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^{3}H-LABELING OF PROKINETIC MOTILIDE ABT-229 FOR BIODISTRIBUTION AND METABOLISM STUDIES

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SUMMARY

The prokinetic drug candidate, ABT-229, has been successfully [^{3}H]-labeled in the macrolactone ring. This was accomplished with [^{3}H]-$NaBH_4$ reduction of the 11-ketone analog in a four step synthetic sequence beginning with the drug candidate. The ^{3}H-labeled drug was obtained with specific activity of 9.0 Ci/mmol and radiochemical purity >99%. This constitutes the first methodology for ^{3}H-labeling of the macrolactone in an erythromycin derivative.

KEY WORDS: tritium, ABT-229, motilin, motilide, prokinetic, erythromycin

INTRODUCTION

ABT-229, 8,9-anhydro-4"-deoxy-3'-N-desmethyl-3'-N-ethylerythromycin B-6,9-hemiacetal[1] **1** is a new gastroprokinetic motilin agonist which is currently entering phase II clinical trials. The compound has potential utility in the treatment of gastrointestinal motility disorders such as gastroesophageal reflux disease, diabetic gastroparesis, and post-operative ileus. As development of the drug candidate has progressed, a radiolabeled analog was required for both *in vitro* and *in vivo* metabolism studies.

The synthetic sequence for compound **1** (Scheme1)[1] presents several opportunities for incorporation of a radiolabel. Use of tributyltin tritium during the deoxygenation at C-4" of

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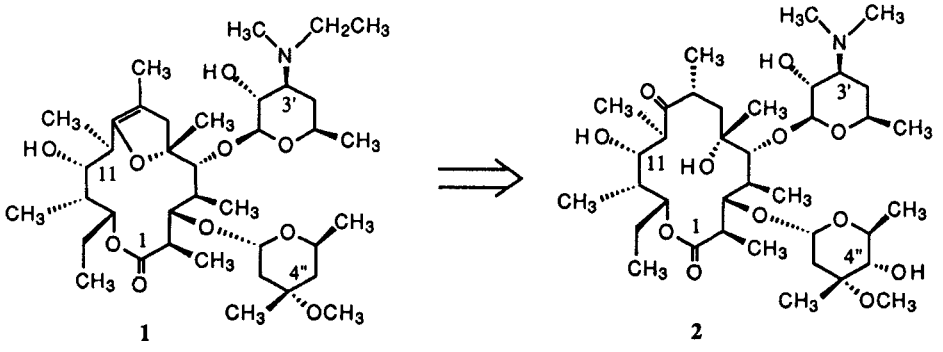
erythromycin B **2** would lead to labeling of the neutral sugar moiety (cladinose). However, that sugar is acid sensitive and will make such a compound an unsuitable substrate for metabolic studies[2,3], as **1** is an orally delivered drug. A second possible site in the synthetic scheme for introduction of a label is the 3'- N-alkylation step. However extensive metabolism studies in the area of erythromycin related antibiotics has shown N-dealkylation to be a major metabolic pathway [4,5]. A third alternative, would be the preparation of a ^{14}C erythromycin B aglycon. By applying techniques similar to those used in the biosynthesis of erythromycin A[6], a labeled precursor could be supplied as substrate to the erythromycin B producing organism. This approach was abandoned due to the lengthy, complex nature of the process, and limited potential for requisite specific activity. We present here a stereoselective method to label the macrolactone of **1** with a tritium *via* an efficient sequence of oxidation followed by reduction with [^3H]-$NaBH_4$. To our knowledge, this is the first report of incorporation of tritium into the backbone of any member of the erythromycin family.

