 mmol) in 5 mL of acetic acid. The solution was heated at 100°C for two hours under an argon atmosphere and the resulting precipitate of mercurous acetate was removed by filtration through Celite. The filtrate was saturated with gaseous hydrogen sulfide, the resulting black precipitate of mercuric sulfide removed by filtration through Celite, and saturated aqueous potassium carbonate was added to the filtrate. The solution was extracted with ether, dried ($MgSO_4$), filtered, and a solution of 1:1 (v/v) ethanol/70% perchloric acid was added slowly to the filtrate. The resulting white precipitate was collected by filtration, washed with ether, and dried *in vacuo*. The precipitate (58 mg) was recrystallized from absolute ethanol to give 28 mg (13%) of **2**: mp 232.5-233°C; perchlorate content: 38.2% (39.1% theoretical for diperchlorate); $^1$H-NMR (DMSO-$d_6$): δ0.82-0.92 (m, 6H), 1.12-1.30 (m, 10H), 1.30-1.48 (m, 2H), 1.48-1.56 (m, 2H), 2.05-2.18 (m, 4H), 2.94 (s, 3H), 2.98 (s, 3H), 3.03-3.10 (m, 2H), 3.86 (t, 2H, J=7.3Hz), 4.18 (t, 2H, J=7.5Hz), 8.91 (s, 1H), 9.32 (s, 1H).

**2-(3-Dimethylamino)propyl-8,8-dipropyl-2-azaspiro[4.5]decane-1-t (1-[$^3$H])** *Method A:* To a vial containing solid sodium borohydride-t (500 mCi at 75 Ci/mmol) was added 3.5 mg of **2** (7 μmol) in 0.75 mL of absolute ethanol. The mixture was stirred at room temperature for 1.25 hours and then the ethanol was removed *in vacuo*. The resulting residue was partitioned between saturated aqueous potassium carbonate and 1:1 (v/v) diethyl ether/ethyl acetate. The organic extract was dried over magnesium sulfate, filtered, and concentrated *in vacuo*. To the resulting residue was added 0.6 mL of 4:1 (v/v) methylene chloride/methanol, and the solution eluted through a silica gel Sep-Pak (Waters) with 4 x 1 mL of 4:1 (v/v) methylene chloride/methanol followed by 8 x 1 mL of 4:1 (v/v) methylene chloride/10% ammonium hydroxide in methanol. The product fractions were combined, concentrated *in vacuo*, and the resulting 19.5 mCi of **1**-[$^3$H] was dissolved in 3 mL of absolute ethanol. The product was obtained at a radiochemical purity of 98.6% (TLC, 4:1 (v/v) diethyl ether/10% ammonium hydroxide in methanol, $R_f$=0.40) and a specific activity of 7.0 Ci/mmol (calculated from the mass and radioactive concentration of the product solution).

*Method B:* A 14.6 mg portion of **3** (2-(3-Dimethylamino)propyl-8-propenyl-8-propyl-2-azaspiro[4.5]decane, 47.7 μmol) was dissolved in 1.4 mL of ethyl acetate. A 2.6 mg portion of 5% Pd/C (Engelhard) was added and the mixture was stirred for two hours

at room temperature under 10 Ci of tritium gas (DuPont NEN). The excess tritium gas was removed and the mixture was filtered through glass wool. The glass wool was rinsed with 1 mL of absolute ethanol, and the combined filtrates were concentrated by vacuum transfer. The residue was dissolved in 1 mL of absolute ethanol, and the solvent was again removed by vacuum transfer. This process was repeated. The resulting clear oily residue was dissolved in 10 mL of absolute ethanol. The ethanol solution contained 3.02 Ci of **1-[$^3$H]** at a radiochemical purity of >93% by TLC (see Method A). A 1 mL portion of the ethanol solution (302 mCi) was purified by preparative TLC (Merck silica gel 60, 20cm x 20cm x 1mm, developed two times with 4:1 (v/v) diethyl ether/10% ammonium hydroxide in methanol). The product band was removed from the silica gel with 9:1 (v/v) ethanol/ammonium hydroxide. The solution was taken to dryness *in vacuo* and the residue was dissolved in 1.4 mL of absolute ethanol. This procedure gave 219 mCi of **1-[$^3$H]** at a radiochemical purity of 99.1% by TLC (see Method A). However, gas chromatographic analysis indicated the presence of ca. 5% olefin **3**. A 91 mCi portion of this material in 2.9 mL of absolute ethanol was hydrogentated with one atmosphere of hydrogen gas over 3 mg of 5% Pd/C at room temperature for two hours. The mixture was filtered through Celite. The resulting ethanol solution contained 88 mCi of **1-[$^3$H]** at a radiochemical purity of 99.0% by TLC. Gas chromatographic analysis indicated the presence of less than 0.5% olefin **3**. Specific activity was 60.2 Ci/mmol as determined by chemical ionization (ammonia) mass spectrometry.

## Acknowledgements

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