Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **43**, 701-710 (2000)

Synthesis of *R*-[*N-Methyl*-^{13}C]SKF 82957 from [^{13}C]Methyl Iodide and [^{13}C]Methyl Triflate

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SUMMARY

With the aim to establish the labeling position in the radiosynthesis of the dopamine D_1 agonist *R*-[$^{11/13}$C]SKF 82957, reaction mixtures containing *R*-SKF 81297, *N*-ethyldiisopropylamine, and [^{13}C]methyl iodide or [^{13}C]methyl triflate, were analyzed using ^{1}H- and ^{13}C-NMR, as well as 2D ^{1}H-^{13}C-NMR. Only *R*-[*N-methyl*-^{13}C]SKF 82957 was obtained using sub-molar quantities of [^{13}C]methyl iodide. In contrast, a second ^{13}C-labeled peak, likely corresponding to an *O*-[^{13}C]methylated derivative, was observed with the highly reactive [^{13}C]methyl triflate. Similar results were obtained in the synthesis of the structurally similar *R*-[^{13}C]SCH 23390.

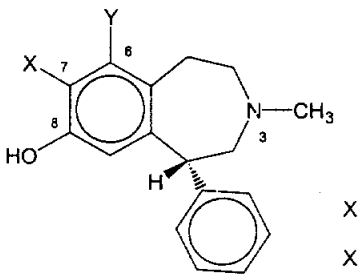
Key Words: carbon-13, *N*-[^{13}C]methylation, *O*-[^{13}C]methylation, *R*-SKF 82957, SCH 23390, positron emission tomography.

INTRODUCTION

R/S-SKF 82957 (*R/S*-3-methyl-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) is a dopamine D_1 agonist that binds with high affinity and selectivity to the high-affinity site (K_i = 0.9 nM) of the D_1 receptor (1,2). The ^{11}C-labeling procedure and *in vivo* rat evaluation were previously reported (3,4). We

Received 17 December 1999
Revised 17 February 2000
Accepted 18 February 2000

have recently demonstrated that *R*-[^{11}C]SKF 82957 provides a more sensitive measurement of D_1 receptors than the racemic mixture (5). *R*-[^{11}C]SKF 82957 is currently used to study living human brain with positron emission tomography (PET) (6), and has potential to image D_1 receptors in neuropsychiatric disorders. However, using a capillary electrophoresis analysis system fitted with a positron detector (7), Gillies et al. have recently suggested that the *O*-[^{11}C]methylation of *R*-SKF 81297 also occurs in addition to *N*-[^{11}C]methylation when using [^{11}C]methyl iodide, but not with [^{11}C]methyl triflate (8). Similar results were also reported following ^{13}C-NMR analyses of the reaction mixtures using [^{13}C]methyl iodide or [^{13}C]methyl triflate (8). The presence of the 7,8-catechol has previously been shown to be essential for agonistic activity in this series of benzazepine analogs. When the 7-hydroxy group is removed, the resulting monohydroxy derivative still binds to D_1 receptors but as an antagonist. Substitution at the 7-position with a halogen (e.g. chloro analog SCH 23390) increases the affinity and produces potent D_1 antagonists (9,10-13). Since the purpose of our work is to develop a high affinity selective D_1 agonist radioligand, it is thus crucial to establish the labeling position on the final $^{11/13}$C-labeled products.



X = OH Y = Cl *R*-SKF 82957

X = Cl Y = H SCH 23390

Figure 1: Structures of *R*-SKF 82957 and SCH 23390.

In this study, similar reaction conditions to those occurring in PET radiochemistry (3,5) were utilized using sub-molar amounts of [^{13}C]methyl iodide or [^{13}C]methyl triflate, in the presence of the weak nucleophilic base *N*-ethyldiisopropylamine. The reactions were then analyzed by 1D ^{1}H- and ^{13}C-NMR, as well as 2D HSQC ^{1}H-^{13}C-NMR. The same conditions were used to prepare and analyze the D_1 antagonist benzazepine [^{13}C]SCH 23390.