Scheme 1



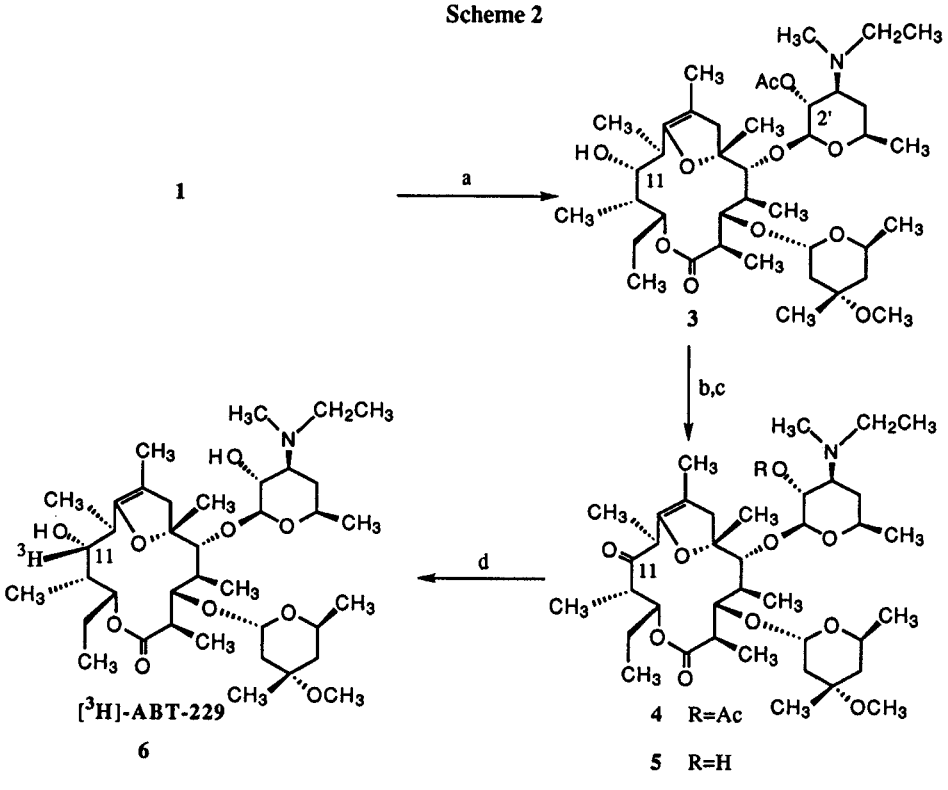
1 **2**

RESULTS AND DISCUSSION

The synthesis of [^3H]-ABT-229 is shown in Scheme 2. Compound **1** was prepared according to published procedures[1]. The 2'-OH of **1** was selectively protected with an acetyl group to provide **3** in excellent yield. Oxidation of the 11-OH *via* the Ley[7] procedure gave compound **4** in 65% yield. After a brief treatment of **4** with methanol, the ketone **5** was obtained in excellent yield. The signal at 207.48 ppm in the ^{13}C NMR and the absence of H-11 signals in the ^1H NMR clearly

indicated the formation of ketone **5**. The latter was treated with [^{3}H]-$NaBH_4$ in ethanol to give the final compound **6**, with the desired stereochemistry at C-11 as the only observed 14-member macrolide product reduced at C-11. In cold experiments, a minor component with a yet to be determined structure was observed, possibly a ring-contracted product.

Scheme 2



(a) Ac_2O / CH_2Cl_2; (b) TPAP / NMO/ 4 A Sieves / MeCN; (c) MeOH; (d) NaB^3H_4/ EtOH

The use of ethanol is of great importance for the outcome of the reduction because of the instability of ketone **5** in solvents such as THF or isopropyl alcohol. Compound **6** was purified by semi-preparative HPLC, to give [^{3}H]-ABT-229 with a specific activity of 9.0 Ci/mmol and radiochemical purity > 99%. In conclusion, [^{3}H]-ABT-229 was obtained with yield, chemical and radiochemical purities, and specific activity sufficient for utilization in *in vitro* and *in vivo* biodistribution and metabolism studies. These studies are currently underway in our laboratory.

