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Research Article

Syntheses of radiolabeled ABT-578 for ADME studies

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Summary

Several isotopomers of ABT-578 (**I**, **II**, **III**, and **IV**) were prepared starting from different labeled precursors: [5'-^{3}H]-tetrazole (**1**), [5'-^{14}C]-tetrazole (**2**), [40-^{3}H]rapamycin (**3**), and [2,11,31-^{3}H]rapamycin (**4**). It was shown that the tritium label at the 40 position of rapamycin is lost during an attempted synthesis of [40-^{3}H]ABT-578 (**III**). Copyright © 2006 John Wiley & Sons, Ltd.

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Key Words: tritium; labeling; ABT-578; rapamycin; ADME

Introduction

ABT-578 is a patented analog of rapamycin and is the active drug ingredient in Abbott's drug-eluting stent device. ABT-578 is more lipophilic than rapamycin so it has the significant advantage of tending to stay at the site of action longer. Since it is a new chemical entity, radiolabeled ABT-578 was needed for animal ADME and human AME studies. Initially, we had it labeled in the tetrazole moiety with both ^{3}H and ^{14}C, but an unacceptably large loss of label in the animal ADME studies was found. Following this, the previously described syntheses of [^{14}C] and [40-^{3}H] rapamycin[1,2] were considered. The [^{14}C] synthesis[1] employs a microbiological process from a complex [^{14}C]precursor which, in turn, must be made by a multi-step synthesis. After careful

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assessment we concluded that this method was prohibitively lengthy and costly.

The somewhat lengthy and low-yielding synthesis of [40-^{3}H]rapamycin (**3**) was carried out[2]. More significantly, the tritium label was lost from the triflate intermediate required to make it. The successful alternative to these was making the [^{3}H]-labeled ABT-578 from [2, 11, 31-^{3}H]rapamycin (**4**) that was outsourced to the International Isotope Clearing House (IICH) to be prepared via a proprietary catalytic exchange method at the Institute of Molecular Genetics of the Russian Academy of Sciences ((IMG RAS) from Moscow, Russia. The tritium NMR analysis of both the labeled rapamycin and the final product showed $\sim$65% at 2, $\sim$20% at 11, and $\sim$15% at 31 position. The animal ADME and human AME studies with [2,11,31-^{3}H]ABT-578 (**IV**) gave excellent results ($<$5% loss of tritium) proving that tritium could also be a viable label to be used in human AME studies.

Results and discussion

[5'-^{3}H]Tetrazole (**1**) and [5'-^{14}C]-tetrazole (**2**) were prepared as shown below (Figure 1). An attempt to prepare [40-^{3}H]ABT-578 (**III**) failed due to the loss of the tritium label from the triflate intermediate (Figure 2) as shown by a comparison of the radio and mass (UV) HPLC traces of the reaction mixture (Figure 3).

[2,11,31-^{3}H]rapamycin (**4**) was made by direct isotope exchange of rapamycin with tritiated water.[3] It's subsequent conversion to the triflate went smoothly and in high yield as shown by the radio and mass (UV) traces of the crude reaction mixture (Figure 4):

Although many by-products (the isomer where condensation occurs on the N-2 position of the tetrazole moiety is the main one) were formed in converting this triflate to (**IV**) the mass (UV) HPLC trace was comparable to that obtained with unlabeled ABT-578. Also, the radio and mass (UV) percentages of the various products were very similar (Figure 5):

In summary, the synthesis of four isotopomers of radiolabeled ABT-578 were attempted, three of which were successful. However, only the isotopomer (**IV**) proved suitable for the required animal ADME and human AME studies (Figure 6).

Experimental

[5'-^{3}H]Tetrazole (**1**), [5'-^{14}C]tetrazole (**2**) (both from GE Healthcare Amersham), [40-^{3}H]rapamycin (**3**) (made at Abbott Laboratories), and [2,11,31-^{3}H]rapamycin (**4**, specific activity of $>$2 Ci/mmol, IICH, IMG RAS) were used as labeled precursors. All solvents and liquid reagents except the triflic anhydride were dried over activated 3 Å molecular sieves prior to use.

