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Proton exchange reactions in isotope chemistry (II) synthesis of stable isotope-labeled LCQ908†

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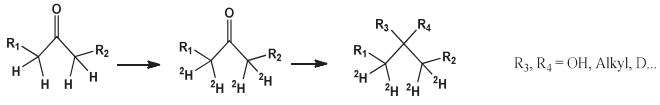
The proton exchange reaction was applied to the preparation of stable isotope-labeled LCQ908. For this synthesis, a suitable intermediate with protons alpha to a carbonyl group was first subjected to the H–D exchange reaction; subsequent coupling of a carbonyl group with [$^{13}C_2$]triethyl phosphonoacetate, followed by hydrogenation and hydrolysis, led to the stable labeled compound. Incorporation of two carbon-13 atoms in the molecule eliminated the presence of undesired M+0.

Keywords: deuterium; exchange; stable isotope

Introduction

Proton exchange reactions on target compounds have been widely used in the preparation of tritium-labeled products[1] but only with limited success in the synthesis of stable labeled materials suitable for bio-analytical studies. Among the reasons are the lack of multiple exchangeable protons in the target molecules, the wide molecule ion mass distribution, and the presence of significant amounts of M+0 due to the incomplete exchange of protons and the presence of labile protons. On the other hand, there are many synthetic intermediates in the synthesis of a particular compound, and some of those intermediates may present opportunities for the introduction of multiple deuterium atoms, for example, a carbonyl group with alpha protons that can be easily replaced by deuterium. But the challenge lies on how to stabilize the newly introduced deuterium because these exchange sites may be labile. One of our strategies is to remove/inactivate those functional groups used in exchange reactions so that those sites are no longer labile:



We have previously reported the direct deuterium exchange approach to labeling target compounds[2] and would like to report the successful application of this new labeling strategy to the synthesis of stable isotope-labeled LCQ908.

Results and discussion

LCQ908 is a novel, small-molecule inhibitor of lipid synthesis that is highly effective in reducing diet-induced weight gain in preclinical models and is under development for the treatment of type-2 diabetes. Stable isotope-labeled LCQ908 was required for bio-analytical studies. Initial experiments with direct H–D exchange reactions failed to provide stable isotope-labeled LCQ908 with mass units greater than or equal to M+3, and we turned our attention to the synthesis of a stable isotope-labeled LCQ908 starting from either carbon-13-labeled or deuterium-labeled commercial starting materials.

The non-labeled LCQ908 is prepared by a linear multistep synthesis (Figure 1).[3] Three key intermediates were identified as potential starting materials for the synthesis of stable isotope-labeled LCQ908: 4-(4-hydroxyphenyl)-cyclohexanone **A**, 2,5-dibromopyridine **B**, and 3-amino-6-trifluoromethylpyridine **C**.

The preparation of carbon-13-labeled **A** can be achieved from [$^{13}C_6$]phenol through a four-step sequence.[4] Carbon-13 labeled **B** can be prepared[5] from [^{13}C]acrylic aldehyde and [^{13}C]acetaldehyde through pyridine[6] and 2-aminopyridine.[7] Carbon-13-labeled compound **C** can also be prepared[8] from [^{13}C]acrylic aldehyde and [^{13}C]acetaldehyde through pyridine,[6] 2-aminopyridine,[7] 2,5-dibromopyridine,[5] and 6-trifluoromethylpyridine-3-carboxylic acid.[9] Routes to **B** and **C** are very long and contain low yields. While deuterium-labeled **B** or **C** can be achieved from relatively inexpensive [$^2H_4$] 2-aminopyridine through similar multistep syntheses, the deuterium on the pyridine ring could be lost during the synthesis because of the harsh reaction conditions.

Compound **A** contains a carbonyl group that allows four protons to be easily exchanged with deuterium. Furthermore, this carbonyl functionality will be replaced by an alkyl group in subsequent reactions, thereby converting the labile deuterium into stable ones (Figure 2). While this looked attractive, it would be an eight-step synthesis, and this was deemed too long both for the overall synthesis and for the reaction steps involving isotopes.