EXPERIMENTAL

NMR spectra were recorded on a GE QE300 at 300 MHz for ^{1}H and 75.48 MHz for ^{13}C with chemical shifts in ppm downfield from an internal TMS standard. Coupling constants are in Hz. Mass spectra were recorded with a Finnigan SSQ700 instrument. All solvents were either distilled or were of analytical reagent quality. The progress of all reactions leading up to the labeling step was monitored by TLC on E. Merck precoated silica gel (0.2 mm layer) plates containing a fluorescent indicator. Detection was first by UV (254 nm) and then by charring with a solution of ammonium molybdate tetrahydrate (12.5 g) and cerium sulfate tetrahydrate (5.0 g) in 10% aqueous sulfuric acid (500 mL). Flash chromatography was performed using silica gel (230-400 mesh, Merck). [^{3}H]-Sodium borohydride was obtained from DuPont/NEN, USA. To determine the extent of the labeling reaction and to purify the product a Gilson HPLC system with a Rheodyne injector (500 µL loop), an IN/US radioactivity flow detector and an ABI UV/VIS (205 nm) detector was used. To determine the radiopurity and specific activity of the purified product, a Hitachi HPLC system, including auto injector and UV detector (205 nm) was used together with a Radiomatic radioactivity flow detector. For both systems YMC Basic C8 columns (5µm, 250 x 4.6 mm, 250 x 20 mm preparative) were used. The mobile phase consisted of phosphate buffer (0.05M KH_2PO_4, pH 6.1: CH_3CN: MeOH, 47:43:10) at a flow rate of 1 mL/min (analytical) and 16 mL/min (preparative).

2'-O-Acetyl-8,9-anhydro-4"-deoxy-3'-N-desmethyl-3'-N-ethylerythromycin B -6,9-hemiacetal (3)

Compound **1** (3.01 g, 4.31 mmol) was dissolved in CH_2Cl_2 (23 mL) under N_2. Acetic anhydride (0.8 mL, 8.5 mmol) was added *via* syringe. The mixture was stirred for 4h at room temperature, diluted with CH_2Cl_2 (150 mL) and extracted sequentially with saturated $NaHCO_3$, H_2O, and brine (40 mL each). The organic phase was dried over Na_2SO_4 and solvent removed *in vacuo* to give an off-white solid which was purified by chromatography (0.5% NH_4OH / 5% MeOH in $CHCl_3$) to afford 2.60g (81%) of **3**: $[\alpha]_D$ -30.0 (c 1.0, $CHCl_3$); mp 110°C; ^{1}H NMR ($CDCl_3$) δ 0.82 (d, J=7.0, 3H), 0.90 (t, J=6.8, 3H), 0.93 (d, J=8.0, 3H), 0.98 (t, J=7.0, 3H), 1.06 (d, J=3.0, 3H), 1.10 (t, J=6.0, 3H), 1.15 (s, 3H), 1.16 (d, J=6.0, 3H), 1.18 (d, J=8.0, 3H), 1.21 (d, J=5.0, 3H), 1.25 (m, 3H), 1.35 (s, 3H), 1.45 (m, 2H), 1.60 (m, 3H), 1.60-1.71 (m, 4H), 1.92-2.00 (m, 2H), 2.01 (s, 3H), 2.20 (s, 3H), 2.21-2.75 (m, 5H), 3.28 (s, 3H), 3.40 (m, 1H), 3.58 (m,

1H), 3.42 (d, J=7.0, 1H), 3.95 (t, J=5.0, 1H), 4.32 (m, 1H), 4.68 (d, J=7.0, 1H), 4.73 (dd, J=10.0, J=7.0, 1H), 5.10 (m, 1H), 5.18 (d, J=6.0, 1H); MS m/e 740 ($M+H^+$). Anal. Calcd for $C_{40}H_{69}NO_{11}$: C, 64.92; H, 9.39; N, 1.89. Found: C, 64.61; H, 9.40; N, 1.89.