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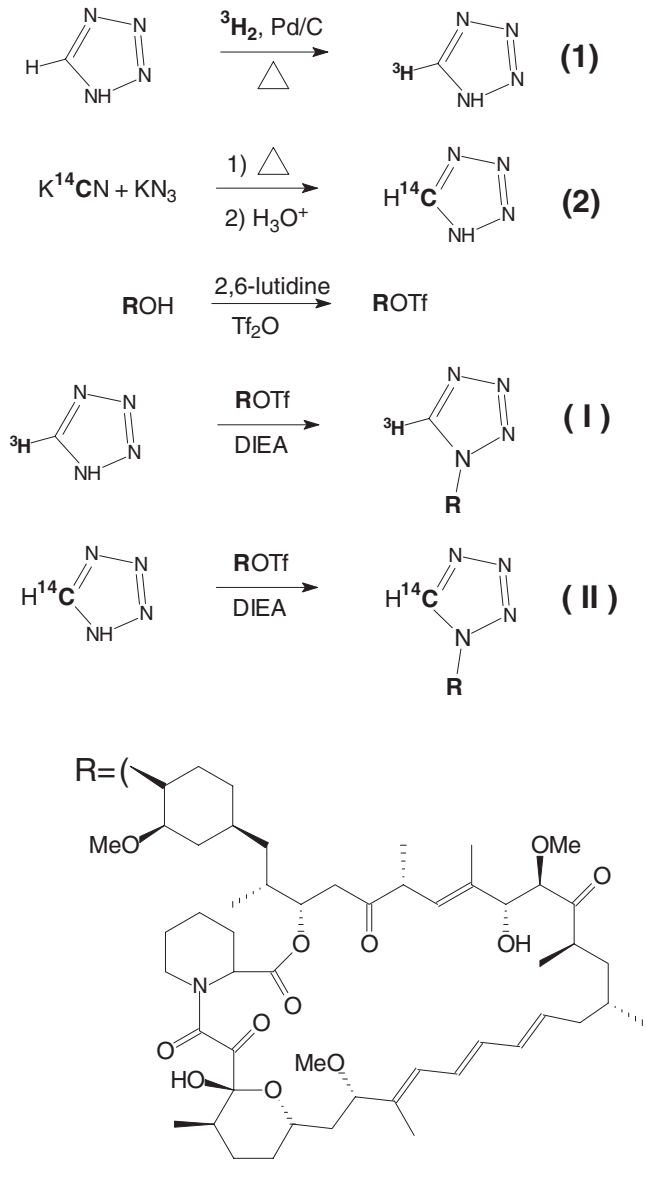

Figure 1. Syntheses of [5'-^{3}H] and [5'-^{14}C] tetrazoles (1 and 2) and their reaction with unlabeled rapamycin triflate

*Preparation of [2,11,31-^{3}H]ABT-578 (***IV***)*

A solution of [2,11,31-^{3}H]rapamycin (**4**, diluted to ~0.2 Ci/mmol, 50 mg, 0.0547 mmol) in acetonitrile (~10 ml) was concentrated *in vacuo* to a volume of ~1 ml then transferred to a 5 ml septum sidearm flask which was attached to a vacuum line. The solution was evaporated to dryness *in vacuo* on the line

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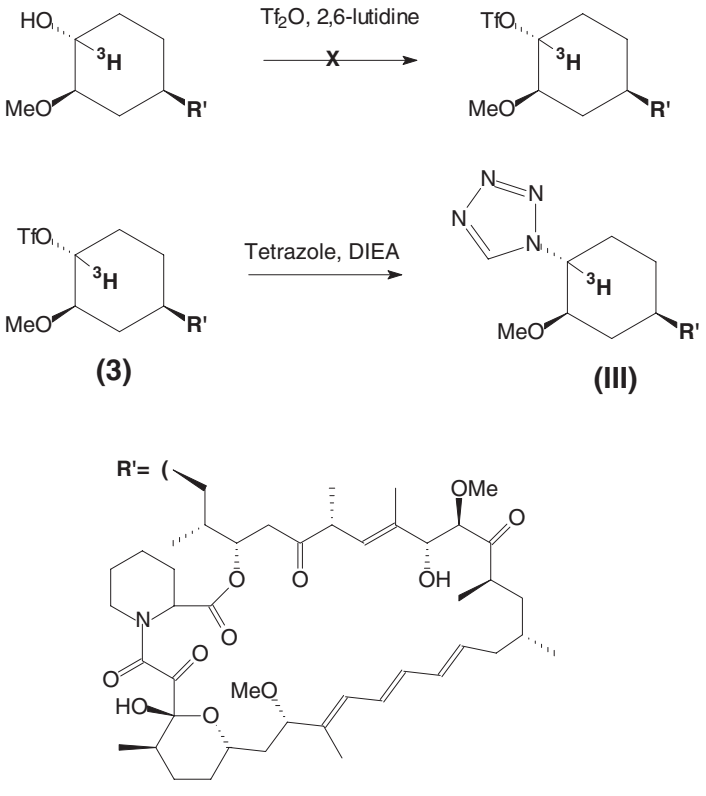