We then decided to reduce the number of reaction steps involving isotopes by starting the reaction from new intermediate **1**. Based upon our past experiences, deuterium exchange reactions do not go to completion, which leaves the final compound with undesired M+0 species in the mass

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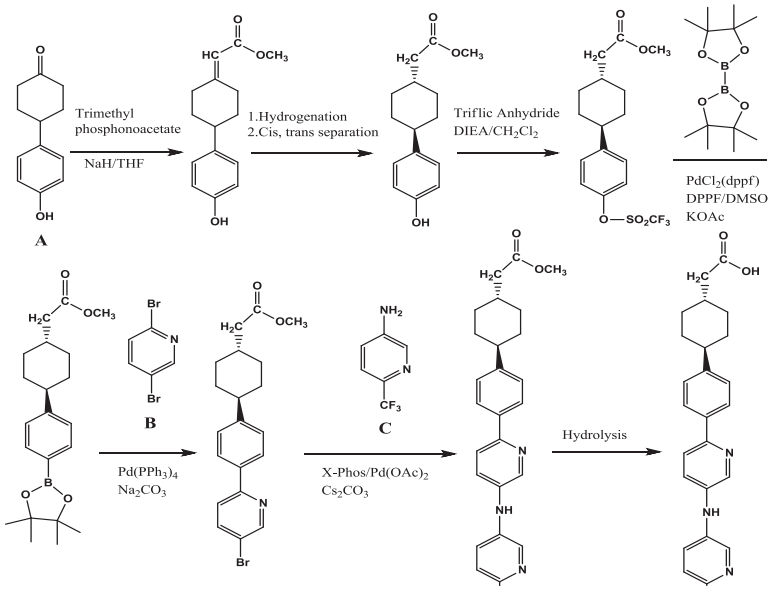

Figure 1. Synthesis of unlabeled LCQ908.

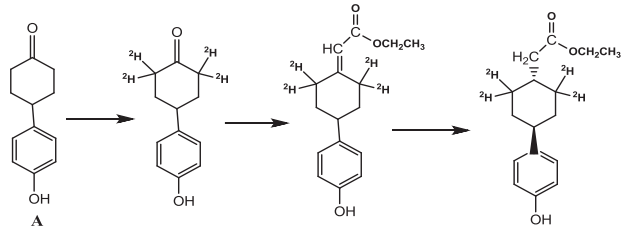

Figure 2. Proposed synthesis of stable labeled intermediate.

spectrum. To avoid this, we decided to incorporate a [$^{13}C_2$] unit into the molecule as well (Figure 3).

Thus, a mixture of ketone **1** in deuterium methoxide containing a catalytic amount of deuterium chloride was refluxed for 6 h. Solvent removal and neutralization with sodium deuteroxide afforded the [$^{2}H_4$]ketone **2** (92%). Mass spectrum analysis showed the presence of M+0 species (0.6%). We decided to carry the material on to the next step because we will eliminate the M+0 species in subsequent reactions. The coupling of **2** with [$^{13}C_2$] triethyl phosphonoacetate yielded olefin **3** in 64% yield. Hydrogenation of compound **3** generated a mixture of *cis* and *trans* isomers that were separated by preparative HPLC. Hydrolysis of the desired *trans* isomer **4** afforded [$^{2}H_4$,$^{13}C_2$]LCQ908 in an overall 22% yield. Although we observed slight deuterium loss during the reactions, mass analysis of [$^{2}H_4$,$^{13}C_2$]LCQ908 showed no detectable M+0 and M+1 species (Figure 4).

