SYNTHESIS, STABILITY, AND RADIOLYTIC DECOMPOSITION OF CARBON-14 LABELED MK0677

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SUMMARY

MK0677 is an orally active growth hormone secretagogue. The crystallized carbon-14 labeled material was found to undergo radiolytic decomposition via a peroxide intermediate which resulted in loss of the benzyl group. The rate was diminished when the tracer was crystallized from nitrogen-degassed solvents. Storage stability was best in aqueous ethanol.

Key Words: carbon-14, stability, radiolysis, synthesis.

INTRODUCTION

MK0677 (**5**) is an orally active growth hormone secretagogue demonstrating high potency *in vitro* and *in vivo*[1]. In support of human and animal metabolic studies, carbon-14 labeled material was synthesized.

However, stability of this tracer became of concern when it was observed that the crystalline solid at both 53 and 10 mCi/mmol degraded from better than 99% radiochemical purity by HPLC to below 95% within one month. There was no apparent differentiation in decomposition rates between the two specific activities. This had not been anticipated; rapid decomposition had not been observed in batches of either sulfur-35 or tritium labeled tracer at much higher specific activity (e.g. up to 1,000 Ci/mmol). However, those lots had been very dilute solutions in ethanol. Therefore, stability studies were undertaken to quantitate the rate of decomposition of the crystalline carbon-14 labeled tracer, estimate its useable lifetime (the point at which it dropped below 98.5%), and elucidate the structure of the degradation species.

DISCUSSION

Synthesis

The synthesis of [^{14}C]MK0677 was carried out as shown in the scheme. [^{14}C]Methane sulfonate **1** was obtained in 75% radiochemical yield via the Strecker reaction[2] of [^{14}C]methyl iodide with aqueous sodium sulfite at room temperature. Slurrying the dried product in acetone removed the sodium iodide byproduct and greatly improved the yield in the subsequent step. Treatment of crude **1** suspended in dichloromethane with oxalyl chloride and dimethylformamide gave

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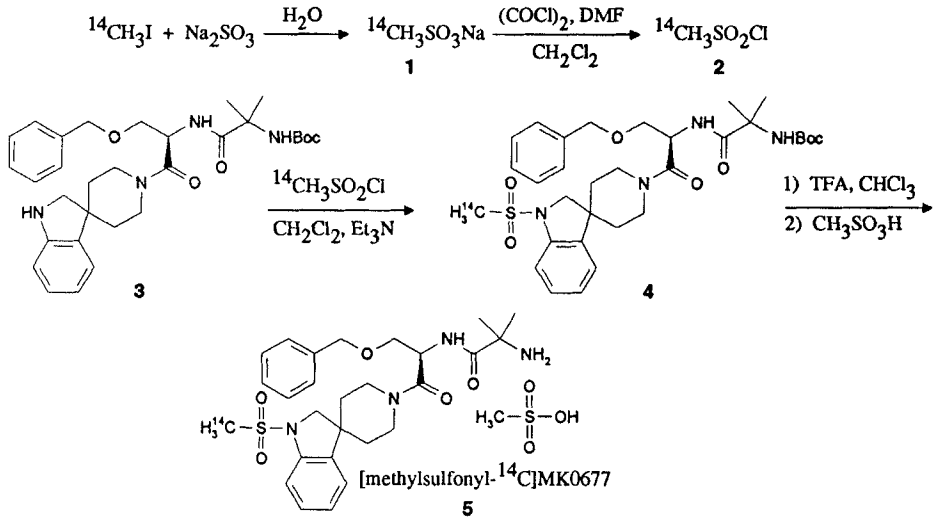


[^{14}C]methanesulfonyl chloride **2**, which was used to sulfonylate the BOC-protected indoline **3** in dichloromethane containing triethylamine. Following workup, the mixture was purified by reverse phase preparative HPLC to afford [^{14}C]sulfonamide **4**. Trifluoroacetic acid in chloroform removed the protecting group, and the crude product purified by reverse phase preparative HPLC. Formation of the mesylate salt, dilution with carrier, and crystallization resulted in [^{14}C]MK0677 with a radiochemical purity of over 99%.