RESULTS AND DISCUSSION

In PET radiochemistry, tracer doses of [^{11}C]methyl iodide or [^{11}C]methyl triflate are used in comparison to the precursor. Therefore, in order to assess the labeling position on *R*-SKF 81297 by NMR, it is important to simulate similar reaction conditions using sub-molar amounts of [^{13}C]methyl iodide or [^{13}C]methyl triflate. Except for the use of 0.5 equivalent [^{13}C]methyl iodide, using similar synthesis and the same HPLC purification conditions to those reported for *R*-[^{11}C]SKF 82957 (3,5), only one ^{13}C-labeled peak (δ_C 47.3 ppm) was found in the HPLC fraction corresponding to *R*-SKF 82957. In order to identify all positions that were labeled with ^{13}C (by *O*-[^{13}C]alkylation and/or *N*-[^{13}C]alkylation), NMR analyses were carried out on the whole reaction mixtures in the NMR tubes. Comparison of 1D ^{1}H-NMR ^{13}C-coupled and ^{13}C-decoupled spectra, proton-decoupled ^{13}C-NMR and 2D HSQC

Table 1. ^{1}H chemical shifts (δ_H in ppm), multiplicity (m), coupling constants (J_{C-H} in Hz) and relative integrations (RI in %) of selected substituents* of the final ^{13}C-labeled products from *R*-SKF 81297 or normethyl-SCH 23390

Reaction Mixture				
Precursor	Reagent# Equiv	*N*-^{13}CH$_3$ (δ_H, m, *J*, RI)	*N*-(^{13}CH$_3$)$_2$ (δ_H, m, *J*, RI)	*O*-^{13}CH$_3$ (δ_H, m, *J*, RI)
R-SKF 81297†	[^{13}C]CH$_3$I 0.75	2.30, d, 133		
R-SKF 81297‡	[^{13}C]CH$_3$I 0.75	2.28, d, 133		
R-SKF 81297‡	[^{13}C]CH$_3$I 4.4		2.99, d, 144, 60	3.43, d, 145, 30 3.70, d, 147, 10
R-SKF 81297†	[^{13}C]CH$_3$Tf 0.75	3.27, d, 139, 70		3.70, d, 147, 30
R-SKF 81297‡	[^{13}C]CH$_3$Tf 0.75	3.27, d, 139, 90		3.70, d, 147, 10
Normethyl-SCH 23390†	[^{13}C]CH$_3$I 0.75	2.30, d, 133		
Normethyl-SCH 23390†	[^{13}C]CH$_3$Tf 0.75	3.27, d, 139, 70		3.70, d, 147, 30

* *N*-^{13}CH$_3$ (δ_H 3.03 ppm) of *R*-SKF 82957•HBr standard. † Equivalents of base = 1.9. ‡ Equivalents of base = 42.7. # Tf = triflate, Equiv = equivalent.

^{1}H-^{13}C-NMR spectra enabled us to identify the carbon-bound protons and assign the positions of the ^{13}C-labeling.

A shift to lower frequency of the final product's *N*-$^{13}CH_3$ proton chemical shift values was noted after reaction with [^{13}C]methyl iodide, as compared to *R*-SKF 82957•HBr standard or to that obtained following use of [^{13}C]methyl triflate (Table 1). This shift is likely due to a charge-transfer complex (14), or a cation-anion interaction caused by an ion pair formation with the iodide counter-ion (15). Addition of sodium bisulfite to the reaction mixture resulted in the breakup of this complex and moved the chemical shifts to similar values as the standard (2D HSQC ^{1}H-^{13}C-NMR δ_H 3.02 ppm, δ_C 44.5 ppm). A slight shift to higher frequency of the final product's *N*-$^{13}CH_3$ signal was observed with [^{13}C]methyl triflate as compared to the SKF 82957 standard (Table 1).

Table 2. Proton-decoupled ^{13}C chemical shifts (δ_C in ppm), multiplicity (m) and coupling constants ($J_{C\text{-}H}$ in Hz) of selected substituents* of the final ^{13}C-labeled products from *R*-SKF 81297 or normethyl-SCH 23390

Reaction Mixture					
Precursor	Reagent# Equiv	Base Equiv#	*N*-$^{13}CH_3$ (δ_C, m, *J*)	*N*-($^{13}CH_3$)$_2$ (δ_C, m, *J*)	*O*-$^{13}CH_3$ (δ_C, m, *J*)
R-SKF 81297	[^{13}C]CH_3I 0.75	1.9	47.0, s		
R-SKF 81297	[^{13}C]CH_3I 0.75	42.7	47.0, s		
R-SKF 81297	[^{13}C]CH_3I 4.4	42.7		42.88, t, 4.2	56.6, s 49.2, s
R-SKF 81297	[^{13}C]CH_3Tf 0.75	1.9	48.8, s		50.4, s
R-SKF 81297	[^{13}C]CH_3Tf 0.75	42.7	48.8, s		50.4, s
Normethyl-SCH 23390	[^{13}C]CH_3I 0.75	1.9	47.0, s		
Normethyl-SCH 23390	[^{13}C]CH_3Tf 0.75	1.9	48.8, s		50.4, s

* *N*-$^{13}CH_3$ (δ_C 44.6 pmm) of *R*-SKF 82957•HBr standard. # Equiv = equivalent, Tf = triflate.