8,9-Anhydro-4"-dideoxy-3'-N-desmethyl-3'-N-ethyl-11-oxoerythromycin B-6,9-hemiacetal (5)

Compound **3** (0.250 g, 0.338 mmol) was dissolved in 10 mL dry acetonitrile with powdered activated 4Å molecular sieves (0.255 g), and N-methylmorpholine-N-oxide (0.082 g, 0.700 mmol) followed by tetrapropylammonium perruthenate (TPAP, 0.013 g, 0.037 mmol). The resulting dark green suspension was stirred overnight under N_2. The acetonitrile was evaporated from the black mixture and the resulting black paste was suspended in ethyl acetate and run through a plug of silica gel, eluting with additional ethyl acetate. The ethyl acetate solution was concentrated under reduced pressure to yield 0.208 g (83%) of a colorless glass **4**. Methanol was added to the 2'-acetyl derivative and the solution stirred overnight. The methanol solution was concentrated under reduced pressure resulting in 0.175 g of colorless glass (90%). This glass was purified by flash chromotography (0.5% NH_4OH / 5% MeOH in $CHCl_3$) to give 0.15 (65%) of **5** as a white solid. $[\alpha]_D$ -37.2 (c 0.7, $CHCl_3$); mp 90-93°C; ^{1}H NMR ($CDCl_3$) δ 0.85 (t, J=7.0, 3H), 1.10 (d, J=7.0, 3H), 1.15 (t, J=7.0, 3H), 1.19 (d, J=7.0, 3H), 1.20 (t, J=7.0, 3H), 1.21 (d, J=8.0, 3H), 1.23-1.53 (m, 9H), 1.55 (s, 3H), 1.67 (s, 3H), 1.72-1.99 (m, 8H), 2.21 (s, 3H), 2.30-2.77 (m, 9H), 3.20 (dd, J=10.0, J=8.0, 1H), 3.25 (s, 3H), 3.62 (m, 1H), 3.92 (m, 2H), 4.31 (m, 1H), 4.49 (d, J=8.5, 1H), 4.91 (d, J=6.5, 1H), 5.45 (dd, J=9.0, J=5.0, 1H); ^{13}C NMR ($CDCl_3$) δ 8.82, 9.47, 10.20, 12.11, 12.19, 13.86, 14.86, 21.19, 21.40, 23.89, 25.80, 26.00, 29.63, 33.52, 36.21, 41.67, 42.24, 43.21, 43.39, 45.94, 47.12, 47.58, 49.35, 61.06, 64.85, 68.34, 70.51, 70.79, 74.25, 75.43, 79.41, 86.65, 95.57, 102.66, 104.15, 146.19, 176.35, 207.48; MS m/e 696 ($M+H^+$). Anal Calcd for $C_{38}H_{65}NO_{10}.0.5H_2O$: C, 65.58; H, 9.41; N, 2.01. Found: C, 64.74, H, 9.43; N, 1.98.

8,9-Anhydro-4"-deoxy-3'-N-desmethyl-3'-N-ethyl-[11-^{3}H]-erythromycin B-6,9-hemiacetal (6).

[^{3}H]-$NaBH_4$ (500 mCi, 56.8 Ci/mmol, 0.0088 mmol) was cooled to 0°C and a solution of the ketone **5** (3.0 mg, 0.0044 mmol) in anhydrous ethanol (300 μL) was slowly added with stirring.

The resulting solution was stirred for 3h at 0°C then allowed to slowly warm to room temperature overnight. The excess borohydride was quenched with 5% KH_2PO_4 / 1% K_2HPO_4 (1 mL, pH 6.1) and the mixture was partitioned between water and CH_2Cl_2. The water layer was washed twice with CH_2Cl_2 and the organic layers were combined, washed with water, dried over Na_2SO_4, filtered, and concentrated to yield 200 mCi. The residue was dissolved in mobile phase (0.05 M KH_2PO_4: MeCN: MeOH, 47:43:10, pH 6.1) and C-8 HPLC analysis of this mixture indicated the presence of desired compound **6**. The crude mixture was purified by HPLC wherein the identified tritiated product co-eluted with unlabeled ABT-229. The overall radiochemical yield of [^{3}H]-ABT-229 was 3.0%. Analysis of isolated **6** by radio-HPLC showed it to be >99% radiochemically pure. Mass spectrometry confirmed the identity of **6** as it gave m/e 698 for **1** and m/e 700 for **6**. Integration indicates 31% labeled compound giving a specific activity of 9.0 Ci/mmol.

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