Experimental

^{1}H-NMR spectra were recorded on a 400-MHz Bruker (Bruker Biospin, Bellrica, MA) NMR spectrometer. Chemical shifts are reported in parts per million relative to TMS (tetra methyl silane). Hydrogenation reaction was carried out on a HEL (Lawrenceville, NJ) Automate Reactor System with pressure control. The LC-MS (Liquid Chromatography-Mass Spectroscopy) analysis was performed on an LCQ-Fleet (ThermoScientific, Madison, WI) ion trap mass spectrometer coupled with a Waters (Waters Corp. Milford, MA) Acquity UPLC system with the following conditions: Waters BEH C18, 1.7 μm, 2.1 × 100 mm; mobile phase A, 0.1% TFA

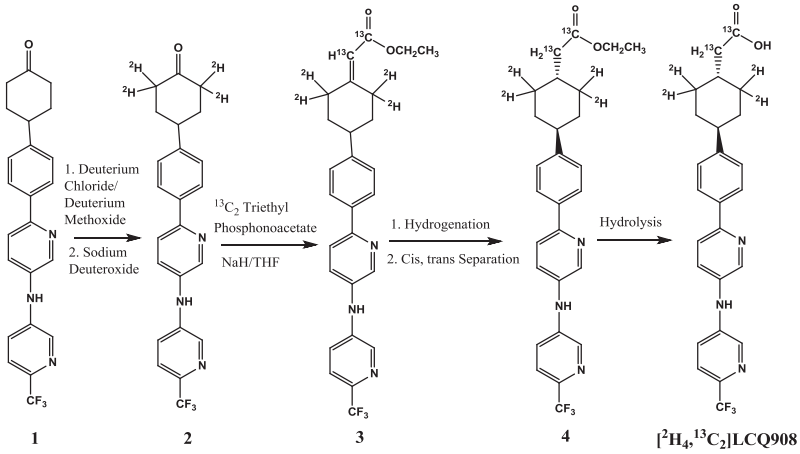

Figure 3. Synthesis of stable labeled LCQ908.

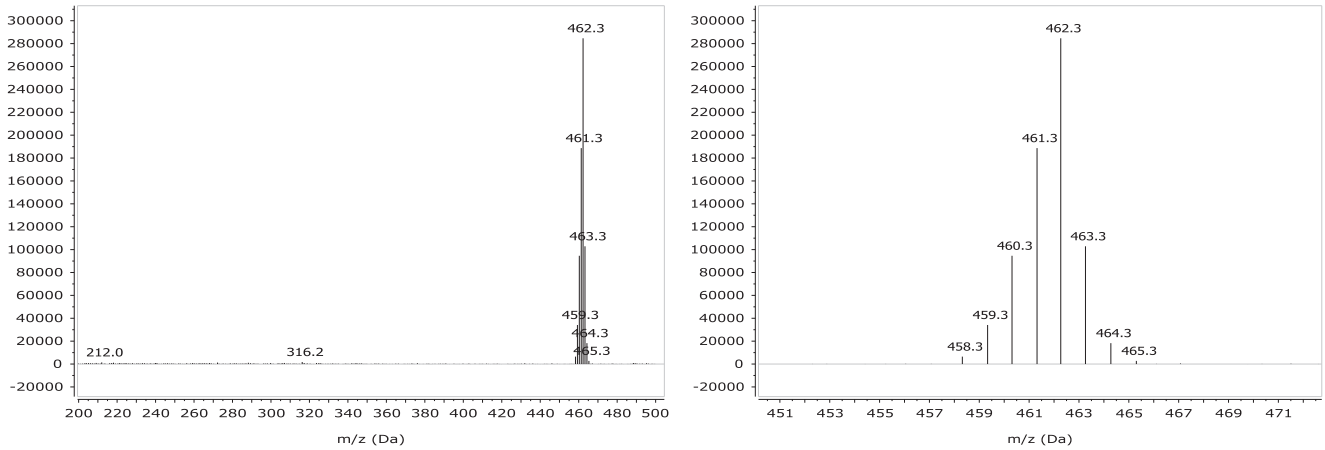

Figure 4. Mass spectrum of stable labeled LCQ908.