The carbon-13 chemical shift values of the *N*-$^{13}CH_3$ peak were mostly unaffected by the counter-ions (Table 2). Therefore, the proton to carbon correlations in the 2D HSQC experiments added invaluable information in this study, and greatly helped in the identification of the produced compounds, especially with the change of the chemical shifts in the ^{1}H-NMR peaks using [^{13}C]methyl iodide.

Using the conditions described in DaSilva et al. (5), with the addition of sub-molar quantities of [^{13}C]methyl iodide and 1.9 equivalents of base to the reaction mixture, the ^{1}H- and ^{13}C-NMR spectra showed only one new ^{13}C-labeled signal for either *R*-SKF 81297•HBr or normethyl-SCH 23390•HCl (Table 1 and 2). 2D HSQC ^{1}H-^{13}C-NMR spectra demonstrated that this new ^{13}C-labeled peak is consistent with an *N*-$^{13}CH_3$ (δ_H 2.30 ppm, δ_C 47.0 ppm). Using large excess of the base (42.7 equivalents), as reported in Gillies et al. (8), and 0.75 equivalent of [^{13}C]methyl iodide resulted in comparable NMR spectra to those obtained with 1.9 equivalents of base. Similar NMR spectra were also observed using the same procedure with *R/S*-SKF 81297. These results contrast those published by Gillies et al. (8). However, using an excess [^{13}C]methyl iodide (4.4 equivalents) and base (42.7 equivalents) with *R*-SKF 81297•HBr produced many new ^{13}C-labeled peaks (Table 1 and 2), with a major signal characteristic of a quaternary ammonium compound centered at δ_C 42.88 ppm (Table 2) in the proton-decoupled ^{13}C-NMR. 2D HSQC ^{1}H-^{13}C-NMR showed three major ^{13}C-labeled peaks (δ_H 2.98 ppm, δ_C 42.9 ppm; δ_H 3.42, δ_C 56.5 ppm; δ_H 3.61, δ_C 49.1 ppm), with the first signal corresponding to $(^{13}CH_3)_2N^+$ and the latter two likely corresponding to *O*-[^{13}C]methylated derivatives. Relative integrations in the 1D ^{1}H-NMR spectrum for peaks at 2.99, 3.43 and 3.70 ppm were 60, 30 and 10%, respectively (Table 1). These results are similar to those reported in Gillies et al. (8), including the triplet centered at δ_C 42.88 ppm in the proton-decoupled ^{13}C-NMR. This triplet is characteristic of a spin-spin coupling between ^{14}N and ^{13}C nuclei (J=4.2 Hz, $(^{13}CH_3)_2N^+$) occurring in seven-membered ring heterocyclic cations, containing a quaternary nitrogen atom (16), not of an *N*-$^{13}CH_3$ group as reported in (8), suggesting that they might have used excess [^{13}C]methyl iodide in the experiment.

As expected, in comparison to [^{13}C]methyl iodide which produced only *N*-[^{13}C]methylation, less selectivity was obtained using sub-molar quantities of

[^{13}C]methyl triflate in the presence of 1.9 or 42.7 equivalents of base. Two new ^{13}C-labeled peaks were observed on the ^{1}H- and ^{13}C-NMR spectra (Table 1 and 2). 2 D HSQC ^{1}H-^{13}C-NMR spectra indicated that one ^{13}C-labeled peak is consistent with an *N*-$^{13}CH_3$ (δ_H 3.27 ppm, δ_C 48.7 ppm), and the other likely corresponding to an *O*-[^{13}C]methylated derivative (δ_H 3.70 ppm, δ_C 50.3 ppm). These results are in agreement with the higher reactivity of methyl triflate as compared to methyl iodide, and were observed with both precursors (*R*-SKF 81297•HBr and normethyl-SCH 23390•HCl). Interestingly, using 1.9 equivalents of base, 70% of the ^{13}C-labeling with [^{13}C]methyl triflate corresponded to an *N*-$^{13}CH_3$, while 30% was an *O*-$^{13}CH_3$ (Table 1). However, using large excess of the weak nucleophilic base (42.7 equivalents), *N*-[^{13}C]methylation of *R*-SKF 81297 preferentially occurred over *O*-[^{13}C]methylation in a ratio of 9/1 (Table 1). These results differ from those published by Gillies et al. (8). However, the results obtained following capillary electrophoresis (8) are difficult to explain, but do not concur with the ones presented in this paper.

