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Research Article

Synthesis of radio- and stable-labelled Fasidotril†

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Introduction

Fasidotril (Figure 1) is a vasopeptidase inhibitor currently under development for treatment of hypertension. Radiolabelled Fasidotril was required for ADME and whole-body autoradiography studies. Stable-labelled Fasidotril (with an appropriately high number of labelled mass units) was also needed as an LC/MS internal standard for use in bioanalytical assays. Details of the synthesis of [^{14}C]Fasidotril (with carbon-14 labelling in the benzylic position of the piperonyl portion of the molecule), and stable-labelled Fasidotril (with deuteriums situated in the benzyl ester moiety) are described below.

Attempted bromination for tritium–halogen interchange

Initially, we envisioned preparing tritium-labelled Fasidotril via a tritium–halogen interchange of a brominated analog of the drug substance. It was thought that the methylene dioxophenyl ring of the drug substance would offer suitable aromatic positions for facile bromination. Unfortunately, attempted bromination of Fasidotril gave none of the desired bromo-analog. Instead, the thiol acetyl group was unstable under all bromination conditions tried, affording mixtures of deactylated products and disulfur-containing dimers.

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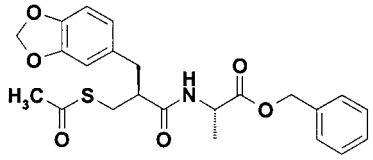
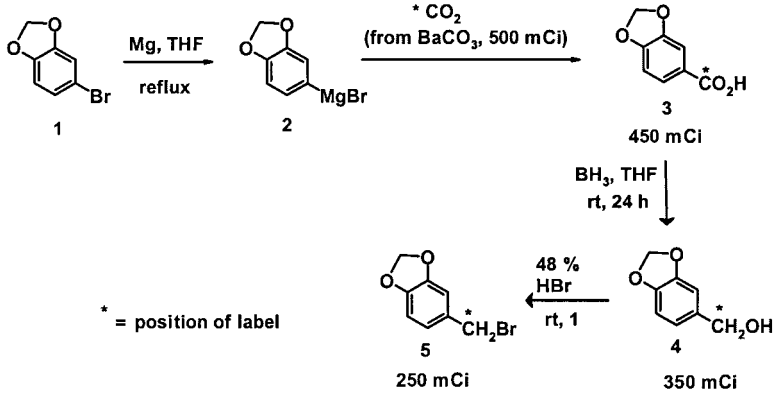


Figure 1.

Synthesis of [^{14}C]Fasidotril

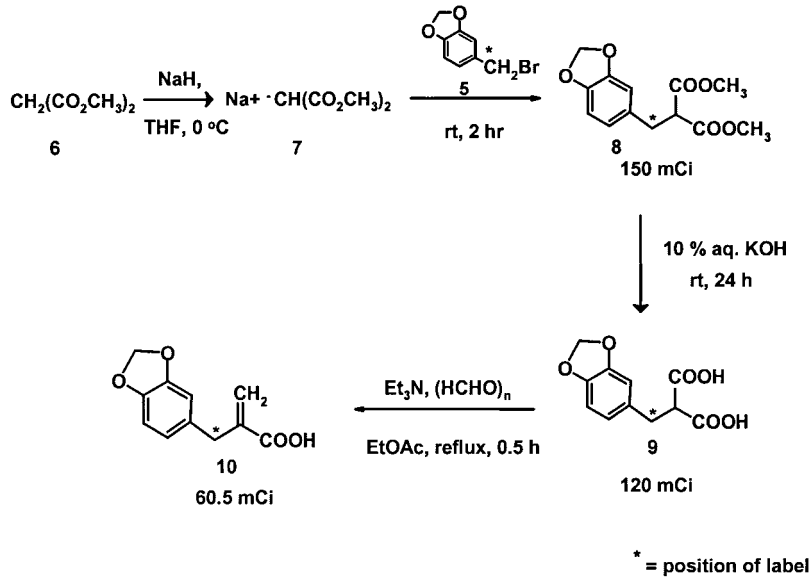
Since tritium–halogen interchange of the drug substance was not a viable option, we developed a synthesis of carbon-14 labelled Fasidotril which was accomplished in 11 steps.