First stability study

For the first set of stability studies, crystalline tracer at a specific activity of 2.5 mCi/mmol was evaluated. It was stored at -70°C, under nitrogen, and protected from light. These conditions were considered most likely to promote stability.

HPLC analysis during this study showed that, over 12 weeks, the radioactive purity dropped to 97.5% (Table), an average decrease of about 0.2% per week. Two major radioactive impurities appeared, with retention times of 0.3 (impurity 1) and 0.8 (impurity 2) relative to the main peak. Linear regression analysis, which gave the best fit to the data, indicated that between weeks six and seven, the radioactive purity of the tracer would drop below the 98.5% minimum.

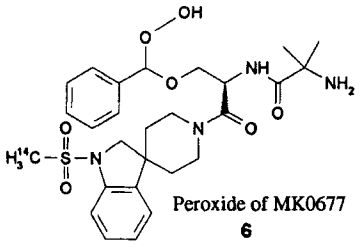
Identification of major impurities

Analysis by LC/MS/MS failed to detect impurity 1, but did detect impurity 2, which gave a parent ion of 561, 32 mass units higher than the starting material. This suggested that a peroxide had formed. Based on the parent structure and evidence suggested by the daughter ion spectra, the peroxide was tentatively assigned to the methylene on the benzyl group (**6**). Consistent with impurity 2 being a peroxide, it decomposed in aqueous acid over about 20 hours, with a concomitant increase in impurity 1. The sum of the two peaks was essentially constant, indicating that they were related.

It seemed reasonable to assume that acid hydrolysis of this peroxide would generate radioactive desbenzyl-MK0677 (**7**), a known metabolite of MK0677, and non-radioactive benzaldehyde.

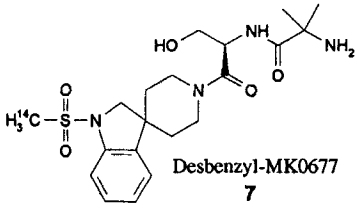
Indeed, chromatography with authentic references showed that impurity 1 co-eluted with desbenzyl-MK0677, and that a UV-only peak corresponded to benzaldehyde.

These results suggest the decomposition of this tracer is due to generation of a free radical on the benzyl methylene, which subsequently reacts with oxygen, whether from exogenous sources, such as incomplete removal during the degassing operation, or trapped in the crystal matrix during crystallization, to form a peroxide (**6**). This is then fairly stable as a solid, but in aqueous acid, it slowly hydrolyses to form **7**. Peroxide formation is a typical decomposition pathway for radiolabeled compounds[3].



Peroxide of MK0677

6



Desbenzyl-MK0677

7

Second stability study

Based on this information, a second batch of crystalline tracer was prepared at 2.1 mCi/mmol, in which all solvents were degassed with nitrogen prior to the final crystallization, and a second set of stability studies undertaken. Samples were stored at room temperature and 5°C, as well as at -70°C, under nitrogen and protected from light. In addition, a 1 mg/mL solution in water containing 2% ethanol was also prepared and stored at 5°C. This solution was chosen because of its biocompatability and anticipated inhibition of free radical formation.

As shown in the table, the stability for this preparation was much greater; it took 17 weeks instead of six before the purity dropped to 98.6%. Storage at -70°C confers some added stability over