EXPERIMENTAL

Equipment and Materials

^{1}H- and ^{13}C-NMR, and 2D ^{1}H- ^{13}C- HSQC NMR spectra were recorded on a Varian Unity 500 MHz spectrometer in deuterated *N,N*-dimethylformamide-d_7 (DMF-d_7) or deuterated chloroform ($CDCl_3$). A 5-mm Varian indirect PFG broadband probe was used for ^{1}H detected measurements, while a Nalorac 5-mm broadband probe was used for ^{13}C detected experiments. Chemical shifts were reported using tetramethylsilane (0.00 ppm) as an internal standard for ^{1}H-NMR, and relative to DMF-d_7 (referencing the first doublet of multiplet at 29.7 ppm and correcting the shifts to tetramethylsilane) or $CDCl_3$ (referencing at 77.0 ppm) for the ^{13}C-NMR. The ^{1}H-^{13}C HSQC experiments were run in the phase sensitive mode using 2x256 increments in F1, linear predicting to 2x512 increments and zero filling to 2x1024. The spectral window in F1 was 20110 Hz and in F2, 4174 Hz. Four scans per increment with a relaxation delay of 1 second was used. Gaussian apodization was used in the processing. Decoupling of ^{13}C was done using the WURST sequence in both 2D HSQC and 1D carbon-decoupled ^{1}H NMR experiments. *R/S*-SKF

81297•HCl and *R/S*-SKF 82957•HCl were generous gifts from SmithKline Beecham Pharm. (King of Prussia, PA, USA). DMF was distilled from BaO and stored over 4 Å molecular sieves. *N*-Ethyldiisopropylamine (Lancaster Synthesis Inc., Windham, NH, USA) was diluted (10% v/v solution) in DMF-d_7 or DMF. The other chemicals were purchased from Sigma-Aldrich (Oakville, Ontario, Canada).

R-SKF 82957

R-SKF 82957•HBr was analyzed by ^{1}H-NMR (DMF-d_7): δ 3.03 (s, 3H, NCH_3), 3.10-3.98 (m, 6H, azepine-H), 5.01 (br d, 1H, 1-H), 6.01 (br s, 1H, 9-H), 7.31 (m, 2H, *o*-C_6H_5), 7.37-7.43 (m, 1H, *p*-C_6H_5), 7.43-7.52 (m, 2H, *m*-C_6H_5), 9.24 (br s, 1H, Ar-OH), 9.74 (br s, 1H, Ar-OH); ^{13}C-NMR (DMF-d_7): δ 26.5 (5-C), 44.6 (NCH_3), 44.9 (1-C), 55.1 (4-C), 60.6 (2-C), 114.2 (9-C), 121.2 (6-C), 126.9 (*p*-C_6H_5), 127.5 (Ar-C), 128.9 (Ar-C), 129.2 (Ar-C), 134.9 (Ar-C), 141.3 (7-C or 8-C), 141.6 (*ipso*-Ar), 145.1 (8-C or 7-C).

R-[^{13}C]SKF 82957

Using [^{13}C]methyl iodide:

NMR analysis after HPLC purification. *R*-[^{13}C]SKF 82957 was prepared using similar conditions as previously described (3,5). [^{13}C]Methyl iodide (10% v/v solution in DMF, 0.5 equivalent) was slowly added to a cooled (–20 °C) solution of *R*-SKF 81297•HBr (2 mg, 5.4 μmol, 1.0 equivalent) and *N*-ethyldiisopropylamine (10% v/v solution in DMF, 2.1 equivalents) in DMF (380 μL). After 5 min at 85°C, the reaction mixture was cooled and quenched with 1 mL of HPLC buffer (3). Analysis of the reaction mixture by analytical HPLC (Phenomenex Prodigy C18 5 μ, 250 x 4.6 mm, eluted with 15% tetrahydrofuran/ 85% 0.1 N CH_3CO_2Na (pH 5) at 1.1 mL/min) showed a 1:1 mixture of *R*-SKF 81297 and *R*-SKF 82957. The product was purified by semi-preparative HPLC (2 injections) using the same conditions as described for [^{11}C]SKF 82957 (3,5). The fractions containing the product were pooled and extracted with dichloromethane (3 x 1 mL). The combined extracts were dried (Na_2SO_4), filtered, and the volume made up to 5 mL with methanol for HPLC analysis. A comparison of the peak areas from injection of aliquots of this solution to a standard solution of *R*-SKF 82957 gave a yield of 28%. The solution was evaporated to dryness and taken up in $CDCl_3$ for NMR analysis.