Figure 2. Attempted synthesis of [40-^{3}H]ABT-578 (III) from [40-^{3}H]rapamycin (3)

and the residue dried under high vacuum for ~½ hour. Toluene (1 ml) was added via syringe and the resulting solution was evaporated to dryness. This step was repeated one more time with toluene and then with methylene chloride (1 ml). The residue was taken up in methylene chloride (2 ml). The reaction mixture was then put under an atmosphere of dry nitrogen. To the stirred solution cooled to −30 °C 2,6-lutidine (24 µl, 0.206 mmol) was added via syringe. Triflic anhydride (freshly distilled, 13 µl, 0.077 mmol) was added via syringe over a 3 min period. The radio-HPLC analysis [from 20% THF/heptane to 80% THF in 35 min then 5 min hold, on YMC Pack-Sil (5 µm, 4.6 × 250 mm) at 1 ml/min, 276 nm UV detection] showed ~86% conversion of the (**4**) to the [^{3}H]triflate. A solution of tetrazole (19 mg, 0.27 mmol) in di-isopropylethyl amine (72 µl, 0.414 mmol) and methylene chloride (0.2 ml) was added via syringe over a 5 min period. The reaction mixture was warmed to 25–27°C and kept there for 30 h. Radio-HPLC analysis (as described above) showed ~40% conversion to (**IV**) (specific activity of 195 mCi/mmol). BHT (2,6-di-t-butyl-4-hydroxytoluene, 0.25 mg) inhibitor was added along with 5.8 ml of methylene chloride in a 10 ml 'V' vial and stored at −70°C.

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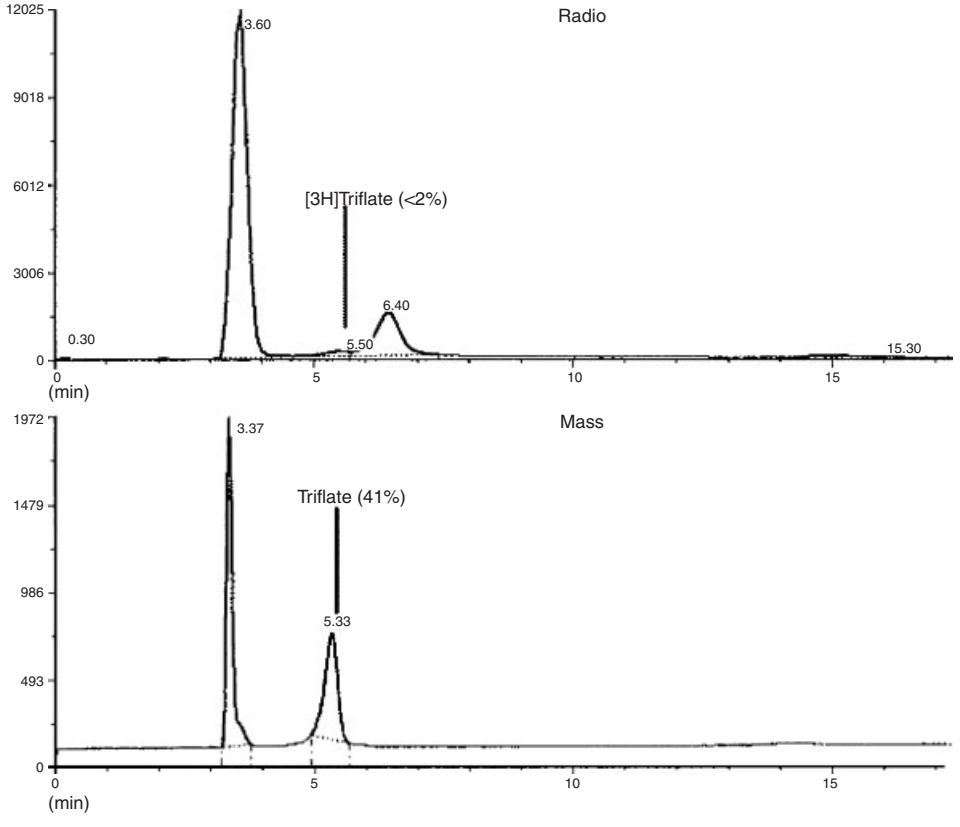

Figure 3. Radio/Mass(UV) HPLC trace of the reaction mixture of Tf_2O with [40-^{3}H]rapamycin (3)

The preparations of (**I**) from [5'-^{3}H]tetrazole (**1**) and preparation of (**II**) from [5'-^{14}C] tetrazole (**2**) were carried out in a similar way. Unlabeled precursor was used in five-fold excess in these reactions.

