Online Supplementary Material

for

Tissue Penetration of a Novel Spectinamide Antibiotic for the Treatment of Tuberculosis

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Radiosynthesis of ^{3}H-1329

Radiolabeling of 1329 [3'-Dihydro-3'-deoxy-3'(R)-(pyridin-2yl) acetylamino spectinomycin trihydrobromide] was performed by adding 3'-Dihydro-3'-deoxy-3'(R)- (5-bromopyridin-2-yl) acetylamino spectinomycin trihydrobromide (50.0 mg, 0.094 mmol; designated as 1516; synthesis and characterization of 1516 are described at (1)) at room temperature under nitrogen (0.094 mmol) to a 10 mL round bottom flask, containing a stirring suspension of 10% palladium on carbon (Pd/C, 10.0 mg) in 2 mL of methanol (**Figure S1**). The flask was placed under a mixed tritium/deuterium environment (T_2/D_2) using a tritium manifold (RC Tritec, Teufen, Switzerland). With the closed-system manifold under high vacuum, incorporation of tritium was accomplished by heating the tritium bound uranium source to 350°C and allowing the measured manifold pressure to stabilize at 247 mbar. Deuterium was then introduced into the manifold from an alternative source to constitute the make-up mixture, with final pressure reached at 1,200 mbar. After subjection of the reaction flask to the T_2/D_2 atmosphere for 30 min, a loss of 124 mbar of gas was observed. Upon recapturing of the diluted tritium gas onto an alternative uranium bed and purging the reaction with nitrogen, the crude reaction mixture was filtered into a 25 mL round bottom flask. One drop of water was added and then the solvent was removed under vacuum and the ^{3}H-1329 (43.7 mg) was collected. The purity and radioactivity of the ^{3}H-1329 was determined by HPLC with radiochromatographic and UV detection (Phenomenex Gemini 3.0 × 50 mm, 5 µm C18 110A column, PerkinElmer LC Series 200 pump with Series 200 UV/Vis detector and Radiomatic 150 TR flow scintillation analyzer controlled by a TotalChrom data system detection, Perkin Elmer, Waltham, MA) and scintillation counter analysis (LS6000TA scintillation counter, Beckman Coulter, Indianapolis, IN). The product had an observed chemical purity of

69.9% (comprising ^{3}H-1329 and unlabeled-1329) and a total radiometric purity of 100%, i.e. all the detected label was associated with the 1329 peak as identified by HPLC with radiometric and UV detection. It had a total activity of 38.4 mCi (specific activity of 0.61 Ci/mmol). A stability study for ^{3}H-1329 in solution at room temperature for 16 days did not identify any appreciable loss of activity for ^{3}H-1329 as assessed by HPLC with radiometric detection.

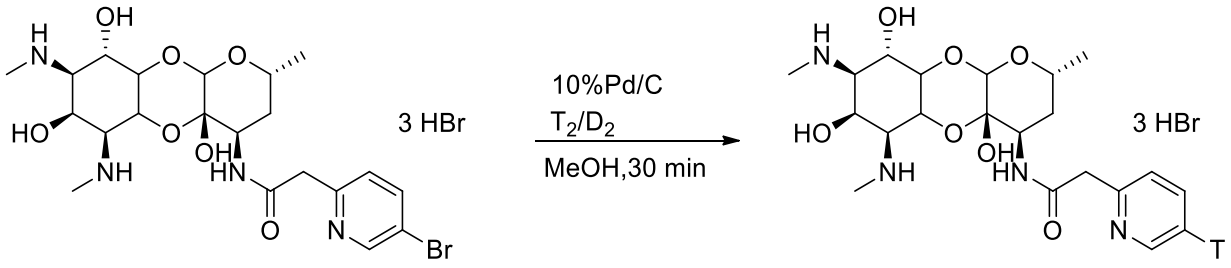

Figure S1: Radiosynthesis of ^{3}H-1329 (Molecular Weight: 697.25 3HBR salt; 454.51 free base)

Sample Preparation for Analysis of ^{3}H-1329 in Different Tissues

Blood: The amount of radioactivity (^{3}H-1329 and any labeled metabolites) in the whole blood was determined by the solubilization method. Briefly, 0.2 mL of blood was added to 1 mL of Solvable™ (aqueous based solubilizer, PerkinElmer, Waltham, MA) in a glass scintillation vial and incubated at 60^{o} C for 1 h. 0.1 mL of 0.1 M EDTA-di-sodium solution was added to reduce foaming due to hydrogen peroxide. 30% hydrogen peroxide (0.5 mL) was added in 0.1 mL increments and gently agitated after each addition to allow reaction and foaming to subside. Hydrogen peroxide treatment helps to reduce the amount of color present, and thus reduces color quench in the final mixture. The mixture was allowed to stand for 30 min at room temperature to complete the reaction and was incubated in a water bath at 60^{o} C for 1 h, venting any slight pressure occasionally. The sample was then cooled to room temperature and 15 mL of liquid scintillation cocktail Ultima Gold™ (Perkin Elmer, Waltham, MA) was added. The samples were light adapted for one hour before counting the radioactivity using a scintillation counter.

Serum and Urine: The amount of radioactivity in serum and urine was determined by the direct addition method. To 0.1 mL of serum or 0.5 mL of urine, 1.5 mL of ethanol and 15 mL of liquid scintillation cocktail Ultima Gold™ was added in a glass scintillation vial and shaken well. The samples were light adapted for at least one hour before scintillation counting.

Organs and Muscle Tissue: Organs (liver, kidney, spleen, lung, heart and brain) and muscle samples were prepared by the solubilization method for the determination of accumulated radioactivity. 50 mg of organs or muscle tissue was weighed in triplicate into a scintillation vial and 2 mL of Solvable™ was added and heated on a water bath at 60^{o}C for 1 - 3.5

h with occasional swirling. After cooling to room temperature, 0.2 mL of 30% hydrogen peroxide was added in two aliquots of 0.1 mL, with swirling between additions, and the mixture was allowed to subside any reaction between additions of the hydrogen peroxide. The mixture was heated again at 60^{o}C for 30 minutes for complete decolorization. 10 mL of liquid scintillation cocktail Ultima Gold™ was added, and the samples were light adapted for at least one hour before scintillation counting.

Feces: The amount of radioactivity excreted in feces was determined by sample combustion with a sample oxidizer (PerkinElmer Model 307 Sample Oxidizer, Waltham, MA) and the recovered ^{3}H was analyzed by scintillation counting. Briefly, feces specimens of each animal collected at different time intervals were combined together and homogenized with 4 volumes of water. Triplicate samples from each animal of approximately 300 mg of homogenate was placed in the sample oxidizer and combusted in an oxygen rich atmosphere to oxidize the tritium presented in the samples to water ($^{3}H_2O$). The water was separated into a scintillation vial and mixed with 10 mL of scintillation cocktail Monophase-S™ (PerkinElmer, Waltham, MA). The samples were light adapted for at least one hour before scintillation counting.

References

1. Lee RE, Qi J, Hurdle JG, Meibohm B, Vaddady PK, Liu J. Spectinamides as anti-tuberculosis agents. US Patent & Trademark Office No. 8,685,978.