(Trifluoroacetic Acid) in water; mobile phase B, acetonitrile; linear gradient: hold 1 min at 90% A–10% B and then ramping to 10% A–90% B in 5 min; 0.5 mL/min; 254 nm. Mass: positive ionization with zoom scan. Prep-HPLC conditions: Cosmosil Cholester, 5 μm, 20 × 250 mm; mobile phase: 80% methanol–20% water; 20 mL/min isocratic; 328 nm. Flash chromatography was conducted on an Analogix BSR system with an Analogix (Madison, WI) SF25-40 g silica gel cartridge (Sepra SI50), eluted with hexane/EtOAc (3/1). The starting material 4-(4-(5-((6-(trifluoromethyl)pyridin-3-yl)amino)pyridin-2-yl)phenyl)cyclohexanone **1** was purchased from Wuxi Pharm. (Wuxi, China), and all other chemicals and solvents were reagent grade obtained from Sigma Aldrich (St. Louis, MO) without further purification. Supporting documents are provided and can be obtained.

[$^2H_4$]4-(4-(5-((6-Trifluoromethyl)pyridin-3-yl)amino)pyridin-2-yl) phenyl)cyclohexanone 2

4-(4-(5-((6-(Trifluoromethyl)pyridin-3-yl)amino)pyridin-2-yl)phenyl)cyclohexanone **1** (600 mg, 1.46 mmol) was taken up in CH_3OD (30 mL) containing DCl (0.2 mL), and the solution was heated to reflux and stirred for 6 h. Excess solvent was removed under reduced pressure. CH_3OD (10 mL) was added and evaporated to dryness. The residue was dried under vacuum for 2 h and then taken up in hexanes. The suspension was filtered, and the filter cake dried under vacuum for 2 h. The resulting solid (the DCl salt of **2**) was taken up in a mixture of EtOAc (20 mL) and 7% sodium deuteroxide (sodium deuteroxide in deuterium oxide, 5 mL) and stirred for 10 min. Layers were separated, and the organic layer was washed with deuterium oxide and then dried over sodium sulfate. Solvent removal afforded 560 mg of **2** (92%). ^{1}H-NMR ($CDCl_3$) δ 1.98 (m, 2H), 2.26 (m, 2H), 3.10 (m, 1H), 6.7 (br. s, 1H), 7.38 (d, 2H, *J* = 7.8 Hz), 7.51 (m, 1H), 7.58 (m, 1H), 7.67 (m, 1H), 7.75 (m, 1H), 7.95 (d, 2H, *J* = 7.8 Hz), 8.48 (s, 1H), 8.66 (s, 1H). MS (ESI): *m/z* 412 (M+0, 0.6%), 413 (M+1, 5.5%), 414 (M+2, 20.9%), 415 (M+3, 57%), 416 (M+4, 100%), 417 (M+5, 20.3%), 418 (M+6, 0.6%).

[$^2H_4$,$^{13}C_2$]Ethyl 4-(4-(5-((6-(trifluoromethyl)pyridin-3-yl)amino) pyridin-2-yl)phenyl)-cyclohexylidene)acetate 3