Purification of [^{3}H]ABT-578 by preparative HPLC

The normal phase preparative HPLC system consisted of a Waters HPLC pump (LC8-A), Shimadzu UV detector (SPD-10Avp) with a high flow rate preparative flow cell and Shimadzu fraction collector (FRC-10A). Two lots of crude [^{3}H]ABT-578 prepared using the method described in the previous section were combined. A portion (about 4 ml) of the combined sample was injected onto a YMC silica column (5 μm, 250 mm × 21.2 mm ID) each time (Figure 1). The mobile phase consisted of a gradient of 20% B to 65% B in 30 min, where mobile phase A was heptane and mobile phase B was THF. The flow rate was set at 20 ml/min, and the UV wavelength of the detector was set at 278 nm. Fractions were collected from 26 to 30 min. The combined collected

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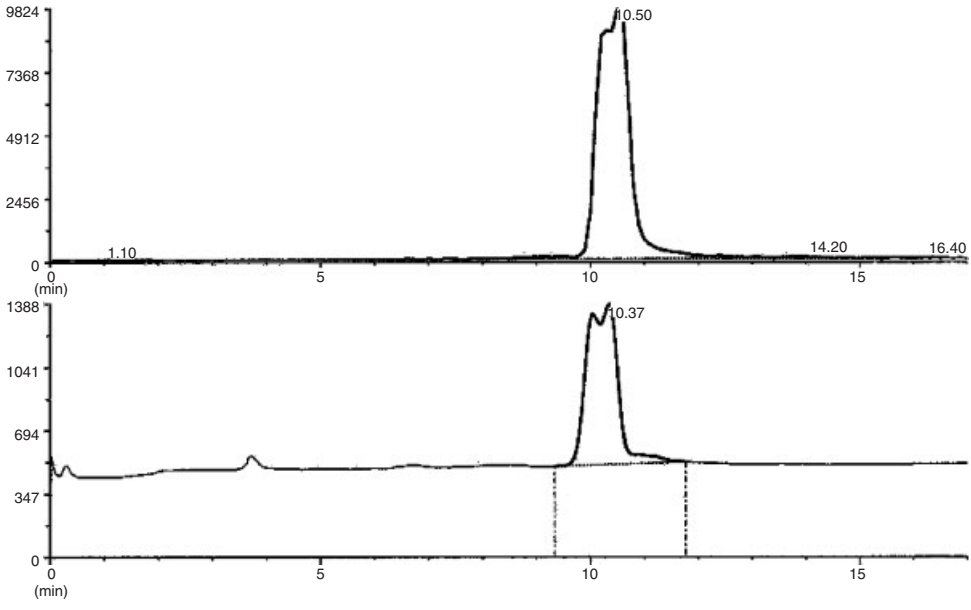

Figure 4. Radio/Mass HPLC trace of the reaction mixture of Tf_2O with [2,11,31-^{3}H]rapamycin (4)

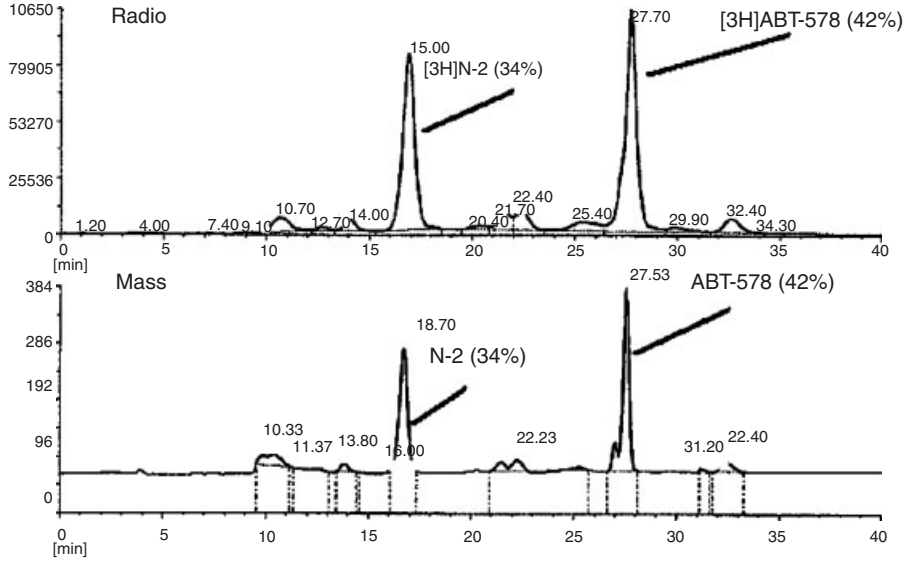

Figure 5. Radio/Mass HPLC trace of a crude reaction mixture from a synthesis of [^{3}H]ABT-578 (IV)

fractions were temporarily stored over dry ice. After the entire sample had been consumed, the combined fractions were evaporated *in vacuo*. The product was dissolved in 10 ml of acetone and stored at −20°C before further