NMR analysis of reaction mixtures. *R*-[^{13}C]SKF 82957 was prepared in a 5 mm NMR tube, using similar conditions as described above (*R*-SKF 81297•HBr, 2 mg, 5.4 μmol, 1.0 equivalent; [^{13}C]methyl iodide, 10% v/v solution in DMF-d_7, 0.75 equivalent; *N*-ethyldiisopropylamine, 10% v/v solution in DMF-d_7, 1.9 equivalents; in DMF-d_7, 380 μL). After 5 min at 85°C, D_2O (50 μL) was introduced and the solution was vortexed. Following addition of DMF-d_7 (300 μL), the reaction mixture was then analyzed by 1D ^{1}H- and ^{13}C-NMR, and 2D ^{1}H-^{13}C-NMR.

This experiment was repeated using the same conditions with *R*-SKF 81297•HBr for reproducibility purposes or with racemic SKF 81297•HCl. This procedure was also repeated with a large excess of the base (40 μL, 42.7 equivalents) as reported in Gillies et al. (8) and 0.75 equivalent of [^{13}C]methyl iodide, or using an excess [^{13}C]methyl iodide (4.4 equivalents, 10% solution in DMF-d_7) and base (42.7 equivalents).

Since the *N*-$^{13}CH_3$ proton chemical shift of the product was shifted to lower frequency following reaction with [^{13}C]methyl iodide, attempts were made to break the putative iodide counter-ion complex by addition of excess sodium bisulfite to the reaction mixture. 1D and 2D NMR analyses were then carried out seven days later.

Using [^{13}C]methyl triflate: Attempts to synthesize *R*-[^{13}C]SKF 82957 in a 5 mm NMR tube were also made with [^{13}C]methyl triflate (10% v/v solution in DMF-d_7, 0.75 equivalent), using the same conditions as described above with 1.9 or 42.7 equivalents *N*-ethyldiisopropylamine, except that the reaction mixtures were heated for 2 min. The reaction mixtures were then analyzed by 1D and 2D NMR.

[^{13}C]SCH 23390

Using [^{13}C]methyl iodide: [^{13}C]SCH 23390 was prepared in a 5 mm NMR tube from normethyl-SCH 23390•HCl using similar conditions as described above (0.75 equivalent [^{13}C]methyl iodide and 1.9 equivalents *N*-ethyldiisopropylamine). The reaction mixture was then analyzed by 1D and 2D NMR.

Using [^{13}C]methyl triflate: [^{13}C]SCH 23390 was also synthesized in a 5 mm NMR tube from normethyl-SCH 23390•HCl as described above (0.75 equivalent [^{13}C]methyl triflate and 1.9 equivalents *N*-ethyldiisopropylamine). The reaction mixture was then analyzed by 1D and 2D NMR.

CONCLUSION

These experiments indicate that only *R*-[*N-methyl*-^{13}C]SKF 82957 is produced using sub-molar quantities of [^{13}C]methyl iodide and *R*-SKF 81297. In contrast, a second ^{13}C-labeled peak, likely corresponding to an *O*-[^{13}C]methylated product, is obtained using sub-molar quantities of the highly reactive [^{13}C]methyl triflate. This study provides strong evidence that, using tracer doses of [^{11}C]methyl iodide, as recently reported by our group, only the *N*-[^{11}C]methylation occurs in the radiosynthesis of the D_1 agonist *R*-[^{11}C]SKF 82957 (5) and D_1 antagonist [^{11}C]SCH 23390.

ACKNOWLEDGEMENTS

The authors are grateful to Professors William F. Reynolds and Hiltrud Grondey of the Department of Chemistry for helpful discussions. This work was funded by the Medical Research Council of Canada (MT-13258).

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