[$^{13}C_2$]Triethylphosphonoacetate (1.0 g, 4.42 mmol) in THF (3 mL) was added dropwise over 10 min to a suspension of 60% sodium hydride (222 mg, 5.55 mmol) in THF (20 mL) at 0 °C. The reaction mixture was stirred at room temperature for 60 min and then cooled to 0 °C before a solution of compound **2** (560 mg, 1.35 mmol) in THF (10 mL) was added dropwise over 30 min. The mixture was stirred for 3.5 h at room temperature, cooled to 0 °C, and quenched with deuterium oxide (7 mL). A mixture of ethyl acetate (10 mL) and hexanes (20 mL) was added, and the layers were separated. The organic layer was dried over sodium sulfate and then concentrated. The residue was purified by flash chromatography to afford 420 mg of **3** (64%). ^{1}H-NMR (DMSO-d_6) δ 1.21 (t, 3H, *J* = 7.1 Hz), 1.56 (m, 2H), 2.01 (m, 2H), 2.86 (m, 1H), 4.09 (m, 2H), 5.71 (d, 1H, *J* = 160 Hz), 7.34 (d, 2H, *J* = 7.8 Hz), 7.62 (m, 1H), 7.72 (m, 2H), 7.89 (d, 1H, *J* = 7.8 Hz), 7.96 (d, 2H, *J* = 7.8 Hz), 8.47 (s, 1H), 8.54 (s, 1H), 9.19 (s, 1H, NH). MS (ESI): *m/z* 484 (M+2, 0.5%), 485 (M+3, 8.3%), 486 (M+4, 27.2%), 487 (M+5, 55%), 488 (M+6, 100%), 489 (M+7, 39%), 490 (M+8, 8.5%).

[$^2H_4$,$^{13}C_2$]*trans*-Ethyl 4-(4-(5-((6-(trifluoromethyl)pyridin-3-yl) amino)pyridin-2-yl) phenyl) cyclohexyl) acetate 4

Olefin **3** (420 mg, 0.86 mmol) and 10% Pd/C (234 mg) in ethyl acetate (20 mL) were stirred under 27 psi hydrogen atmosphere for 3 days. The mixture was filtered through a thin layer of celite and concentrated. The *cis* and *trans* isomers were separated by preparative HPLC to afford 201 mg of the *trans* isomer (48%) and 105 mg of the *cis* isomer (25%).

^{1}H-NMR (DMSO-d_6) δ 1.2 (t, 3H, *J* = 7.2 Hz), 1.48 (m, 2H), 1.8 (m, 3H), 2.23 (dm, 2H, *J* = 128 Hz), 2.5 (m, 1H), 4.07 (m, 2H), 7.32 (d, 2H, *J* = 7.8 Hz), 7.6 (m, 1H), 7.7 (m, 2H), 7.89 (d, 1H, *J* = 8.6 Hz), 7.95 (d, 2H, *J* = 7.8 Hz), 8.46 (s, 1H), 8.54 (s, 1H), 9.19 (s, 1H, NH). MS (ESI): *m/z* 486 (M+2, 1.4%), 487 (M+3, 12.8%), 488 (M+4, 21%), 489 (M+5, 62%), 490 (M+6, 100%), 491 (M +7, 39.5%), 492 (M+7, 7%).

[$^2H_4$,$^{13}C_2$]LCQ908

The *trans*-ester **4** (201 mg, 0.41 mmol) was taken up in THF (4 mL), sodium hydroxide (93 mg, 2.3 mmol) in water (1 mL) was added, and the mixture was stirred at 50 °C for 12 h and then at room temperature for 3 days. Solvent was removed, and the residue was taken up in water (5 mL), acidified with 1N HCl, and extracted with EtOAc (3 × 20 mL). The combined EtOAc extracts were washed with water and dried over Na_2SO_4. Solvent removal and drying under vacuum afforded 147 mg of product (78%). ^{1}H-NMR (DMSO-d_6) δ 1.49 (m, 2H), 1.74 (m, 1H), 1.83 (m, 2H), 2.16 (dm, 2H, *J* = 127 Hz), 2.51 (m, 1H), 7.33 (d, 2H, *J* = 7.8 Hz), 7.71 (m, 3H), 7.9 (d, 1H, *J* = 8.6 Hz), 7.96 (d, 2H, *J* = 7.8 Hz), 8.47 (s, 1H), 8.55 (s, 1H), 9.19 (br. s, 1H, NH), 12.01 (s, 1H). MS (ESI): *m/z* 458 (M+2, 2.5%), 459 (M+3, 10.3%), 460 (M+4, 30.4%), 461 (M+5, 71%), 462 (M+6, 100%), 463 (M+7, 39%), 464 (9%).

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