Synthesis of the key precursor [^{14}C]piperonylacrylic acid (10, Schemes 1 and 2). [^{14}C]Piperonylacrylic acid (**10**) was a key intermediate in the radiosynthesis. It was synthesized following the route displayed in Schemes 1 and 2. Initially, benzylbromide **5** (Scheme 1) was prepared starting with 5-bromo-1,3-benzodioxazole (**1**) which reacted with magnesium in THF to form the Grignard reagent **2**. Reagent **2**, without isolation, was treated with $^{14}CO_2$ generated from $Ba^{14}CO_3$ to furnish acid **3**. Reduction of the carboxylic group of **3** afforded piperonyl-α-*^{14}C*-alcohol (**4**). Substitution of the hydroxyl group with bromine was achieved by treatment of **4** with a 48% aqueous solution of HBr yielding **5**.



Scheme 1. Synthesis of [^{14}C] benzyl bromide (5)

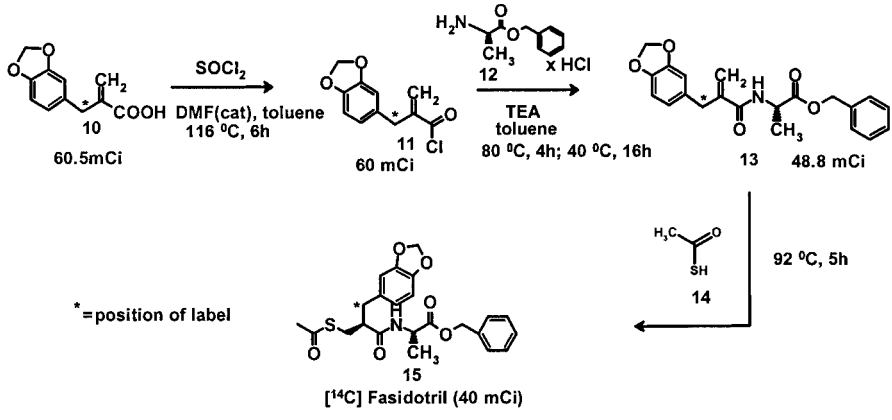
Carbanion **7** (Scheme 2), which was generated from malonic acid (**6**), reacted with **5** to furnish **8**. Hydrolysis of the ester groups was achieved by treatment of **8** with a 10% aqueous solution of KOH forming diacid **9**. Finally, [^{14}C]Piperonylacrylic acid (**10**) was obtained by the reaction of **9** with



Scheme 2. Synthesis of piperonyl acrylic acid (10)

formaldehyde in the presence of triethylamine. Overall, 60.5 mCi of **10** was obtained starting from 500 mCi of [^{14}C] barium carbonate.

Completion of the synthesis of [^{14}C] Fasidotril (15, Scheme 3). The synthesis of labelled Fasidotril was completed in four steps as shown in Scheme 3. [^{14}C]Piperonylacrylic acid (**10**) was converted to acid chloride (**11**) by treatment with thionyl chloride in DMF. The resulting [^{14}C]Piperonylacryloyl chloride reacted with L-alanine phenylmethyl ester (**12**) in the presence of triethylamine in toluene, affording ester **13**. Treatment of **13** with thiolacetic