MK0677 STABILITY

	Batch 1 (-70°C)[a]		Batch 2[b] (-70°C)		Batch 2[b] (25°C)		Batch 2[b] (5°C)		Batch 2[b,c] (5°C)	
Week	Impurity[d]	MK0677	Impurity[d]	MK0677	Impurity[d]	MK0677	Impurity[d]	MK0677	Impurity[d]	MK0677
0	0.17%	99.69%	0.10%	99.70%	0.10%	99.70%	0.10%	99.70%	0.10%	99.70%
1	0.32%	99.62%			0.20%	99.63%	0.25%	99.58%	0.15%	99.67%
2	0.58%	99.26%			0.24%	99.57%	0.27%	99.62%	0.10%	99.76%
4	0.71%	99.20%			0.32%	99.46%	0.34%	99.60%	0.18%	99.60%
6	1.11%	98.72%			0.48%	99.40%	0.47%	99.48%	0.11%	99.69%
7			0.48%	99.46%						
10					0.70%	98.72%	0.92%	98.56%	0.17%	99.51%
12	1.85%	97.53%	0.93%	99.07%						
15					1.08%	98.67%	0.89%	98.78%	0.19%	99.56%
17			1.24%	98.61%						
21					1.30%	97.74%	1.19%	98.58%	0.28%	99.13%
24			1.93%	97.86%						
27	7.35%	92.65%								
32	8.48%	91.21%								
39	9.62%	89.64%								

[a]Specific activity 2.5 mCi/mmol. [b]Specific activity 2.1 mCi/mmol. [c]In a solution of 5% ethanol in water. [d]Impurity is the sum of the radioactivity in the desbenzylMK0677 and hydroperoxyMK0677 peaks, since the latter slowly converts to the former over time. The sum of the two is constant during the analysis.

storage at warmer temperatures, but the difference is marginal. Water containing ethanol confers the best stability, since under these conditions the rate of formation of a free radical is greatly inhibited.

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EXPERIMENTAL

HPLC conditions

HPLC systems were either a Varian Vista 5500 equipped with a Kratos Spectraflow 757 UV detector, or a Varian Star system equipped with autosampler, pump, UV detector, and data station. Radioactivity data was collected on-line with a Packard Radiomatic Flo-one/Beta radioactivity flowmonitor equipped with a data station.

Typical HPLC conditions were: acetonitrile/water/trifluoroacetic acid 35/65/0.1 at 1 mL/min. on a Zorbax RX-C8 column (250 x 4.6 mm) at 35°C, with UV monitoring at 230 NM. The flowmonitor, operated in the TRLSC = low mode, contained a 500 uL liquid cell. Cocktail (Packard Ultima Flo M) was pumped at 3.0 mL/min. Data was updated every 3 seconds. Samples were prepared for analysis by dissolution at 1 mg/mL in 50:50 acetonitrile/water containing 1% trifluoroacetic acid, except solutions, which were injected neat; 10 or 20 uL were injected. For stability data time points, either 3 or 4 injections were made of each sample. Using the arithmetic feature of the flowmonitor data system, the multiple runs were averaged into one run, which was then analyzed. This had the benefit of reducing noise and increasing real signal in low-activity samples, giving more consistent results.

Synthesis (typical)

[^{14}C]methanesulfonyl chloride (2). Na_2SO_3 (124 mg, 0.998 mmol) was dissolved in H_2O (1.5 mL) and frozen with liquid nitrogen. [^{14}C]CH_3I (2 x 28 mCi, 0.996 mmol) was added by vacuum line transfer and the solution allowed to warm to room temperature. HPLC analysis after 1 h (Zorbax RX C-8, 60/40 A/B, A = CH_3CN, B = H_2O, [^{14}C]CH_3I, r_t = 8.0 min.: [^{14}C]methanesulfonic acid sodium salt , r_t = 2.5 min.) showed no remaining methyl iodide. The mixture was concentrated by nitrogen stream at 90°C and dried overnight under vacuum at 80°C to afford a white solid. The [^{14}C]methanesulfonate was slurried in dry acetone (with sonication; 2 x 1 mL), then filtered to afford [^{14}C]methanesulfonic acid sodium salt (150 mg, 42.5 mCi, 283 uCi/mg). In a screw cap test tube, crude **1** (60.6 mg, 17.2 mCi, 0.513 mmol) was slurried in CH_2Cl_2 (2 mL). Following the addition of DMF (0.5 uL), the mixture was cooled to 0°C and oxalyl chloride (65.2 mg, 45 uL) was added. The mixture was aged 3h at room temperature to provide a crude solution of **2** in dichloromethane.