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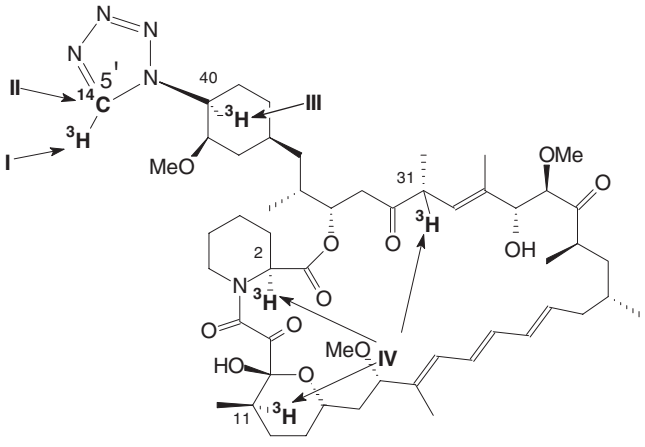

Figure 6. Positions of labeling of ABT-578 for ADME/AME studies

purification by reverse phase HPLC. The reverse phase preparative HPLC system was an Agilent 1100 series HPLC system consisting of a quaternary pump, autosampler, UV detector and fraction collector.

The [^{3}H]ABT-578 acetone solution after normal phase purification was dried *in vacuo*. About 3 ml of a 1:1 mixture of CH_3CN/NH_4OAc buffer (0.01 M, pH = 4.0) was added to dissolve the [^{3}H] ABT-578. A portion (0.3 ml) of the sample above was injected onto a Phenomenex Luna C18(2) column (5 μm, 250 mm × 10 mm ID). The mobile phase consisted of a gradient of 55% B to 80% B in 28 min, where mobile phase A was NH_4OAc buffer and mobile phase B was acetonitrile. The concentration of mobile phase B was increased to 95% in 2 min and held at 95% for 6 min after the gradient. The column was kept at 45°C by using a column heater. The UV wavelength was set at 278 nm and the flow rate at 4 ml/min. Fractions were collected and temporarily stored over dry ice. After the entire sample had been consumed, the combined fractions were evaporated *in vacuo*. The product was dissolved in 5 ml of acetone. The total radioactivity was assayed by liquid scintillation counting as 4.66 mCi. In order to be consistent with the bulk drug manufacturing process, 0.5% w/w BHT was added to the product. BHT (11.15 mg) was accurately weighed in a vial and dissolved in 10 ml of acetone. Using a pipette, 0.102 ml of this BHT solution was added to the [^{3}H]ABT-578 for storage.

Determination of purity and specific activity

[2,11,31-^{3}H]ABT-578 (**IV**) was analyzed using an Agilent 1100 series HPLC system consisting of a quaternary pump, an autosampler and a photodiode array UV detector. A Packard Radiomatic A500 radioactivity detector was

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connected to the HPLC system. A 500 µl flow cell and a 3:1 ratio of Ultima-Flo M scintillation cocktail to HPLC mobile phase were used. The analyses were performed using a Zorbax Eclipse XDB-C8 (3.5 µm, 75 mm × 4.6 mm ID). The mobile phase consisted of a gradient of 50% B to 60% B in 20 min, where mobile phase A is NH_4OAc buffer (0.01 M, pH = 4.0) and mobile phase B is acetonitrile. The flow rate was set at 1 ml/min and the UV wavelength at 278 nm. The column was kept at 45°C by using a column heater. The radiochemical purity of [^{3}H]ABT-578 (**IV**) was found to be > 99.8% (includes ~7–10% of the oxepin isomer of slightly longer retention time). The specific activity was determined to be 195.1 mCi/mmol by measuring the mass and radioactivity concentrations of [^{3}H]ABT-578 (**IV**) dissolved in mobile phase. The concentration of radioactivity was determined by liquid scintillation counting of an accurately measured aliquot. The mass concentration was measured by comparing the HPLC UV peak area of an accurately measured aliquot to a standard sample preparation.

Acknowledgements

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