Scheme 3. Completion of the synthesis of [^{14}C]Fasidotril

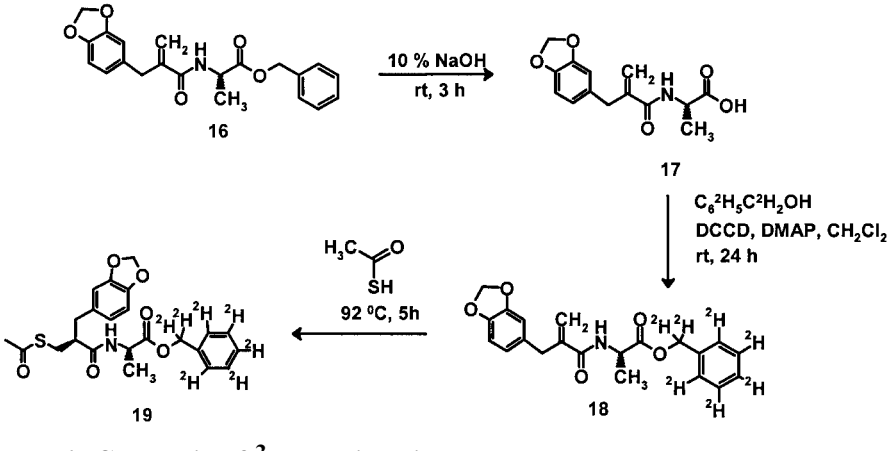
acid (**14**) afforded crude **15** (via a stereoselective Michael addition), which was purified by crystallization from isopropyl alcohol yielding 40 mCi of [^{14}C]Fasidotril.

Analysis of the diastereomeric purity of [^{14}C] Fasidotril by HPLC. In order to establish the enantiomeric excess associated with the second chiral center formed from the reaction of thiolacetic acid with **13**, a reverse phase chiral HPLC method was developed. This method utilized an acetonitrile/water mobile phase with a Chiralcel AS-RH column to maximise the separation of the diastereomers. Using this method, the diastereomeric purity of the desired (S,S) labelled drug substance was established as $>97\%$.

Synthesis of [$^{2}H_7$]Fasidotril

Attempted synthesis via direct hydrolysis and reesterification. Initially, it was envisioned that [$^{2}H_7$] Fasidotril could be directly synthesized from unlabelled Fasidotril by first hydrolyzing the ester groups, followed by reesterification with labelled benzyl alcohol affording the $^{2}H_7$ benzyl group and replacing the acetyl group. This approach was unsuccessful since the resulting acid after base hydrolysis (containing the free thiol) was extremely unstable and dimerized during work-up.

Preparation of [$^{2}H_7$]Fasidotril (Scheme 4). The synthesis of [$^{2}H_7$]Fasidotril was achieved (starting from intermediate **16**) in three steps. The benzyl group of **16** was removed by stirring a solution of **16** in methanol with 10% aqueous NaOH at room temperature for three hours. After purification, the resulting acid **17** was tested for the absence of any benzyl alcohol. Acid **17** was then converted to the [$^{2}H_7$]benzyl ester by stirring with [$^{2}H_7$]benzyl alcohol in the



Scheme 4. Synthesis of $^{2}H_7$-Fasidotril

presence of 1,3-dicyclohexylcarbodiimide and 4-(dimethylamino)pyridine in methylene chloride at room temperature for 24 h. Finally, the labelled ester was converted to [$^{2}H_{7}$]Fasidotril (**18**) via reaction with thiolacetic acid at 90 °C for 5 h.

Conclusions

1. The vasopeptidase inhibitor Fasidotril with carbon-14 labelling was efficiently synthesized in an 11-step synthesis starting with [^{14}C]barium carbonate.
2. Deuterium-labelled Fasidotril suitable for use as an MS internal standard was prepared via a three-step synthesis starting from a readily available intermediate.
3. The intrinsic instability of the thiol acetyl group can offer considerable synthetic challenge in the synthesis of isotopically labelled compounds containing this group. Thiol acetyl groups are often unstable to hydrolysis and/or halogenation and the resulting free thiol groups readily dimerize. Therefore, these common reactions may need to be circumvented when designing a successful syntheticroute for labelled thiol acetyl-containing drug substances.