N-[(1R)-[1,2-Dihydro-1-[^{14}C]methanesulfonylspiro[3H-indole-3,4'piperdin]1'-yl)carbonyl]-2-phenylmethoxy)ethyl]-2-*tert*-butyloxycarbonylamino-2-methylpropanamide; [^{14}C]BOC-MK0677 (4). Indoline **3** (273 mg, 0.513 mmol) and Et_3N (180 uL, 1.26 mmol) were dissolved in CH_2Cl_2 (15 mL) and cooled to 0°C. A solution of [^{14}C]methanesulfonyl chloride (17.2 mCi) in CH_2Cl_2 was added dropwise by cannula (leaving behind most of solids) and the mixture aged at 0 °C for 30 min. followed by 1 h at room temperature. The mixture was concentrated, and the yellow residue redissolved in 20 mL of ethyl acetate, washed with 1.0 N HCl (10 mL), saturated aqueous $NaHCO_3$ (2 x 10 mL), and brine (10 mL). The organic layer was dried over $MgSO_4$ and concentrated to afford 8.6 mCi (45%) of crude solid BOC-[^{14}C]MK0677 **4**. This reaction was repeated to afford an additional 7.1 mCi of crude solid **4**. Purification was effected by preparative HPLC (Zorbax RX C-8, 25 cm x 22 mm, step gradient of 40% A to 50% A, A = CH_3CN, B = H_2O) to afford a total of 130 mg (11.5 mCi) of 99.7% radiochemically pure BOC-[^{14}C]MK0677 **4** as a white solid.

N-[(1R)-[1,2-Dihydro-1-[^{14}C]methanesulfonylspiro[3H-indole-3,4'piperdin]1'-yl)carbonyl]-2-phenylmethoxy)ethyl]-2-amino-2-methylpropanamide methane sulfonic acid; [^{14}C]MK0677. BOC-[^{14}C]MK0677 **4** (31.6 mg, 0.052 mmol, 2.9 mCi) was dissolved in 2 mL of $CHCl_3$ and cooled to 0°C. Trifluoroacetic acid (1.5 mL) was added and the mixture aged for 1 h at room temperature at which time HPLC (Zorbax-RX C-8, 65/35 A/B, A = CH_3CN, B = H_2O, 0.1% H_3PO_4) showed no starting material remained. The mixture was concentrated *in vacuo*, redissolved in ethyl acetate (5 mL) and the organic layer washed with 10% aqueous Na_2CO_3.

The layers were separated and the aqueous layer was extracted with EtOAc (2 x 4 mL). The combined organic layer was washed with brine (2.0 mL), dried over Na_2CO_3, and concentrated. This reaction was repeated on an additional 90 mg of 4 to afford a total of 11.3 mCi of crude [^{14}C]MK0677. Crude [^{14}C]MK0677 was purified by preparative HPLC (Zorbax RX C-8, 25 cm x 22 mm, step gradient 20% A to 30% A, A = CH_3CN, B = H_2O, 0.1% TFA) to afford a total of 70.8 mg (6.8 mCi) of 99.7% radiochemically pure [^{14}C]MK0677 as a white solid. The solid was dissolved in ethanol and 10 uL of methanesulfonic acid was added followed by 1.7 g of unlabeled MK0677. The ethanol was removed and the solid slurried into 35 mL of 8% ethanol in ethyl acetate. 100 mg of seed MK0677 was added and the mixture aged at 55°C for 3 h. The mixture was cooled to room temperature, filtered, and dried *in vacuo* at 35°C for 3h to afford 1.689 g, 5.96 mCi of [^{14}C]MK0677 at 99.6% radiochemical purity and a specific activity of 3.52 uCi/mg.

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